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RESEARCH PAPER

Enhancing water use efficiency and phytochemical responses of fenugreek plants cultivated under drought stress using superabsorbent hydrogel

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Abstract

This study investigates the impact of two different superabsorbant hydrogels (SAH) on the growth and development of fenugreek plants cultivated under sandy soil conditions. The hydrogels used in this study were (Polyacrylic acid and Vinyl alcohol sodium polyacrylate (and were applied with rates of 0.05, 0.1, 0.2, and 0.4% of the soil weight. The amended soil with SAH could improve the growth of fenugreek plants, and the enhancement level was simultaneous with the added hydrogel rate. Morphologically, the highest concentration (0.4%) of SAH treatments recorded the highest values of plant height, root lengths, fresh weights, and shoot and root dry weight. SAH enhanced water use efficiency by modulating relative water content (RWC) in plants compared to the control. Application SAH improved transpiration rates, stomatal conductance, Water use efficiency, Photosynthesis rate, and chlorophyll contents compared to non-treated. In contrast, antioxidant enzyme activities Superoxide dismutase (SOD), peroxidase (POD), Ascorbate peroxidase (APX), Catalase (CAT), protein contents, carbohydrate content, amino acids, and proline content were decreased when SAHs were applied as the plant was still far from the wilting point. This study shows that applying SAH in newly reclaimed desert soils was advantageous for those who had suffered from water shortage conditions.

Keywords

Sandy soil, Hydrogels, Water shortage, Fenugreek, antioxidant enzymes, WHC, WUE

Introduction

The increasing global demand for water, compounded by climate change, is leading to water shortages in various regions, especially in arid. Competition for limited water resources among urban, industrial, and agricultural sectors is intensifying (Oladosu et al. 2019). On the other hand, abiotic stresses such as drought and salinity are significant factors affecting agricultural activities. Water irrigation is becoming scarcer, necessitating more water-efficient agricultural practices (Calcagnile et al. 2019). Agriculture faces the challenge of meeting the food demands of a growing population. Food security is threatened due to rising food consumption and diminishing water supplies. Climate change impacts the agricultural economy, manifested in water scarcity and desertification (Abdelkader et al. 2022a). Plant survival is crucial for crop growth, and irrigation is vital to reducing water stress on plants. Given water scarcity and a focus on environmental preservation, there is significant interest in researching hydrogels for use in the agricultural sector (Kreye et al. 2009; Oladosu et al. 2022).

A superabsorbent hydrogel (SAH) is a long chain of molecules that absorb water molecules. Certain materials can absorb irrigation water up to 500 times their weight. These highly absorbent hydrogels play a beneficial role in addressing water retention. Water deficit is a significant environmental challenge in arid regions such as Egypt, and extensive research has been conducted on applying polymeric soil conditioners to address this issue. Arid soils commonly exhibit limited water retention capabilities, susceptibility to evaporation, low organic matter content, and unfavorable physical characteristics (Al-Darby 1996; Bryan 1992). In the case of sandy soils, plant productivity faces constraints due to their diminished capacity to retain water, low fertility, and excessive losses through deep percolation. These factors collectively lead to decreased efficiency in water absorption and fertilizers by plants (Sabrah 1994; Sen et al. 1995; Sivapalan 2006). Cultivated plants or crops in these regions often suffer from water shortage due to drought conditions, reducing productivity (Rigas et al. 1999).

Polymeric soil conditioners have a significant positive impact by delaying fertilizer dissolution, increasing sorption capacity, and enhancing plant nutrient uptake (Jhurry 1998). The efficacy of soil improvement is contingent upon factors such as hydrolysis degree, molecular mass, chain length of polymer, and application rates (Chan et al. 1996). Hydrogels composed of polyacrylamide and potassium polyacrylate have been proven effective in enhancing the quality of sandy soils for cultivation. These hydrogels exhibit an impressive water-absorbing capability, often retaining water at a rate one thousand times their weight. By doing so, they contribute to a reduction in the watering frequency and improve water retention capacity. Importantly, it has been observed that the use of this hydrogel does not lead to salt accumulation. Application SAH alleviates the impact of drought stress, thereby reducing plant losses attributed to water deficits (El-Rehim et al. 2004) because the polymer's stored water was available to plants, enhancing water use efficiency in high yields (Sivapalan 2006). The time it takes for seedlings to emerge and the height of the plants exhibit a linear dependence on the extent of polymer swelling. (Rigas et al. 1999); due to adding the conditioner, the time it took for sunflowers to emerge decreased by 32%. Additionally, their growth was enhanced by more than 50%, while the time to wilt was improved more than three times. The soil's field capacity (FC) was boosted two times, and water availability was improved by up to 244% compared to the control treatment.

