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Synthesis and characterization of xanthan gum and carboxymethylcellulose sodium salt based ionic crosslinked hydrogels for agricultural application

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Ever-growing world population is creating immense pressure on the agriculture sector leading to the overexploitation of resources, soil degradation, and water scarcity which in turn significantly reduces crop yield. To break this destructive loop, a current investigation is initiated aiming at the development of new materials, carboxymethylcellulose sodium salt (NaCMC)-based ionic (Fe^{3+}) cross-linked hydrogels (XG-$Fe_{1\text{-}5}$, $NaCMC_{1\text{-}5}$, and XG-Fe-$NaCMC_{1\text{-}5}$). The hydrogels were characterized using Fourier-transform infrared spectroscopy, thermogravimetric analysis, scanning electron microscopy, and X-ray diffraction analysis. The study confirms that the hydrogel, XG-Fe-NaCMC with maximum thermal stability and swelling index, has potential application in agriculture improving the water holding and retention capacity of the soil. Moreover, a significant positive effect on the growth of wheat grass *(Triticum aestivum)* was recorded in a pot experiment. It showed that the plants grew faster in the soil treated with urea-loaded XG-Fe-NaCMC than the plants raised in soil treated directly with urea. The current investigation, for the first time, approached the development of hydrogels by crosslinking dual anionic natural polysaccharides *via* trivalent ions for agricultural applications.

Keywords: Hydrogel; NaCMC; Xanthan gum; Agriculture

INTRODUCTION

The efficient use of water resources for crop production is a major step toward sustainable agriculture. In this context, the Government of India has sponsored a scheme "per drop more crop." It promotes water management mainly through micro irrigation (Drip and Sprinkler irrigation systems) and the adoption of micro-level water storage systems in agricultural fields [1]. The application of 3-D polymeric matrices (hydrogels, HGs), with the ability to absorb and hold a large amount of water, to agricultural fields is a chemical approach to support the scheme. These hydrogels act as on-the-spot tiny water reservoirs, releasing up to 95% of adsorbed water into the soil during drought water stress [2-4]. Also, they amend the properties of the soil in favor of plant growth [2]. Most synthetic and semi-synthetic hydrogels employed in agriculture today contain acrylic acid derivatives and polyacrylamide in their basic skeletal framework [6, 7]. Their persistence in the soil is a serious environmental problem. Moreover, the residual acryl amide in polyacrylamide is a potential soil and food contaminant [3]. It necessitates the utilization of benign materials to design safer hydrogels for agricultural applications [8-11].

The use of cellulose (a natural polysaccharide) for the synthesis of hydrogels has been rising in popularity day-by-day as it is ubiquitous in nature, less expensive, and environmentally safe. Agro-wastes [12, 14], office waste papers [15, 16], and waste products of cotton industries (cotton linters, and knitted rag) [4,5] are rich sources of cellulose. The presence of many reactive hydroxyl groups on polymeric chains facilitates their structural modifications thereby introducing a new set of physicochemical properties [6]. Carboxymethyl cellulose (CMC), is one such derivative of cellulose synthesized by reacting sodium mono chloroacetate and cellulose in an alkaline medium [20, 21]. Its sodium salt (NaCMC) is non-toxic, biocompatible, bioadhesive, biodegradable, and water-soluble. More COO^- functional groups increase the water storage ability of polymers due to an increase in oxygen content in the framework. The introduction of carboxylate functional groups also allows the polymeric chains to link together *via* multivalent ionic crosslinkers to develop 3-D network of hydrogels [22-26]. It is evident that trivalent iron (Fe^{3+}) is a safe and non-toxic crosslinking agent.

Xanthan gum (XG) is a water-soluble, anionic, exocellular microbial polysaccharide (PS). It is produced by the microbe *Xanthomonas campestris*. It consists of β(1→4) linked glucan backbone with alternating charged trisaccharide side chain containing a glucuronic acid residue between two mannose units [7]. XG plays diverse roles in food, medical, cosmetic industries and oil fields [8].

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Presently, it is attracting enormous attention of scientists by exhibiting ~~positive~~ synergistic action with different polysaccharides such as partially hydrolyzed polyacrylamide (HPAM) [9], galactomannans (guar gum and locust bean gum) [30, 31], gum Arabic [10], Konjac gum [11]. It is now well established that the synergistic combination brings about additional functional properties to the mixture, compared to polysaccharide alone. Studies also confirm that positive results are obtained with XG/PS in 1:1 ratio (w/w) [11,12].

