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Hydrogel polymer improves plant biomass and leaf relative water content in dill and fenugreek

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Summary: Drought is the most important abiotic stress in arid and semi-arid regions of the world and causes a decrease in the yield of agricultural plants. One of the ways to deal with drought is the use of hydrogels. Two pot experiments were conducted to study the effect of hydrogel polymer on the growth and the physiological traits of fenugreek and dill plants. The study was carried out as a factorial experiment in a completely randomized design. The experiment included two factors: hydrogel polymer rates (0, 0.02, 0.04, and 0.08 grams per kilogram of soil) and irrigation intervals (irrigation intervals of 3 and 6 days). Results showed that leaf, stem and seedling fresh and dry weight were increased by hydrogel polymer application in both plant species. Hydrogel polymer increased leaf relative water content but decreased chlorophyll content. Plants treated with the polymer applied at 0.08 g kg^{-1} produced the highest dry matter. The improvement was probably due to the increased leaf relative water content. The highest dry weight of the seedling was measured in plants treated with the polymer applied at 0.08 g kg^{-1}, whereas the lowest seedling dry weight was found in plants that were not treated with the polymer. The effect of irrigation interval on growth characteristics of both plant species was not significant. Overall, the use of hydrogel improves plant biomass in dill and fenugreek. Application of amounts higher than 0.08 g/kg of hydrogel on dill and fenugreek is recommended for further research.

Keywords: *Anethum graveolens* L., chlorophyll, hydrogel, medicinal plants, relative water content, *Trigonella foenum-graecum* L.

Introduction

Fenugreek (*Trigonella foenum-graecum* L.) and dill (*Anethum graveolens* L.) are plant species belonging to the Fabaceae and Apiaceae family, respectively. They are medicinal plants cultivated in many countries (Saberali & Moradi, 2019). Dill is native to parts of Europe (Portugal, Spain and Turkey) and Asia (Iran), and is used as a vegetable, soup or medicinal plant (Stefanaki & Andel, 2021). Fenugreek is cultivated in parts of the world such as America, China and Egypt and has medicinal properties such as antidiabetic, antioxidant and immunological activities (Filipović et al., 2013; Wani & Kumar, 2018). Although water is the most abundant compound on earth and is vital in all chemical reactions, its scarcity is the most important limiting factor in crop yields worldwide (Fahad et al., 2017). In arid areas, in addition to water scarcity, more than 97% of the water is somehow lost before it becomes available to the plant (Alizadeh, 1999). Many strategies have been proposed and tested to control water deficit stress and increase water use efficiency such as irrigation methods (Wang et al., 2021), soil management (Hatfield et al., 2001) and, recently, hydrogel polymers (Savi et al., 2014; Wei et al., 2016; Kenawy et al., 2021; Liu et al., 2021).

Moisture absorbing compounds are known as water-superabsorbent polymers, hydrogels, hydrophilic gels, and polymer-based hydrogels (Heidari et al., 2016; Yang et al., 2020). Hydrogel polymers are special polymeric materials that can absorb large amounts of water in their main chain structure. Such technology can be used as a water reservoir in agriculture for use in arid areas, where water scarcity is a major problem (Ai et al., 2021). For this purpose, the hydrogel polymer is mixed dry in the soil near the plant roots. The soil is irrigated and the unabsorbed water by the plants is stored in a swollen hydrogel polymer, and as soon as the soil dries, water is released and made available for the plant. This process makes it possible to store water resources (Sannino, 2008).

In a previous study about the effect of drought stress on morphological characteristics and yield components of fenugreek, it was reported that plant height and yield components (pod number per plant, seed number per pod, and 1000-seed weight) decreased due to drought stress (around -43% in both biomass and grain yield; Bazzazi et al., 2013). In a study on dill comparing different irrigation intervals (5, 7, 9, 11, and 13 days), the irrigation intervals of 5 and 7 days induced the highest grain yield and improved the morphological characteristics (Gholinezhad, 2017). Berenguer & Faci (2001) reported that water deficit stress reduced dry matter production and plant height in sorghum. Irrigation compared to the control (without irrigation) caused a 79% and 47% increase in dill biomass yield in the first and second year, respectively (Popović et al., 2019).