Sandy soils have undesirable properties that contribute to water deficit conditions in the Egyptian deserts. Employing soil conditioners as a potential solution becomes crucial to address reclamation and cultivation challenges in desert regions due to the lack of information regarding the response of fenugreek cultivation in sandy soils treated with superabsorbent hydrogels. Therefore, the principal objective of the present investigation is to study the impact of superabsorbent hydrogel (SAH) treatments on plant growth, photosynthetic pigment, gas exchange, oxidative stress, antioxidant enzymes activities, and biomass accumulation of Fenugreek (*Trigonella foenum-graecum* L.) plants.

Materials and methods

Fenugreek was employed as a model during the experiments conducted in this research. The seeds were supplied from ARC, Ministry of Agriculture, Giza, Egypt. Newly reclaimed sandy soil of more than 80% particles >20 μ was collected, then further air-dried, sieved by a 2 mm sieve, and kept preparing the culture mixture. The main characteristics of the soil are presented in Table 1.

The applied SAHs were imported from the Soil, Water and Environment Research Institute, ARC, Ministry of Agriculture, Giza (Table 2). The water absorption capacity of the used SAH was 261 g water / g in AQUAKEEP hydrogel and 106 g water / g in IGETAGEL hydrogel (Mazen et al. 2015). A non-heated greenhouse experiment was conducted at Taif University, Taif Governorate, Saudi Arabia. SAHs were mixed with the soil at 0.05%, 0.1%, 0.2%, and 0.4% of soil, while non-mixed soil served as the control treatment.

Table 1. Characteristics of the loamy sand soil that was used in the experiment.

Property	Value	Property	Value
Coarse Sand < 200 μ %	42.4	Available K ppm	99
Fine Sand 200 – 20 μ %	41.9	Total N ppm	469
Silt 20 – 2 μ %	12.2	Total P ppm	823
Clay > 2 μ %	3.5	Total K ppm	1025
Soil Texture	Loamy Sand	Bulk density kg m−3	1.59
pH 1:2.5	7.6	Total porosity (%)	38.4
EC 1:5 dSm^{-1}	0.85	Water holding capacity (%)	21.5
CaCO3%	3.5	Field capacity (%)	4.56
CEC cmol Kg^{-1}	3.9	Wilting percentage	1.38
Organic Matter %	0.3	Hydraulic conductivity m day−1	7.6
Available N ppm	68	Mean diameter of soil pores μ	16.1
Available P ppm	11		

Table 2. The main components of superabsorbent hydrogels that were added to the soil.

No.	SAH	(Abbr.)	The effective material	Company
1	AQUAKEEP	(AKG)	Polyacrylic acid	Sumitomo Fine Chemicals
2	IGETAGEL	(IGG)	Vinyl alcohol sodium polyacrylate	Sumitomo Chemicals

Abbreviations

AKG: Aquakeep hydrogel; **IGG**: IGETAGEL; **SAH**: superabsorbant hydrogels; **RWC**: relative water content; **SOD**: Superoxide dismutase; **POD**: peroxidase; **APX**: Ascorbate peroxidase; **CAT**: Catalase; **FW**: Fresh weight; **DM**: Dry Matter; **Dw**: Dry weight; **WHC**: water holding capacity; **WUE**: Water use efficiency.

Experimental design

Ten fenugreek seeds were planted in each pot filled with soil-hydrogel mixtures. Each pot contains eight kilograms of the respective soil blend, measuring 25 cm in diameter and 30 cm in depth. The soil was saturated with water by placing the pots in trays filled with water for 24 hours. Following this, the pots were elevated to allow excess water to drain, and their weights were measured gravimetrically. Subsequently, the pots were transferred to a growth chamber and weighed regularly until no observable weight loss was recorded. If signs of seedling wilting appeared, pots were uniformly watered. Additionally, a half-strength Hoagland nutrient solution was supplied to the plants weekly. (Hoagland et al. 1950). Growth chamber temperature and humidity conditions were controlled via a sensor set to a range from an average temperature of 27(±2) °C during the day to 16 (±2) °C during the night. Humidity was maintained at around 42 (±5) %. After 15 weeks, samples were collected for physio-chemical analyses.

Growth parameters

Plant height was assessed five weeks post-cultivation. Upon completion of the experimental timeframe, the fresh weight (shoots and roots) was calculated. The separated fresh shoots and roots underwent drying in a ventilated oven at 70 °C, with successive weighings performed until a constant dry weight was achieved. The water content of both shoots and roots was computed using the following equation:

$$\text{RWC}\ (\%) = \{(\text{FW}-\text{DM}) / \text{FW}\} * 100$$

RWC is relative water content, FW is fresh weight (g), and DM is dry matter (g) of the samples.

