Egypt. J. Chem. **Vol. 66**, No. 10 pp. 355 - 362 (2023)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Using Cellulose-Based Hydrogel to Alleviate the Effect of Drought Stress of Sunflower Plant

Ahmed Salama[1], Mohamed A. Ismail[2], Mohamed A. Amin[2], Medhat E. Owda[3], Ragab E. Abouzeid[1*], H. Abd El-Wahab[3]

[1]*Cellulose and Paper Department, National Research Centre, 33 El-Behouth St., Dokki, P.O. 12622, Giza, Egypt*
[2]*Department of Botany and Microbiology, Faculty of Science, Al-Azhar University, Nasr City 11884, Cairo, Egypt*
[3]*Chemistry department, Faculty of Science, Al-Azhar University, Nasr City 11884, Cairo, Egypt*

Abstract

In the current study, bioadsorbent based cellulose, CMC-g-P(DMEMA) hydrogel, was prepared, characterized and investigated for alleviating the bad effects of drought stress on the growth and biochemical traits of sunflower plants. The impact of water stress on plant pigments, total phenol, proline content, enzymatic responses and some growth characteristics of sunflower plants was investigated. The results showed that CMC-g-P(DMEMA) hydrogel decreased the drought stress on growth characters, chlorophyll content and total phenol contents. The lengths and fresh weight of shoot, pigments and phenol contents was highly improved. These results displayed that CMC-g-P(DMEMA) hydrogel had good water retention and is expected to have potential applications in sustainable modern agriculture.

Keywords: Cellulose – Hydrogels – Drought stress – Sunflower plants

1. Introduction

Sunflower (Helianthus annuus L.) is an important crop and ornamental plant in the world. It is used for animal feed and it is the second most important crop producing edible oil after soybean [1]. Water is a vital factor that enhances crop production. Several factors hinder the agricultural growth such as rising water and irrigation costs, water shortages and drought [2]. Due to these problems, it is important to use water resources efficiently. Recently, the use of super absorbents hydrogels as water storage materials for agricultural applications has attracted growing interest. Hydrogels are presented as biocompatible materials due to their high water content which make them like natural extracellular matrices [3]. Moreover, their porous structure and high swelling% are particularly appropriate properties for loading water-soluble compounds, like therapeutically active proteins and peptides [4]. These unique properties of hydrogels have gained growing attention for applying these materials as reservoir systems for active ingredients that are slowly released from the hydrogel matrix in a controlled fashion to preserve the needed concentration of the active materials in the surrounding environment. Hydrogel based natural polymers are emerging as a potential alternative method for preparing highly swellable, biocompatible and biodegradable 3D porous materials for loading and releasing active ingredients. These three-dimensional cross-linked polymeric networks have many advantages such as the facility of the incorporation of different chelating groups and high internal specific surface area [5]. Hydrogels obtained from bioresources, such as cellulose, starch, chitosan and alginate have applied for loading and delivery of active materials in specific environment with controlled release [3,6–8]. Moreover, polysaccharides-based hydrogels were reported as eco-friendly, cost effective swellable materials in biomedical and environmental applications [9].

*Corresponding author e-mail: r_abouzeid2002@yahoo.com.;(Ragab E. Abouzeid).
EJCHEM use only: Received date 25 April 2022; revised date 02 July 2022; accepted date 19 February 2023
DOI: 10.21608/EJCHEM.2023.135796.5982

Carboxymethylcellulose, hydrophilic anionic polysaccharides, has gained increasing interest as a bio-adsorbent for the preparation of pH responsive hydrogels due to its biocompatibility, biodegradability and anionic properties [10]. Moreover, cellulose is a biodegradable, safe, andhydrophilic polymer, and the prepared hydrogels with ionic moieties can be applied as plant growth regulators [11,12].

The water retention property of hydrogels applied in agriculture and horticulture is a promising strategy for soil water saving. The hydrogels can improve soil quality, raising the survival rate of seedlings, and supporting the growth of plants. The aim of this work was to develop a promising and high-loading bioadsorbent based on cellulosic materials (carboxymethylcellulose) for alleviate the bad effects of drought stress on growth and biochemical traits of sunflower plants.

2. Experimental part

2.1. Materials

Carboxymethyl cellulose sodium salt (> 99.5%) was obtained from FlukaBiochemikawith high viscosity. Ethylene glycol dimethacrylate (Sigma-Aldrich) and (2-dimethylaminoethyl) methacrylate monomer (Merck) were used without purification. Other chemicals were analytical grade and used without further purification.

