Formaldehyde Cross-Linked Poly (acrylate-co-acrylamide) Superabsorbent in Organism-Ecosystem Interactions

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ABSTRACT: *Superabsorbent polymers (SAPs) have recently been designed to meet the requirements of various applications such as agriculture. In this study, a new formaldehyde cross-linked poly (acrylate-co-acrylamide) (FCP(Ac-co-Am)) SAP was successfully synthesized using the solution polymerization and based on the co-polymerization of acrylic acid and acrylamide in the presence of potassium persulfate as an initiator and formaldehyde as a crosslinking agent. Fourier Transform InfraRed (FT-IR) and Proton Nuclear Magnetic Resonance (HNMR) spectroscopy along with ThermoGravimetric Analysis (TGA) were applied to determine the molecular structure and characterization of the synthesized SAP. The swelling properties of the (FCP(Ac-co-Am)) SAP such as free absorbency, wicking capacity, and swelling rate were evaluated, and the values of 15000 ± 650 %, 0.12 ± 0.004 g/g, and 270 ± 12 s were obtained respectively. Moreover, the impact of the synthesized SAP in the improvement of soil properties was examined using real-time-polymerase chain reaction (RT-PCR), MTT, fluorescent microscopic, and, DNA Ladder assays. The obtained results showed that the (FCP(Ac-co-Am)) SAP did not inhibit germination, beneficial soil microbes and, normal plant growth. The effects of the synthesized SAP on soil and agricultural achievements in Iran, an arid country were tested and satisfactory results were obtained.*

KEYWORDS: *Superabsorbent polymer; Formaldehyde crosslinking; Swelling behavior; Agriculture; Soil properties.*

INTRODUCTION

Water plays a critical role in agricultural production. However, water shortages and droughts have caused soil desertification and salinization, which also challenge the sustainable development of agriculture as well as food security. Therefore, improving the utilization efficiency of water is of great importance in agriculture [1]. In such a situation,

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reducing water consumption and avoiding waste and pollution is vital. After a report on the manufacture of the first commercial superabsorbent in 1970, special attention was paid to the uses of these compounds in various fields around the world [2]. Superabsorbent polymers are cross-linked polymer networks that consist of water-soluble building blocks. Generally, SAP is composed of ionic monomers and is characterized by low cross-linking density, resulting in high liquid absorption. Interestingly, these polymeric networks can absorb and retain aqueous solutions several hundred times their weight while retaining them even under pressure [3]. The osmotic pressure forces water into the polymer due to a higher ionic concentration inside the polymer compared to the surrounding solution because of the presence of charged and hydrophilic moieties onto the ionic monomers. The combination of these charged groups and additional polar moieties in the SAPs attracts water and induces hydrogen bondings [4]. Also, changing the pH of the aqueous environment can lead to the (de)protonation of acidic or basic groups of SAPs. When the pH is higher (lower) than the pKa, acids become negatively (base becomes positively) charged which results in an increased swelling due to an increased affinity for water. Additionally, the repulsion of the charges leads to an increased free volume, in which water can accumulate [5]. SAPs have natural or synthetic origins. Some of the natural polymers used to make SAPs are chitosan, alginate, collagen, dextran, cellulose, chitin, etc., whilst the materials such as acrylic acid, acrylates, polyethylene glycol, polyvinyl alcohol, etc. are applied for synthetic polymers preparation [6]. Synthetic polymers have a higher hydrophobicity and mechanical strength compared to natural ones. Most current synthetic SAPs are prepared from the copolymerization of the acrylic acid (AA), hydrophilic nonionic monomer, or its salts and the acrylamide (AM), an ionic co-monomer, by the solution or inverse-suspension polymerization methods [3], [6]. Due to special features like high water absorption capacity, different water absorption rates according to the desired application, non-toxic, odorless, cost-effective, excellent swelling behavior, biodegradability, biocompatibility, good durability, and stability in swelling environments, SAPs have broad applications [7]. They are found applicable in various fields like waste-water treatment, healthcare products, hygienic (disposable diapers and feminine napkins), gel actuators, drug delivery systems [8, 9], sealing composites, drilling fluid additives, artificial snow, agriculture, and horticulture (holding soil moisture) [10]. In agriculture, SAPs can be used as mixtures with soil, seeds, agricultural chemicals, and fertilizers especially in the form of granules to maintain soil moisture in arid areas. They can affect many properties of the soil, such as structure, density, permeability, erosion resistance, evaporation, and microbial activity. As well as, they can significantly decrease the frequency of irrigation by improving the water retention capacity of the soil, enhancing the survival rates of plants, reducing pesticide residues, and improving microbial activity in the soil. [11–13]. Ameliorating the environmentally friendly properties of SAPs is a hot research topic today [14–18]. Therefore, while preparing a superabsorbent polymer with a wide agricultural application perspective, consideration should be given not only to its water absorption / retention in the soil, but also to its reusability, degradability, safety, etc..[1]. Although practically, it is not possible to have a superabsorbent that exhibits all the above mentioned properties at the same time, therefore depending on the application, the formulation and process conditions must be designed and optimized in such a way that a desirable balance between the product properties is achieved [18]. The most well-known synthetic SAP is a copolymer of acrylic acid and acrylamide [19], whereas cross-linked polyacrylates are the main commercially SAPs. To further improve its environmentally friendly characteristics and for the first time in this study, we synthesized a new SAP based on the co-polymerization of acrylic acid and acrylamide in the presence of formaldehyde as a cross-linking agent via the solution polymerization method. It is worthy to note that although formaldehyde which is a very harmful compound is used as a crosslinking agent, but fertilizer that incorporates formaldehyde slowly releases nitrogen over time, which is necessary for plant growth. Therefore it allows to faster germination and thus greater growth in crops. Furthermore, formaldehyde concentrates have been used in fertilizer to protect plants and control afflictions such as potato disease [20]. Evaluation of the agricultural applications of (FCP(Ac-co-Am)) SAP in the germination of a group of monocot plants (wheat, biotic turf) and a dicotyledonous leguminous plant (alfalfa and beans) using RT-PCR, MTT, fluorescent microscopic, and DNA Ladder assays demonstrated satisfactory results.