Chlorophyll content and photosynthetic gas exchange analyses

For measuring chlorophyll, leaves samples were collected and ground in 85% acetone. Chlorophyll contents were measured spectrophotometrically (Abdelkader et al. 2023b; Metzner et al. 1965). Chlorophyll contents of plants treated with hydrogels were compared to the control. Photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, and transpiration rate were measured using an infrared analyzer (LI- 6400 System, Li-COR Company Lincoln, NE). Leaf gas exchange was recorded on two leaves per individual plant, with an open flow gas exchange system (Li-6400, Li-Cor, Lincoln, NE, USA). Leaves were inserted into the chamber and photosynthesis was induced for about 40 min at 25 °C, at a CO_2 mole fraction of 390 µmol mol^{-1}, a photosynthetic photon flux density of 1200 µmol m^{-2} s^{-1}, and an air flux of 300 µmol s^{-1}. Light saturated net CO_2 assimilation rate and stomatal conductance for water vapour were recorded and intrinsic water use efficiency (WUE) was computed.

Carbohydrates

Determination of carbohydrates was carried out by hydrolyzing a known weight of the dried powdered tissue (0.5 g) in the distilled water for two hours in a boiling water bath to extract soluble carbohydrates, but HCl was used for the extraction of total carbohydrates. After cooling, the hydrolyte in all samples was filtered then, a known volume of filtrate was used for determination of water-soluble saccharides and total carbohydrates by the anthrone sulphuric acid method (Fales 1951).

Proteins

To estimate the soluble proteins, 0.5 g from powdered tissue samples were boiled in distilled water for two hours. After cooling, the extract was centrifuged, and supernatant was separated and completed to known volume with distilled water, but, Total protein extracted by NaOH solution. The plant protein content was determined according to Lowery et al. (1951) using bovine serum albumin as standard.

Proline

Proline was extracted and estimated according to the method of Bates et al. (1973).

Free amino acids

Free amino acids were extracted from the plant tissues, where, a known weight of dry matter samples (0.5 g) was prepared for determination by Atomic Absorption flame Spectrophotometer (Perkinelmer 3300), according to the method of Moore and Stein (1948).

Antioxidant enzyme activity analyses

The leaves were homogenized in a buffer solution specific to each enzyme. The homogenate samples were filtered and then centrifuged. The supernatants were used for enzyme assays.

Catalase (CAT) and peroxidase (POD) determination

The reaction mixture contains 50 mM potassium phosphate buffer, 1% guaiacol, 0.4% H_2O_2, and enzyme extract. An increase in the absorbance due to guaiacol oxidation was measured at 470 nm. Enzyme activity was calculated regarding mmol of guaiacol oxidized min^{-1} g^{-1} fresh weight. Catalase activities (CAT) were determined by measuring the reduction of absorbtion at 240 nm of a reaction mixture (Chandlee and Scandalios 1984; Scandalios 1984; Zhang 1992).

Ascorbate peroxidase (APX)

The reduction in ascorbate concentration was followed by a decline in Abs at 290 nm, then APX activities were calculated using the extinction coefficient (E = 2.8 mM^{-1} cm^{-1} at 290 nm) for ascorbate (Hernández et al. 2015).

Superoxide dismutase (SOD)

The assay mixture contained 50 mM phosphate buffer (pH 7.8), 9.9 mM L-methionine, 57 lM NBT, 0.025% (w/v) Triton X-100 and 0.0044% (w/v) riboflavin. The photo-reduction of NBT was estimated at 560 nm. One unit of the SOD was defined as extract volume that caused 50% inhibition of the photo-reduction of NBT (Beauchamp and Fridovich 1971).

Statistical analysis

The data underwent analysis of variance (ANOVA), and two conventional analysis methods were employed for comparing means (Duncan 1951; Sokal and Braumann 1980). Statistical analyses were conducted utilizing the SPSS program (We analyzed the data using IBM SPSS Statistics 28.0.), and each treatment was replicated four times.

Results

In this experiment, the results in Table 3 showed that the addition of AKG and IGG hydrogels to the soil in which the fenugreek plants are grown had improved the growth of the fenugreek plants, represented by the plant height and root length, which has improved compared to the control plants. Plant height and root length were improved in fenugreek plants by increasing the SAHs rates.

The non treated fenugreek plants with any of hydrogels were the shortest plants and were less developed and growth than hydrogel-treated plants throughout the trial period. The illustrated data in Table 3 show that in all hydrogel treatments of fenugreek plants, the increase rate in root length was lower than that of plant height. The highest increase in plant height was detected at the highest concentration (0.4% AKG and IGG) of hydrogels. Plant height of fenugreek was significantly increased by 117.4% and 110.6% compared to control plants, respectively. With all concentrations of hydrogels, the plant height of fenugreek was higher concerning AKG than the same level of IGG hydrogel. Regarding the root length of fenugreek, the results were similar to those of plant height, where, at higher hydrogels concentration (0.4%), the root length increased by 80.4% and 84% compared with control plants, respectively (Table 3). When applying hydrogels, fenugreek plants' relative water content (RWC) moderately increased. AKG and IGG increased RWC by 6–10% for shoots and 5–9% for roots compared to control.

