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Development of Urtica dioica-Functionalized Tragacanth Hydrogels for Improving Early Growth, Physiological Performance, and Drought Tolerance in Basil (*Ocimum basilicum*)

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Abstract

Drought stress is one of the major environmental factors limiting plant growth and productivity. In this study, tragacanth-based hydrogels functionalized with Urtica dioica extract (p(Tragacanth-co-Urtica)) were synthesized and evaluated for their potential to improve drought tolerance in basil (*Ocimum basilicum L.)* under different irrigation regimes (100%, 60%, and 40% of full irrigation). Plant growth parameters, physiological traits, antioxidant enzyme activities (catalase, superoxide dismutase, and ascorbate peroxidase), non-enzymatic antioxidants (total phenolic content and FRAP), and oxidative stress markers (malondialdehyde and proline) were analyzed. In addition, hydrogel swelling behavior, FTIR characteristics, and multivariate relationships among biochemical parameters were investigated.

The developed p(Tragacanth-co-Urtica) hydrogel exhibited enhanced swelling stability and water-retention properties compared with the unmodified hydrogel. Drought stress significantly reduced growth and altered biochemical responses in basil plants, whereas hydrogel application mitigated these adverse effects. In particular, p(Tragacanth-co-Urtica) improved shoot and root growth, reduced oxidative damage, and promoted more balanced antioxidant responses under deficit-irrigation conditions.

Principal component analysis explained 74.7% of the total variance and revealed treatment-dependent differences in biochemical responses. A positive but statistically non-significant relationship was observed between total phenolic content and FRAP activity ($r = 0.50$, $p = 0.167$). Overall, the results demonstrate that functionalization of tragacanth-based hydrogels with Urtica dioica extract provides a multifunctional bio-based material that combines water-retention capacity with biologically relevant plant-supporting effects. The developed hydrogel showed

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promise for improving early-stage growth and physiological performance of basil under water-limited conditions.

Keywords: *Ocimum basilicum; Urtica dioica*; drought stress; biopolymer hydrogel; water deficit; antioxidant enzymes; physiological responses; sustainable agriculture.

Introduction

Basil (*Ocimum basilicum* L. var. Nufar), member of the Lamiaceae family, is an important medicinal and aromatic plant widely utilized in the culinary, pharmaceutical, and cosmetic industries. Its leaves are consumed in both fresh and dried forms [1-4]. Basil is rich in essential oils and diverse bioactive compounds exhibiting antimicrobial, antifungal, antioxidant, anti-inflammatory, antiviral, and wound-healing properties[4]. These phytochemicals also contribute to the plant's resistance against biotic and abiotic stresses [5-8]. Owing to its high content of phenylpropanoids and terpenes, which constitute the major components of its essential oils, basil has considerable economic value in the food, fragrance, cosmetic, and traditional medicine sectors [5, 9].

Despite being adapted to semi-arid environments, basil is highly sensitive to water deficit conditions. [10, 11]. Drought stress adversely affects plant growth and development by disrupting physiological and biochemical processes, ultimately leading to reduced productivity [12-14].

To cope with drought-induced oxidative stress, plants activate a range of defense mechanisms, including reactive oxygen species scavenging, regulation of cellular redox balance, stabilization of proteins, and accumulation of osmoprotective compounds[15-17]. Phenolic compounds constitute an important component of the antioxidant defense system and contribute significantly to plant adaptation under adverse environmental conditions such as drought, salinity, extreme temperatures, heavy metal toxicity, and ultraviolet radiation [1, 18-20].

Recent studies have highlighted the substantial impact of water deficit on plant growth and productivity, emphasizing the need for sustainable strategies to improve crop performance under limited water availability. Various approaches, including stress-mitigating compounds, soil amendments, and advanced agricultural materials, have been investigated to enhance plant adaptation to drought conditions. These findings support the development of innovative materials capable of improving water management and sustaining plant growth under water-limited environments [21, 22].

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Hydrogels are three-dimensional crosslinked polymeric networks capable of absorbing and retaining large amounts of water while maintaining their structural integrity. Due to their high water-holding capacity, biodegradability, and controlled-release characteristics, hydrogels have attracted considerable attention in agricultural applications aimed at improving soil moisture retention and reducing the adverse effects of drought stress [23, 24]. Among natural polymers, tragacanth gum, a polysaccharide obtained from Astragalus species, is particularly attractive because of its biodegradability, biocompatibility, non-toxicity, and abundance of hydrophilic functional groups[25]. These properties make tragacanth a suitable matrix for the development of environmentally friendly hydrogel systems designed to enhance water availability in the rhizosphere[26, 27].