EXPERIMENTAL SECTION

Materials

Acrylic acid (stabilized with hydroquinone monomethyl ether), acrylamid, Sodium hydroxide (pellets), and Formaldehyde solution (37 % v/v) were all purchased from Merck Millipore and potassium persulfate (purity > 99 %) were prepared from Merck (Germany). All the chemicals used in the synthesis process were of analytical grade. It should be noted that deionized water was used in the whole process of SAP synthesis and swelling measurements.

Preparation of the (FCP(Ac-co-Am)) SAP

In a 50-mL beaker, the acrylic acid monomer (10.0 g) was neutralized to an appropriate pH using sodium hydroxide (11.0 M) at a low temperature. After the neutralization, 5.0 g of acrylamide together with the partially neutralized acrylic acid was transferred into a reactor equipped with a mechanical stirrer and a nitrogen line. Under a nitrogen atmosphere, the solution was stirred at medium speed (850 rpm) for 30 min. 2.0 g of formaldehyde as a cross-linker and 0.5 g of potassium persulfate as an initiator (dissolved in 2 mL of distilled water) were respectively added to the above solution. The reactor was then placed in a thermostat water bath at 45 °C for 3 h and then transferred to another thermostated water bath at 60 °C for a further 1 h. The product was dried at 110 °C. A superabsorbent particles were obtained by grinding and sieving through a 100-mesh sieve.

FT-IR spectrum, HNMR and TGA analysis

Fourier Transform InfraRed (FT-IR) spectroscopy analysis was done using an ABB Bomem FT-IR spectrometer (model MB-100). KBr pellets were applied to record the IR spectrum of the synthesized SAP within the wavenumbers ranging from 4000 to 400 1/cm. HNMR spectra were recorded in deuterated dimethyl sulfoxide (d6-DMSO) using a Bruker AVANCE AV 400 MHz. Thermal decomposition study was also carried out using a thermogravimetric analyzer (model PL-1500, UK) from 0 °C to 800 °C under a nitrogen atmosphere and a heating rate of 10 °C/min.

Measurement of free-absorbency capacity (Se)

The centrifuge method was used to determine the swelling capacity of the synthesized SAP [11, 21, 22]. A nonwoven fabric bag (6060 mm) was filled with 0.2 g (W_1) of the synthesized SAP. The bag is immersed in 100 mL of saline solution for 30 min at room temperature. Then it was taken out and the excess solution is removed using centrifugation (250 g, 3 min). The weight of the bag (W_2) is measured. The same steps were carried out with an empty bag and its weight was measured (W_0). The free-absorbency capacity is calculated according to Eq. (1).

$$\mathrm{Se} = \frac{W_2 - W_0 - W_1}{W_1} \tag{1}$$

Measurement of the Swelling Rate (SR)

The vortex method was applied to calculate the SAP swelling rate [23–25]. Water or saline solution (50.0 g) was poured into a 100 mL beaker and heated to 30 °C. A magnetic stirrer was used to stir at 600 rpm. The SAP sample (W_0 = 0.50-2.0 g, mesh 50-60) was added and a stopwatch was started. The time that elapsed from the addition of SAP into the liquid until the vortex disappeared (tvd, sec) was measured. Finally, the swelling rate (g /g.s) was calculated according to Eq. (2).

$$\mathrm{SR} = \frac{(50/W_0)}{\mathrm{tvd}} \tag{2}$$

Measurement of the Wicking Capacity (WC)

To measure the WC [11, 26], SAP (W_1 = 0.0500.0005 g) was added to a folded filter paper cone made from a precisely tarred circle of 9 cm Whatman 54 paper. The cone was tapped lightly to set the sample in the tip, and the tip of the cone was then placed in a 9 cm petri dish with 25 mL of water for 60 s. Water soaks up the entire length of the paper in one minute. Excess water is then allowed to drain from the paper by bringing the tip into contact with a circle of dry filter paper on a square absorbent cloth for 60 s. The weight of wet paper plus swollen polymer was determined (A) and then the absorbency of the sample (g /g) was calculated after correcting the weight of the dry paper and the amount of water which, under identical conditions, was removed from the paper alone in the absence of the SAP (Eq. 3). Each test is preferably repeated 3-5 times and the results were averaged.

$$\mathrm{WC} = \frac{(A - B - W_1)}{W_1} \tag{3}$$

Where B is wet paper with no polymer. Assuming monotonous absorption for 60 s, the wicking rate (g/g.s) of the SAP can be obtained by dividing the WC value by 60.

