



Synthesis of Cellulose-based, Azolla Extract Infused Hydrogel Fertilizer and its Effect on the Early Growth of Maize (*Zea mays L.*)

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Received: 21 Aug 2025; Revised: 10 July 2026; Accepted: 13 Jul 2026; Published online: 31 Jul 2026

Abstract

The growing demand for sustainable agriculture inputs has increased interest in biodegradable materials. In this study, a novel approach combining cellulose-based hydrogel with azolla extract infusion was used to assess its effect on the early-stage growth of maize (*Zea mays L.*). Cellulose was isolated through alkaline, acid, and bleaching treatments and confirmed via Fourier Transform Infrared Spectroscopy (FTIR) analysis. Hydrogels were synthesized by free-radical polymerization and loaded with Azolla extract. Pot experiments with loamy soil were conducted to assess the effect of applications. H4 and H5 treatments increased shoot height by ~65–75% and total chlorophyll by ~180% compared to the control ($p \leq 0.05$). This approach highlights the potential of waste-derived cellulose hydrogels as eco-friendly, slow-release biofertilizers to improve plant growth, reduce chemical fertilizer use, and support sustainable agriculture in nutrient-deficient and water-limited environments.

Keywords: Sustainable Agriculture, Cellulose, Free Radical Polymerization, Fourier Transform Infrared Spectroscopy (FTIR), water retention.

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Introduction

Meeting global food demands requires sustainable agricultural practices; however, soil degradation through nutrient depletion, erosion, compaction, and loss of organic matter continues to limit productivity. [1]. With the global population expected to surpass 9.5 billion by 2050 [2], enhancing soil water retention (SWR) becomes increasingly important, particularly in drought-prone regions where water scarcity directly hinders crop development [3]. Since agriculture accounts for nearly 92% of global freshwater consumption [4] Improving soil-water dynamics is essential for reducing water use and enhancing the resilience of agricultural systems under these growing environmental pressures. [5].

Hydrogels are three-dimensional networks of hydrophilic polymers that can absorb and retain over 100 grams of water per gram of dry weight. [6] Their exceptional water-holding capacity is attributed to the presence of functional groups such as carboxyl, sulfonic, hydroxyl, and amide on the polymer backbone [7]. When incorporated into soil, hydrogels enhance moisture retention and nutrient availability, thereby reducing irrigation frequency, alleviating water stress, and promoting plant growth. [8]. Additionally, they modify soil structure, enhance root development, and reduce nutrient loss. Acting as water reservoirs, hydrogels support seed germination, enhance soil aeration, and

increase fertilizer efficiency, all while reducing water consumption, operational costs, and labour requirements. (Shivakumar et al., 2019).

Sugarcane (*Saccharum officinarum*), a tropical plant of the Gramineae family, serves as a valuable source of cellulose and lignin. [10]. Cellulose, along with hemicellulose and lignin, forms the primary structural components of lignocellulosic biomass and is present in wood, plants, algae, and certain microorganisms. [11]. Structurally, cellulose is a linear polymer composed of β -D-glucopyranose units linked by β -(1 $\rightarrow$ 4) glycosidic bonds and contains three hydroxyl groups, two secondary at the C-2 and C-3 positions, and one primary at C-6, each exhibiting distinct reactivity. [12]. These structural features contribute to cellulose's polymorphic nature and functional versatility, making it an important renewable material for biomass valorisation. [13].

Azolla is an aquatic fern with significant agricultural applications as a biofertilizer, animal feed, and weed suppressant. [14]. Azolla is rich in essential vitamins and nutrients, including Vitamin A, Vitamin B12, beta-carotene, calcium, phosphorus, potassium, iron, magnesium, copper, zinc, and various amino acids. [15], Azolla forms a symbiotic association with *Anabaena azollae*, enabling it to fix up to 1.1 kg N ha⁻¹ day⁻¹ and supply 20–40 kg N ha⁻¹ within 25 days. Remarkably, it fixes nearly three times more nitrogen annually than

legumes. [16]. Beyond nitrogen fixation, Azolla improves soil organic matter, enhances nutrient availability, reduces ammonia volatilization, and contributes to sustainable agriculture through soil remediation. [17].