To our knowledge, combined effect of XG/NaCMC has been rarely investigated. The current investigation, therefore set out to develop a series of hydrogels (XG-Fe-NaCMC 1-5) by crosslinking XG, NaCMC (1:1) through Fe^{3+} ions for agricultural applications. Swelling test demonstrated the optimum concentration for crosslinker. Thus, optimal hydrogel XG-Fe-NaCMC was characterized by Fourier Transform infrared (FTIR) spectroscopy, scanning electron microscopy, energy dispersive X-ray analysis (SEM/EDX), and thermogravimetric analysis (TGA). We also report herein the impact of hydrogel on the growth of wheat grass (*Triticum aestivum*) in terms of average number of germinated seeds, average shoot height (cm), average root length (cm), total fresh weight (g) and total dry weight (g) of wheat grass raised for 15 days in normal environmental conditions.

MATERIALS AND METHODS

Chemicals

Commercial XG powder was purchased from the local market, anhydrous iron (III) chloride was procured from Merck Lifesciences Pvt Ltd, NaCMC and urea were purchased from CDH Pvt. Ltd., Milli Q grade water was used to prepare aqueous solutions.

Synthesis of hydrogels (HGs)

Three sets of HGs were prepared using varied amounts of Fe^{3+} as crosslinker; i) xanthan gum hydrogel (XG-Fe 1-5), ii) carboxy methylated cellulose hydrogels (NaCMC-Fe 1-5), and iii) xanthan gum, carboxy methylated cellulose (1:1) hydrogels (XG-Fe-NaCMC 1-5) (Table 1). Briefly, the desired amount of xanthan gum and /or sodium carboxy methylated cellulose was stirred in 40 mL of distilled water with the help of magnetic stirrer until a uniform thick solution is obtained. 40 ml of $FeCl_3$ solution with requisite amount of $FeCl_3$ was added to this aqueous-viscous solution and kept for 2 days at room temperature. The resultant hydrogels were washed thoroughly in distilled water to avoid unreacted materials and then dried in an oven at 70°C until constant weight.

Swelling study

The swelling of HGs was investigated by gravimetric analysis. Dried samples of hydrogels were accurately weighed and immersed in 250 mL of distilled water. At predetermined time intervals, swollen gels were taken out and blotted with filter paper to remove extra water. Weight of the gel was recorded and it was placed in water again. This process was repeated until a constant weight was reached. The % swelling index (SI) of hydrogels was calculated using equation 1 [13,14]

$$SI\ (\%) = \frac{(Ws - Wd)}{Wd} \times 100 \qquad \text{Eq. 1}$$

where W_s and W_d are the weights of swollen and dry hydrogels, respectively.

Table 1. Swelling indices of hydrogels of different formulations

Formulation	Xanthan Gum(g)	NaCMC (g)	Distilled Water(ml)	Crosslinker-$FeCl_3$(g/40 ml)	Swelling Index (%)
$XG\text{-}Fe_1$	0.4	0	40	0.04	ns
$XG\text{-}Fe_2$	0.4	0	40	0.08	702.1
$XG\text{-}Fe_3$	0.4	0	40	0.12	521.5
$XG\text{-}Fe_4$	0.4	0	40	0.16	479.7
$XG\text{-}Fe_5$	0.4	0	40	0.20	413.5
$NaCMC\text{-}Fe_1$	0	0.4	40	0.04	ns
$NaCMC\text{-}Fe_2$	0	0.4	40	0.08	364.8
$NaCMC\text{-}Fe_3$	0	0.4	40	0.12	343.2
$NaCMC\text{-}Fe_4$	0	0.4	40	0.16	241.4
$NaCMC\text{-}Fe_5$	0	0.4	40	0.20	231.3
$XG\text{-}Fe\text{-}CMC_1$	0.2	0.2	40	0.04	ns
$XG\text{-}Fe\text{-}NaCMC_2$	0.2	0.2	40	0.08	526.4*
$XG\text{-}Fe\text{-}NaCMC_3$	0.2	0.2	40	0.12	412.2
$XG\text{-}Fe\text{-}NaCMC_4$	0.2	0.2	40	0.16	360.3
$XG\text{-}Fe\text{-}NaCMC_5$	0.2	0.2	40	0.20	310.2

* XG-Fe-NaCMC formulation with maximum % SI represents optimum concentration of crosslinker and therefore selected for detailed analysis. ns- not synthesized

Characterization

The XRD analysis of XG-Fe-NaCMC, XG-Fe, and NaCMC-Fe hydrogels was carried out by Perkin Elmer spectrum version 10.5.3 model in solid-state form. FTIR spectroscopy was performed using Perkin Elmer spectrum two. Thermo-gravimetric analysis (TGA) of XG-Fe-NaCMC hydrogel was carried out using a PerkinElmer, TGA 4000 in N_2 atmosphere in the temperature range from 25°C to 800°C with 10°C/min of uniform heating rate. The surface morphology of XG-Fe-NaCMC hydrogel was analyzed by SEM on EVO 18 research, Zeiss, instrument.