In a study on *Pinus halepensis* Mill. about the effect of five doses of a Stockosorb® modifier, a hydrogel, on seed germination under drought conditions, it was concluded that, in a soil with clay-loam texture, the application of 0.4% of the modifier increased the survival rate of the pine plants more than the application of the same modifier at 0.2% (Hüttermann et al., 1999). In the study of six canola (*Brassica napus* L.) genotypes under drought stress and application of hydrogel polymer, it was found that under stress, chlorophyll content and photosynthesis decreased. Leaf greenness in the presence of hydrogel polymer was significantly increased by water uptake and retention (Tohidi-Moghaddam et al., 2009). Sivapalan (2001) studied the effect of hydrogel polymers in coarse-textured soils on soybean (*Glycine max* (L.) Merr.) plants and showed that dry matter production, plant height, and yield in hydrogel treatments were higher than untreated control. Anionic acrylic copolymer increased dry matter production, grain yield, and plant height in soybean. Acrylamide copolymer hydrogel increased wheatgrass (*Agropyron cristatum* (L.) Gaertn.) seedling establishment, biomass, and the emergence and overcame soil moisture restrictions. This method can be used for steppe revegetation (Mangold & Sheley, 2007). Hydrogel addition to soil increased soil moisture retention in both sandy loam and loam soils compared to the untreated soil. Chickpea (*Cicer arietinum* L.) germination was increased by gel application compared to control and hydrogel delayed wilting of seedlings compared to the untreated soil (Akhter et al., 2004).

Many studies focused on the effect of hydrogel polymer on the whole plant, but research on seedling establishment and growth is scarce. Consumption of hydrogel polymer in drought conditions is likely to reduce the adverse effects of drought. It is expected that the use of hydrogel polymer can allow a modification of the irrigation strategy adopted by increasing the irrigation interval. Therefore, this research aimed to investigate the effect of hydrogel polymer on growth traits of fenugreek and dill seedlings under drought.

Materials and Methods

Two pot experiments were conducted in 2015 to study the effect of hydrogel polymer on fenugreek (*Trigonella foenum-graecum* L.) and dill (*Anethum graveolens* L.) growth and physiological traits. The research was carried out in a cold frame greenhouse at the Campus of Agriculture and Natural Resources, Razi University, Kermanshah. Ten seeds were sown in a pot (7 cm in diameter, 7.5 cm in depth) filled with field soil, and then hydrogel polymer was placed around the seed. A200 hydrogel polymer is a type of acrylamide, acrylic acid and potassium acrylate. This hydrogel is a product of the Polymer and Petrochemical Research Institute of Iran. Its moisture absorption capacity (g/g) is 220. To measure the effect of two irrigation intervals and four amounts of hydrogel in three replications, 24 pots (2 × 4 × 3) were used. Considering that two plants were used in this study, a total of 48 pots were used. The seeds sown were twice the number of plants needed so that if some seeds did not germinate, enough seedlings would remain for the experiment, but after seedling emergence only five seedlings per pot were left. The experiment was conducted according to a complete randomized design with two factors—hydrogel polymer rates (0, 0.02, 0.04, and 0.08 grams per kilogram of soil) and irrigation intervals (irrigation intervals of 3 and 6 days)—and three replicates.

After three weeks, leaf chlorophyll content was measured by the Spad device (SPAD-502 Plus Chlorophyll Meter, Minolta, Japan). The last developed leaf of the plant was used to measure leaf chlorophyll. Chlorophyll was measured at three leaf points (Rasheed et al., 2003). The last developed leaf of the plant was used to measure leaf relative water content to reduce the effect of age. Leaf sampling was done at noon with a sharp razor. To measure the leaf relative water content, each the leaves were first weighed (fresh weight), then placed in distilled water for 24 hours, and re-weighed (turgid weight). After that, the sample was placed in the oven at 85 °C for 24 hours and dry weight was measured. The leaf relative water content was measured using the following equation (Turner & Kramer, 1980):

$$\text{Leaf relative water content (\%)} = \frac{(\text{Leaf fresh weight} - \text{Leaf dry weight})}{(\text{Leaf turgid weight} - \text{Leaf dry weight})} \times 100 \qquad \text{(eq. 1)}$$

At the five-leaf stage, plant height was measured with a ruler. Right after this measurement, plants were cut, their leaves and stems were separated, and their fresh weight was measured with digital scale (Quintix224, Sartorius, Germany). To measure the dry weight of stems and leaves, these organs were separately dried in an oven at 85 °C for 24 hours. Five plants per pot were randomly selected to measure these traits.

