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EFFECT OF MONTMORILLONITE BASED HYDROGELS APPLICATION ON MORPHOLOGICAL CHARACTERISTICS OF LETTUCE SEEDLINGS (*LACTUCA SATIVA*)

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Abstract

The agricultural sector and food production have witnessed persistent expansion in the past few decades. This occurrence has resulted in the excessive utilization of intensive production methodologies, leading to the unsustainable depletion of soil nutrients and water resources. Numerous research endeavors have been undertaken with the aim of examining the influence of hydrogels on the enhancement and optimization of agricultural inputs. The aim of this study is to assess the impact of four different compositions of hydrogels based on montmorillonite on the morphological characteristics of lettuce seedlings (Lactuca sativa) subsequent to the transplanting procedure, in the greenhouse conditions. The effect of the tested hydrogels on the development of the plants was regularly monitored through evaluation of the overall height, number of leaves and the relative content of chlorophyll. The results show no noticeable differences for the height parameters of the samples, meanwhile the total number of leaves and relative content of chlorophyll proved to be significantly higher for the samples cultivated using hydrogels compared to the control samples.

Key words*: hydrogels, lettuce, montmorillonite, morphological parameters.*

INTRODUCTION

Agriculture in the contemporary era encounters numerous obstacles such as the growing population, the reduction of cultivable land, and the swift process of urbanization. Consequently, the development of advanced technologies has become an essential requirement, leading to a transition from traditional farming methods to safeguarded cultivation, specifically soilless cultivation (Thomas et al., 2021).

Lactuca sativa, commonly referred to as lettuce, is a plant of considerable economic value in terms of human consumption on a global scale. This plant is cultivated primarily for its palatable flavour, its abundant nutritional content, and its potential medicinal properties. Lettuce serves as a crucial ingredient in the creation of various dishes, such as salads, soups, and vegetable curries, and serves as a vital source of phytonutrients (Güzel Murat Erdem, 2021). Furthermore, it has been extensively investigated for its pharmacological capabilities, which include its ability to combat microbial agents, its antioxidant properties, its capacity to protect neurological health, and its potential sedative effects (Naseem & Ismail, 2022). Additionally, lettuce contains indispensable components, such as vitamins, minerals, and organic substances (Shi et al., 2022). Beyond its culinary and medicinal applications, lettuce has garnered considerable attention in the realm of biotechnology and the process of *in vitro* regeneration (Farina et al., 2021).

The results obtained by Ekka et al. (2022) imply that the integration of hydrogel and inorganic manure in the cultivation of lettuce has the potential to augment the development and output of lettuce crops. The outcomes of the investigation offer significant knowledge for maximizing lettuce production in agroforestry systems based on citrus, which could potentially result in enhanced economic gain and ecological durability in lettuce farming.

Hydrogels have been used in the field of agriculture for the past five decades, demonstrating their efficacy as a reservoir for water retention and a facilitator of nutrient mobilization within the soil. The use of hydrogels composed of superabsorbent polymers has become prevalent in agricultural industry due to their significant contributions towards soil improvement, enabling plant growth in arid regions, and facilitating the process of seed germination (Palanivelu et al., 2022).

Hydrogels possess the remarkable ability to absorb water at a rate of 400 times their own weight while gradually releasing it (Darban et al., 2022), thereby reducing the leaching of herbicides and fertilizers (Kaur et al., 2023).

Montmorillonite is a type of mineral clay that is commonly found in soils and sediments. It has various applications in agriculture and horticulture due to its ability to retain water and nutrients, improve soil structure and enhance plant growth, and has recently been used in the production of hydrogels, such as the nanocomposite hydrogel with calcium montmorillonite (NC-MMt) that can enhance the growth and development of seedlings without adverse effects (Melo et al., 2019).

The growth of *Lactuca sativa* can be affected by a range of factors and technologies. A particular investigation revealed that the manipulation of the time difference between cycles of light and temperature can exert control over the development of seedlings of *L. sativa* (Masuda et al., 2022).

In this study, four different compositions of hydrogels based on montmorillonite were tested, in order to assess the effects on the morphological characteristics of lettuce seedlings (*Lactuca sativa*) subsequent to the transplanting procedure, in greenhouse conditions.