RESULTS AND DISCUSSION

Preparation of the (FCP(Ac-co-Am)) SAP

The solution polymerization was used to prepare the (FCP(Ac-co-Am)) SAP [27–30]. It was synthesized by cross-linking poly (acrylate-co-acrylamide) using formaldehyde as a cross-linking agent. The polymerization was initiated by potassium persulfate. The neutralization technique played a key role here which was done using NaOH solution; in the case of polyacrylates, the carboxylic acid groups were partially neutralized before (pre-neutralization) or after (post-neutralization) the polymerization. A rapid exothermic reaction gave a gel-like elastic product which was then dried and the macroporous mass pulverized and sieved to obtain the required particle size. For general production of a SAP with acceptable swelling properties, manufacturers may often prefer the cheaper and faster technique such as the solution method [31].

Characterization of the (FCP(Ac-co-Am)) SAP

FT-IR, HNMR and TGA analyses were conducted to identify and characterize the synthesized SAP. FT-IR study is a useful technique in providing valuable information about the presence or absence of specific functional groups that will lead to the confirmation of the proper synthesis of the desired compound. It was used to illustrate the nature of the bond formation in the synthesized SAP structure. FT-IR spectrum of the (FCP(Ac-co-Am)) SAP is demonstrated in Fig. 1. According to this spectrum, the peaks at 3194 1/cm and 1663 cm^{-1} can be attributed to the N-H and carbonyl stretching vibrations of carboxamide functional groups respectively [32]. Another broad peak at 3354 cm^{-1} is corresponding to the asymmetrical stretching vibration of the NH_2 group of acrylamide [29]. A sharp peak at 2933 1/cm corresponds to the CH_2 stretching in the polymer backbone and crosslinking bridges. Another weak peak at 1347 1/cm can be attributed to CH_2 waging and CN stretching vibrations and, CH bending mode is seen at 1318 1/cm. C-C stretching vibrations are seen as two small peaks at 1189 1/cm and 1122 1/cm. In the spectra of polymers containing acrylic acid, there is a peak between 1046 1/cm - 1044 1/cm assigned to the coupled asymmetrical stretching of C-C-O bending. The all obtained data can confirm the successful synthesis of the (FCP(Ac-co-Am)) SAP. Fig. 2. presents the HNMR spectrum of FCP (Ac-co-Am) copolymers. The peak at 2.9 ppm (or peak 1) is corresponded to the proton of Ac unit of FCP (Ac-co-Am) copolymers. The peak at 2.3 ppm (or peak 4) may be ascribed to Am unit of FCP (Ac-co-Am) copolymers. The peak at 3.3 ppm may correspond to water (H_2O). Several peaks at around ~6.0 ppm indicated the remnant of Ac monomer. ThermoGravimetric Analysis (TGA) of polymers is conducted to measure weight changes as a function of temperature and time which provides noteworthy data that can be applied to improve product quality, predict product performance and select materials for specific end-use applications. TGA diagram of the synthesized SAP is illustrated in Fig. 3. Considering this diagram, four decomposition peaks appear. A weight loss at a temperature < 200 °C is related to the dehydration of the water content of the SAP. The second peak between 200 °C -280 °C, is corresponding to the branches of the graft copolymer. Degradation of formaldehyde within the graft co-polymer happened in the temperatures between 280 °C - 410 °C (the third peak). And, a weight-loss above 410 °C is attributed to the degradation of the polymer chain. Thus, the relative thermal stability of the composite in comparison with the (Ac-co-Am) copolymer proved by TGA demonstrated that, a non-oxidative degradation occurs at above 410 °C. These results indicate the cross-linking effect of formaldehyde does not bad effect on the thermal and mechanical properties of the final copolymer [33].

Study the swelling properties of the (FCP(Ac-co-Am)) SAP

The free-absorbency capacity

When a superabsorbent composite is free to swell without stress, this is known as free absorbency capacity [34]. Superabsorbent polymers have the capability of enhancing the capacity of water storage in the soil through the reduction of water evaporation from the surface of the soil. The absorbency capacity of the synthesized SAP was evaluated according to the mentioned procedure in section 2.4 (Eq. (1)) and its value was 15000 ± 650 %.

Swelling rate

Swelling rate is one of the most important properties of SAPs. The vortex method (the fastest and easiest method for determining the SAP swelling rate), is widely used in R&D and technical laboratories. The swelling time can also easily be determined with this method. Using Eq. (2) (section 2.5), the value for the swelling rate of the (FCP(Ac-co-Am)) SAP was calculated to be 270 ± 12 s.

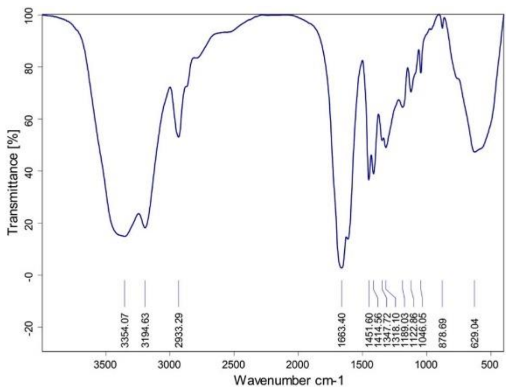


Fig. 1: FT-IR spectrum of the (FCP(Ac-co-Am)) SAP.