2.2. Preparation of CMC-g-P(DMEMA) hydrogel

The current hydrogel was prepared according to our previous study[13]. In brief, 2 gm CMC were dissolved in100 mL double-distilled water to prepare 2% (w/v) solution. The solution was heated at 70 oC and purged with nitrogen for 30 minutes. Potassium persulphate (0.45 g, 0.017 mol/L) was added, followed DMEMA monomer (6.28 g, 0.4 mol/L) and ethylene glycol dimethacrylate (0.13 gm, 0.0065 mol/L) were added and the reaction mixture was stirred until complete gelation. The formed hydrogel was cooled and then poured into excess water with continuous stirring to remove the unreacted molecules. The gel product was chopped to small pieces and dried in oven (at 50 °C) for 24 h. After grinding, the powdered superabsorbent hydrogel was stored in dry atmosphere [14,15]. The hydrogel prepared was denoted by H1.

2.3. Characterization

2.3.1. ATR-FTIR

Attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR) was done on a Thermo Nicolet FT-IR Nexus 470 with a diamond crystal. Spectra were recorded from 500 to 4000 cm-1 with a resolution of 2 cm-1.

2.3.2. SEM

Scanning electron microscopy was done on a JEOL JXA-840A Electron probe

2.4. Swelling properties

The swelling% of the prepared CMC-g-P (DMEMA) hydrogel in different mediums acidic, nutral and in alkaline solutions were calculated. The swelling percent was calculated by the following equation:

$$\text{Swelling\%} = (W_t - W_o)/W_o \times 100$$

where W_o is the initial weight and W_t the weight of the hydrogel at time t [16].

2.5. Pots experiment on the use of CMC-g-P(DMEMA) hydrogel to treatment drought stress

Sunflower Seeds (*Helianthus annuus* L.) were obtained from the Agricultural Research Centre (ARC), Ministry of Agriculture, Giza, Egypt. Soil moisture and the ability of hydrogel to keep water for a long time were achieved by growing sunflower - (Helianthus annuus L.) plant in drought stress conditions. Two states were studied: first one was the soil without hydrogel and the second was containing CMC-g-P(DMEMA) hydrogel. The state 1 of the experiment, the soil without a hydrogel, lacks soil water retention. The state II, a mixture of soil/CMC-g-P(DMEMA) hydrogel, was spreader on the surface of the soil. The swelling of hydrogel increased soil porosity and allowed good aeration which returned on the plant growth. With respect to plant growth, irrigation times were gradually decreased and adjusted according to precipitation (every 8 days). The plant samples of sunflower were collected to analyze their morphological characters and biochemical analysis (pigments, phenol, proline and enzymes contents).

2.5.1. Growth Measurements

The length of five shoots was grouped randomly for each treatment and the measurements were made from the soil surface to the end of terminal bud of the plants and written in centimeters. The plants in every treatment were weighted immediately after collection and determination of the fresh weight of the shoots.

2.5.2. Biochemical analysis

Chlorophylls contents were estimated using the method of Vernon and Seely[17]. With respect to carotenoid pigments, the concentration was estimated according to Lichtentahler[18]. The total phenolic constituents were estimated by using Folin-Ciocalteu method[19]. The plant content of free proline can be estimated by the method used in reference[20].

2.6. Extraction and estimation of enzymes catalase, peroxidese, polyphenol oxidase and superoxide dismutase

2.6.1. Extraction

The tissue parts of plant used for calculation of antioxidant enzymes, catalase, peroxidase, polyphenol oxidase and superoxide dismutase enzymes were the terminal buds in addition to young leaves.

The procedure include, 2 g of the plant buds were ground with 10 ml of phosphate buffer pH 6.8 (0.1 M), then centrifuge at 2 ºC for 20 min at 20000 rpm in a cooling centrifuge. The clear supernatant (containing the enzymes) was taken as the enzymes source[20].

