

EFFECTS OF MONTMORILLONITE-ENRICHED HYDROGELS ON LETTUCE MORPHOLOGY UNDER DROUGHT STRESS

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Abstract

Lettuce is a widely cultivated leafy vegetable that is sensitive to drought stress. Drought stress is a significant environmental factor that adversely affects the growth and development of lettuce. Montmorillonite-infused hydrogels are emerging to mitigate the effects of drought by reinforcing soil moisture retention and facilitating plant growth. This study investigates the impact of different montmorillonite-enriched hydrogel samples (0%, 0.25%, 0.5%, and 1% montmorillonite) on lettuce growth under irrigated (every 3 days) and drought-stressed (no watering) conditions. The study examined various morphological characteristics, including total plant length, leaf count, root length, root weight, root volume, stem diameter, and aerial biomass. The results revealed that the hydrogel formulation with the highest montmorillonite content (1%), produced the most favorable results under irrigated and drought-stressed conditions, promoting enhanced root development (11.90% under drought and 4.17% with irrigation compared to the control sample) and leaf production. The findings underline the potential of montmorillonite-enriched hydrogel formulations to improve lettuce growth under different water conditions, presenting a promising approach for sustainable approaches.

Key words: drought, hydrogel, *Lactuca sativa*, montmorillonite.

INTRODUCTION

Lettuce is a widely cultivated leafy vegetable sensitive to drought stress (Chaorlina et al., 2021). Drought is a significant environmental factor that adversely affects lettuce's growth and development, leading to stunted growth, reduced leaf size, and impaired root development (Paim et al., 2020).

In recent years, hydrogels have attracted substantial attention for their potential applications in sustainable agricultural practices (Mazloom et al., 2020).

Hydrogels are polymeric materials that can absorb and retain large amounts of water, which makes them useful for various agricultural applications, such as water-holding agents, controlled-release carriers for nutrients and pesticides, and soil amendments to improve water-holding capacity and nutrient availability (Yu et al., 2017).

Montmorillonite-infused hydrogels are emerging to mitigate the effects of drought by reinforcing soil moisture retention and facilitating plant growth (Uddin, 2018).

The incorporation of montmorillonite, a clay mineral with high water absorption capacity,

into hydrogel formulations has shown promising results in enhancing the water retention properties of the hydrogels and improving plant growth under water-limited conditions (Montesano et al., 2015).

Hydrogels with different montmorillonite contents may have varying effects on the morphological characteristics of lettuce under irrigated and drought-stressed conditions. On lettuce, as with many crops, identifying optimal hydrogel formulations can help improve plant performance and yield under different water regimes. The effects on the lettuce morphology with varying montmorillonite-enriched hydrogels remain to be thoroughly explored.

Root length, volume, and weight are crucial indicators of a plant's ability to access water and nutrients (Zhang et al., 2020). Changes in these parameters under drought stress can reveal the effectiveness of hydrogels in promoting root development. Drought stress often leads to reduced leaf area, affecting photosynthesis and overall plant growth. Measuring leaf area, length, width, and the number of leaves can provide insights into the hydrogel's impact on mitigating these effects (Seleiman et al., 2021). Stem diameter and height can be indicators of

overall plant vigor. (Kim et al., 2010). Measuring both above-ground (aerial) and below-ground (root) biomass can provide a comprehensive assessment of plant growth (Madec et al., 2017).

Previous studies have examined the effects of hydrogels on various crops, including lettuce, but the specific impact of montmorillonite-enriched hydrogels on lettuce morphology under drought stress remains understudied. The objective of this study was to investigate the effects of montmorillonite-enriched hydrogels on the morphological characteristics of lettuce under irrigated and drought-stressed conditions.

MATERIALS AND METHODS

HYDROGEL SYNTHESIS

The hydrogel samples utilized in this study were synthesized at the National Institute for Laser, Plasma and Radiation Physics, Măgurele, employing the electron beam radiation method with potassium persulfate as the activator. Four distinct hydrogel formulations were developed, varying in their compositions of sodium alginate, acrylic acid, polyethylene oxide, and montmorillonite at concentrations of 0%, 0.25%, 0.5%, and 1%, respectively, as presented in Table 1.