With increasing hydrogel concentrations in the soil, the leaf area of fenugreek plants increased to 102% and 74% compared to control when AKG and IGL were mixed with the sandy soil by 0.4%, respectively. Also, amending sandy soils improved the dry weight of shoot (59–153%) and root (14–90%) in AKG hydrogel, while the impact of IGG ranged from 53–141 and 7–76% in dry shoot and rood respectively compared to control (Table 4).

The net photosynthesis rate (Pn) was enhanced by increasing the hydrogel rate to reach 116.5% and 95% when the maximum dose (0.4%) of AKG and IGG were applied, respectively. When AKG was used by 0.2% and 0.4%, the highest chlorophyll contents were registered in fenugreek leaves. In contrast, the highest dose of IGG gave the most considerable amount of chlorophyll content (0.67 mg g^{-1} FW) compared to all other treatments.

Transpiration rate (mmol $m^{-2}S^{-1}$) significantly ($P < 0{\cdot}05$) increased by applying AKG and IGG hydrogels concerning the corresponding control plants (Fig. 1A). It was noted that treated pots generated more holding capacity, leading to a higher leaf transpiration rate than those of the non-treated with hydrogel pots. Despite the highest AKG rate producing the maximum transpiration rate (12.3), 0.4 IGG rate reduced the transpiration rate compared to 0.2%. A Steady increase in stomatal conductance was observed when hydrogels were applied compared to control, and AKG had a more positive impact than IGG (Fig. 1B).

The intercellular CO_2 concentration of plant leaves (mol/mol) is a significant factor in photosynthesis (Tominaga et al. 2018). The highest rates (0.2 and 0.4%) of AGK and IGG hydrogels decreased carbon dioxide on fenugreek tissues

Table 3. Effect of superabsorbent hydrogels AQUAKEEP (AKG) and IGETAGEL (IGG) on plant height, root length, root &shoot water content of fenugreek plants cultivated in sandy soil.

Treatments	Plant height (cm)	Root length (cm)	RWC, shoot (%)	RWC, root (%)
Control	20.7 ± 0.9 e	13.1 ± 0.7 d	83.2 ± 1.1 b	82.5 ± 0.8 b
AKG, 0.05%	28.3 ± 1.5 d	17.6 ± 1.5 bc	89.1 ± 0.9 a	87.3 ± 0.7 a
AKG, 0.10%	36.1 ± 2.6 c	19.2 ± 1.3 b	90.5 ± 0.6 a	88.1 ± 0.2 a
AKG, 0.20%	40.4 ± 3.4 b	22.5 ± 1.1 ab	91.9± 0.7 a	89.4 ± 0.5 a
AKG, 0.40%	45.0 ± 3.9 a	23.7 ± 0.9 a	91.5 ± 1.5 a	89.3 ± 2.2 a
IGG, 0.05%	26.9 ± 1.7 d	16.6 ± 1.1 c	88.6 ± 1.4 a	85.9 ± 1.4 a
IGG, 0.10%	33.7 ± 2.1 c	18.3 ± 0.9 bc	89.7 ± 1.7 a	87.9 ± 1.3 a
IGG, 0.20%	42.2 ± 2.5 b	21.5 ± 1.2 b	90.6 ± 0.9 a	88.4 ± 0.6 a
IGG, 0.40%	43.6 ± 3.3 ab	24.1 ± 1.5 a	91.2 ± 1.1 a	89.5 ± 0.8 a

Different letter(s) in each column indicate significant variations between the treatments ($p \leq 0.05$).

Table 4. Effect of superabsorbent hydrogels AQUAKEEP (AKG) and IGETAGEL (IGG) on leaf area, shoot, and root dry weight, net photosynthesis rate, and chlorophyll contents of fenugreek plants cultivated in sandy soil.

Treatments	Leaf area (cm^2/plant)	Shoot dry weight (g)	Root dry weight (g)	Net photosynthesis rate (Pn) ($\mu mol\ m^{-2}\ s^{-1}$)	Chlorophyll content (mg g^{-1} FW)
Control	53.6 ± 3.4 g	0.32 ± 0.21 f	0.29 ± 0.12 e	7.9 ± 1.8 f	0.33 ± 0.03 d
AKG, 0.05%	71.2 ± 3.1 e	0.51 ± 0.26 e	0.33 ± 0.08 d	11.3 ± 2.1 d	0.41 ± 0.05 c
AKG, 0.10%	90.9 ± 2.7 c	0.62 ± 0.09 c	0.41 ± 0.09 c	13.5 ± 2.2 c	0.59 ± 0.08 b
AKG, 0.20%	95.3 ± 2.6 b	0.78 ± 0.15 ab	0.52 ± 0.14 b	16.6± 2.6 a	0.66 ± 0.02 a
AKG, 0.40%	108.2 ± 4.8 a	0.81 ± 0.29 a	0.55 ± 0.20 a	17.1 ± 0.9 a	0.65 ± 0.04 a
IGG, 0.05%	65.7 ± 2.5 f	0.49 ± 0.11 e	0.31 ± 0.05 d	8.8 ± 1.5 e	0.39 ± 0.01 c
IGG, 0.10%	74.6 ± 4.3 e	0.56 ± 0.20 d	0.33 ± 0.07 d	10.8 ± 1.7 d	0.56 ± 0.02 b
IGG, 0.20%	85.3 ± 3.9 d	0.80 ± 0.22 a	0.53 ± 0.18 ab	15.3 ± 2.0 b	0.58 ± 0.03 b
IGG, 0.40%	93.4 ± 1.7 bc	0.77 ± 0.31 b	0.51 ± 0.11 b	15.4 ± 2.7 b	0.67 ± 0.07 a