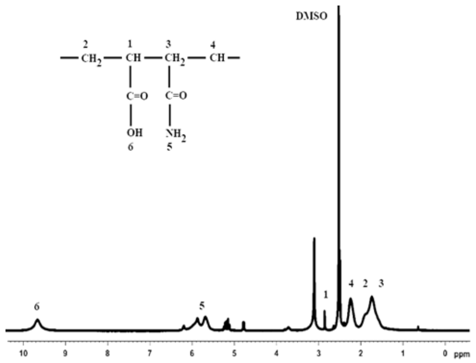


Fig. 2: HNMR spectra of the (FCP(Ac-co-Am)) SAP.

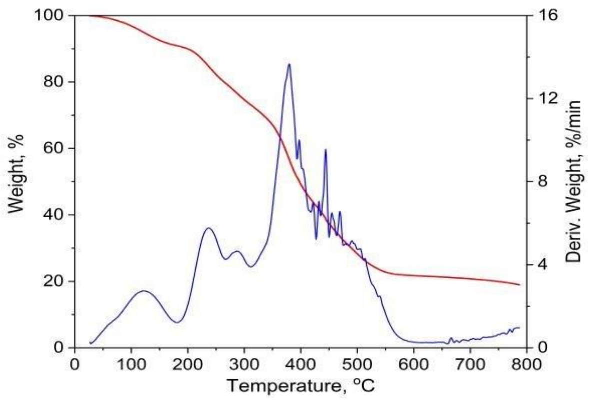


Fig. 3: TGA profile of the (FCP(Ac-co-Am)) SAP.

Wicking Capacity

A simple test was proposed by Fanta and Doane to determine the wicking capacity of SAP materials [26]. As discussed in section 2.6 (Eq. 3), for a certain time, 0.12 ± 0.004 g / g was calculated as the wicking capacity of the (FCP(Ac-co-Am)) SAP.

Study the agriculture applications of the (FCP(Ac-co-Am)) SAP

In agriculture, SAPs are utilized as soil water retention agents in soilless cultivation, artificial turf, seed coatings, etc. They act as micro-water reservoirs on plant roots. They are capable of absorbing water to several hundred times their weight and slowly releasing it again due to the root capillary suction mechanism, which prevents water loss in the soil through leaching and evaporation. The water released in this way can provide optimal moisture for rapid germination and maturation of the seedlings. Benefiting from this advantage, SAP coating seeds emerge quickly and give the plants an early and healthy start. It also has strong and good disease resistance. Such SAP-coated seeds are very useful for plants in drought areas [17,18]. Therefore, studying the effects of the synthesized SAP on various soil properties and micro-organisms seems to be very important.

Determination of non-inhibitory activity of SAP on germination and plant growth

Due to the utilization of the produced SAP in agricultural soils, it should not negatively affect plant germination and growth. This adverse effect may be due to the toxicity of the synthesized SAP, a change in the soil pH, or the failure in the release of the necessary water for germination. Therefore, a group of monocot plants (wheat, biotic turf) and a dicotyledonous leguminous plant (alfalfa and beans) are usually selected to study germination and growth conditions via applying the synthesized SAP. In this study, the seeds of alfalfa legume, as well as the seeds of wheat and turf, were selected as candidates to evaluate the germination process applying the (FCP(Ac-co-Am)) SAP. Fig. 4 shows the influence of the synthesized (FCP(Ac-co-Am)) SAP and another similar foreign (Netherlands) SAP sample on the germination of wheat, leguminous, and turf seeds. It should be noted that for a better assessment of the synthesized SAP properties in soil and seeds germination, besides using a control, all experiments were conducted using a similar foreign SAP sample (SF) SAP too.

As demonstrated in Fig. 4, the (FCP(Ac-co-Am)) SAP showed a better performance compared to (SF) SAP.