Table 1. Sample codification used in this study

Sample code		Montmorillonite dose (%)
Bead Hydrogel	Granular Hydrogel	
Hb1	Hg1	0
Hb2	Hg2	0.25
Hb3	Hg3	0.5
Hb4	Hg4	1
C		Control sample with no hydrogel

Two methods of hydrogel administration were evaluated in this study: granular form (Hg) and beads (Hb). Each hydrogel composition was applied in 10 replicates, with the weight of a single hydrogel bead maintained at 0.2 g, which was also the weight used for the granular form. The hydrogels were incorporated into 50 g of soil, and 100 mL of water was added to each sample to facilitate the swelling of the polymeric material.

Biological material

The lettuce (*Lactuca sativa*) seedlings placed in alveolar tray were acquired from VDRS Buzău (Figure 1).



Figure 1. Lettuce seedling

Working protocol

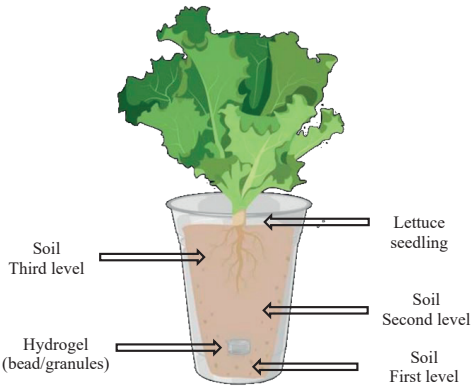


Figure 2. Working protocol graphic illustration
Created with BioRender.com

For this experiment, cups were used, in which soil (first level) and the hydrogel samples as beads (a) and granules (b), weighing approximately 0.2 g (Figures 2 and 3) were placed.

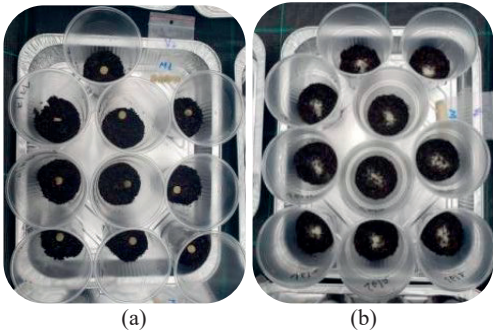


Figure 3. Application of hydrogel: (a) Beads and (b) Granules

The second level of soil was then added, and hydration was carried with 100 ml water/each sample to assure the maximum swelling degree of the hydrogels. Further, after 24h, lettuce seedlings were transplanted, and the third level of soil was then added (Figure 4). Ten repetitions for each sample were prepared.



Figure 4. Lettuce seedlings in cups with hydrogels

After 3 weeks, the seedlings were transplanted from cups into pots (Figure 5).



Figure 5. Lettuce plants in pots with hydrogels

The samples were monitored for approximately 5 weeks, after which the seedlings were transplanted from pots into a greenhouse setting (Figure 6). The lettuce samples were transplanted according to a predetermined protocol with 2 equidistant rows established with a drip irrigation system. The samples were planted in the soil at a distance of approximately 50 cm, with 4 replications of each sample at the level of each row. One row consisted of lettuce samples maintained under irrigated conditions throughout the experimental period. The other with lettuce plants maintained for 1 week under irrigation conditions, after which drought conditions were imposed by shutting off the irrigation system for the entire experimental period.



Figure 6. Lettuce plants in greenhouse

MORPHOLOGICAL CHARACTERISTICS ANALYSIS

TOTAL PLANT LENGTH

The method involved measuring with a ruler at 7-day intervals to determine the total plant length. The measurement consisted of determining the distance from the soil level to the total length of the tallest leaf.

The mature size of lettuce (*Lactuca sativa*) can vary depending on the cultivar and growing conditions. Mature lettuce plants might reach a height of about 15-30 cm (Concepcion et al., 2020).