Maize (*Zea mays* L.) is an important annual grain crop of the Poaceae family, widely cultivated across tropical and temperate regions. [18]. Although 70% of maize production occurs in temperate zones, it is a key food source in arid and semi-arid regions facing climate stress. [19]. Over 1 billion people depend on maize for more than half of their daily caloric intake. Nutritionally, maize is rich in anthocyanins, calcium, phosphorus, iron, vitamins, and dietary fibre. (Poole et al., 2020). Its starch, which meets rising demand from the food and pharmaceutical industries, is also widely used in paper, textile, and poultry feed sectors. [21].

This study aimed to extract and characterize the cellulose using FTIR, through which hydrogel fertilizers are synthesized. Then to load the Azolla pinnata extract through absorption and assess its effect of growth of the *Zea mays* L. plant.

Materials and Methodology

Isolation and Activation of Cellulose from Bagasse

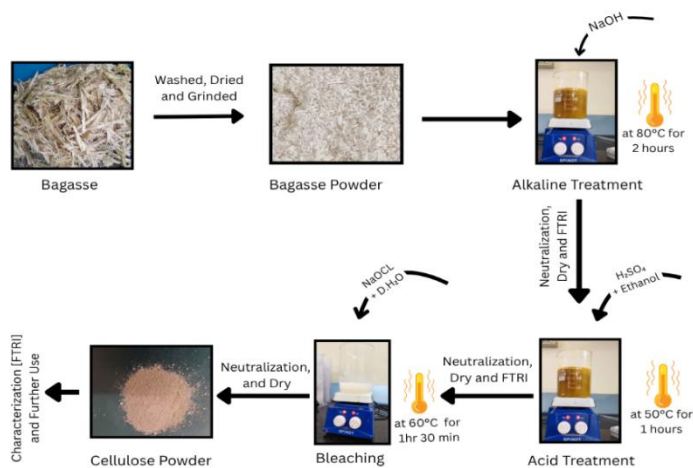


Figure 1. Extraction of cellulose derived from bagasse

Bagasse was thoroughly washed with distilled water, air-dried for 3 days, and ground into a fine powder using a mechanical grinder. The powder was sieved through a 100 μ m mesh and stored in an airtight container. Cellulose extraction followed a three-step chemical process. First, 30 g of the powder was treated with 1,400 ml of NaOH at 80°C for 2 hours to remove lignin and hemicellulose, followed by vacuum filtration and washing to neutral pH, then drying at 50°C for 12 hours. [22]. The dried material was then subjected to acid hydrolysis using 140 ml concentrated H₂SO₄ and 500 ml

ethanol at 50°C to further eliminate hemicellulose and pectin. Ethanol was used as a diluent to moderate the reactivity of concentrated H₂SO₄ and reduce excessive cellulose degradation [23]. Finally, the residue was bleached with 120 ml NaOCl and 90 ml distilled water at 60°C for 1.5 hours to remove residual lignin and improve cellulose purity. [24]. FTIR analysis was conducted after each step to assess component removal and cellulose integrity.

Preparation of Cellulose-Based Hydrogel

Cellulose-based hydrogels were synthesized using varying concentrations (0.11, 0.22, 0.32, 0.43, and 0.54 wt%) of cellulose extracted from sugarcane bagasse. For each formulation, 5 mL of acrylic acid (AA) was mixed with 40 mL of distilled water. Separately, pre-activated cellulose, 0.25 g of N, N'-methylenebisacrylamide (MBA), and 0.75 g of ammonium persulfate (APS) were blended, and then added to the AA solution. The reaction mixture was sealed with aluminium foil and stirred at 60 °C for 45 minutes to initiate free radical polymerization. APS generated radicals, while MBA enabled crosslinking between cellulose and AA chains via graft copolymerization. All formulations were synthesized using identical acrylic acid, APS, and MBA concentrations; only cellulose content varied. After polymerization, the mixtures were poured into petri plates and dried at 60°C for 12 hours until constant weight, ensuring moisture removal and hydrogel stabilization. This procedure was repeated for all cellulose concentrations under identical conditions. [25].

