# PHYTOTOXICITY EVALUATION OF RICE STRAW BIOPOLYMER-BASED HYDROGELS

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The disposal of rice crop residue, *i.e.* straw, from fields *via* burning is a major concern because of its hazardous effects on the environment. Therefore, it is essential to find other ways for effective valorization of such unavoidable residues available in abundant amounts in the field. As an alternative to burning, major components of rice straw, *i.e.* such as cellulose, hemicelluloses and lignin, could be utilized for the production of various high-value biomaterials. The current study presents the synthesis of hydrogels from rice straw biopolymers, *i.e.* microcrystalline cellulose (MCC) and lignin, using glutaraldehyde/epichlorohydrin crosslinkers, followed by the evaluation of their effects on seed germination. Hydrogels were characterized by Fourier transform infrared (FT-IR) spectroscopy and scanning electron microscopy (SEM) analyses. The phytotoxicity of hydrogels was evaluated by using them as media for germinating monocot (wheat) and dicot (moong bean) seeds. The growth of seedlings on all hydrogels was observed to be better than that of control seedlings, which confirmed the positive role of the hydrogels on the germination of monocot and dicot seeds. Hence, they could be further explored for improving plant growth, especially in drought areas.

***Keywords***: microcrystalline cellulose, lignin, glutaraldehyde, epichlorohydrin, phytotoxicity

## INTRODUCTION

In India, about 23 million tons of rice residue, also called rice straw, is produced annually, out of which 80% is burnt in order to create space for the next crop owing to the short window period between rice harvesting and wheat sowing.[1] Burning of rice straw releases toxic gases, such as carbon dioxide, nitric oxide, carbon monoxide, sulfur oxides, black carbon *etc*., which have a serious impact on the environment, being responsible for the formation of intense haze and melting of Himalayan glaciers. Additionally, it releases heat, which penetrates one centimeter into the soil and elevates temperature. Higher temperature kills the beneficial bacterial, fungal population or other micro-organisms, as well as deteriorates organic quality, which is critical for the fertility of soil.[2] Therefore, the rice straw burning issue needs to be urgently addressed.

There is much need to find potential alternatives for utilization of this unavoidable biomass for the production of high-value materials. The biopolymers constituting the major part of rice straw are cellulose, hemicelluloses and lignin, which have been extensively exploited for the production of various value-added products, such as ethanol, furfural, adhesives and many other useful aromatic compounds with commercial and industrial importance.[3,4] Recently, the exploration of these polymers for the synthesis of bio-based hydrogels has come into notice. More research needs to be done in this area in order to investigate the potential application of such hydrogels in agriculture.

Hydrogels are cross-linked hydrophilic polymeric networks, with three-dimensional configurations, capable of absorbing high amounts of water or biological fluids, almost a hundred times greater than their own weight. The synthesis of bio-based hydrogels can be carried out based on lignin and cellulose by chemical crosslinking reactions. Generally, hydrogels can be synthesized *via* two methods, *i.e.* physical crosslinking and chemical crosslinking. Physical crosslinking involves interaction between polymers by van der Waals forces, electrostatic forces and hydrophobic interactions, while chemical crosslinking can be done by forming covalent bonds between polymers.[5] Marketing of hydrogels has been increased due to their unique properties, such as flexibility, versatility, soft surface and rapid swelling in water. Owing to their swelling and mechanical properties, hydrogels have distinguishable uses, such as therapeutic uses, in medical devices, in the controlled release of drugs,[6] in cartilage reconstruction and regeneration,[7] artificial organs,[8] wound dressings providing the humid environment beneficial for wound healing.[9] Additionally, hydrogels have wide applications in industrial fields, for the production of sanitary napkins, baby diapers and cosmetics *etc*.[5]

Recently, hydrogels have gained greater research attention for use in agriculture, soil improvement and plant growth.[10,11,12] Hydrogels of polyethylene oxide crosslinked *via* radiation have been demonstrated to boost seedling survival, plant growth under water stress.[13] Hydrogels can also be used as a reservoir of water and nutrients in soil that encounters impacts of dehydration, to control soil erosion, humidity in crops and runoff reduction in steep slope areas.[14] The biopolymer based superabsorbent hydrogels, based on cellulose derivatives, can be utilized for an optimized use of water resources in agriculture and horticulture, by achieving a smart water consumption.[15] Chitin biopolymer based hydrogels are suitable for use as soilless culture media for plant growth. Hydrogels have been reported to trigger the germination process in coffee seedlings by acting as water and nutrients reservoir in soil,[16] thus combating moisture stress during water shortage. Considering these aspects, the present study aimed to synthesize rice straw based hydrogels and evaluate their phytotoxicity for use as media for seed germination of monocot (wheat) and dicot (moong bean) seedlings.