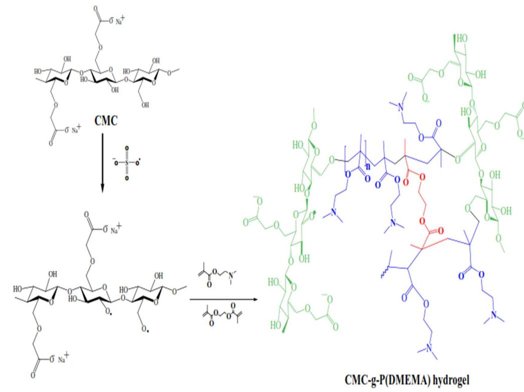
2.6.2. Calculation

Catalase activity was calculated as described in the literature[21] . Activities of peroxidase were determined using the method of Castillo[20].The activity of PPOand SOD enzymeswere calculated from the method described by [22]. Results were statistically analyzed according to Snedecor and Cochran [23].

3. Results and discussion

Scheme 1 shows the suggested preparation process involved for producing CMC-g-P(DMEMA) cationic hydrogel through free radical polymerization method.

The existence of graft copolymer having amino groups - cationic spots - on the designed hydrogel may be responsible for improving the swelling property of the hydrogel. The formed CMC-g-P(DMEMA) hydrogel was prepared through free radical polymerization of DMEMA as monomer, ethylene glycol dimethacrylate as cross-linker.



Scheme 1: Proposed mechanism for synthesis of CMC-g-P(DMAEMA) hydrogel

3.1. FTIR-Spectroscopy

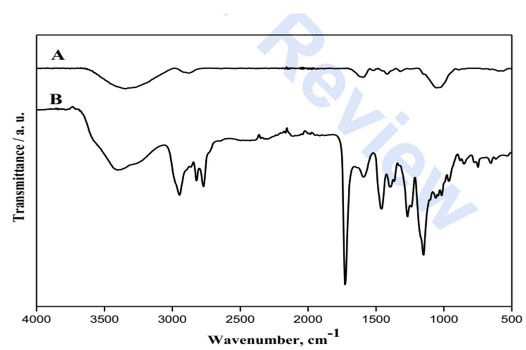


Figure 1: FTIR spectra of CMC (A) and CMC-g-P (DMEMA) hydrogel (B)

The graft crosslinking of CMC-g-P(DMEMA) hydrogel using ethylene glycol diacrylate as crosslinker was investigated by FTIR spectroscopy. Figure 1A exhibits the distinctive bands at 1623, 2890, and 3441 cm^{-1} which assigned to carboxyl group, asymmetric C-H stretching and O-H stretching vibration in CMC, respectively [24,25]. Moreover, characteristic bands at 1727 and 1149 cm^{-1} may assign to ester stretching carbonyl and C-N stretching bonds. Additionally, the specific peaks at 2770 and 2821 cm^{-1} may be ascribed to –CH2- groups which are adjacent to the nitrogen atom [26]. These FT-IR results affirm that the CMC-g-P(DMEMA) hydrogel was successfully prepared.

3.2. Morphology

The morphology of CMC-g-P(DMEMA) hydrogel is illustrated in Figure 2. It is clear that the CMC-g-P(DMEMA) hydrogel showed well defined macroporous and organized three-dimensional framework construction with a pore diameter ~20 µm. Moreover, the porous structure seems homogenous and uniform which permits the access of aqueous solutions inside the hydrogel.

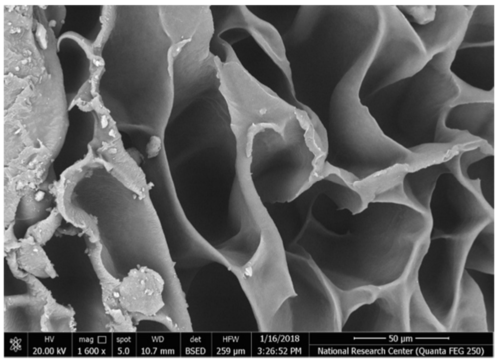


Figure 2: Surface morphology of CMC-g-P(DMEMA) hydrogel

3.3. Swelling study

Swelling study is a vital factor to investigate the prepared materials as carriers for drugs or other active ingredients. The pH sensitivity of CMC-g-P(DMEMA) hydrogel was studied at different pHs (3, 6 and 8) and the results were exhibited in Figure 3. The calculated results showed significant changes in the swelling properties depending on the pH of the solution. The swelling% in acidic solutions (pH 3 and 6) is higher than that calculated results in slight alkaline medium (pH 8). This behavior may be attributed to the presence of charged sites of protonated tertiary amino groups in CMC-g-P(DMEMA) hydrogel which yields strong electrostatic repulsion in acidic aqueous solution. This repulsion forces enhance the polymer chains expansion, creating a macroporous arrangement. In neutral and alkaline pHs, the repulsive forces between the tertiary amino groups are reduced causing a contraction of the designed hydrogel and consequently reducing the total swelling capacity. The equilibrium swelling was reduced from 1700 in in acidic medium (pH 3) to 1470 and 701 in case of slight acidic and alkaline solutions (pH 6 and 8, respectively). Swelling study showed that CMC-g-P(DMEMA) hydrogel are stable and may be efficient systems for loading and delivery of active ingredients.