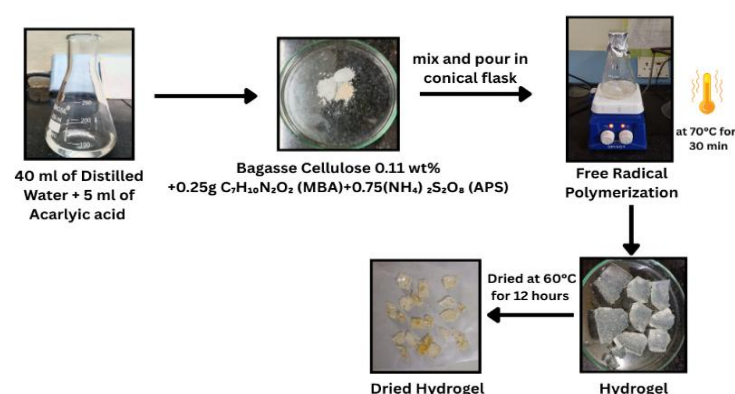


Figure 2: Preparation of Cellulose-based Hydrogel derived from Bagasse.

Preparation of Azolla Extract

Fresh Azolla biomass of 500 g was washed thoroughly to remove debris and surface contaminants, then boiled in 1 L of distilled water for 45 minutes to extract soluble bioactive compounds. After cooling, the mixture was

filtered using a fine mesh to obtain the 100% raw Azolla extract. Serial dilutions (5–50%) were prepared using distilled water, and all extracts were stored in airtight containers at 4 °C to preserve their bioactivity. [14].

Infusion of Azolla Extract into Hydrogel

Pre-dried cellulose-based hydrogels (0.11–0.54 wt% cellulose) were immersed in a 1:1 diluted Azolla extract for 48 hours under controlled conditions to facilitate swelling and absorption of bioactive compounds. After infusion, the Azolla-loaded hydrogels were oven-dried at 60°C until a constant weight was reached, ensuring moisture removal and stabilization for further application. (26).

Water Holding Capacity (WHC) in Soil

Water holding capacity (WHC) refers to the maximum quantity of water that a material can hold, and in the case of hydrogels, it demonstrates their ability to keep moisture within the soil. To determine the WHC, the soil was dried at 45°C until a consistent weight was achieved. Mix 2g of dry hydrogel (about 5mm diameter) with 50g of dried soil. The soil-hydrogel mixture was transferred to a plastic container, and the starting weight was recorded as W1. A small hole was drilled at the bottom of the pot to allow surplus, unretained water to drain.

The control was pure dry soil, with a weight of W0. After adding 50 mL of distilled water to each pot, the resultant weight was recorded as W2. The water-holding capacity was determined using the following formula:

$$\text{Eq. (1) Water Holding Capacity (\%)} = (W2 - W1/W0) \times 100$$

Where W1 = Weight of the soil-hydrogel mixture before water addition, W2 = Weight of the pot after water addition, and W0 = Weight of pure dried soil used as a control.

Water Retention in Soil

Water retention (WR) is the ability of a material, such as soil or hydrogel, to hold water over time and prevent rapid moisture loss. It is an important criterion for determining the long-term effectiveness of hydrogels in retaining soil moisture. To assess water retention, the samples prepared for the water holding capacity (WHC) study were kept at room temperature (RT) and their weights were measured daily for several days until no further weight loss was observed, indicating complete evaporation or drainage of retained water. This approach enabled the measurement of how much water remained in the hydrogel-soil mixture over time. The water

retention percentage was calculated using the following equation:

$$\text{Eq. (2) Water Retention (\%)} = (Wt - W) / (Wi - W) \times 100$$

Where, Wt = Weight of the sample at a given time interval, Wi = Initial weight of the sample after water was added, and W = Final weight of the completely dried sample without water.



Figure 3: Azolla-Infused Hydrogel.



Figure. 4: Zea. Mays L. seeds.



Figure 5: Experimental setup for maize growth.