LEAF COUNT

The total number of leaves was determined over 40 days. The counting of lettuce leaves was carried out after removing any dried leaves.

Quantifying the total number of leaves on lettuce plants offers insights into the progress and density of the foliage. This metric aids in evaluating the overall health and vigor of the plant, as a greater leaf count often indicates enhanced growth and productivity (Singh et al., 2018).

ROOT LENGTH

The root length was determined by measuring the root system from the top of the root to the apical meristem.

The roots play a critical role in providing water and nutrients to the lettuce plant, and their morphological characteristics, such as length, volume, and weight, are key indicators of the plant's overall vigor and ability to withstand stress (Gregory & Kirkegaard, 2016).

ROOT WEIGHT AND AERIAL BIOMASS

After separating the root and aerial portions of the plants, which consisted of the stem and the rosette of leaves, the root and aerial biomass were determined using a technical balance.

The observed changes in root and aerial biomass under drought stress and the different hydrogel treatments can reveal the effectiveness of the hydrogels in supporting plant growth and development (Tomadoni et al., 2020).

ROOT VOLUME

The root volume was determined by immersing the root system in a graduated cylinder containing a known volume of water and calculating the root volume based on the difference in water level observed compared to the initial level.

The root volume is a useful indicator of the plant's ability to absorb water and nutrients from the soil, which is crucial for maintaining growth and productivity under limited water conditions (Raviteja et al., 2021).

STEM DIAMETER

The stem diameter was measured using a ruler following the separation of the aerial portion from the root system.

The stem diameter can provide insights into the overall vigor and health of the lettuce plant, as it reflects the plant's ability to transport water and nutrients effectively (Zhang et al., 2020).

RESULTS AND DISCUSSIONS

TOTAL PLANT LENGTH

All tested samples, including the control, exhibited relatively uniform total plant lengths (~40-42 cm) prior to the drought stress treatment, suggesting that the hydrogels did not significantly influence early plant growth.

The results demonstrated that the hydrogel bead samples consistently exhibited superior performance in supporting lettuce growth under both drought-stressed and irrigated conditions (Figure 7). Although the control sample performed well under irrigation, the **Hb1**, **Hg2**, and **Hb4** hydrogel formulations demonstrated superior results under drought conditions compared to the control sample. Consequently, the **Hb1** hydrogel can be considered the most suitable overall choice for promoting stable and

resilient growth in lettuce plants across varying water availability scenarios.

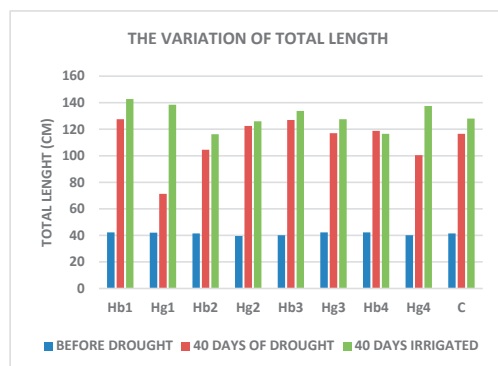


Figure 7. Total length of the lettuce samples after 40 days of testing

TOTAL NUMBER OF LEAVES

The results demonstrate that the hydrogel formulations, in both bead and granular forms, substantially enhanced lettuce leaf production (>50 leaves) under both drought-stressed and well-irrigated conditions. Importantly, the efficacy of the bead and granular hydrogel samples exhibited minimal variation, implying that both formulations were equally capable of supporting leaf development under environmental stress conditions. Considering the superior initial leaf count and sustained performance across both drought-stressed and well-irrigated conditions, the **Hb4** and **Hb2** samples could be considered the most dependable choices for reliably supporting abundant leaf production in lettuce plants under variable water availability conditions (Figure 8).