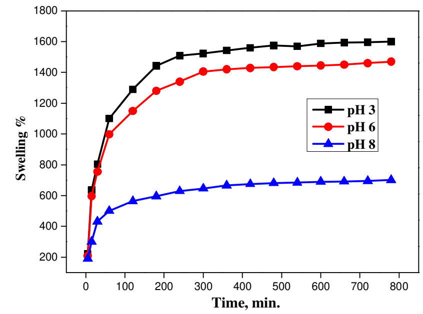


Figure 3: Swelling % of CMC-g-P(DMEMA) at different pHs (3,6 and 8)

3.4. Morphological characters and agronomic characteristics of sunflower using CMC-g-P(DMEMA) hydrogel

Water drought stress is considered an important type of abiotic factors causing adverse effects on growth and productivity of most plants. In the case of water stress (control), as displayed in figure 4A, showed significant decrease for shoot lengths and percentage of germination. The decline in growth parameters was displayed in our previous study on common bean plants [27], where showed a significant decrement in growth parameters (shoot length and both of fresh and dry weight of shoots and roots) in response of drought stress. Hydrogels are explained as “water storing granules” or “root- water supplied crystals” as result of absorbing a large quantity of water. Consequently, the field capacity of the soil was increased, and the irrigation times were reduced. Soil without hydrogels, lead to water lost rapidly (phase I), where considered as control for the experiment. The soil which contains the prepared hydrogel, CMC-g-P(DMEMA), was irrigated with water enough to intake water for reaching the maximum water absorbance degree (phase II). Figure 4B shows the direct relation between shoot length and fresh weight of shoot in phase I and II. In phase II, the shoot increased up to 65.68%, as comparison to shoot lengths in phase I, and the fresh weight of shoot increased about 86.05% in phase II rather than phase I. The present results from the sunflower cultivation trial revealed as general, improvement in growth parameters of plant treated with CMC-g-P(DMEMA) hydrogel substances during the time of analysis (figure 4B).

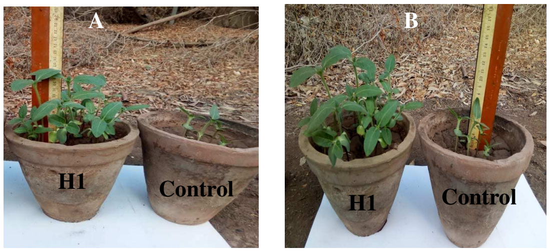


Figure 4: A) Shows the growth of sunflower at 20 days after sowing) B) Shows the growth of sunflower (at 25 days after sowing) without hydrogel (phase I) as control, with CMC-g-P(DMEMA) (Phase II). The chart showed shoot lengths and fresh weight of shoot in the two phases of sunflower

Results from figure 4B showed an overall improvement in growth measurement of plants. The shoot lengths with adding CMC-g-P(DMEMA) hydrogel became longer than those of water stress treatment plants, increased from 13.9 to 23.03 cm. Moreover, the shoot fresh weight of CMC-g-P(DMEMA) hydrogel was increased from 0.98 to 1.82 gm. These results displayed positive benefits of CMC-g-P(DMEMA) hydrogels through a clear increase in vegetative growth of sunflower plants and alleviation of harmful effects of water stress. The continuous presence of hydrogel in the soil saved some water for plants even in case of decreasing times of irrigation, which caused high turgidity of the leaves.