Different letter(s) in each column indicate significant variations between the treatments ($p \leq 0.05$).

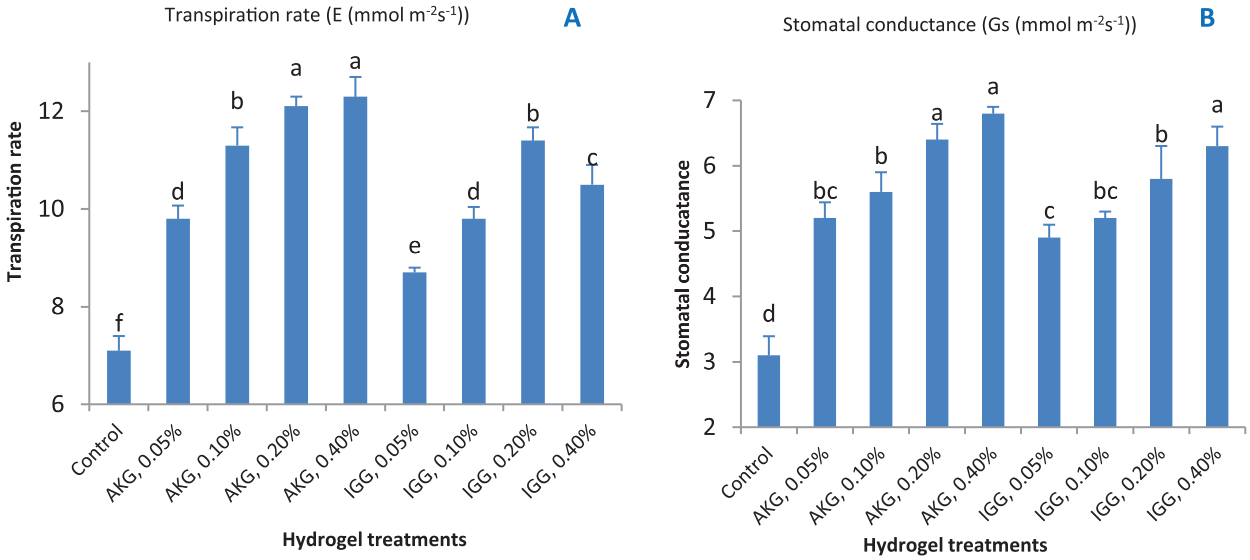

Figure 1. Effect of Hydrogel AQUAKEEP (AKG) and IGETAGEL (IGG) on the transpiration rate (**A**) and stomatal conductance (**B**) of fenugreek plants cultivated in sandy soil. Different letter(s) indicate significant variations between the treatments ($p \leq 0.05$).

compared to the control treatment, which registered 250 Ci mol mol^{-1}. In contrast, the two minimum concentrations of IGG hydrogel (0.05 and 0.1%) generate high CO_2 gaseous in fenugreek leaves (Fig. 2A). Water use efficiency (WUE) Measures the capacity to convert water into plant biomass. Increased WUE leads to organized usage of stored water during the growing season (Fig. 2B). In this investigation, both hydrogel concentrations increased WUE compared to the control treatment, and the maximum WUE (1.66) was generated from 0.4% AKG while 0.2% IGG produced the best WUE correspond to other IGG concentration.

Peroxidase (POD) and other antioxidants (APX, CAT, and SOD) are involved in various cellular processes such as plant development and stress response (Abdelkader et al. 2022a). These enzymes regulate plant growth by controlling hormonal and cell wall metabolism and antioxidant defense. Increasing peroxidase activities refer to the plant being exposed to stress conditions. Compared to the control treatment, superabsorbent hydrogels (AKG and IGG) mitigate the drought stress on fenugreek plants (Table 5). In AKG treatments, POD concentration reduction ranged from 28%–67%, APX reduced by 57%–94%, CAT decreased by 57–78%, and SOD by 69–94% compared to control treatment. The reduction generated by IGG treatments was as follows: 33%–72% of POD, 66–92% at APX, 61–86% at CAT, and 73–96% at SOD compared to control treatment. From the illustrated data

Table 5. Effect of hydrogel AQUAKEEP (AKG) and IGETAGEL (IGG) on POD, APX, CAT, and SOD activities of fenugreek plants cultivated in sandy soil.