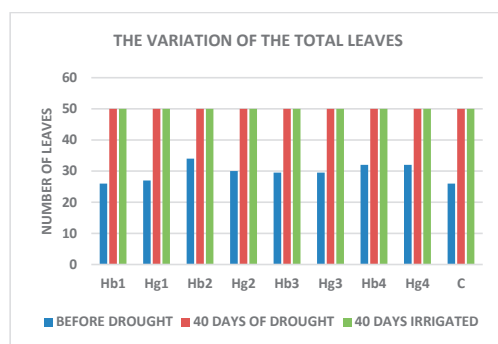


Figure 8. Total number of leaves of the lettuce samples after 40 days of testing

ROOT LENGTH

The results indicate that root length diminished across all samples under drought stress compared to the pre-drought period (Figure 9). The **Hb4** sample demonstrated the most effective performance, with a root length of 23.5 cm, outperforming the control samples at 21 cm under drought stress. This represents an 11.90% increase, suggesting enhanced root resilience under water-limited conditions. Furthermore, under irrigated conditions, the **Hb4** hydrogel maintained a root length of 25 cm compared to the control's 24 cm, indicating a 4.17% improvement with the hydrogel application. The **Hb4** sample consistently produced the longest root systems, providing the most effective support for root growth and development under both drought-stressed and irrigated conditions.

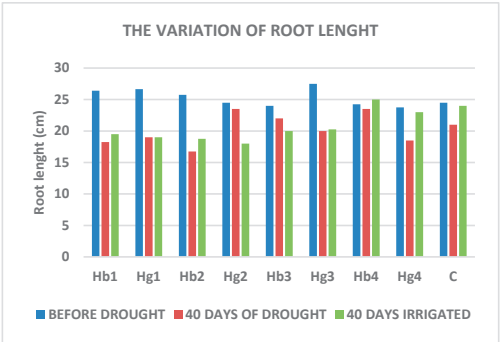


Figure 9. Root length of the lettuce samples after 40 days of testing

ROOT WEIGHT AND AERIAL BIOMASS

The lettuce sample **Hg1** exhibited the highest aboveground biomass of 265.23 g under drought stress, exceeding the control sample's 232.12 g by 14.26% (Figure 10). However, under irrigated conditions, the **control** sample attained the greatest aboveground biomass of 314.45 g, surpassing the 304.67 g achieved by the **Hg1** sample, a 3.11% decrease compared to the control. While the control demonstrated superior aboveground biomass production under optimal irrigation, the **Hg1** sample proved to be the most effective in supporting robust plant growth and biomass accumulation under water-limited conditions.

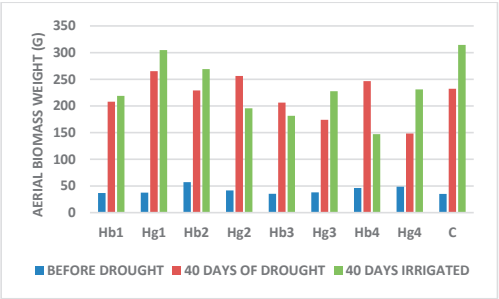


Figure10. Aerial biomass of the lettuce samples after 40 days of testing

The **Hg1** sample demonstrated the most favorable root weight performance under drought stress, with a root weight of 23.82 g, which exceeded the control sample's 20.35 g by 17.02% (Figure 11). Under irrigation, the **Hg1** hydrogel treatment exhibited the highest root weight performance, with a root weight of 30.43 g, which exceeded the control sample's 29.58 g by 2.87%. The **Hg1** sample demonstrated the most favorable performance, consistently surpassing the control sample in terms of root biomass under both drought-stressed and well-irrigated conditions. This suggests that the **Hg1** hydrogel formulation is the most effective choice for fostering resilient root growth under diverse water conditions.