MATERIALS AND METHODS

Polymeric Material

The polymeric materials used in this study were received from The National Institute for Laser, Plasma and Radiation Physics, Măgurele. They were obtained utilizing the electron beam radiation technique and, in their composition, potassium persulfate was added as a catalyst. Four distinct compositions of hydrogels, based on montmorillonite were used in this study. Further, two methodologies for the application of the hydrogels were investigated, specifically the granular form (Hg) and beads (Hb) (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

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)

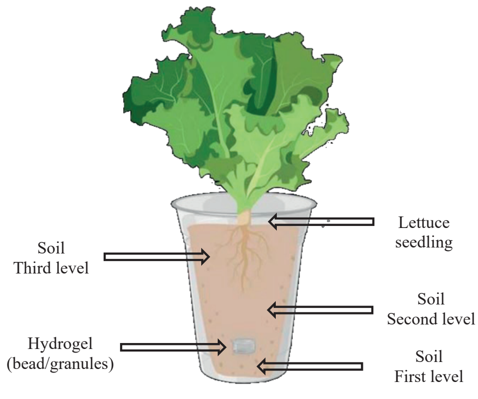
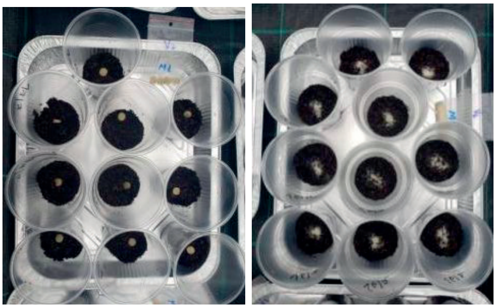


Figure 2. Working protocol graphic illustration
Created with BioRender.com

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, 3) were placed.



(a) (b)

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

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 24 h, 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
in the presence of hidrogel samples

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

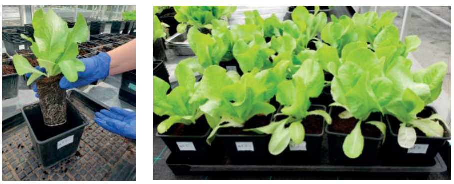


Figure 5. Lettuce plants in pots
in the presence of hidrogel samples

The samples were maintained in greenhouse conditions: between 32 and 100% relative air humidity, at temperature between 14.1°C and 37.92°C and watered regularly. The morphological analyses: the overall height, number of leaves and relative content of chlorophyll of lettuce were further conducted for a total of 5 weeks.

Overall height

The overall height of the samples was determined weekly measuring from the ground level to the greatest height of the highest leaf of each sample (Figure 6).

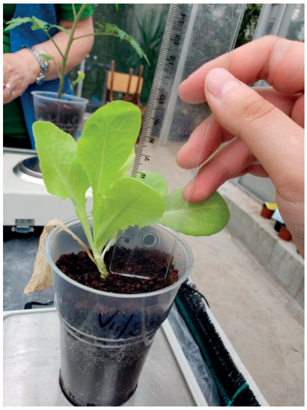


Figure 6. The determination of the height of samples

Number of leaves

The number of leaves of the samples was determined weekly by counting every leaf after the removal of the dry ones.

Relative content of chlorophyll

Relative content of chlorophyll of the samples was determined after transplanting the samples to pots and after an accommodation period of 14 days using the BIOBASE Portable Chlorophyll Meter CM-B. The chlorophyll content was determined at a specific time, in the morning, protected from direct sunlight, from the most developed leaf of each sample, and was calculated using the formula (99*SPAD)/(144-SPAD) and related to μg/cm^2 (Cerovic et al., 2012)

RESULTS AND DISCUSSIONS

The morphological analyses were conducted weekly and the relative content of chlorophyll was determined during the last 2 weeks of the experiment after the accommodation period subsiding the transplantation process.

Overall height

The evaluation of plant height is conducted in order to investigate the vertical growth of lettuce plants, as well as their overall dimensions and progress. This method provides valuable insights into the growth rate, vigor, and efficiency of nutrient absorption and utilization by the plants (Camen et al., 2022).

Overview results showed no significantly high differences between the samples containing montmorillonite and the control sample over the 5 weeks trial.

However there proved to be some differences between the different proportions of montmorillonite from hydrogel composition.

After 35 days trial, the lowest height (12.53±1.33 cm) was recorded for the the sample Hb2 (lowest concentration of montmorillonite) and the highest height value (17.06±4.27 cm) was recorded for Hb3 (medium concentration of montmorillonite) (Figure 7).