Conduct and design of the experiment

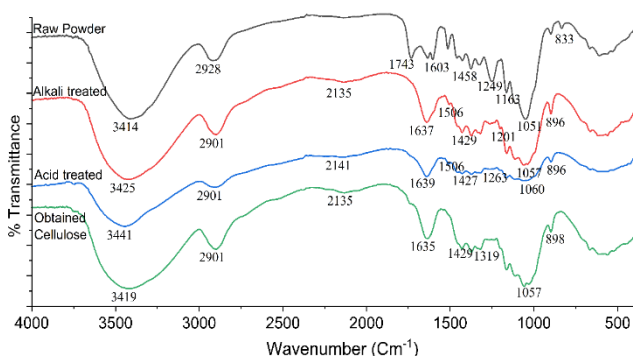
Each treatment consisted of three independent replicates ($n = 3$), with one plant per bag. Treatments were arranged in a completely randomized design. Air-dried, sieved agricultural soil was placed into six polythene grow bags for a maize growth experiment. The control (C) contained 2 kg of plain soil, while treatments H1 to

Table 1: Composition of Soil and Azolla Infused Hydrogel Combination for Maize Growth Experiment

Treatment	Condition
Hc	2kg Agricultural Soil
H ₁	2kg Agricultural Soil + 0.11Wt% of Cellulose-based Hydrogel 4g
H ₂	2kg Agricultural Soil + 0.22Wt% of Cellulose-based Hydrogel 4g
H ₃	2kg Agricultural Soil + 0.32Wt% of Cellulose-based Hydrogel 4g
H ₄	2kg Agricultural Soil + 0.43Wt% of Cellulose-based Hydrogel 4g
H ₅	2kg Agricultural Soil + 0.54Wt% of Cellulose-based Hydrogel 4g

H5 consisted of 2 kg of soil mixed with 4 g of Azolla-infused cellulose hydrogel at 0.11, 0.22, 0.32, 0.43, and 0.54 wt%, respectively. *Zea mays* L. (SMH-34 hybrid) seeds were sown 40 mm deep in each bag, which were then maintained outdoors under natural conditions. Irrigation was performed every three days with 300 mL of water per bag. Plant growth parameters-height, stem diameter, total leaf area, and leaf count-were recorded on days 7, 14, and 21 before carefully uprooting the plants for further analysis. Data were analysed using one-way analysis of variance (ANOVA), followed by Tukey's HSD post-hoc test at $p \leq 0.05$ using Origin (2025b) [27]

Fig.6: FTIR Spectra of Bagasse



Estimation of Chlorophyll Content

Fresh maize leaves (1.5 g) were ground and soaked in 25 mL of 95% ethanol. The mixture was incubated at room temperature for 24 hours for complete pigment extraction. The extract was filtered using Whatman filter paper. The filtrate's absorbance was recorded at 663 nm and 645 nm using a UV-Vis spectrophotometer. (Kamble

et al.,2015). Chlorophyll content was calculated using Arnon's equations:

$$\text{Chlorophyll A } \frac{\text{mg}}{\text{g}} = (12.7 \times A_{667} - 2.69 \times A_{645}) \times \left(\frac{V}{1000} \times W\right)$$

$$\text{Chlorophyll B } \frac{\text{mg}}{\text{g}} = (22.9 \times A_{645} - 4.68 \times A_{663}) \times \left(\frac{V}{1000} \times W\right)$$

$$\begin{aligned} \text{Total Chlorophyll } \frac{\text{mg}}{\text{g}} \\ = (20.2 \times A_{645} - 8.02 \times A_{663}) \times \left(\frac{V}{1000} \times W\right) \end{aligned}$$

Where:

A_{663} = Absorbance at 663nm

A_{645} = Absorbance at 645nm

V= Volume of extract (25mL)

W = Fresh weight of leaf sample (1.5 g)