## EXPERIMENTAL

### Materials and methods

Rice straw of crop variety "Pusa Basmati PR 121" was collected from the fields of Punjab Agricultural University, Ludhiana. It was oven-dried at 60 °C, ground, passed through 1 mm sieve and stored in plastic bags at room temperature in an air-tight container. Seeds of wheat and moong bean were procured from the Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India.

### Extraction of biopolymers and synthesis of hydrogels

Microcrystalline cellulose (MCC) and lignin were extracted from rice straw using standard methodologies.[17,18] Hydrogels were synthesized from rice straw biopolymers, *i.e.* MCC and lignin, using 1% glutaraldehyde/epichlorohydrin crosslinker. Polymeric solutions were prepared independently, *i.e.* 5% MCC solution in 7% NaOH + 12% urea solution by keeping at -20 °C for 5 min, 5% lignin solution in 2% NaOH; and 5% polyvinyl alcohol (PVA) solution in distilled water. Biopolymer-based hydrogels with different compositions were prepared and denoted as detailed in Table 1.

A PVA solution was mixed in MCC or MCC + lignin solution with vigorous stirring for 2-3 hours. In the case of lignin, solutions were stirred for 5-8 min.[19] Afterwards, the crosslinker was added to the homogenous solution, with continuous stirring at 70 °C for 25-45 min, to convert it into a gelatinous solution. The synthesis of MCC incorporating hydrogels involved an extra freeze-thawing step to settle down the crosslinking between polymeric networks.[20] The synthesized hydrogels were structurally and morphologically characterized by Fourier transform infrared (FT-IR) spectroscopy and scanning electron microscopy (SEM) analyses.

### Phytotoxicity evaluation

Five seeds of each wheat and moong bean were sown separately in a 10 cm³ beaker filled with different hydrogels till 2 cm³. For the control, moist filter paper was used instead of hydrogels. All the beakers containing wheat and moong bean seeds were kept in an incubator at 13 ± 2 °C and 25 ± 5 °C, respectively, for 7 days. The seeds were considered germinated, when the tip of the radicle was grown out of seed up to 1-2 cm.

Table 1
Composition of biopolymer-based hydrogels

| Hydrogel | Polymers involved | Ratio | Crosslinker |
|---|---|---|---|
| MG | MCC: PVA | 1:2 | Glutaraldehyde |
| ME | MCC: PVA | 1:2 | Epichlorohydrin |
| HG | MCC: lignin: PVA | 1:1:2 | Glutaraldehyde |
| HE | MCC: lignin: PVA | 1:1:2 | Epichlorohydrin |
| LG | Lignin: PVA | 1:1 | Glutaraldehyde |
| LE | Lignin: PVA | 1:1 | Epichlorohydrin |

**Germination percentage**

The germination percentage was measured by dividing the number of seeds germinated at the end of 7 days by the total number of seeds sown.[21]

Germination (%) = (No. of seeds germinated/ /total number of seeds) ×100 (1)

**Root and shoot length**

Monocot and dicot seedlings were picked off from the hydrogels and the control. The roots of the seedlings were washed for the removal of any attached loosened hydrogel media and gently placed on the filter paper to remove surface moisture. Root and shoot length of the seedlings was measured in cm, with the help of a scale. The area below the hypocotyl to the root tip was considered as root length[22] and the area above the hypocotyl to the terminating bud was considered as shoot length of dicot plants. While in monocot plants, the fibrous part below the leaf sheath was considered as root length and shoot length was considered from the leaf sheath to the terminating part of the plant.

**Root to shoot length ratio**

The root to shoot length ratio was calculated by dividing the root length measured by the shoot length of each plant:[23]

R:S = Root length / Shoot length (2)

**Seedling vigour index**

Seedling vigour index was calculated by multiplying seedling length (root length + shoot length) by germination percentage:[24]

Seedling vigour index = seedling length (cm) × × germination (%) (3)

**Germination index**

The germination index was recorded after 7 days by using the following formula:[25]

$$G_{index} = [(G / G_o) \times (L / L_o)] \times 100 \quad (4)$$

where G = no. of germinated seeds and L = root length in hydrogel, $G_0$ and $L_0$ = number of germinated seeds and root length in distilled water, respectively.