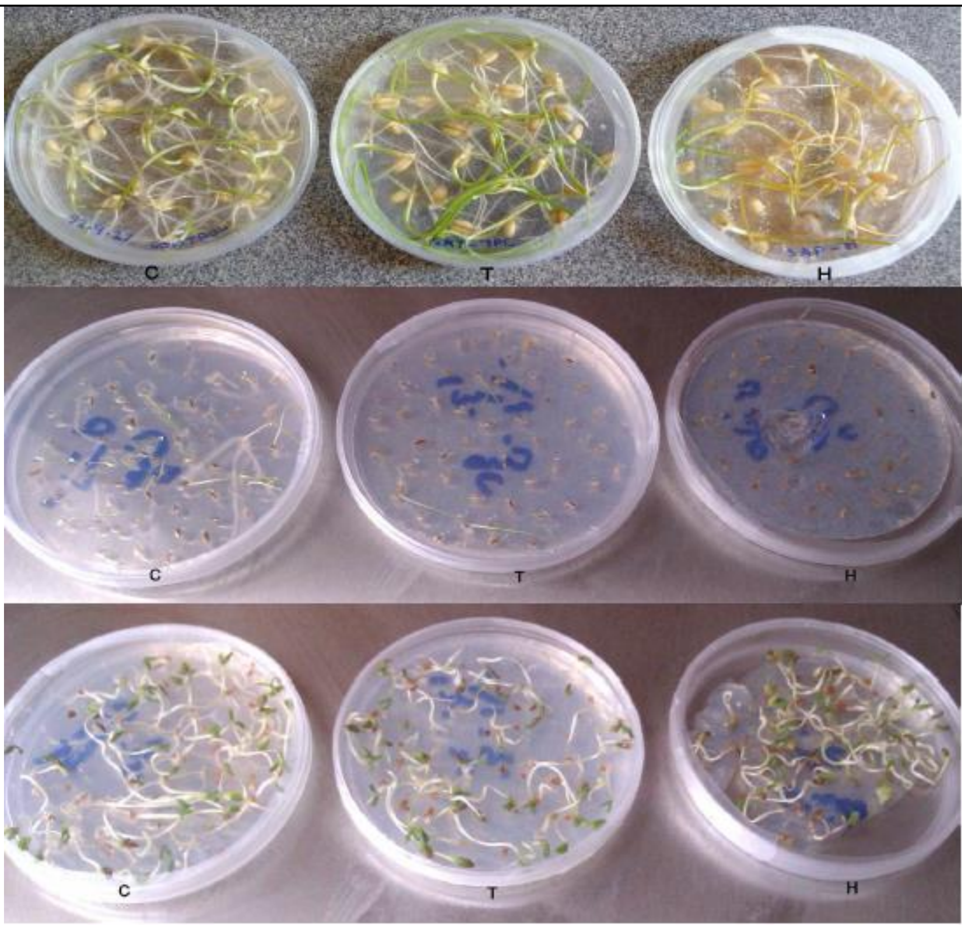


Fig. 4: Effect of the (FCP(Ac-co-Am)) SAP (T) and (SF) SAP (H) compared to control (C) on germination of wheat (top plates), turf (middle plates), and leguminous (bottom plates) seeds.

For instance, by considering the germ plates of wheat, it is observed that, no germination reduction was occurred using the synthesized SAP (T) compared to the control one (C). However, about the foreign sample (H), reduced germination of the wheat seeds besides the yellowing of young seedlings was observed indicating the adverse effect on plant growth and germination that can reduce the crop yield. About the alfalfa germination compared to the control one, the results are also more obvious; the synthesized SAP did not affect the germination, while the similar foreign sample significantly reduced the germination rate. Furthermore, in the case of the turf plant, the result confirmed the far greater superiority of the (FCP(Ac-co-Am)) SAP sample over the similar foreign one. Table 1 also shows the results of the measurement of germination percent, root, and stem length related to the wheat, turf, and alfalfa after 5 days.

Determination of the non-inhibitory and lethal effects on beneficial soil microbes

Bacteria are the bedrock of basic soil reactions and play an important role in the biodiversity of soil invertebrates. The number and activity of these bacteria strongly depend on the pH of the environment. The addition of SAP may have an adverse influence on soil pH or the bactericidal effect. Thus, it is necessary to test the antibacterial effect of the synthesized SAP on beneficial soil bacteria. Two types of bacteria were selected; Streptomyces is a bacterium of the actinomycetes and not only supports soil decay and fertility through the biological production of antifungal and bacterial substances but also combats bacterial and fungal diseases and even pests that attack plant roots. This bacterium is also gram-positive and so the results of the action of the proposed synthetic SAP on the biological activity of this bacterium can be extended

Table 1: Measurement of germination percent, root, and stem length of three cultivars after 5 days.

SAP sample	Plant property	Wheat	Alfalfa	Turf
(FCP(Ac-co-Am)) SAP	Germination	91	88	85
(SF)		82	84	81
Control		96	94	90
(FCP(Ac-co-Am))	Root length	37	14	17
(SF)		23	12	15
Control		47	17	21
(FCP(Ac-co-Am))	Stem length	37	24	31
(SF)		22	19	30
Control		43	30	36

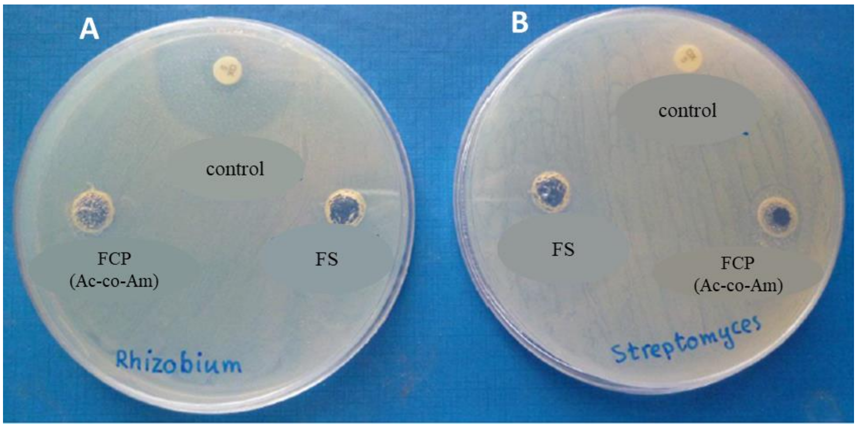


Fig. 5: Grass culture of Rhizobium (A) and Streptomyces (B) bacteria. The disk diffusion method was applied to study the antibacterial effects of the synthesized SAP.

to the other soil's gram-positive flora. The other selected bacteria was Rhizobium, which is important for soil fertility, especially for legumes. The results are shown in Fig. 5. As it can be seen from Fig. 5, none of the (SF) and the (FCP(Ac-co-Am)) SAPs had an unfavorable effect on Streptomyces and Rhizobium bacteria.