Results and Discussion

Table. 2: Interpretation of FTIR Spectra

Wave number (cm^{-1})	Functional Group	Literature Support	Interpretation
1743	C=O stretching of hemicellulose esters	[10]	Confirms removal of hemicellulose during treatment
1724	Carbonyl (C=O) stretching of non-cellulosic components	[10]	Indicates purification and removal of non-cellulosic residues
1603–1506	Aromatic skeletal vibrations of lignin	[29]	Confirms breakdown and removal of lignin
1502	Aromatic skeletal vibrations (lignin)	[29]	Successful delignification
1635	H–O–H bending of adsorbed water	[30]	Indicates moisture due to hydrogen bonding
1641	O–H bending of water molecules	[30]	Confirms water absorption by cellulose hydroxyl groups
1429	–CH ₂ scissoring / symmetric CH ₂ bending	[31]	Confirms cellulose crystalline structural groups
1319	Phenolic O–H bending (residual lignin)	[30]	Indicates partial/trace lignin retention or modification
3440–3446	Broad O–H stretching of cellulose	[32]	Confirms intact hydroxyl groups and a hydrogen-bonded cellulose network
2900	C–H stretching of aliphatic cellulose	[33]	Aliphatic cellulose backbone preserved
898–899	β-glycosidic linkage (C–O–C stretch)	[32]	Indicates cellulose polymer backbone remains unaltered

Effect of hydrogel on Water holding capacity and Water retention

Table. 3: Effect of hydrogel on Water holding capacity and Water retention.

Treatments Code	WHC (%)	WR (%)
HC	25.04 ± 0.19 ^f	36.99 ± 0.28 ^f
H1	75.88 ± 0.23 ^b	74.16 ± 0.21 ^c
H2	72.33 ± 0.19 ^d	79.80 ± 0.22 ^b
H3	69.93 ± 0.22 ^c	69.82 ± 0.21 ^c
H4	75.14 ± 0.13 ^c	73.09 ± 0.20 ^d
H5	77.93 ± 0.23 ^a	83.95 ± 0.30 ^a

Note: Values are expressed as mean ± standard deviation (SD). Means followed by the same lowercase letter (a, b, c) within a column are not significantly different, whereas means followed by different letters differ significantly at $p \leq 0.05$ according to Tukey's HSD test after one-way ANOVA.

Hydrogel application significantly improved water holding capacity (WHC) and water retention (WR) when compared to control soil (Table 3). All treated soils had significantly greater WHC and WR, demonstrating the hydrogels' robust water absorption and moisture retention capacity. Among the treatments, H5 had the highest WHC (77.93%) and WR (83.95%), indicating superior water storage and long-term moisture release, while the control had the lowest values (25.04% and 36.99%). The increased WHC is due to the hydrogels' hydrophilic polymer network, which absorbs and holds water inside its three-dimensional structure. Higher WR values suggest less moisture loss over time. Statistical analysis revealed significant differences across treatments ($P < 0.05$), indicating the efficiency of hydrogel additions in preserving soil moisture.

Impact of Azolla-Infused Hydrogel on Maize Growth Parameters

Shoot Height

Over the 21-day observation, shoot height increased steadily across all treatments, indicating effective vegetative development. The control (H_c) reached 27.3 cm by day 21, while hydrogel treatments significantly outperformed it. Notably, H5 exhibited the highest growth, progressing from 10.2 cm (day 7) to 47.9 cm (day 21), followed closely by H4 (9.52 to 47.6 cm). Early and sustained growth in H4 and H5 suggests improved nutrient availability and water retention via hydrogel-mediated delivery. These findings align with Abg Ahmad et al. (2023), who reported enhanced plant height in rice using encapsulated urea hydrogels. (26).

Stem Diameter

Stem diameter, a key indicator of structural strength, increased with time across all treatments. While the control achieved 5.67 mm by day 21, treatments H4 and H5 showed the greatest thickening, reaching 9.1 mm and 9.2 mm, respectively. The consistent improvement suggests enhanced vascular development and

mechanical support under hydrogel treatment. These results are supported by Albalasmeh et al. (2022), who reported that hydrogels reduce evaporation and improve water availability. [34].