The height parameter after 35 days for lettuce in the presence of hydrogel beads was recorded as:

Hb2< Hb4< Hb1< C< Hb3

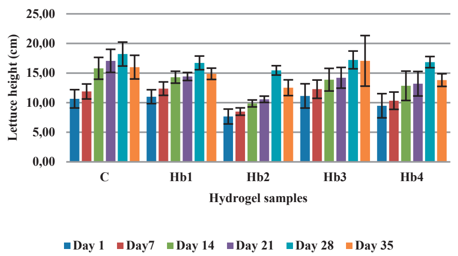


Figure 8. Overall height of the lettuce samples with bead hydrogels

Regarding the granular type of hydrogels, after 35 days trial, the lowest height (12.88±1.38 cm) was recorded for the the sample Hg4 (highest concentration of montmorillonite) and the highest height values were recorded for the control sample C and Hg2 (low concentration of montmorillonite) (Figure 8).

The height parameter after 35 days for lettuce with hydrogel granules in the presence of recorded as:

Hg4< Hg1< Hg3< Hg2< C

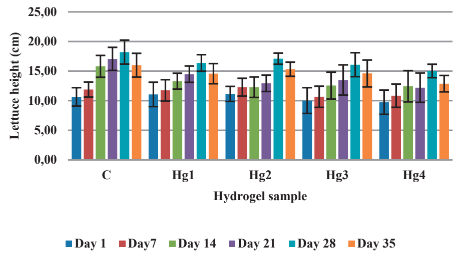


Figure 8. Overall height of the lettuce samples with granular hydrogels

The average height of *Lactuca sativa* varies depending on different factors. According to a particular investigation, an optimal crop height of 21.05cm was achieved by the application of 360 kg/ha of Ammonium Sulphate (Ponce-Lira et al., 2022). Another study showed that the height span per individual plant ranged from 15.18cm to 30.2 cm subsequent to a 60-day period of growth (Pereira de Oliveira et al., 2017).

Number of leaves

Measuring the total number of leaves present on lettuce plants provides insights into the progress and density of leaves. This process aids in assessing the general well-being and vitality of the plant, as a higher number of leaves frequently implies increased growth and productivity (Camen et al., 2022).

The number of leaves of the lettuce samples over the 35 days trial prove to increase overtime regardless of the dried base leaves that were periodically removed.

For both forms of hydrogels, the number of leaves proved to be higher then the control sample.

After 35 days trial, for the bead hydrogels, the lowest number of leaves resulted on the control sample (C) with 19.75±1.91 while the hydrogels with the lowest concentration of montmorillonite (Hb2) showed the greatest number of leaves with 25.75±1.28 (Figure 9).

The number of leaves after 35 days for lettuce in the presence of hydrogel beads was recorded as:

C<Hb1<Hb3<Hb4<Hb2

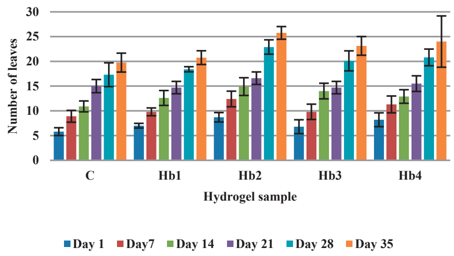


Figure 9. Total number of leaves of the lettuce samples with bead hydrogels

For the granular form, the sample with the highest concentration of montmorillonite hydrogel (Hg4) demonstrated the greatest number of leaves (24.50±3.66) after 35 days and the lowest value was recorded for the control sample (19.75±1.91) (Figure 10).
The number of leaves after 35 days for lettuce in the presence of hydrogel granules was recorded as:

C<Hg1<Hg3<Hg2<Hg4

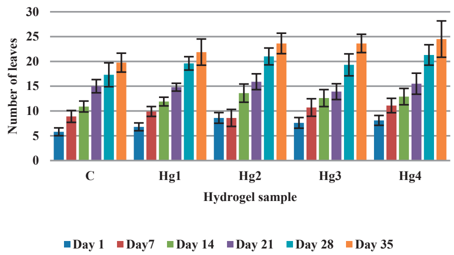


Figure 10. Total number of leaves of the lettuce samples with granular hydrogels

The average number of leaves of *Lactuca sativa* varies depending on each condition and treatment applied. In the study conducted by Chowdhury and Rahman (2021), the maximum leaf number was 27 using Approshika organic manure. Overall, the utilization of hydrogels has exhibited advantageous impacts on parameters associated with leaves in lettuce; however, the precise impact on the quantity of leaves may differ contingent upon the experimental circumstances such as applied treatments. Ponce-Lira et al. (2022) tested a new eco-fungicide on lettuce and the maximum number of leaves recorded was only 15.50.