## RESULTS AND DISCUSSION

The obtained yield of lignin from rice straw was 6.8%, with 85% recovery. The obtained MCC yield was 30.24%, with 79% recovery. The FT-IR spectra of all the hydrogels showed a strong absorption band between 1150-1080 $cm^{-1}$ due to C-O stretching vibrations, which confirmed the formation of ether bonds corresponding to crosslinking in polymeric networks[26] (Fig. 1). SEM images of all hydrogels showed the crosslinked 3-D network of polymers with different density and porosity. MCC hydrogels present a denser crosslinked network of polymers, with small sized pores. This may be due to the fibrous nature of the MCC biopolymer. Lignin hydrogels have a homogenous polymeric network, with large pore size. SEM images of the hybrid MCC and lignin hydrogels revealed the crosslinked matrix of the polymers, with intermediate density and porosity, compared to those of hydrogels based on individual biopolymers (Fig. 2). The morphology of different hydrogels varied not only as a function of the biopolymers used, but also according to the type of crosslinker used to some extent. Epichlorohydrin based hydrogels have a looser crosslinked network than glutaraldehyde based hydrogels. The crosslinking density of hydrogels is important as it can further impact their water absorbing, holding and releasing capacity.

It was observed that glutaraldehyde based hydrogels have more stretchability and biocompatibility than epichlorohydrin based hydrogels. Hydrogels crosslinked with epichlorohydrin absorb and release water with more ease, due to their loose crosslinking. Glutaraldehyde based hydrogels absorb water in a lesser quantity, but show higher water retaining efficiency (*i.e.* controlled release) due to their stable crosslinked network.[27] Further, the study of these hydrogels was carried out by conducting the germination experiment.

### Germination percentage

The germination percentage of wheat and moong seeds was higher in the presence of the LG hydrogel, but the increase was not significant (1.07 fold), as compared to the control (Figs. 3, 4, 5A). As regards all other hydrogels, the germination percentage was the same as for the control. The results confirmed that the hydrogels provided sufficient water content to the seeds for the germination. Seeds absorb water from the hydrogel and hydrate the cotyledons to activate the physiological process of germination.[28] This proves that such hydrogels could be applicable in monocot and dicot seed germination as culture media.

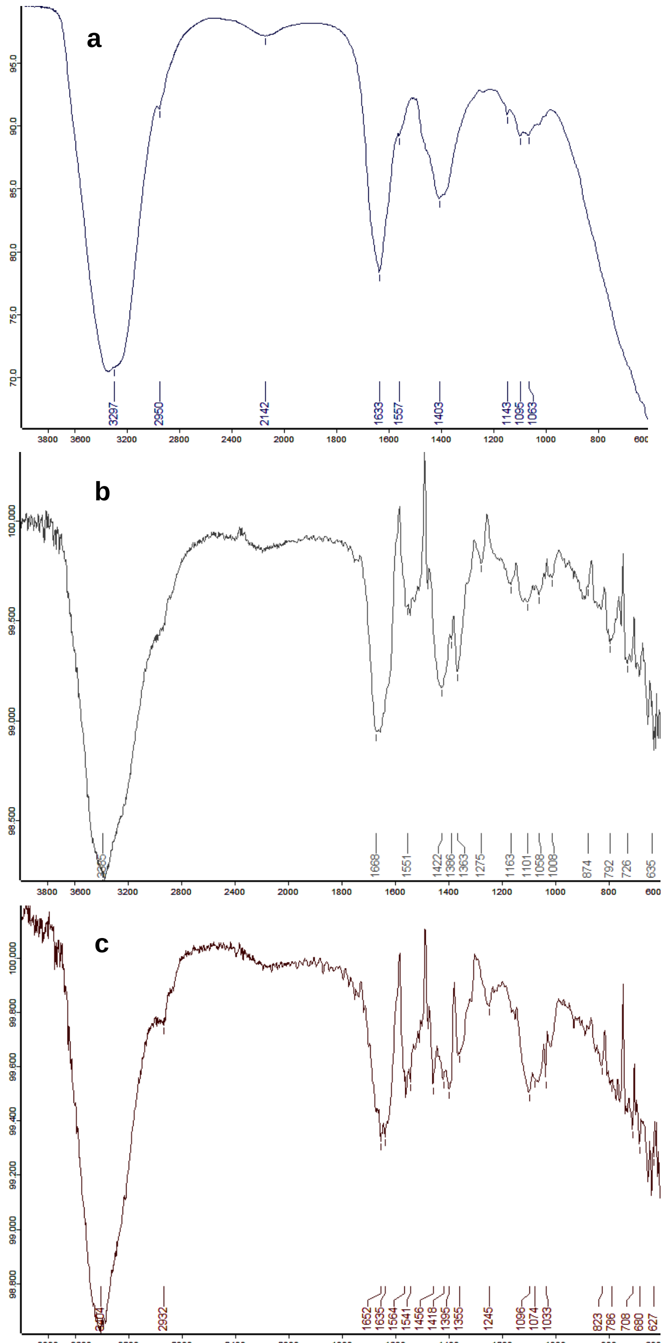



Figure 1: FT-IR spectra of different biopolymer-based hydrogels, a) MCC, b) lignin, c) MCC+lignin