Leaf Area

Leaf area expanded significantly in hydrogel-treated plants compared to the control. H_c reached only 17.12 cm² by day 21, whereas H₅ peaked at 42.85 cm² and H₄ at 41.6 cm², indicating enhanced photosynthetic capacity. This increase in leaf surface area underlines the hydrogel's role in promoting metabolic activity and biomass accumulation. Montesano et al. (2015) reported similar trends in hydrogel-treated cucumber plants [35].

Number of Leaves

Leaf count was lowest in the control (1–4 leaves by day 21), while H4 and H5 achieved 6 and 5 leaves, respectively. The greater leaf number reflects improved nutrient uptake and cellular activity. This observation supports Sasmal and Patra (2022), who noted a significant increase in leaf number following hydrogel application. The positive correlation between hydrogel concentration and leaf production suggests potential for improving crop canopy density and photosynthetic output. [27].

Root growth:

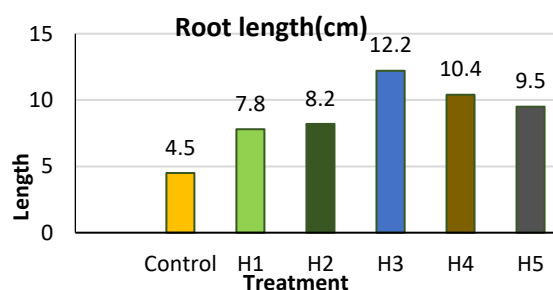


Figure 7: Graphical Representation of Root Length in Maize (*Zea mays* L) after 21 days

At the end of 21 days, root length—a key factor for plant stability and nutrient uptake—was measured. The control (H_c) had the shortest roots at 4.5 cm, while hydrogel treatments showed significant improvement: H1 (7.8 cm), H2 (8.2 cm), and H3 had the longest roots at 12.2 cm, highlighting the strong effect of hydrogel combined with Azolla. H4 and H5 also showed increased root lengths of 10.4 cm and 9.4 cm, respectively. This trend reflects how hydrogels enhance soil moisture retention and nutrient availability, promoting better root growth and resilience. These results align with Rajanna et al. (2022), who reported improved root development with hydrogels, especially under water-limited conditions. [36].

Table.4: Growth parameter of Zea mays plants after 7,14 and 21 days.

	After 7 days				After 14 days				After 21 days			
Treatment	Shoot height (cm)	Stem diameter (mm)	Plant leaf area (cm ²)	No. of leaves	Shoot height (cm)	Stem diameter (mm)	Plant leaf area (cm ²)	No. of leaves	Shoot height (cm)	Stem diameter (mm)	Plant leaf area (cm ²)	No. of leaves
H _C	6.00 ± 0.10 ^c	2.55 ± 0.06 ^c	6.30 ± 0.14 ^c	1.00 ± 0.00 ^c	20.50 ± 0.58 ^c	3.92 ± 0.09 ^c	12.07 ± 0.31 ^c	3.00 ± 0.00 ^c	27.30 ± 0.52 ^c	5.67 ± 0.11 ^c	17.12 ± 0.36 ^c	4.00 ± 0.00 ^c
H ₁	7.20 ± 0.12 ^b	3.92 ± 0.08 ^b	7.43 ± 0.19 ^b	2.00 ± 0.00 ^b	35.20 ± 0.71 ^b	6.68 ± 0.15 ^b	22.50 ± 0.52 ^b	4.00 ± 0.00 ^b	44.12 ± 0.64 ^b	8.02 ± 0.17 ^b	33.50 ± 0.61 ^b	5.00 ± 0.00 ^b
H ₂	7.40 ± 0.10 ^b	3.85 ± 0.07 ^b	7.82 ± 0.18 ^b	2.00 ± 0.00 ^b	35.50 ± 0.66 ^b	7.36 ± 0.18 ^a	24.75 ± 0.55 ^a	4.00 ± 0.00 ^b	44.50 ± 0.59 ^b	8.50 ± 0.19 ^a	36.75 ± 0.68 ^a	5.00 ± 0.00 ^b
H ₃	8.20 ± 0.14 ^a	4.27 ± 0.09 ^a	9.12 ± 0.22 ^a	2.00 ± 0.00 ^b	36.80 ± 0.74 ^a	7.48 ± 0.16 ^a	25.30 ± 0.57 ^a	4.00 ± 0.00 ^b	46.10 ± 0.62 ^a	8.62 ± 0.18 ^a	38.20 ± 0.71 ^a	5.00 ± 0.00 ^b
H ₄	7.90 ± 0.13 ^a	4.10 ± 0.08 ^a	8.85 ± 0.21 ^a	2.00 ± 0.00 ^b	36.10 ± 0.69 ^a	7.30 ± 0.17 ^a	24.90 ± 0.53 ^a	4.00 ± 0.00 ^b	45.60 ± 0.60 ^a	8.42 ± 0.16 ^a	37.10 ± 0.66 ^a	5.00 ± 0.00 ^b
H ₅	7.60 ± 0.11 ^b	3.98 ± 0.07 ^b	8.10 ± 0.20 ^b	2.00 ± 0.00 ^b	35.80 ± 0.67 ^b	6.95 ± 0.14 ^b	23.40 ± 0.50 ^b	4.00 ± 0.00 ^b	45.00 ± 0.58 ^b	8.10 ± 0.15 ^b	34.80 ± 0.63 ^b	5.00 ± 0.00 ^b