Relative content of chlorophyll
The assessment of the nutritional status of plants can be facilitated by its utilization, as the availability of crucial nutrients has an impact on the production of chlorophyll. The presence of alterations in the relative concentration of chlorophyll may potentially serve as an indicator of stress or physiological issues within plants, including deficiencies in sustenance or environmental factors. The measurement of the relative chlorophyll concentration offers a non-invasive and easily accessible approach to assess the overall photosynthetic performance and well-being of lettuce plants (Camen et al., 2022).
The relative content of chlorophyll (μg/cm^2) of the samples proved to be higher for the lettuce plants in the presence of montmorillonite based hydrogels.
Regarding the bead form, after 35 days, the highest relative content of chlorophyll was registered for the lettuce plants in the presence of hydrogels with the lowest and highest concentration of montmorillonite - Hb2 (28.42±1.61μg/cm^2) and Hb4 (27.10± 6.14 μg/cm^2). The lowest relative content of chlorophyll was registered for the lettuce samples in the presence of hydrogels with no montmorillonite Hb1 (19.15±1.22 μg/cm^2).
The relative content of chlorophyll after 35 days for lettuce in the presence of hydrogel beads was recorded as:

Hb1<Hb3<C<Hb4<Hb2

For the granular form, the highest relative content of chlorophyll values were recorded for the lettuce plants in the presence of hydrogel containing medium and higher concentrations of montmorillonite, Hg4 (25.27±7.78 μg/cm^2) and Hg3 (23.21±5.24μg/cm^2). The lowest values were recorded for the samples Hg2 (19.93±4.34μg/cm^2) and Hg1 (19.96±3.29 μg/cm2) (Figure 11).
The relative content of chlorophyll after 35 days for lettuce in the presence of hydrogel granules was recorded as:

Hg2<Hg1<C<Hg3<Hg4

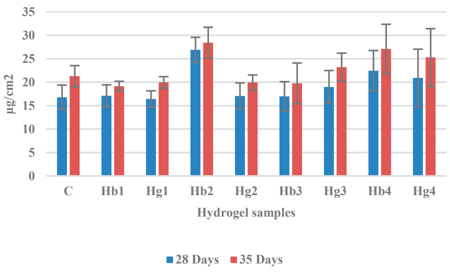


Figure 11. Relative content of chlorophyll of the lettuce samples

A study conducted by Lu et al. (2019) showed that the range of the chlorophyll content varied from 7.61 to 44.62µg/cm² for the Romaine lettuce.

CONCLUSIONS

The changes in lettuce plant over time were influenced by various factors including growth stage, environmental conditions, and nutrient availability (Ahmed et al., 2020).

The results of this study showed no noticeable differences regarding the height of lettuce in the presence of hydrogels compared to the control sample with a few exceptions, such as Hb3 (hydrogel with medium concentration of montmorillonite). The remaining samples registered close of slighly lower values than the control.

Meanwhile, the total number of leaves and relative content of chlorophyll proved to be significantly higher for lettuce cultivated in the presence of hydrogels (bead and granules) compared to the control sample. The hydrogels with a high (Hg4 and Hb4) and medium (Hg3 and Hb3) concentration of montmorillonite proved to enhance these two parameters for the lettuce plants in both forms of administration (bead and granules). Therefor, the montmorillonite based hydrogels proved to enchance some morphological parameters of lettuce.

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REFERENCES

Ahmed, H.A., Yu-Xin, T. & Qi-Chang, Y. (2020). Optimal control of environmental conditions affecting lettuce plant growth in a controlled environment with artificial lighting: A review. South African Journal of Botany, Volume 130, May 2020, Pages 75-89.

Camen, D., Moatăr, M.M., Ciulca, A., Popescu, S. & Ciulca, S. (2022). Assessment of some morphological and physiological parameters in lettuce (*Lactuca sativa* L.) cultivated in hydroponic system. Columella, 9(2): 83-89. doi: 10.18380/szie.colum.2022.9.2.83.

Cerovic, Z.G., Masdoumier, G., Ghozlen, N.B. & Latouche, G. (2012). A new optical leaf-clip meter for simultaneous non-destructive assessment of leaf chlorophyll and epidermal flavonoids. Physiologia Plantarum 146: 251–260. 2012.

Chowdhury, S. & Rahman, M. (2021). Influence of different organic manures on growth, yield and mineral nutrient accumulation in lettuce (*Lactuca sativa* L.). Dhaka University Journal of Biological Sciences, 30(2): 159-168. doi: 10.3329/DUJBS.V30 I2.54642.

Darban, Z., Shahabuddin, S., Gaur, R., Ahmad, I. & Sridewi, N. (2022). Hydrogel-Based Adsorbent Material for the Effective Removal of Heavy Metals from Wastewater: A Comprehensive Review. Gels. 2022 Apr 22, 8(5): 263. doi: 10.3390/gels8050263. PMID: 35621561; PMCID: PMC9140941.