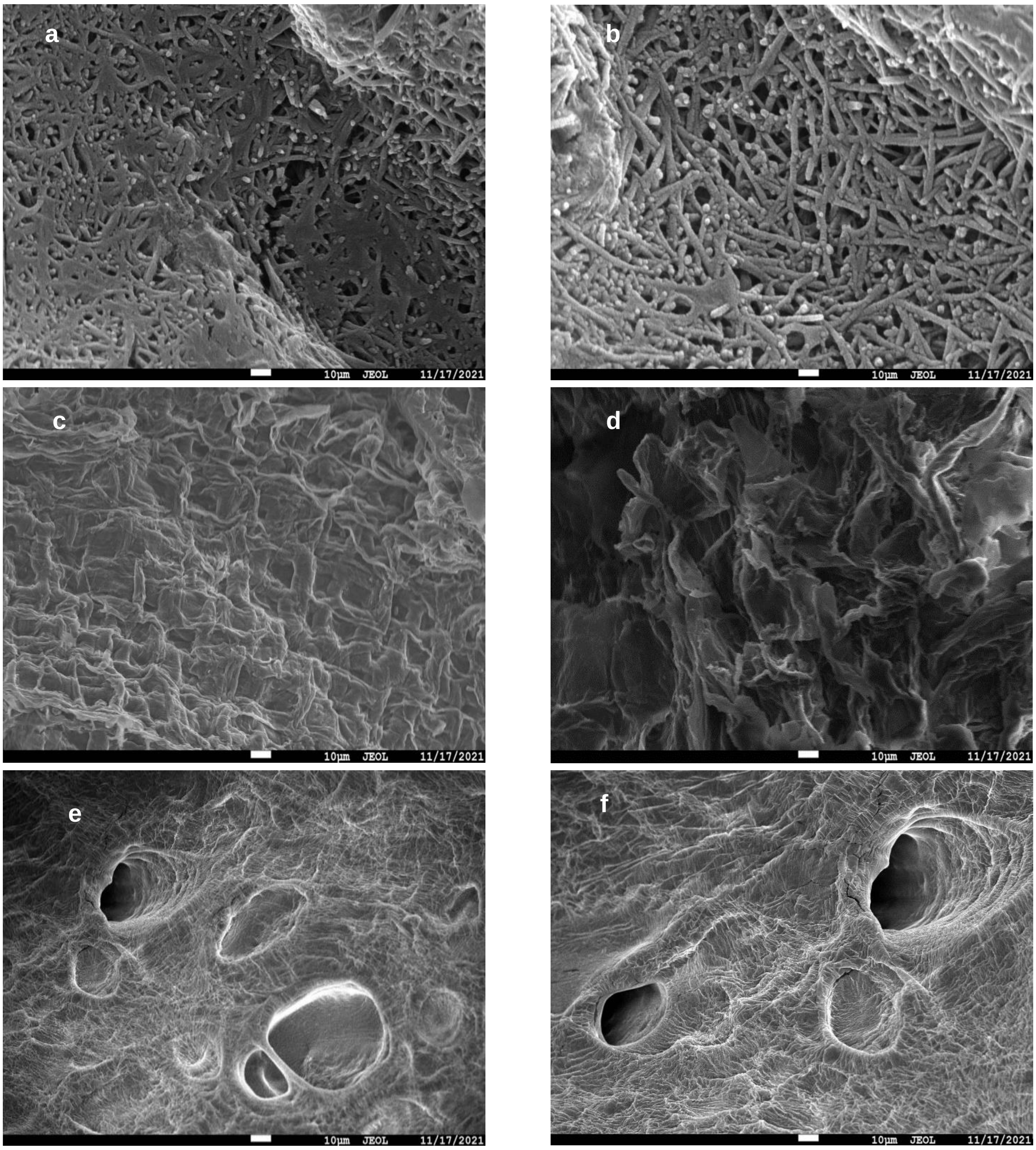



Figure 2: SEM images of biopolymer-based hydrogels: a) MG, b) ME, c) HG, d) HE, e) LG, f) LE

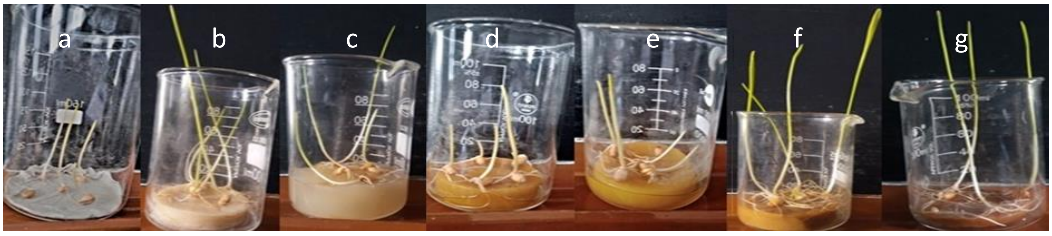
**Root and shoot length**

The root length of wheat seedlings decreased in the presence of hydrogels, compared to the control, except in the case of hydrogels LG and LE, where root length increased significantly – 1.93 and 1.65 fold, respectively. The root length of wheat seedlings in the presence of MG, ME, HG and HE hydrogels were at par with that of the control (Figs. 3, 5B).