Before running the ANOVAs, outliers were identified by Minitab software (version 14; Minitab, LLC, State College, Pennsylvania, USA) and, if necessary, deleted, and the normality test was performed on the data. Means were compared using the Duncan test at a probably level of 5%. Data were analyzed by SAS software (version 9.1.3; SAS Institute Inc., Cary, NC, USA).

Results

In both plant species, the analysis of variance showed that the application of hydrogel polymer significantly affected all the measured vegetative traits (plant height, stem fresh weight, leaf fresh weight, seedling fresh weight, stem dry weight, leaf dry weight, and seedling dry weight) and physiological parameters (leaf relative water content and leaf chlorophyll content), whereas the irrigation treatment and the interaction between hydrogel polymer and irrigation treatment did not affect all the measured parameters in both fenugreek and dill (Tables 1 and 2).

Hydrogel polymer increased plant height, stem fresh weight, leaf fresh weight, seedling fresh weight, stem dry weight, leaf dry weight, seedling dry weight, and leaf relative water content in both fenugreek and dill (Tables 1 and 2). The highest plant height, stem fresh weight, leaf fresh weight, seedling fresh weight, stem dry weight, leaf dry weight, seedling dry weight, and leaf relative water content was measured in plants treated with the hydrogel polymer rate of 0.08 g (Tables 1 and 2). The lowest plant height, stem fresh weight, leaf fresh weight, seedling fresh weight, stem dry weight, leaf dry weight, seedling dry weight, and leaf relative water content were found in plants with the hydrogel polymer rate of 0 g (Tables 1 and 2). Hydrogel polymer application reduced leaf chlorophyll content in both fenugreek and dill (Tables 1 and 2). The highest leaf chlorophyll content was found in plants with the hydrogel polymer rate of 0 g. The lowest leaf chlorophyll content was measured in plants treated with the hydrogel polymer rate of 0.08 g (Tables 1 and 2).

The lack of difference between the irrigation intervals in terms of the studied traits may be due to the appropriate tolerance of fenugreek and dill to drought at the early stages of plant growth. Another reason is that the irrigation intervals applied in this research did not cause severe stress for these plants.

These results show that hydrogel can improve the growth characteristics and plant biomass of dill and fenugreek and can be considered by farmers as an agricultural input. Application of 0.08 g/kg of hydrogel increased the dry weight of the seedling by 265 and 75% in fenugreek and dill, respectively, which is significant.

Table 1. Effect of irrigation interval (I), hydrogel polymer (P) and I×P interaction on growth traits of fenugreek

Source of variation	Plant height (cm)	Stem fresh weight (g)	Leaf fresh weight (g)	Seedling fresh weight (g)	Stem dry weight (g)	Leaf dry weight (g)	Seedling dry weight (g)	Leaf relative water content (%)	Chlorophyll (Spad index)
Irrigation (I)									
Drought (6 days)	9.1a	82.4a	195.3a	277.8a	25.5a	75.5a	101.1a	82.8a	44.3a
Control (3 days)	9.4a	83.5a	196.1a	279.6a	25.8a	75.9a	101.7a	81.3a	44.7a
Significance	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Polymer (P)									
0 g/kg (control)	6.3d	44.4d	100.7d	145.1d	17.2d	29.1d	46.2d	75.8b	45.3a
0.02 g/kg	8.9c	68.2c	128.8c	197.0c	21.8c	54.0c	75.8c	77.8b	44.8ab
0.04 g/kg	10.1b	100.9b	265.5b	366.4b	28.1b	106.7b	134.8b	86.7a	44.1bc
0.08 g/kg	11.7a	118.4a	287.9a	406.3a	35.5a	113.2a	148.7a	88.0a	43.7c
Significance	**	**	**	**	**	**	**	**	**
I×P	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*

** and ns indicate significance at the probability level of 1% and non-significance, respectively.

Table 2. Effect of irrigation interval (I), hydrogel polymer (P) and I×P interaction on growth traits of dill