Maximum water holding capacity of soil (MWHC)

Air-dried soil, collected from DTU garden, was filtered through a 3 mm sieve. 0.1 g of hydrogel was mixed in 50 gm of this soil. The mixture was then placed in a PVC tube (diameter - 2.5 cm, length - 10 cm) with small holes at the bottom covered with filter paper inside the lower surface. Weight of this set up is recorded as W1. The soil samples were then gradually soaked with distilled water from the top of the tube until the water began to leak out of the bottom of the tube. Once there was no longer leakage of water at the bottom, the tube was weighed again and recorded as W2. For control, soil was taken without hydrogel. % MWHC of the soil was calculated using equation 2 [15,16].

$$\text{MWHC}\ (\%) = \frac{(W2-W1)}{50}\times 100 \qquad \text{Eq. 2}$$

Plant growth study

The effects of the hydrogel-treated soil on the growth of *T. aestivum* were investigated in terms of i) average number of seeds germinated; ii) total fresh and dry weight and iii) average shoot height and root length. In this process, 300 g of soil, collected from DTU garden, was taken in different pots. 0.1 g of urea-loaded hydrogels (XG-Fe, NaCMC-Fe and XG-Fe-NaCMC) was placed at a depth of 5 cm in soil in all the ~~different~~ pots. For control, soil without hydrogels was directly treated with urea solution. Fifteen wheat seeds were sowed in each pot and exposed to natural environmental conditions with occasional irrigation. Number of seeds germinated in each set was recorded. The appearance of radicle alone was considered as a sign of seed germination. After a period of 15 days, the whole plant was removed from the soil, cleaned, weighed and length of the shoots and roots was carefully measured. In order to determine the dry biomass, the plant material was dried for 24 hours at 70°C [17].

RESULTS AND DISCUSSION

Synthesis

Formation of water-insoluble mass from water soluble precursors, XG and/or NaCMC, in the presence of Fe^{3+} ions with the ability to hold large amount of water clearly indicates the creation of a hydrogel. Both XG and NaCMC contain anionic carboxyl functional groups. Thus, ionic crosslinking arises *via* Fe^{3+} ions to develop 3D polymer network (Fig. 1). The extent of crosslinking is significantly influenced by the concentration of Fe^{3+}, which is also demonstrated by the % swelling index. 0.04 g/40 ml of $FeCl_3$ fails to develop efficient interaction between the chains in the identical synthetic conditions, hence, hydrogel is not obtained. With gradual increase in $FeCl_3$ concentration from 0.08 to 0.2 g/40 ml aq. solution, a regular decrease in % swelling index was observed indicating reciprocal correlation between crosslinking and water storage capacity of the hydrogels. Based on these findings the optimal XG-Fe-NaCMC hydrogel (XG:NaCMC in 1:1 ratio and 0.08 g/40 ml of aqueous $FeCl_3$ solution) was selected for detailed analysis.

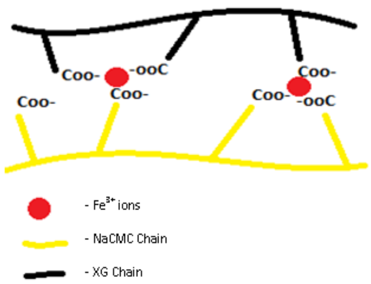

Fig. 1. Binding of carboxylate groups of XG and NaCMC with Fe^{3+} ions.

Characterization of synthesized hydrogels

X-ray diffraction

X-ray diffraction pattern (Fig. 2) confirms that the crystallinity of XG and NaCMC is adversely affected by the crosslinking. Thus, XG-Fe and NaCMC-Fe have more disordered structure than that of their precursors. It may be assumed that polymeric chains undergo the order → disorder transition to form ionic bonds with trivalent Fe ions [18]. This transition is more prominent in XG-Fe-NaCMC hydrogel. It has a completely amorphous structure. Carboxylate functional groups are attached to two different polymeric chains in a different way. Probably, for crosslinking either the electrostatic force of attraction between $Fe^{3+}$and COO^- and/or the repulsive interaction between XG and NaCMC, both being anionic polysaccharides, causes rearrangement of basic polysaccharide network leading to loss of crystallinity.