Treatments	POD activity (unit g^{-1} FW)	APX (unit g^{-1} FW)	CAT activity (unit g^{-1} FW)	SOD activity (unit g^{-1} FW)
Control	181.1 ± 11.9 a	50.2 ± 2.1 a	82.2 ± 2.3 a	48.3 ± 1.7 a
AKG, 0.05%	120.9 ± 7.5 b	47.6 ± 1.6 b	64.3 ± 0.8 c	45.2 ± 0.9 b
AKG, 0.10%	90.2 ± 8.6 d	40.2 ± 1.7 d	55.1 ± 1.7 d	44.5 ± 1.1 b
AKG, 0.20%	60.7 ± 6.4 e	31.5 ± 3.1 ef	52.4 ± 1.7 e	38.9 ± 0.8 d
AKG, 0.40%	51.4 ± 5.9 f	28.4 ± 2.9 f	46.9 ± 2.5 f	33.1 ± 1.2 e
IGG, 0.05%	130.8 ± 9.7 b	46.3 ± 1.5 b	71.2 ± 2.1 b	46.4 ± 1.3 ab
IGG, 0.10%	118.7 ± 5.1 b	43.2 ± 2.9 c	65.9 ± 1.2 c	45.1 ± 1.0 b
IGG, 0.20%	111.1 ± 10.5 c	39.1 ± 3.3 d	53.1. ± 2.8 de	41.4 ± 0.7 c
IGG, 0.40%	60.3 ± 4.3e	33.2 ± 2.6 e	50.2 ± 0.6 e	35.1 ± 0.2 e

Different letter(s) in each column indicate significant variations between the treatments ($p \leq 0.05$).

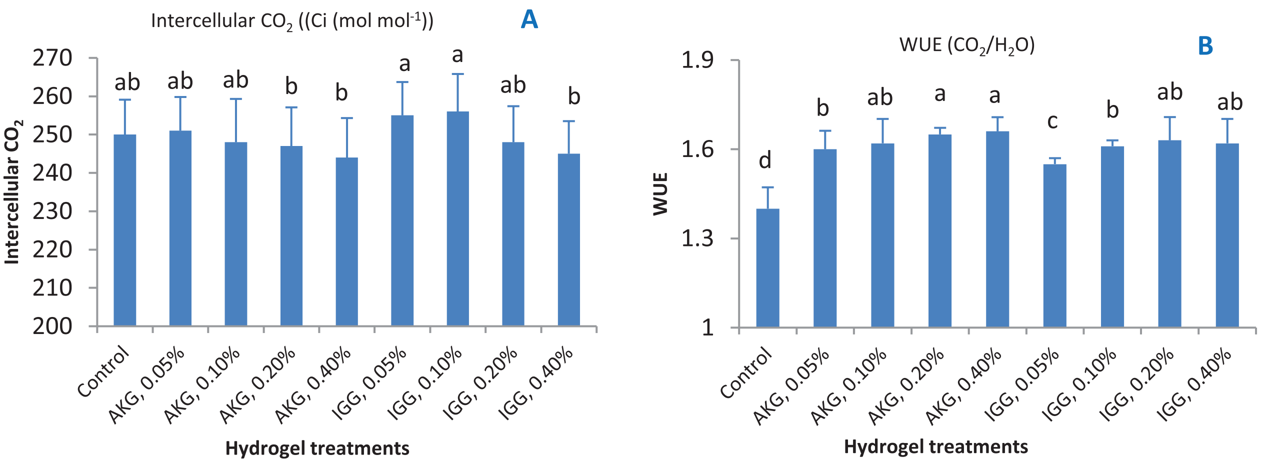

Figure 2. Effect of Hydrogel AQUAKEEP (AKG) and IGETAGEL (IGG) on intercellular CO_2 (**A**) and water use efficiency (**B**) of fenugreek plants cultivated in sandy soil. Different letter(s) indicate significant variations between the treatments ($p \leq 0.05$).

Table 6. Effect of hydrogel AQUAKEEP (AKG) and IGETAGEL (IGG) on proteins and carbohydrates contents of fenugreek plants cultivated in sandy soil.