Similar results were observed for root length in moong bean seedlings. A significant 1.66 fold increase in root length was observed for LE and a non-significant increase was observed for LG. A significant decrease in root length was observed for MG, ME and HE hydrogels, while HG showed results at par with those of the control (Figs. 4, 5B).

The shoot length of wheat and moong bean seedlings increased in the presence of all the hydrogels, as compared to the control. The shoot length of wheat seedlings grown in LG, LE, MG, ME, HE and HG hydrogels showed a significant increase by 2.30, 1.92, 1.45, 1.41, 1.32 and 1.29 fold, respectively, compared to the control (Figs. 3, 5C). In moong bean seedlings, LG and LE hydrogels showed the maximum increase and a significant one (4.54 and 7.83 fold, respectively) in shoot length, as compared to the control. Meanwhile, in the other hydrogels, *i.e.* MG, ME, HG and HE, the increase in shoot length was not significant (1.48, 3.23, 1.29 and 2.66 fold, respectively) (Figs. 4, 5C). These results indicate that the hydrogels offered the necessary moisture to the seedlings – decreased root length indicates that the culture medium provides sufficient water content to the seeds for their growth. On the other hand, increased root length indicates growth of seeds under inadequate water availability, *i.e.* in the case of the control.[29]

Still, the lignin-based hydrogels, *i.e.* LG and LE, contributed to higher root length as well as shoot length of both wheat and moong bean seedlings, compared to the control. This could be due to the macroporous nature of lignin hydrogels,[30] which results in fast evaporation of surface water, as compared to other hydrogels.

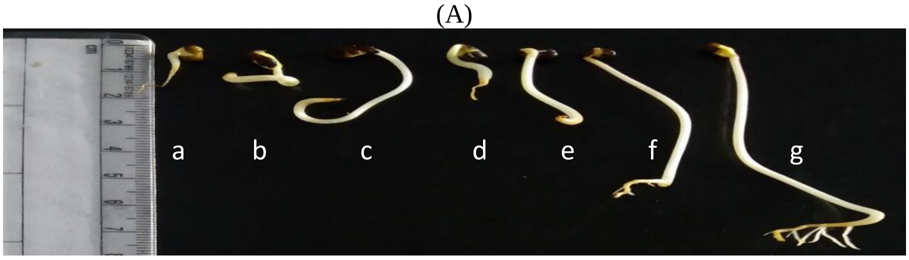



(A)


(B)

Figure 3: Wheat germination in different media (A) and their scale length (B): a) control, b) MG, c) ME, d) HG, e) HE, f) LG, g) LE

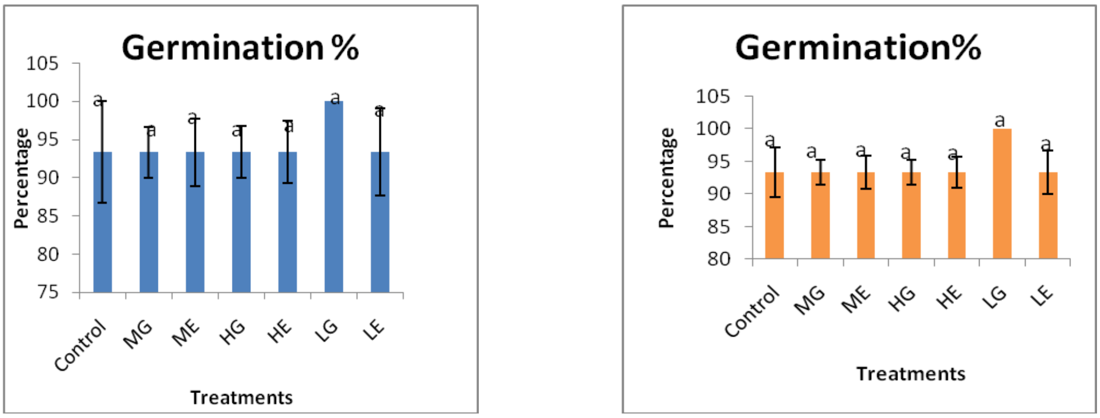

(A)