Real Time-Polymerase Chain Reaction (RT-PCR) analysis

The abundance and diversity of soil microbes can be studied in different ways. Considering that, thousands of bacteria will make up the soil microbial flora and affect soil fertility and balance, isolation and reduction of the microbial diversity in the soil to study the changes in microbial populations was practically impossible. Molecular technology eliminates the need for a bulky culture of soil bacteria, many of which are uncultivable or undetected, as well as the smallest microbial changes in the soil can be measured with great accuracy. As one of the famous methods, the RT-PCR technique [35] was utilized in this part of the study, which can detect the abundance and concentration of soil microbes before and after using SAP samples. The results are presented in Fig. 6.

As shown in Fig. 6, the presence of peaks of an appropriate slope, as well as appropriate CT values, demonstrated the accurate performance of this technique for assessing changes in the microbial population of soils treated with synthesized and foreign SAP samples compared to the control one. To normalize the results, a standard diagram was subsequently drawn using extracted DNA from the control soil sample (without using SAP) and the generally designed 16S rDNA gene primers for bacteria (Fig. 7). According to this figure, it can be concluded that the standard curve is well-plotted and is reliable for comparing changes in the microbial flora populations.

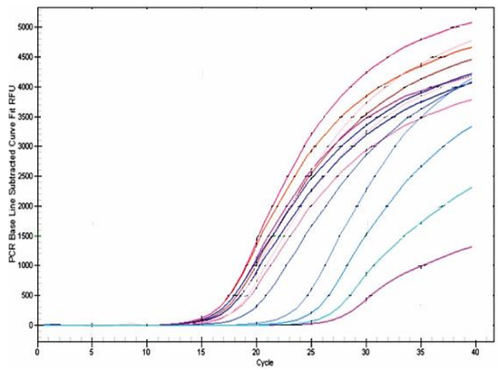


Fig. 6: The RFU curve obtained from quantitative PCR analysis of the changes in genomic DNA extracted from soil.

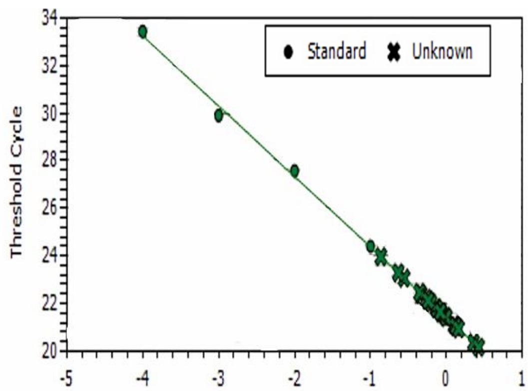


Fig. 7: Standard diagram for the evaluation of the quantitative PCR results.

The results of the experiment were analyzed based on the ΔCT formula and using the REST software. Demographic alterations of the soil microbial flora are given in Fig. 8. Compared to control soil samples, the experiments showed that the amount of microbial flora was reduced while using both (FCP(Ac-co-Am)) and (SF) SAPs in this study. Given that one percent of the soil was mixed with a SAP sample, the decrease of the microbial flora per volume unit or percentage of the soil was predictable. However, the soil sample treated with the (FCP(Ac-co-Am)) SAP had a higher microbial flora relative to the soil treated with the (SF) SAP. Therefore, the synthesized SAP has a better performance compared to the (SF) SAP as the (SF) SAP had further reduced the microbial flora in the soil.

MTT assay

SAPs should not have any toxic or harmful effects on human cells that can be considered as a main feature of

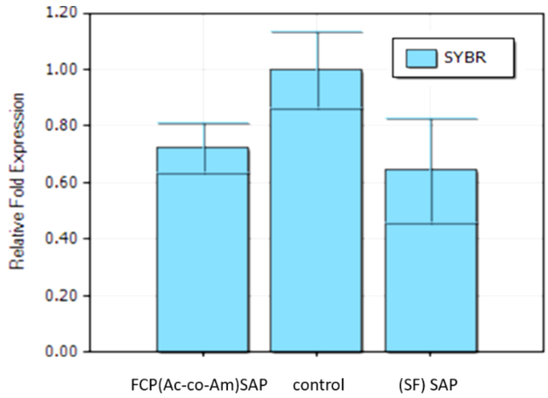


Fig. 8: Influence of the (FCP(Ac-co-Am)) and (SF) SAPs on microbial demographic alterations of the soil obtained by PCR.

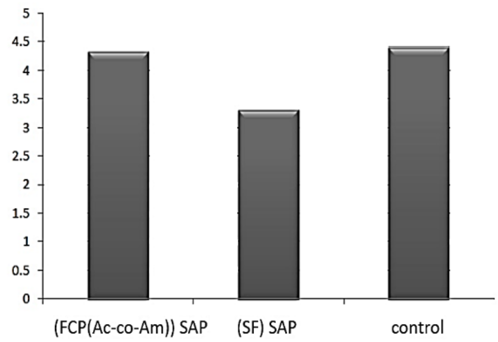


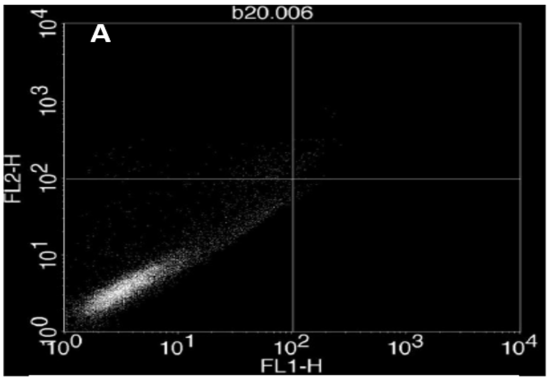
Fig. 9. The proliferation rate of the HUVES cell and the influence of SAPs on cells necrosis.