Source of variation	Plant height (cm)	Stem fresh weight (g)	Leaf fresh weight (g)	Seedling fresh weight (g)	Stem dry weight (g)	Leaf dry weight (g)	Seedling dry weight (g)	Leaf relative water content (%)	Chlorophyll (Spad index)
Irrigation (I)									
Drought (6 days)	8.0a	21.3a	41.2a	62.5a	7.9a	14.9a	22.9a	84.0a	27.4a
Control (3 days)	8.1a	22.4a	42.7a	65.5a	8.6a	15.6a	24.2a	82.0a	27.9a
Significance	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Polymer (P)									
0 g/kg (control)	6.6c	17.4c	30.7d	48.0d	6.6c	10.3d	16.9d	77.3c	29.0a
0.02 g/kg	7.7b	19.9bc	38.1c	58.0c	7.8bc	13.9c	21.8c	81.5bc	27.8b
0.04 g/kg	8.7a	22.7b	45.9b	68.6b	8.9ab	17.1b	25.9b	85.5ab	27.2b
0.08 g/kg	9.0a	27.4a	53.1a	80.5a	9.8a	19.7a	29.5a	87.7a	26.7b
Significance	**	**	**	**	**	**	**	**	**
I×P	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*

** and ns indicate significance at the probability level of 1% and non-significance, respectively.

Discussion

Hydrogel polymer increased leaf, stem, and seedling dry weight in fenugreek and dill. Farahmand et al. (2007) stated that consumption of zeolite mixed with soil increased leaf area, leaf fresh weight, leaf dry weight, root fresh weight, root dry weight, chlorophyll content, flowering stem diameter, flowering stem fresh weight, and flowering stem dry weight in *Narcissus tazetta* L. plant. Hydrogel increased the shoot dry weight of roselle (*Hibiscus sabdariffa* L.) under severe drought stress (Besharati et al., 2021). Hojjati et al. (2007) reported that consumption of zeolite has increased the root number, root length, root diameter, root fresh weight, root dry weight, leaf area, shoot fresh weight and shoot dry weight of fenugreek. The increase of biomass in dill and fenugreek with hydrogel in the present research is consistent with the results of the mentioned researchers. Hydrogels are polymers that absorb large amounts of water during expansion being able to absorb an average of 10 times their own weight of water (Ahmed, 2015). Water holding capacity in sandy soil with hydrogel polymer increased from 16.8% to 27%. The water holding capacity of clay increased from 37.5% to 44% with the help of hydrogel polymer (Peyrusson, 2021). In the study of the effect of different amounts of Pusa hydrogel on ginger (*Zingiber officinale* Rosc.), the highest amount of hydrogel (5 kg ha^{-1}) had the highest plant height, leaf number, number of tillers, yield per hectare and essential oil percentage (Rakshith et al., 2018). This is consistent with our research results about the stem weight, leaf weight, seedling weight, and plant height in dill and fenugreek.

The positive effect of hydrogel polymer on plant growth and biomass production was reported by many researchers (Johnson & Piper, 1997). Hydrogels can improve many soil properties such as CEC, organic matter, fertility, and water holding capacity (El-Hady & Abo-Sedera, 2006). Maize (*Zea mays* L.) yield was increased by soil natural and synthetic conditioner due to improved soil moisture retention (Yangyuoru et al., 2006). Many more benefits from hydrogels were achieved under drought and sandy soils. Urea-formaldehyde foam soil amendment extended time to wilting of water deficit stressed *Flindersia schottiana* saplings in sand and loam soil but not in the clay soil (Chan & Joyce, 2007). One gram of synthetic hydrogel polymer can absorb up to 900 g of water (Krul et al., 2000). This ability can save water around the root and improve plant growth.

Hydrogel application increased leaf relative water content in both fenugreek and dill plants and improving plant water status resulted in a stimulation of plant growth (Tables 1 and 2). Similarly, the hydrogel improved the leaf relative water content of guava (*Psidium guajava* L.) seedling under drought stress (Abdallah, 2019) which is consistent with the results of increasing the leaf relative water content in dill and fenugreek under the influence of hydrogel in the present research.