3.5 Responses of green pigments, phenols, proline and enzyme activities

The green pigments of sunflower plants (chlorophylls) constituent is one of the main factors affecting photosynthetic process. Our results presented in worktable 1 revealed that water drought stress caused a significant decrement in chlorophyll and carotenoid constituents in the sunflower leaves as comparison to soil that mixed with CMC-g-P(DMEMA) hydrogels (under the same circumstances). The deficient in chlorophyll constituent recorded in the present work perhaps explained by the strong reduction in the water content of sunflower plant. The rate of photosynthetic process in sunflower plants decreases due to the loss of water in the guard cells. This is due to the decrement in photosynthetic enzymes. The data recorded in table 1 proved that in water stress conditions, mixing CMC-g-P(DMEMA) hydrogel with the soil improved the chlorophyll content as compared to control experiment (phase I). Also, the present data displayed that the used hydrogel can mitigate the harmful effects on chlorophyll contents caused by water stress in sunflower plants.

Table 1
Chlorophyll a, b, a+b and carotenoids content (mg/g fresh leaves) in sunflower plants in both cases, control (phase I) and with CMC-g-P(DMEMA) (phase II), values given are the means of five replicates ± SD

Treatments	Chlorophyll a	Chlorophyll b	Chlorophyll a+b	Carotenoids
Controlexperiment	7.32±0.24	6.26±1.54	13.59±0.65	**3.89±0.15**
Soil with CMC-g-P(DMEMA)	9.79±0.09	10.57±1.49	19.86±0.68	**5.30±0.09**
LSD at 0.05	0.35	0.71	1.83	**0.33**

Note: Different letters within the columns show significant differences ($P < .05$) from T-test

3.6. Enzymes, Phenol and proline contents

The presented results in table 2 show the effect of drought stress on activities of antioxidant enzymes (catalase, peroxidase, polyphenol oxidase and superoxide dismutase) which take part in removing reactive oxygen species (ROS). The data indicated high content of the antioxidant enzymes taken from the terminal buds of sunflower plants under water stress conditions. Water deficiency is ultimately related to acceleration of oxidative stress which increases the accumulation of ROS, essentially O2− and H_2O_2 in chloroplasts, mitochondria, and peroxisomes. The high content of antioxidant enzymes is a general adaptation mechanism for plants to resist oxidative stresses [28]. Table 2, also, demonstrates that the hydrogel can mitigate the harmful effects resulted from oxidative stress as water stress occurred in sunflower plants. The supplement of hydrogel exhibited significant decrease in antioxidant enzymes. The addition of CMC-g-P(DMEMA) hydrogel improved the water reserving capability of the soil which alleviate the adverse effects of water stress.

Table 2

Antioxidant enzymes (unit/g fresh weight / hour) of leaves of the apical tip) of sunflower plants in response to stress and hydrogel supplement, values given are the means of five replicates ± SD

Treatments	catalase	peroxidase	Polyphenol oxidase	Superoxide dismutase
Control	160.21±1.20a	774±2.21a	186±1.25ba	65.25±2.32a
Soil with CMC-g-P(DMEMA)	141.32±2.32b	588±1.54b	152±0.96c	52.21±1.25b
LSD at 0.05	5.01	24.32	9.21	6.32

Note: Different letters within the columns show significant differences (P < .05) from T-test

Figure 5 describes the effect of stress only and the interaction between stress and CMC-g-P(DMEMA) hydrogel on phenol and proline contents. Proline dissolves to high extent in water and found in a zwitterionic (dipolar) state. Proline participates in this property with other substances and all these compounds collectively called "compatible solutes" that are exited in large number of organisms to adjust osmotic pressure in living cells [29]. Our results appear significant increase in proline content in response to drought stress. The interaction effect between drought stress and CMC-g-P(DMEMA) hydrogel shows significant decrease in proline content and vice versa in phenol contents. These results demonstrate the significant role of CMC-g-P(DMEMA) hydrogel in reducing the harmful effect of water stress on sunflower plants.