Ekka, P.A., Daniel, S., Larkin, A., Kishore, P. & Singh, H. (2022). Effect of hydrogel and inorganic manure on the growth and yield of lettuce (*Lactuca sativa* L) under citrus-based agroforestry system. International Journal of Farm Sciences, 12(1):37-40. doi: 10.5958/2250-0499.2022.00009.x.

Farina, P., Fambrini, M., Pugliesi, C. & Viviani, A. (2021). Expression of homeobox genes during in vitro culture of *Lactuca sativa*. Plant Biosystems, 155(3): 609-621. doi: 10.1080/11263504.2020.1762793.

Güzel, M.E. (2021). Leaf Micromorphogy of Cultivated Lettuce (*Lactuca sativa* L.) and Its Wild Relatives. Bağbahçe Bilim Dergisi, 8(1) 2021: 121-133.

Kaur, P., Agrawal, R., Pfeffer, F.M., Williams, R. & Bohidar, H.B. (2023). Hydrogels in agriculture: Prospects and challenges. Journal of Polymers and the Environment 31(9): 1-18, doi:10.1007/s10924-023-02859-1.

Lu, F., Bu, Z. & Lu, S. (2019). Estimating Chlorophyll Content of Leafy Green Vegetables from Adaxial and Abaxial Reflectance. Sensors (Basel). 2019 Oct; 19(19): 4059. doi: 10.3390/s19194059.

Masuda, K., Yamada, T., Kagawa, Y. & Fukuda, H. (2022). Application of time lags between light and temperature cycles for growth control based on the circadian clock of *Lactuca sativa* L. seedlings. Frontiers in Plant Science, 13 doi: 10.3389/fpls.2022.994555.

Melo, R.A., Jorge, M.H., Bortolin, A., Boiteux, S.L., De Oliveira, C.R. & Marconcini, J.M. (2019). Growth of tomato seedlings in substrates containing a nanocomposite hydrogel with calcium montmorillonite

(NC-MMt). Horticultura Brasileira, 37(2):199-203. doi: 10.1590/S0102-053620190210
Naseem, S. & Ismail, H. (2022). In vitro and in vivo evaluations of antioxidative, anti-Alzheimer, antidiabetic and anticancer potentials of hydroponically and soil grown *Lactuca sativa*. BMC complementary medicine and therapies, 22(1) doi: 10.1186/s12906-022-03520-5.
Palanivelu, S.D., Armir, N.A.Z., Zulkifli, A., Hair, A.H.A., Salleh, K.M., Lindsey, K., Che-Othman, M.H., Zakaria, S. (2022). Hydrogel Application in Urban Farming: Potentials and Limitations - A Review. Polymers 2022, 14, 2590. https://doi.org/10.3390/polym14132590.
Pereira de Oliveira, I., Bruna, V. S. G., Moura de Deus., J.M., Araújo de Castro, W. & Aquino, M.M. (2017). Ammonium sulfate and lettuce (*lactuca sativa* l.) plant growth relationship. Global Science and Technology, 9(3).
Ponce-Lira, B., Aguilar-Arteaga, K., López-Perea, P., & Landero-Valenzuela, N. (2022). Ecofungicides as growth stimulants in *Lactuca sativa* L.. Revista de ingeniería biomédica y biotecnología, 18-26. doi: 10.35429/jbeb.2022.15.6.18.26.
Shi, M., Gu, J., Wu, H., Rauf, A., Emran, T.B., Khan, Z., Mitra, S., Aljohani, A.S.M., Alhumaydhi, F.A. & Al-Awthan, Y.S. (2022). Phytochemicals, Nutrition, Metabolism, Bioavailability, and Health Benefits in Lettuce - A Comprehensive Review. Antioxidants 2022, 11, 1158.
Sung, J-S., Hur, O-S., Ryu, K-Y., Baek, H-J., Choi, S., Kim, S.G., Luitel, B.P., Ko, H-C., Gwak, J-G. & Rhee, J-H. (2016). Variation in Phenotypic Characteristics and Contents of Sesquiterpene Lactones in Lettuce (*Lactuca sativa* L.) Germplasm. 29(6):679-689. doi: 10.7732/KJPR.2016.29.6.679.
Thomas, T., Biradar, M.S., Chimmad, V.P. & Janagoudar, B.S. (2021). Growth and physiology of lettuce (*Lactuca sativa* L.) cultivars under different growing systems. 26(3): 526-534. doi: 10.1007/S4 0502-021-00591-3.