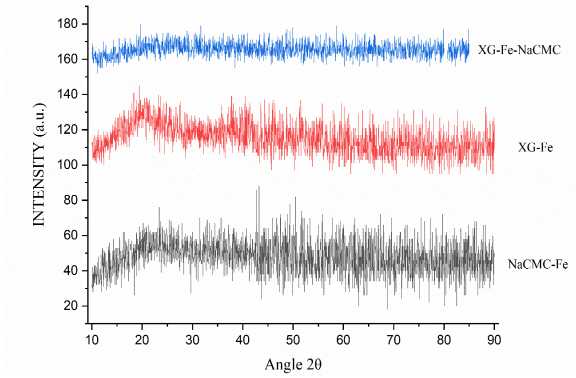

Fig. 2. XRD spectra of hydrogels.

Fourier Transform infrared spectroscopy

Fig. 3 represents the FTIR spectra of XG-Fe-NaCMC, XG-Fe and NaCMC-Fe. The absorption band in the range of 3200-3300 cm^{-1} confirms the presence of OH groups in the hydrogels. Two broad bands between 1609 and 1390 cm^{-1} arise from the asymmetrical and symmetrical stretching mode of –COO^{-} groups. The absorption bands at 2920 and 2850 cm^{-1} of XG-Fe hydrogel correspond to the asymmetric and symmetric methylene C-H stretching of XG, respectively [19]. Absorption band in the range of 1050-1030 cm^{-1} shows the presence of symmetrical stretching of -COO^{-} group of glucuronic acid and at 1037 cm^{-1} is due to the C-O-C stretching of the ether group.

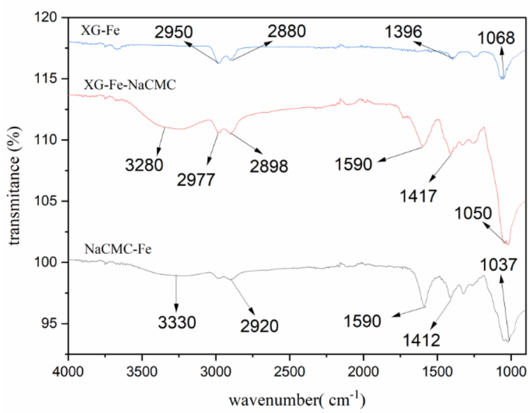

Fig. 3. FTIR spectra of hydrogels

Thermogravimetric analysis

Thermogravimetric assessment of hydrogels is presented in Fig. 4. The first phase in the range of 50-200°C is observed in all samples, probably due to desorption of moisture bound to the saccharide structure through water hydrogen [20]. The significant weight loss observed between 250-800 °C is attributed to breakdown of main polymeric chain [20]. In NaCMC-Fe, the primary degradation began at ~240°C, with weight loss from the inorganic portion followed by pyrolysis occurred after 335°C. XG-Fe followed a multi-step degradation pattern. TGA also confirms that hydrogel (XG-Fe-NaCMC) is thermally more stable than the hydrogels prepared using single natural polymeric chains (XG-Fe or NaCMC-Fe).

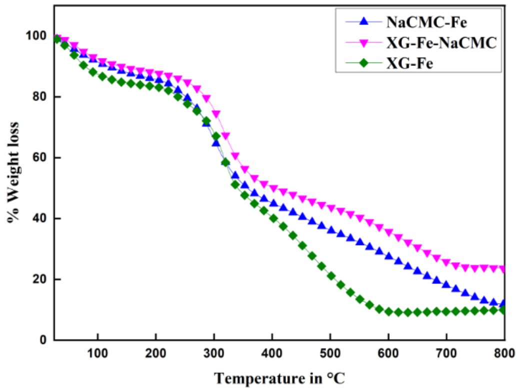

Fig. 4. TGA thermograms of XG-Fe, NaCMC-Fe and XG-Fe-NaCMC

Scanning electron microscopy

The surface morphologies of XG, NaCMC, and their hydrogels and EDX were studied (Fig. 5). The XG is found to have a smoother surface than its hydrogel XG-Fe which has a relatively rougher and coarser surface. NaCMC too has a smooth surface but NaCMC-Fe has smooth surface with abrupt ruptures and cracks exposing rough and irregular morphology [21]. The porosity of XG-Fe-NaCMC is surprisingly very high. It supports our assumption that repulsive interaction between two different anionic polysaccharide chains and their order → disorder transition during crosslinking favors the development of highly porous 3D network of hydrogel.