Note: Values are expressed as mean ± standard deviation (SD). Means followed by the same lowercase letter (a, b, c) within a column are not significantly different, whereas means followed by different letters differ significantly at $p \leq 0.05$ according to Tukey's HSD test after one-way ANOVA.

Chlorophyll Content in Maize Leaves

Table 5. Chlorophyll Content in Different Treatments in Maize Leaves After 21 Days (mg/g).

Treatment	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll (mg/g)
HC	0.0924 0.0015 ^f	± 0.0755 0.0015 ^f	± 0.1679 0.0030 ^f
H1	0.2136 0.0028 ^d	± 0.2499 0.0030 ^a	± 0.4634 0.0041 ^b
H2	0.2393 0.0032 ^c	± 0.2039 0.0029 ^d	± 0.4430 0.0045 ^c
H3	0.2649 0.0026 ^a	± 0.1579 0.0023 ^c	± 0.4228 0.0031 ^d
H4	0.2348 0.0023 ^c	± 0.2421 0.0025 ^b	± 0.4767 0.0045 ^a
H5	0.1969 0.0025 ^c	± 0.2195 0.0022 ^c	± 0.4163 0.0033 ^c

Values are mean ± SD (n = 3). Means followed by different superscript letters within a column differ significantly at P ≤ 0.05 according to Tukey's HSD test.

Chlorophyll content in maize leaves varied significantly across treatments, measured using Arnon's method (1949). The untreated control (Hc) had the lowest chlorophyll a (0.0924 mg/g), chlorophyll b (0.0755 mg/g), and total chlorophyll (0.1679 mg/g), indicating limited photosynthetic activity. In contrast, all Azolla-infused hydrogel treatments (H1-H5) showed notably higher chlorophyll levels, with H4 recording the highest total chlorophyll (0.4767 mg/g), chlorophyll a (0.2348 mg/g), and chlorophyll b (0.2421 mg/g), followed closely by H1 and H2. These findings align with Ms et al. (2020), who reported similar chlorophyll improvements due to enhanced soil moisture retention and stress reduction. [37].

Conclusion

This study demonstrated a sustainable method for converting sugarcane bagasse into cellulose-based hydrogels infused with Azolla extract for agricultural use. Cellulose was successfully extracted and verified by FTIR, then used to synthesize hydrogels via free-radical polymerization. The study was limited to short-term pot experiments and did not assess nutrient release kinetics under field conditions. When applied to maize (*Zea mays* L.), the Azolla-infused hydrogels, especially H4 and H5, significantly improved shoot height, stem diameter, leaf area, root length, leaf number, and chlorophyll content. These results highlight the hydrogel's ability to enhance moisture retention and nutrient delivery. The approach offers a low-cost, eco-friendly supplement or partial replacement of chemical fertilizers, promoting sustainable crop growth while addressing water and soil fertility challenges, with strong potential for dryland and organic farming systems.

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