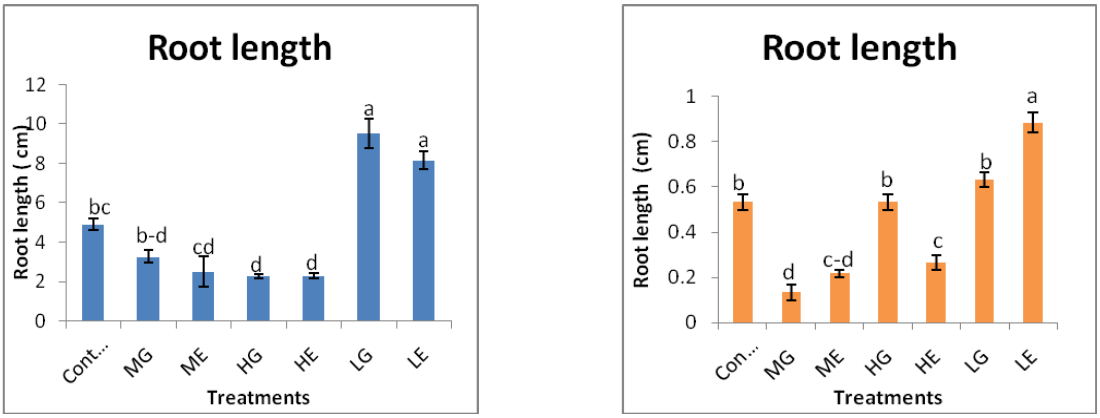

(B)

Figure 4: Moong bean germination in different media (A) and their scale length (B): a) control, b) MG, c) ME, d) HG, e) HE, f) LG, g) LE

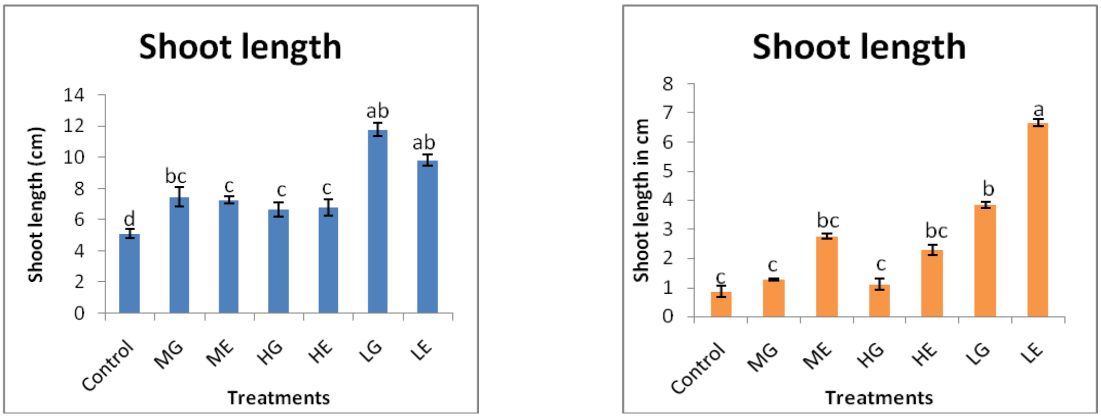
**A**


Germination %
Percentage
Treatments
Control
MG
ME
HG
HE
LG
LE
Germination%
Percentage
Treatments

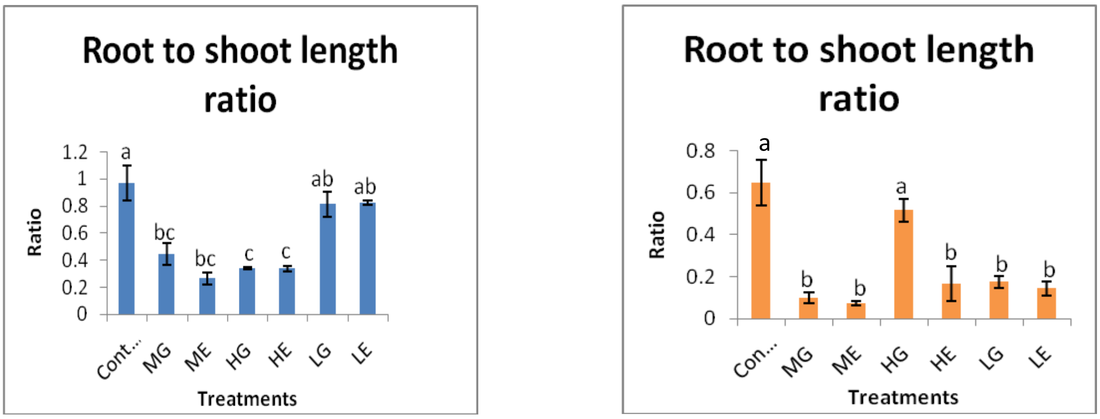
**B**


Root length
Root length ( cm)
Treatments
Cont…
MG
ME
HG
HE
LG
LE
Root length
Root length (cm)
Treatments
Con…