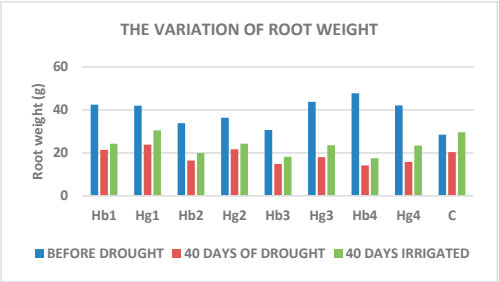


Figure 11. Root weight of the lettuce samples after 40 days of testing

ROOT VOLUME

For the root volume of the lettuce samples, the **Hg1** sample exhibited the most favorable root volume performance under drought stress conditions, with a root volume of 27.5 cm³, which surpassed the control sample at 20 cm³, representing a 37.5% increase (Figure 12).

However, under well-irrigated conditions, the **control** sample demonstrated the highest root volume at 30 cm³, exceeding the **Hg1**'s 25.3 cm³ by 15.67%. These results suggest that while the control sample provided superior root volume under optimal water availability, the **Hg1** sample was the most effective in enhancing root growth and resilience in drought-stressed environments.

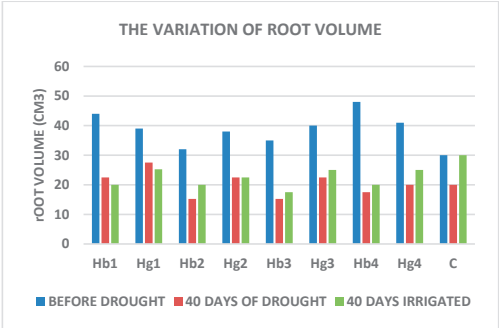


Figure 12. Root volume of the lettuce samples after 40 days of testing

STEM DIAMETER

The lettuce sample with hydrogel bead formulation **Hb2** exhibited the most favorable root diameter under drought stress, with a value of 1.6 cm compared to the control at 1.3 cm, representing a 23.08% improvement (Figure 13). Conversely, under irrigated conditions, the hydrogel granule **Hg1** demonstrated the highest root diameter at 1.5 cm, surpassing the control's 1.3 cm by 15.38%. Overall, **Hg1** provided the most consistent enhancement in root diameter across both drought and irrigation.

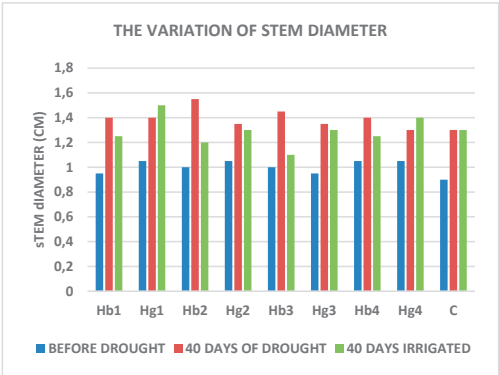


Figure 13. Stem diameter of the lettuce samples after 40 days of testing

CONCLUSIONS

The results of the study indicate that the incorporation of montmorillonite-enriched hydrogels into the soil substrate positively impacted the growth and development of lettuce plants under both drought stress and optimal irrigation conditions, outperforming the control samples.

The lettuce samples with hydrogel beads (**Hb4**) exhibited the most favorable outcomes for total plant length and root length and the number of leaves, suggesting their effectiveness in supporting overall plant development under water-limited conditions. **Hg1** hydrogel granules excelled in promoting aerial biomass, root weight, and root volume, demonstrating their capability to uphold root and biomass-related parameters in drought-stressed plants. Under consistent watering, the control samples generally outperformed the hydrogel treatments in terms of total plant length and root volume, indicating that regular water availability can be more beneficial for these morphological factors. However, the **Hg1** sample maintained the highest root weight and stem diameter among the treatments, suggesting their potential to enhance these specific characteristics even under optimal moisture conditions.

In summary, the **Hb4** and **Hg1** hydrogel formulations appear to be the most balanced and effective in supporting lettuce growth and resilience across varying water availability scenarios. While the control samples exhibited the best overall performance under irrigation, the hydrogel treatments, particularly **Hg1**, demonstrated the capacity to consistently improve key morphological parameters, highlighting their value in enhancing the sustainability and productivity of lettuce cultivation, especially in regions prone to drought.

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