Treatments	Soluble protein (mg g^{-1} DW)	Total protein (mg g^{-1} DW)	Soluble carbohydrate (mg g^{-1} DW)	Total carbohydrate (mg g^{-1} DW)
Control	134.9 ± 15.7 a	301.7 ± 21.2 e	65.8 ± 2.0 a	129.3 ± 6.2 d
AKG, 0.05%	105.4 ± 5.1 bc	314.8 ± 22.8 d	58.9 ± 1.3 b	144.0 ± 8.1 c
AKG, 0.10%	100.7 ± 6.5 c	362.4 ± 7.5 b	55.8 ± 2.8 c	160.2 ± 6.6 a
AKG, 0.20%	79.1 ± 2.0 e	349.6 ± 17.7 bc	54.1 ± 1.7 c	158.8 ± 7.4 ab
AKG, 0.40%	61.0 ± 1.4 f	395.2 ± 15.5 a	45.6 ± 1.9 e	163.7 ± 11.5 a
IGG, 0.05%	90.6 ± 14.6 d	307.9 ± 10.4 de	60.4 ± 1.8 b	145.4 ± 5.3 c
IGG, 0.10%	111.2 ± 8.6 b	315.6 ± 12.4 d	53.1 ± 5.3 cd	154.1 ± 7.6 b
IGG, 0.20%	84.7 ± 11.5 de	351.3 ± 14.0 bc	55.0. ± 2.6 c	157.9 ± 10.0 ab
IGG, 0.40%	84.2 ± 10.7 e	339.2 ± 12.6 c	52.2 ± 2.1 d	161.5 ± 9.8 a

Different letter(s) in each column indicate significant variations between the treatments ($p \leq 0.05$).

in Table 6, AKG has a more positive influence on the expression of antioxidant enzymes than IGG.

Soluble protein contents (mg/g DW) and total protein (mg/g DW) were determined in fenugreek leaves (Table 6). The data indicate that soluble protein has an inverse relationship with total protein content in all studied treatments. In contrast, the highest soluble protein produced from the control treatment was the highest total protein generated when AKG was applied at 0.4%. The same trend was observed when soluble carbohydrates and total carbohydrates were measured in fenugreek samples (Table 6). The least soluble protein amount (45.6 mg/g DW) and the highest total carbohydrates (163.7 mg/g DW) were obtained from the same treatment (AKG 0.4%).

As it is known, one of the plant's strategies when it is exposed to drought stress is that it adjusts its osmosis by increasing soluble substances such as soluble proteins and soluble carbohydrates, as well as increasing proline and free amino acids, and this is clear with plants not treated with hydrogel (control). But when hydrogel is added and the condition of the plant improves and it is not exposed to drought stress, it does not need to adjust the osmosis and increase solutes, so it decreases in this case. The increase in total carbohydrates and total protein is due to an increase in the process of photosynthesis, as well as an increase in the growth rate in plants treated with the hydrogel and an improvement in their biochemical properties.

Amino acids play crucial roles in plant tissues, serving as signal molecules, controlling root and shoot structure, influencing flowering time, and managing stress defense (Abdelkader et al. 2023c, 2023a). Fenugreek plants' total free amino acids concentration decreased by applying hydrogels compared to non-treated plants (Fig. 3A).

The most negligible content (31.9 mg/g DW) was observed when AKG was added by 0.4% to the soil. The same trend was observed when proline content was determined (Fig. 3B), whereas non-treated soils gave the highest amount of proline (11.3 mg /g DW) compared to all applied hydrogel treatments (AKG and IGG).

Discussion

Limited soil water content is the most critical problem that faces desert area reclamation, which restricts plant growth under drought conditions. Consequently, insufficient irrigation water and rainfall in arid areas can determine plant status (Huang and Gao 1999; Wang et al. 2003). A way to

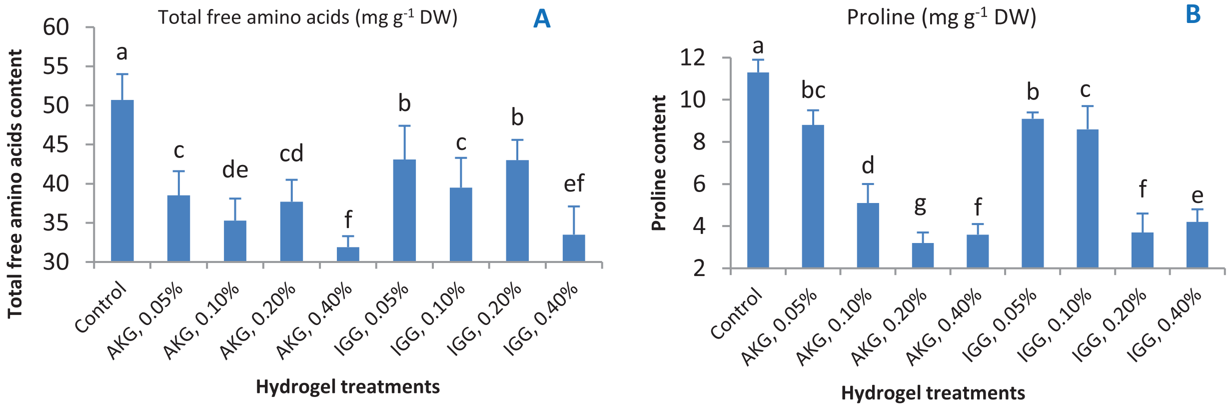

Figure 3. Effect of Hydrogel AQUAKEEP (AKG) and IGETAGEL (IGG) on total free amino acids (**A**) and proline contents (**B**) of fenugreek plants cultivated in sandy soil. Different letter(s) indicate significant variations between the treatments ($p \leq 0.05$).