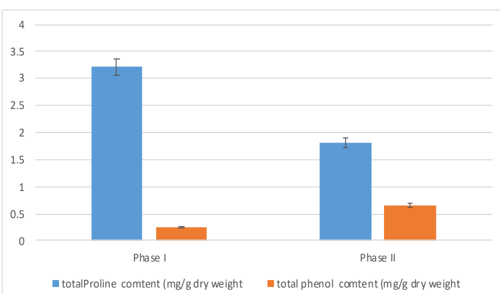


Figure 5: Phenol and proline contents in the two phases

4. Conclusion

New cellulose-based hydrogel composed of CMC-g-P(DMEMA) appropriate for the reducing the detrimental effects of drought was reported. The supplement of hydrogel into the soil leads to an increase in water holding capacity of the soil with excellent water retention property of the soil which enhanced the vigor of sunflower plants grown under drought stress conditions. The prepared hydrogel significantly improved shoot length, chlorophyll and phenol contents. Chlorophyll content of sunflower plants under stress conditions decreased as compared to sunflower grown in soil treated with CMC-g-PDMEMA hydrogel which reflect the low rate of photosynthesis and bad anabolism. The rate of photosynthesis which also depends on the water and chlorophyll content are reflected in the leaf surface area and the length of the shoot system of sunflower plants under stress conditions. The results in the current article displayed that CMC-g-PDMEMA hydrogel had high water retention which is suggested to have possible applications in sustainable modern agriculture.

5. References

[1] D. Fairless, Biofuel: The little shrub that could - maybe, Nature. 449 (2007) 652–655. https://doi.org/10.1038/449652a.

[2] D. Cheng, Y. Liu, G. Yang, A. Zhang, Water- and Fertilizer-Integrated Hydrogel Derived from the Polymerization of Acrylic Acid and Urea as a Slow-Release N Fertilizer and Water Retention in Agriculture, (2018). https://doi.org/10.1021/acs.jafc.8b00872.

[3] A. Salama, Carboxymethyl cellulose-g-poly (acrylic acid)/calcium phosphate composite as a multifunctional hydrogel material, Mater. Lett. 157 (2015) 243–247. https://doi.org/10.1016/j.matlet.2015.05.088.

[4] A. Salama, M. El-Sakhawy, Preparation of polyelectrolyte/calcium phosphate hybrids for drug delivery application, Carbohydr. Polym. 113 (2014) 500–506. https://doi.org/10.1016/j.carbpol.2014.07.022.

[5] Y. Bao, J. Ma, N. Li, Synthesis and swelling behaviors of sodium carboxymethyl cellulose-g-poly(AA-co-AM-co-AMPS)/MMT superabsorbent hydrogel, Carbohydr. Polym. 84 (2011) 76–82. https://doi.org/10.1016/j.carbpol.2010.10.061.

[6] Y. Hu, X. Dong, L. Ke, S. Zhang, D. Zhao, H. Chen, X. Xiao, Polysaccharides/mesoporous silica nanoparticles hybrid composite hydrogel beads for sustained drug delivery, J. Mater. Sci. 52 (2017) 3095–3109. https://doi.org/10.1007/s10853-016-0597-x.

[7] A. Salama, Chitosan based hydrogel assisted spongelike calcium phosphate mineralization for in-vitro BSA release, Int. J. Biol. Macromol. 108 (2018) 471–476. https://doi.org/10.1016/j.ijbiomac.2017.12.035.

[8] I.M. El-Sherbiny, A. Salama, A.A. Sarhan, Ionotropically cross-linked pH-sensitive IPN hydrogel matrices as potential carriers for intestine-specific oral delivery of protein drugs, Drug Dev. Ind. Pharm. 37 (2011). https://doi.org/10.3109/03639045.2010.495754.

[9] R.E. Abou-Zeid, N.S. Awwad, S. Nabil, A. Salama, M.A. Youssef, Oxidized alginate/gelatin decorated silver nanoparticles as new nanocomposite for dye adsorption, Int. J. Biol. Macromol. 141 (2019) 1280–1286. https://doi.org/10.1016/j.ijbiomac.2019.09.076.

[10] A. Salama, Cellulose/calcium phosphate hybrids: New materials for biomedical and environmental applications, Int. J. Biol. Macromol. 127 (2019) 606–617. https://doi.org/10.1016/j.ijbiomac.2019.01.130.

[11] R.E. Abouzeid, R. Khiari, A. Salama, M. Diab, D. Beneventi, A. Dufresne, In situ mineralization of nano-hydroxyapatite on bifunctional cellulose nano fi ber / polyvinyl alcohol / sodium alginate hydrogel using 3D printing, Int. J. Biol. Macromol. 160 (2020) 538–547. https://doi.org/10.1016/j.ijbiomac.2020.05.181.

[12] A. Salama, R.E. Abou-Zeid, I. Cruz-Maya, V. Guarino, Soy protein hydrolysate grafted cellulose nanofibrils with bioactive signals for bone repair and regeneration, Carbohydr. Polym. 229 (2020) 115472. https://doi.org/10.1016/j.carbpol.2019.115472.