Hydrogel application decreased chlorophyll content in dill and fenugreek. Immediately after irrigation, soil moisture is at its maximum, but over time, soil moisture gradually decreases (Zand-Parsa et al., 2018). Therefore, in the interval between irrigation times, the plants may experience temporary drought stress. The use of hydrogel can prevent drought stress by maintaining soil moisture between watering times. Drought stress may even occur temporarily in well-watered treatment. It has been reported that stresses such as drought can increase leaf chlorophyll (Rustioni & Bianchi, 2021) which is not consistent with the results of the present research that drought had no effect on leaf chlorophyll. Increased leaf chlorophyll under stress is the plant's defense mechanism against stress (Agathokleous et al., 2020). Assuming that in well-water treatment the plant did not suffer from water deficit stress, again it can be said that with increasing plant growth and plant leaf area by hydrogel, chlorophyll concentration per unit area decreased, so the use of hydrogel polymer reduces chlorophyll per unit area. If the concentration of chlorophyll multiplied by biomass, it can be said that the use of hydrogel polymer in the plant can produce more chlorophyll.

Usually, the growth characteristics of the plant are related to each other. In this research, it is also observed that the hydrogel, by improving the moisture status of the plant through the leaf relative water content, improved the plant height, the weight of the stem and leaf, and finally the seedling dry weight of dill and fenugreek.

Among the weak points of this research, we can point out the treatment of irrigation interval, if the irrigation interval was considered longer, perhaps the effect of drought would appear in the plant, although the use of irrigation interval that reduces water consumption but does not reduce the biomass of the plant is also valuable. One of the strengths of this work is the evaluation of agricultural traits such as plant biomass under the influence of drought and hydrogel, which is important for the farmer and determines the final performance of the plant and economic performance, especially in dry areas. Considering that little research has been done on the agricultural needs of medicinal plants such as dill and fenugreek, this research that evaluates the reaction of these two plants to drought in the conditions of using hydrogel has innovation and originality.

Conclusions

Hydrogel polymer improved plant biomass in fenugreek and dill. The improvement was probably due to soil moisture retention that induced an improvement in plant water status. Interestingly, this positive effect was found independently of the irrigation frequency adopted. In addition, our study suggests that the best effects were recorded applying the polymer at a rate of 0.08 grams per kilograms of soil. Additional research will be necessary to test the effects of application rates higher than 0.08 grams per kilogram of soil. The use of a longer irrigation interval than the interval studied in this research at different stages of dill and fenugreek plant growth can be evaluated in future research.

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Hidrogel za poboljšanje biljne biomase i relativnog sadržaja vode u listovima kod mirođije i piskavice

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Suša je najvažniji abiotički stres u sušnim i polu-sušnim područjima sveta koji utiče na smanjenje prinosa poljoprivrednih kultura. Jedan od načina borbe protiv suše je upotreba hidrogela. Dva ogleda u saksijama su sprovedena u cilju ispitivanja uticaja hidrogela na rast i fiziološke osobine piskavice i mirođije kao faktorijalni eksperiment koristeći potpuno slučajni dizajn. Ogled je uključivao dva faktora: doze hidrogela (0, 0,02, 0,04 i 0,08 grama po kilogramu zemljišta) i intervale navodnjavanja (3 i 6 dana). Rezultati su pokazali povećanje sveže i suve mase listova, stabljike i klijanaca pri upotrebi hidrogela kod obe biljne vrste. Hidrogel je povećao relativni sadržaj vode u listovima ali je smanjio sadržaj hlorofila. Biljke koje su tretirane hidrogelom u količini 0,08 g kg^{-1} pokazale su najveći sadržaj suve materije. Poboljšanje je verovatno posledica povećanog relativnog sadržaja vode u listovima. Najviša suva masa klijanaca je izmerena kod biljaka koje su tretirane hidrogelom u količini 0,08 g kg^{-1}, dok je najmanja suva masa klijanaca izmerena kod biljaka koje nisu tretirane hidrogelom. Uticaj intervala navodnjavanja na karakteristike rasta obe biljne vrste nije bio značajan. Uopšteno govoreći, upotreba hidrogela poboljšava biljnu biomasu kod mirođije i piskavice. Za dalja istraživanja preporučuje se primena hidrogela kod mirođije i piskavice u količinama preko 0,08 g/kg.

Ključne reči: *Anethum graveolens* L., hydrogel, hlorofil, lekovito bilje, relativni sadržaj vode, *Trigonella foenum-graecum* L.