solve such a problem is to apply water-retentive polymers (SAH) as a recent tool for solving water shortages in arid and semi-arid zones (Kant et al. 2008). Moreover, it was reported that hydrogels could chelate more water to plants by enhancing water contents in the rhizosphere (Davies Jr et al. 1987; Nwonwu 1987; Hüttermann et al. 1999, 2009; Oscroft et al. 2000; Salas and Reznicek 2001; Viero et al. 2002). With an increase in the hydrogel level, there was a corresponding rise in average plant height, leaf area, dry matter, and wilting time (El-Rehim et al. 2004).

In this study, applying SAH into the soil alleviated the negative impact of drought on both shoot and root dry matter. The AKG hydrogel exhibited more significant influence than the IGG hydrogel concerning the RWC of fenugreek shoots and roots, as presented in Table 4. During drought stress, soluble proteins revert to high amino acid levels as plants accumulate proteins with smaller molecular mass. High protein content could contribute to enhanced drought tolerance (Azevedo Neto et al. 2010). Previous studies have indicated that the presence of polymers extends plant survival, improves WUE, and promotes dry matter accumulation under drought conditions (Azzam 1983; Woodhouse and Johnson 1991).

Applying SAH enhanced dry matter of roots, stems, and leaves compared to those grown in SAH-free soils (Orikiriza et al. 2009). Applying soil conditioner enhanced maize plants' dry matter, reflecting the total yield (Yangyuoru et al. 2006). SAH creates better water retention and nutrition, increasing plant water availability and yield (Tohidi-Moghadam et al. 2009) that positively reflected on the fenugreek plant. Simultaneously, the control treatment produced the shortest seedlings than the other treatments during the water deficit. The development in fenugreek plants continued to improve by increasing SAH rates in the soil (0.4%).

The SAH, such as AKG and IGG cross-linked three-dimensional polymer networks to absorb and retain irrigation water and solute molecules in a swollen state because of the various hydrophilic groups such as carboxyl groups, amino groups, hydroxyl groups attached to their polymeric backbone (Spagnol et al. 2012; Feng et al. 2014; Saha et al. 2020b). The hydrophilic polymer can improve the WHC of the soil and reduce drought conditions. Generally, one gram of hydrogels, as those used in our investigation, can absorb from 100–500 g water (Akhter et al. 2004; Koupai et al. 2008; Saha et al. 2020a; Mahmood et al. 2023).

Using SAH as a soil additive can enhance water retention and availability, decreasing infiltration, particularly in soils with a coarse texture. Consequently, the superabsorbent can reduce irrigation requirements and promote improved crop growth in drought conditions. Beyond these benefits, the crucial significance of employing hydrogels lies in their ability to support plant survival during water-stress situations (Mazen et al. 2013). Water stress directly impacts the soil ecosystem and leads to soil degradation. The enduring impact of drought on the soil ecosystem is so profound that recovery is often slow, even after substantial rainfall or rehabilitation efforts (Geng et al. 2015). Therefore, there is a need to restrict excess moisture loss during water stress conditions to prevent soil degradation. It was reported that SAH amendment could delay the negative impact of water shortage stress on plants by extending their survival time (Darini et al. 2015; Ekebafe et al. 2011; Islam et al. 2011; Johnson and Piper 1997; Moslemi et al. 2011; Nazarli et al. 2010; Orikiriza et al. 2013). A significant increase in plant survival time when SAH amendment was applied under drought conditions resulted from decreasing hydraulic conductivity and controlling water loss from the rhizosphere (Chirino et al. 2011).

Conclusion

The findings suggest a significant enhancement in fenugreek plants facilitated by applying hydrogels. The improvement in plant growth was evident through assessing various growth parameters, including plant height which increased by 117.4% and 110.6%, root length also increased by 80.4% and 84% by adding 0.4% of the used hydrogel compared to control plants. Relative water content

(RWC) moderately increased in both shoots and roots and biomass, measured in terms of fresh weight and dry matter of shoots and roots. The percentage of increasing of these parameters was 59–153% for shoot, 14–90% for root dry weight, 116.5% for net photosynthetic rate. Furthermore, increased hydrogel concentration in the soil correlated with higher plant growth. However, the overall promotion of plant growth was contingent upon the hydrogel-to-soil ratio. Notably, the challenge of drought stress appeared to be effectively addressed through applying hydrogels (AKG and IGG). The parameter of Transpiration rate, stomatal conductance, intercellular CO2 concentration, Water use efficiency, total proteins and total carbohydrates are enhanced, but total free amino acids and proline decreased. This implies that hydrogels offer valuable benefits in newly reclaimed desert soils facing drought stress conditions.

Conflict of Interest

The Author has no conflict of interest.

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