SAPs. Because food contamination poses irreparable dangers for humans. Therefore, the nontoxicity of SAPs on human cells should be investigated. MTT assay is the best way to assess the toxicity. The tetrazolium-based MTT test relies primarily on the enzymatic conversion of the dye to formazan crystals, which occurs in numerous organelles including the mitochondria and endoplasmic reticulum. The results of this test are shown in Fig. 9.

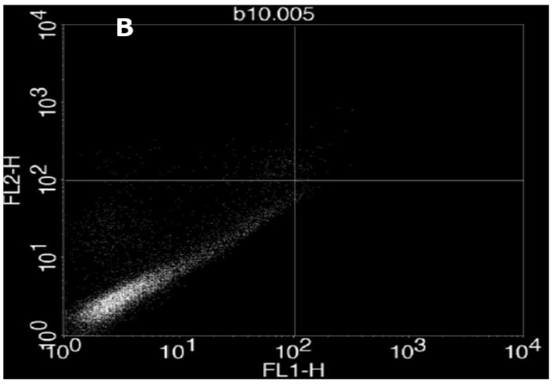
According to Fig. 9, the rate of change while applying the synthesized (FCP(Ac-co-Am)) SAP sample is less than that of the (SF) SAP compared to the control, implying that the proposed SAP has no toxic effect on human cells.

Flow Cytometry study

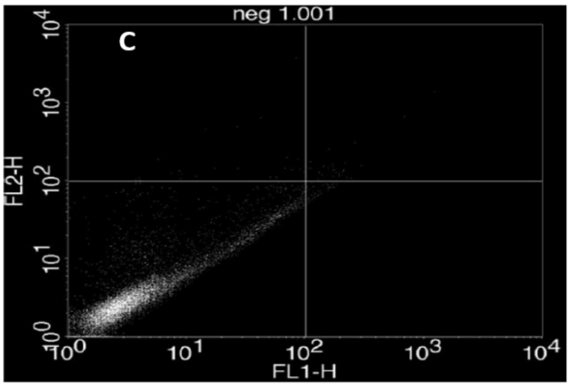
The pattern of cell death (primary and secondary apoptosis or necrosis) was determined by flow cytometry using Annexin V-FICT staining and Propidium Iodide (PI)



Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	163	1.63	1.63	49.21	33.24	152.99	146.86
UR	49	0.49	0.49	144.43	140.39	231.13	204.00
LL	9774	97.74	97.74	6.37	3.95	6.51	4.33
LR	14	0.14	0.14	122.86	121.88	71.30	70.59



Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	145	1.45	1.45	50.48	33.21	174.07	165.54
UR	41	0.41	0.41	147.80	140.75	229.84	194.52
LL	9783	97.83	97.83	6.16	3.72	6.17	3.84
LR	31	0.31	0.31	116.50	115.65	71.05	69.95



Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	23	0.23	0.23	37.35	21.97	352.89	185.43
UR	26	0.26	0.26	236.71	194.97	218.24	166.34
LL	9909	99.09	99.09	6.15	3.49	5.40	3.24
LR	42	0.42	0.42	133.80	131.48	76.73	75.65

Fig. 10: Human angiogenic epithelial cells A) treated with the (FCP(Ac-co-Am)) SAP, B) treated with the (SF) SAP, and C) with no treatment.

technique (Fig. 10). The signal corresponding to Annexin V provides a very sensitive method for the detection of cellular apoptosis, while PI is used for the detection of necrotic or late apoptotic cells, which are characterized by the loss of the integrity of the plasma and nuclear membranes. The results of the flow cytometry were analyzed using FCS Express 3.0 software. According to the results, no apoptosis was observed. Treatment of human angiogenic epithelial cells with the (FCP(Ac-co-Am)) SAP indicates no necrosis of cells and thus the SAP's safety (Fig. 10, A). However, PE staining showed necrosis occurred in a small fraction of cells when using the (SF) SAP sample ((Fig. 10, B), which was consistent with MTT assay results. It is worth mentioning that Fig. 10, C shows the human angiogenic epithelial cells without any treatment (control). Accordingly, it can be concluded that the proposed SAP is safe and can be applied for the betterment of the soil in agricultural applications especially in arid areas such as the lands of Iran.

Fluorescent microscopic assay

DAPI staining was also conducted to examine whether the cells are necrotic and apoptosis is possible or not. DAPI as a nuclear counterstain (diamidino-2-phenylindole), is a blue fluorescent probe that fluoresces brightly upon selectively binding to the minor groove of double-stranded DNA, where its fluorescence is approximately 20-fold greater than in the non-bounded state. One of the properties of cells involved in apoptosis is the fragmentation of genomic DNA. DAPI stains the nucleus, and therefore the small variations within the nucleus can be detected using a microscope (Fig. 11).