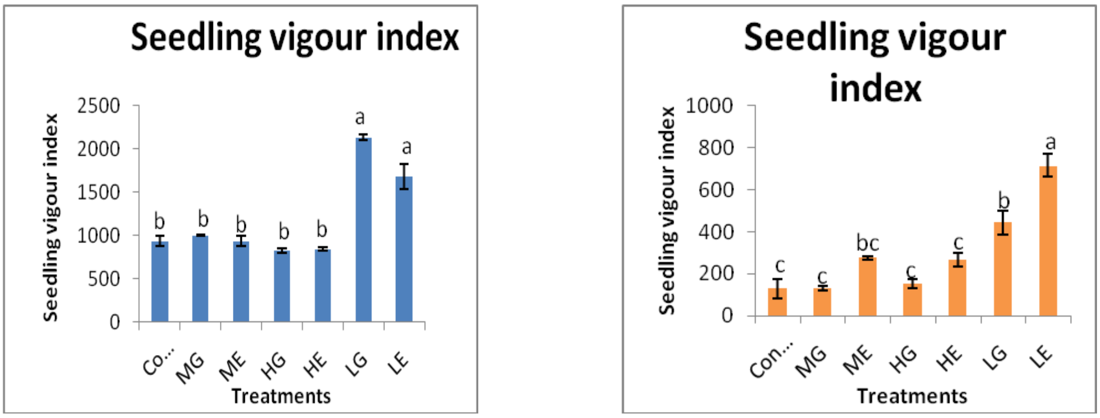
**C**


Shoot length
Shoot length (cm)
Treatments
Control
MG
ME
HG
HE
LG
LE
Shoot length
Shoot length in cm
Treatments

**D**


Root to shoot length ratio
Ratio
Treatments
Cont…
MG
ME
HG
HE
LG
LE
Root to shoot length ratio
Ratio
Treatments
Con…

E




F

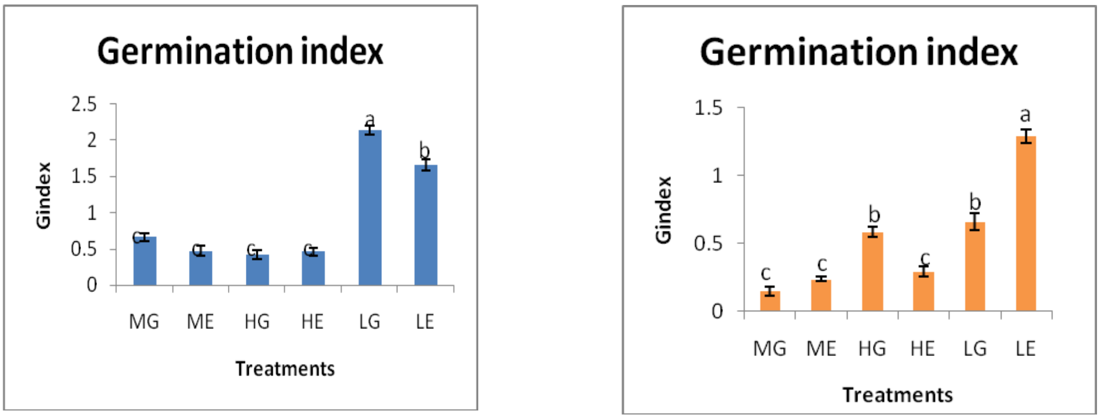



Figure 5: Germination percentage (A), root length (B), shoot length (C), root to shoot length ratio (D), seedling vigour index (E) and germination index (F) of wheat (blue – left) and moong bean (orange – right) seedlings in different media (error bars denote mean ± standard error of three replicates; bar with different small letter(s) denote significant difference in media at P<0.05: analysed by Duncan's post hoc test)

Zhou (2020) stated that macropores increased evaporation of water between soil surface and ground water.[31] This might have forced the seedlings to penetrate the surface and absorb water from its pores, to acquire higher moisture content to fulfil the requirements for fast growing shoots. In the case of the other hydrogels, the growth of root was decreased, which indicates sufficient water availability to the seeds. However, because of their lower porosity, the seedlings were unable to efficiently penetrate the surface of the hydrogels, which resulted in lesser growth of the seedlings, compared to that for the lignin-based hydrogels.