Swelling study

The amount of crosslinker was varied from 0.04 to 0.20 g/40 ml aq. solution of $FeCl_3$ for the synthesis of different sets of hydrogels. In general, it was observed in each case that the % swelling index decreases with increase in Fe^{3+} concentration. Maximum % SI was attained in about 25 hours (Fig. 6 a, b). The extensive analysis of % SI of different formulations (Table 1) revealed a significant fact: the % SI of single polysaccharide hydrogel, NaCMC-Fe_2, is 364.5 with 0.08 g/40 ml of aq. $FeCl_3$ solution, whereas, with the same amount of crosslinker % SI is enhanced to 526.4 when dual polysaccharide frame work is developed using XG:NaCMC in 1:1 ratio (formulation XG-Fe-$NaCMC_2$). Similar trend was exhibited by other formulations also confirming the positive interaction between XG and NaCMC.

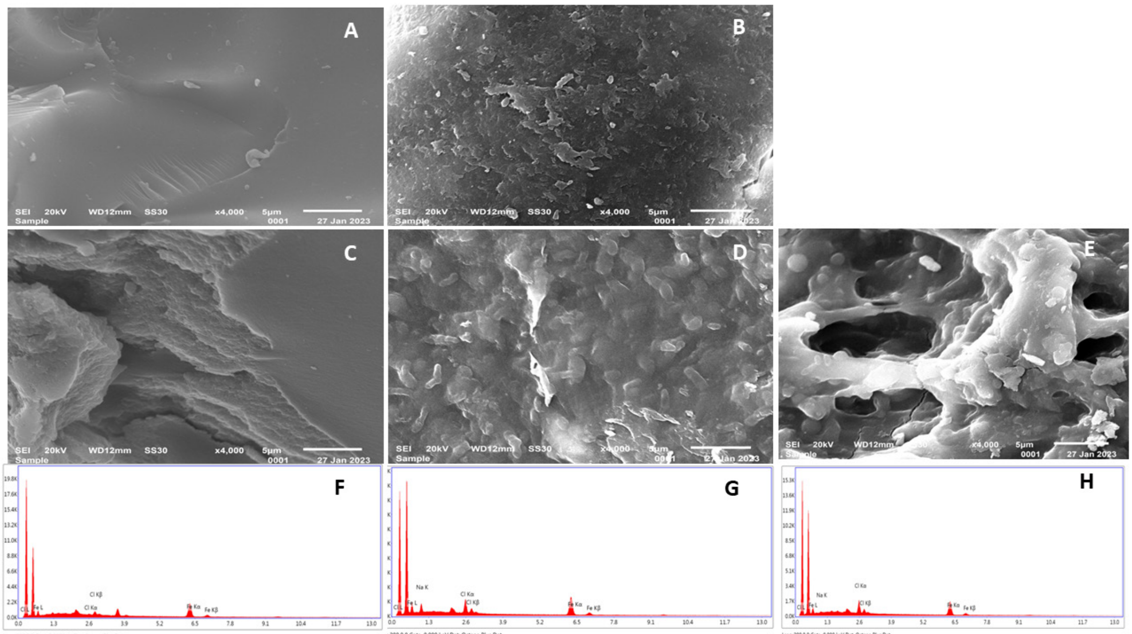

Fig. 5. SEM images and EDX graphs of hydrogels, (A) XG, (B) XG-Fe hydrogel, (C) NaCMC, (D) NaCMC-Fe hydrogel, (E) XG-Fe-NaCMC hydrogel (F) EDX of XG-Fe, (G) EDX of NaCMC-Fe, and (H) EDX of XG-Fe-NaCMC.

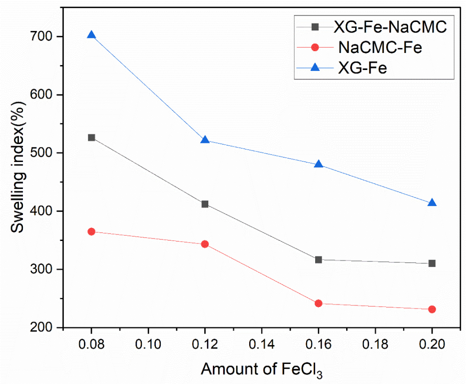

Fig. 6 (a). % Swelling index with different amounts of $FeCl_3$.