According to Fig. 11, fluorescence microscopic imaging showed no apoptosis using both (FCP(Ac-co-Am)) and (SF) SAPs treated HUVEC cells, indicating the applicability of the synthesized SAP.

DNA ladder assay

A defect in apoptotic signaling pathways plays a key role in the progression of cancer. The DNA ladder assay is a fast, simple, and inexpensive method for estimating apoptosis in single cells to check for DNA damage through using SAPs, (if damaged, conversion to the cancerous cells is probable). In this regard, followed by treatment of the HUVEC cells with the synthesized SAP, DNA extraction was carried out for 24 hours and then an electrophoresis

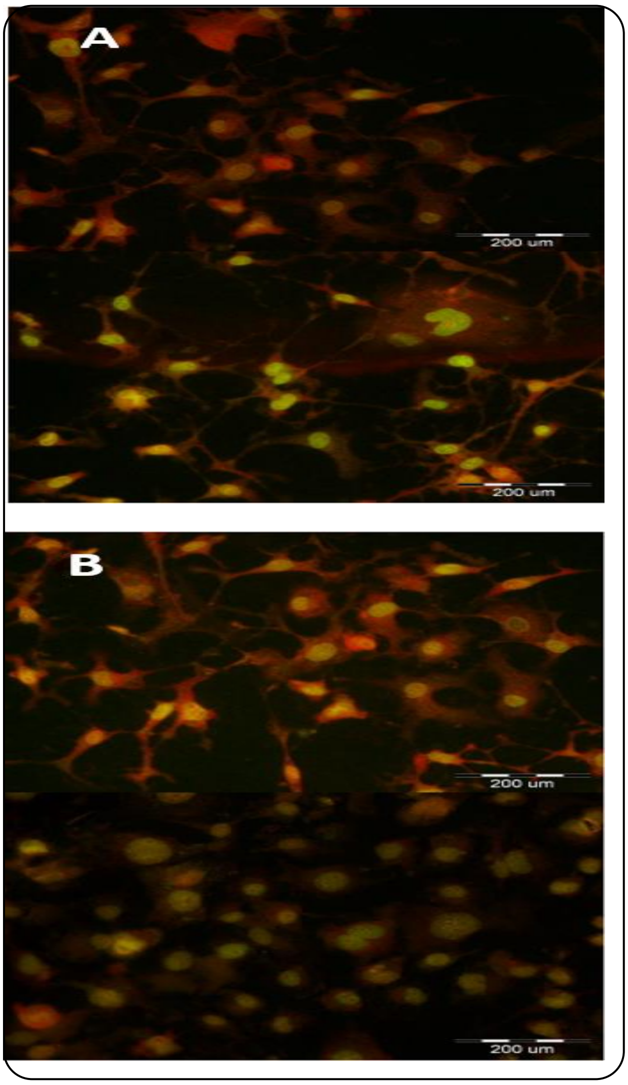


***Fig. 11:** Fluorescence microscopic assay of the HUVEC cells after treatment with A) (FCP(Ac-co-Am)) SAP, B) (SF) SAP via DAPI staining. The upper images are related to the no treated cell (control)*

test was done. Fragmentation of the DNA during electrophoresis would be visible if the DNA was damaged. Fig. 12 represents the electropherograms of the extracted DNA. Considering the obtained result, no cracks or fragmentation were observed in either the synthesized (FCP(Ac-co-Am)) SAP or the (SF) SAP treated cells.

CONCLUSIONS

A novel superabsorbent polymer has been prepared by copolymerization of acrylic acid and acrylamide and using formaldehyde as a cross-linking agent. The FT-IR, HNMR

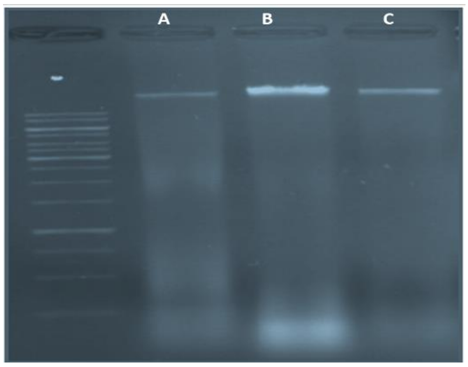


***Fig. 12:** Electropherograms of the HUVEC cells treated with A) (FCP(Ac-co-Am)) SAP, B) (SF) SAP, and C) no SAP.*

results showed that the novel (FCP(Ac-co-Am)) SAP was successfully synthesized. The prepared superabsorbent polymer had good free absorbency of 15000 ± 650 %, wicking capacity of 0.12 ± 0.004 g/g, and the swelling rate of 270 ± 12 s. besides, the TGA results showed that, the synthesized SAP has appropriate thermal stability. Various assays (RT-PCR, MTT, flow cytometry, fluorescent microscopy, DNA ladder) were carried out to assess the proposed SAP capability in agricultural applications. The experimental results proved the ability of the synthesized SAP to positively impact the germination of wheat, turf, and legumes due to its desirable water retention capacity while it has no adverse influence on soil bacterial flora. All these functional properties proved the best solution for the global upcoming water issue with concerning plant growth in agricultural field. So, it can be deduced that the formaldehyde cross-linked poly (acrylate-co-acrylamide) SAP with different properties can be used as a soil conditioner in agriculture, particularly in arid lands such as the lands of Iran.

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