The control showed an increased root length, with the lowest shoot length, which means that the seedlings were growing under water scarcity conditions. This confirms that the water content in the moist filter paper evaporated quickly, providing insufficient growth conditions for the seedlings. In contrast, the hydrogels possess a three-dimensional condensed network of pores, which can hold the water and provide the required moisture to the seedlings.

**Root to shoot length ratio**

The control was observed to have the highest root to shoot length ratio (*i.e.* 0.97) for wheat seedlings, out of all the samples. All the hydrogels led to a decrease in root to shoot length ratio, as compared to the control. Specifically, HE, HG, ME and MG hydrogels showed a significant decrease in root to shoot ratio by 2.93, 2.85, 2.69 and 2.20 fold, respectively, as compared to the control. However, this ratio decreased non-significantly, by 1.19 fold, for both LE and LG hydrogels, compared to the control (Figs. 3, 5D).

In the case of moong bean seedlings, all the hydrogels led to a decrease in root to shoot length ratio, compared to the control (Figs. 4, 5D). The HG hydrogel showed a non-significant decrease in the R:S ratio, while all other hydrogels, *i.e.* MG, ME, HE, LG and LE, showed a significant decrease – by 6.40, 9.14, 4.00, 3.76 and 4.57 fold, respectively.

Higher root to shoot length ratio indicates higher root penetration into the culture media to take up water. Under water deficient conditions, the root:shoot ratio increases to enhance soil exploration and nutrient uptake.[32] This explains the maximum value of R:S in the case of the control, indicating sufficient water availability and proper porosity of the hydrogels, due to their 3D network, allowing the roots to explore and contributing to high shoot length. This supports the application of these hydrogels as media for seed germination and seedling growth in agriculture.

**Seedling vigour index**

Seedling vigour is an important index of seed quality, which analyses the potential for rapid and uniform emergence of plants.[33] The seedling vigour index of wheat seedlings grown in all types of hydrogels was at par with that of the control, except those for hydrogels LG and LE, which were significantly higher (2.27 and 1.79 fold, respectively) than that of the control (Figs. 3, 5E). Moong bean seedlings also showed similar results for seedling vigour index. Seedling vigour was similar in the case of all types of hydrogels to that of the control, except for hydrogels LG and LE, which showed a 3.38 fold (LG) and a 6.02 fold (LE) increase compared to the control (Figs. 4, 5E).

Wheat and moong bean seeds exhibited their full capacity to germinate in all the hydrogels, while the highest seedling vigour index was observed in LG and LE. It might be explained by their highest porosity, among all of the hydrogels in this study, as observed in SEM images (Fig. 2). Large porosity helps to retain more water for successful seed germination. If the growth medium has sufficient moisture, the seeds will complete their germination.[34] This confirms that LG and LE hydrogels were able to provide the required moisture to the seeds for their germination and growth of seedlings up to a certain limit.

**Germination index (GI)**

Germination index (GI) was measured to know the speed of germination. The higher is the value of the germination index, the higher is the rate of germination.[35] The LG hydrogel showed the highest value of wheat germination index, *i.e.* 2.14, among all the hydrogels used as growth media (Figs. 3, 5F), while LE, MG and ME hydrogels showed a lesser rate of germination. However, hydrogel HG showed the lowest value of wheat germination index, *i.e.* 0.42, which indicates the lowest rate of germination.

In the case of moong bean seedlings, the highest germination index was observed in hydrogel LE, *i.e.* 1.72, indicating the highest rate of seed germination (Figs. 4, 5F), and the lowest – in MG – of 0.25.

## CONCLUSION

The valorization of rice straw in the synthesis of hydrogels is a contributing step towards a green and sustainable environment. SEM images of epichlorohydrin and glutaraldehyde based hydrogels showed slight difference in their crosslinking densities, which further impacted their water retention capacity, while in the comparison of biopolymers, lignin based hydrogels showed larger pores than the others. The prepared hydrogels were tested to check their possible phytotoxicity by using them as germination media for wheat and moong bean seeds. The germination rate was found to be maximum for both lignin hydrogels, *i.e.* LG and LE, as compared to the control and the other hydrogels. However, the other hydrogels also performed well, as compared to the control. Overall, the health of the seedlings was calculated by measuring the root to shoot length ratio, the germination index and the seedling vigour index parameters. The results revealed maximum growth in both lignin hydrogels, as compared to the control and the rest of the hydrogels. Lignin hydrogels proved as the best media for seed germination for wheat and moong bean in the present study. The results also suggested that all the prepared hydrogels efficiently meet the moisture requirements for seed germination, without causing any toxicity. These hydrogels can be further explored to be used as an ingredient of culture soil under water deficient conditions and can be beneficial in coping with the serious issue of water crisis in Northern India.

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