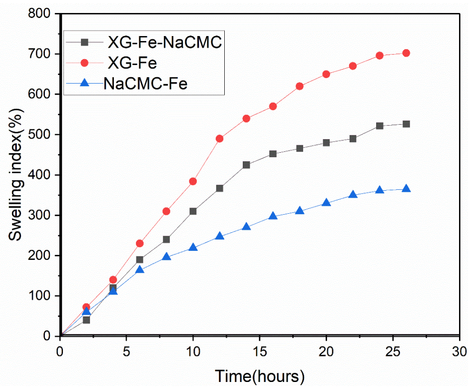

Fig. 6 (b). % Swelling index with time

Maximum water holding capacity (MWHC)

MWHC of soil was determined in control (without applying hydrogels) and in soil treated with hydrogels: XG-Fe, NaCMC-Fe and XG-Fe-NaCMC. In control, MWHC of soil was 20 ml per 50 g of soil, which increased by 10% (22 ml per 50 g of soil) by application of NaCMC-Fe hydrogel. Application of XG-Fe-NaCMC and XG-Fe demonstrated an increase in MWHC by 30% (26 ml per 50 g of soil) and 40% (28 ml per 50 g of soil), respectively [15].

Plant growth

The influence of hydrogel (0.1 g per 300 g of soil) on growth of wheat grass is shown in Table 2 and Fig. 7. Water is a vital input in agriculture. It not only triggers the seed germination, but is also responsible for the utilization of nutrients in fullest by plants [22]. It is also well known that seeds grow better when urea is available for a longer period. Current investigation confirms that hydrogel XG-Fe-NaCMC is a potential component of agricultural field for better growth of the plant by maintaining urea and water level in soil.

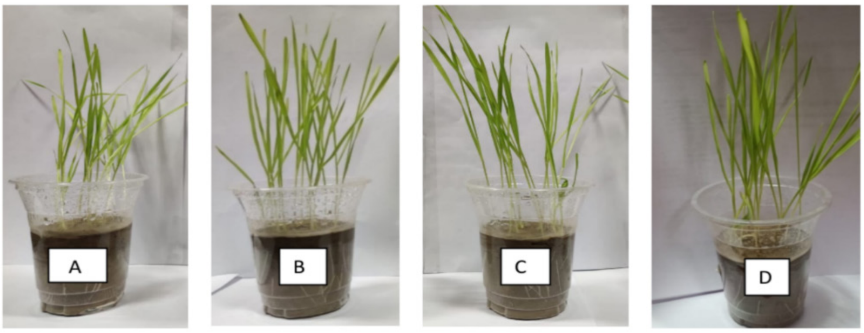

Fig. 7. Plant growth, (A)- Control (soil without hydrogel), (B)- soil with NaCMC-Fe, (C)- soil with XG-Fe, and (D)- soil with XG-Fe-NaCMC.

Table 2. Wheat grass growth assessment by pot experiment

Sample	Total fresh weight (g); % increase	Total dry weight (g); % increase	Average number of germinated seeds; %	Average shoot height (cm); % increase	Average root height (cm); % increase
XG-Fe	2.2696; 52.07%	0.2403; 56.24%	14; 93.33%	18.92; 18.69%	6.48; 15.50%
NaCMC-Fe	1.7680; 18.46%	0.1814; 17.95%	14; 93.33%	17.11; 7.34%	5.88; 4.81%
XG-Fe-NaCMC	2.6164; 75%	0.2745; 78.48%	15; 100%	19.14; 20.07%	6.71; 19.60%
Ctrl	1.4924	0.1538	13; 86.67%	15.94	5.61

CONCLUSION

In current time the efficient use of available water in agriculture sector is a worldwide serious challenge. To fulfil the food demand of ever-growing population and make agriculture sustainable and future-ready multidirectional efforts are urgently needed, including development of new technologies and effective management of agriculture practices. With this view the current study explored the use of gainful interaction between two different polysaccharides (xanthan gum and carboxymethylated cellulose) crosslinked through Fe^{3+} ions for agricultural applications. An optimal formulation (XG:NaCMC in 1:1 ratio and 0.08 g/40 ml of aqueous $FeCl_3$ solution) exhibited promising benefits on seed germination (100 % seed germination), plant growth (up to 75% increase in total fresh weight and 78.84% increase in total dry weight) and MWHC of soil. Besides, carboxymethylated cellulose can be obtained from waste plant materials, thus, offers a benign way for agro-waste management.

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