[13] A. Salama, N. Shukry, M. El-Sakhawy, Carboxymethyl cellulose-g-poly(2-(dimethylamino) ethyl methacrylate) hydrogel as adsorbent for dye removal, Int. J. Biol. Macromol. 73 (2015) 72–75. https://doi.org/10.1016/j.ijbiomac.2014.11.002.

[14] H.-M. Kang, Y.-L. Cai, P.-S. Liu, Synthesis, characterization and thermal sensitivity of chitosan-based graft copolymers., Carbohydr. Res. 341 (2006) 2851–7. https://doi.org/10.1016/j.carres.2006.08.001.

[15] G.R. Bardajee, Z. Hooshyar, Novel potentially biocompatible nanoporous hydrogel based on poly ((2-dimethylaminoethyl) methacrylate) grafted onto salep: synthesis, swelling behavior and drug release study, J. Polym. Res. 20 (2012) 67. https://doi.org/10.1007/s10965-012-0067-3.

[16] A. Salama, Cellulose/silk fibroin assisted calcium phosphate growth: Novel biocomposite for dye adsorption, Int. J. Biol. Macromol. 165 (2020) 1970–1977. https://doi.org/10.1016/j.ijbiomac.2020.10.074.

[17] L.P. Vernon, G.R. Seely, The Chlorophylls, Elsevier, 1966. https://doi.org/10.1016/C2013-0-12536-6.

[18] H.K. Lichtenthaler, Photosynthesis, Elsevier, 1982. https://doi.org/10.1016/B978-0-12-294301-0.X5001-5.

[19] S. Žilić, A. Serpen, G. Akıllıoğlu, M. Janković, V. Gökmen, Distributions of phenolic compounds, yellow pigments and oxidative enzymes in wheat grains and their relation to antioxidant capacity of bran and debranned flour, J. Cereal Sci. 56 (2012) 652–658. https://doi.org/10.1016/j.jcs.2012.07.014.

[20] L.S. Bates, R.P. Waldren, I.D. Teare, Rapid determination of free proline for water-stress studies, Plant Soil. 39 (1973) 205–207. https://doi.org/10.1007/BF00018060.

[21] H. Aebi, Catalase in vitro, in: Methods Enzymol., 1984: pp. 121–126. https://doi.org/10.1016/S0076-6879(84)05016-3.

[22] A. Matta, A.E. Dimond, Symptoms of Fusarium wilt in relation to quantity of fungus and enzyme activity in tomato stems, Phytopathology. 53 (1963) 574.

[23] W.G. Snedecor, G.W.. Cochran, Statistical methods 7 edition, The Iowa State University Press; Seventh Edition, 1987.

[24] A. Salama, Preparation of CMC-g-P(SPMA) super adsorbent hydrogels: Exploring their capacity for MB removal from waste water, Int. J. Biol. Macromol. 106 (2018) 940–946. https://doi.org/10.1016/j.ijbiomac.2017.08.097.

[25] A. Salama, S. Etri, S.A.A. Mohamed, M. El-Sakhawy, Carboxymethyl cellulose prepared from mesquite tree: New source for promising nanocomposite materials, Carbohydr. Polym. 189 (2018) 138–144. https://doi.org/10.1016/j.carbpol.2018.02.016.

[26] L. Jin, Y. Deng, J. Hu, C. Wang, Preparation and characterization of core-shell polymer particles with protonizable shells prepared by

oxyanionic polymerization, J. Polym. Sci. Part A Polym. Chem. 42 (2004) 6081–6088. https://doi.org/10.1002/pola.20453.

[27] M.M. Ghobashy, B.K. El-Damhougy, H.A. El-Wahab, M. Madani, M.A. Amin, A.E.M. Naser, F. Abdelhai, N. Nady, A.S. Meganid, S.A. Alkhursani, D.M. Alshangiti, Controlling radiation degradation of CMC solution to optimize the swelling of acrylic acid hydrogel as water and fertilizer carriers, Polym. Adv. Technol. 32 (2021) 514–524. https://doi.org/10.1002/pat.5105.

[28] C.H. Foyer, G. Noctor, Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria, Physiol. Plant. 119 (2003) 355–364. https://doi.org/10.1034/j.1399-3054.2003.00223.x.

[29] P.H. Yancey, Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses, J. Exp. Biol. 208 (2005) 2819–2830. https://doi.org/10.1242/jeb.01730.