In addition to water-retention properties, the incorporation of plant-derived bioactive compounds may further improve the functionality of hydrogel systems. *Urtica dioica* is known to contain phenolic compounds and antioxidant constituents associated with various biological activities. Therefore, the incorporation of *U. dioica* extract into a tragacanth-based hydrogel may provide a multifunctional material that combines efficient water management with biologically relevant plant-supporting effects [28-30]. Despite increasing interest in agricultural hydrogels, limited information is available regarding *U. dioica*-functionalized tragacanth hydrogels and their potential effects on basil grown under drought stress conditions.

Although numerous hydrogel systems have been reported for agricultural water management, most function primarily as passive water reservoirs. In contrast, the hydrogel developed in the present study integrates the water-retention capacity of a natural tragacanth matrix with the bioactive constituents of *U. dioica* extract. This multifunctional design is expected not only to improve swelling performance and water availability but also to support plant physiological responses under drought stress.

Therefore, the present study aimed to investigate the effects of tragacanth-based hydrogels on the growth, physiological characteristics, and oxidative stress responses of basil plants exposed to different irrigation regimes. In addition, a *U. dioica*-functionalized tragacanth hydrogel (p(Tragacanth-co-Urtica)) was developed and evaluated as a sustainable biomaterial for improving water availability and supporting plant performance under deficit-irrigation conditions.

Materials and Methods

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Reagents

Ethylene glycol dimethacrylate (EGDMA), sodium hydroxide (NaOH), and hydrochloric acid (HCl, 36.5–38% v/v) were purchased from Sigma-Aldrich (USA). Sodium chloride (NaCl) was obtained from Isolab (Germany). Ammonium persulfate (APS, 98%) and N,N,N′,N′-tetramethylethylenediamine (TEMED, 99%) were supplied by Merck (Germany). All chemicals and reagents were of analytical grade and used without further purification. *Urtica dioica* plants used for hydrogel synthesis were collected from the campus of Van Yuzuncu Yil University, Van, Türkiye. Tragacanth gum was obtained from a local herbal supplier. Distilled water (DI water, 18.2 MΩ·cm; Human I) was used throughout all experimental procedures.

Preparation of Plant Extract

U. dioica samples used for hydrogel preparation were collected from the campus of Van Yuzuncu Yil University (Van, Türkiye). **Plant identification was performed by Dr. Yakup Yapar from Bingöl University. The altitude of the orchards where the figs were collected is approximately 700 m. Samples were taken for identification on 19.00.2026 from the orchards where the figs were collected. These samples were identified at the Herbarium within Van Yüzüncü Yıl University and given the herbarium number F14132.**

The collected plant materials were thoroughly washed to remove surface contaminants, air-dried at room temperature, and finely ground into powder form.

For extraction, 1 g of powdered plant material was mixed with 30 mL of distilled water in a suitable container. The mixture was then incubated in the dark at room temperature for 15 days to allow natural fermentation and extraction. Following the extraction period, the samples were centrifuged at 9000 rpm for 20 min, and the supernatants were separated by filtration. The obtained *U. dioica* extracts were collected and stored at 4°C until further use.

Previous studies have demonstrated that a 15-day natural fermentation process significantly enhances the tannin, flavonoid, and total phenolic contents of *U. dioica* extracts, while also improving their antioxidant activity compared with fresh plant material [28, 29].

Synthesis of Tragacanth–*Urtica dioica* Functionalized Hydrogels

Tragacanth-based hydrogels and their functionalized variants containing Urtica dioica extracts were synthesized using a conventional free-radical polymerization according to the methods

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described by Alpaslan et al., [31-33]. The *U. dioica* extracts were prepared according to the extraction procedure described in the previous section.

Briefly, 0.5 g of tragacanth gum was dispersed in 40 mL of distilled water and continuously stirred at 50°C for 24 h until a clear and homogeneous solution was obtained. For the synthesis of pristine tragacanth hydrogels, 2 mL of the prepared tragacanth solution was transferred into a reaction vessel and mixed with 2 mL of distilled water. The mixture was homogenized at 2500 rpm to ensure uniform dispersion. Subsequently, 200 µL of ethylene glycol dimethacrylate (EGDMA) was added as a crosslinking agent, followed by the addition of 20 µL of N,N,N′,N′-tetramethylethylenediamine (TEMED) and 0.03 g of ammonium persulfate (APS) dissolved in 100 µL of deionized water to initiate polymerization. The reaction mixture was poured into Petri dishes and allowed to polymerize at room temperature for 4 h. After polymerization, the hydrogels were removed from the molds, cut into 6 mm discs, thoroughly washed with deionized water to eliminate unreacted reagents, and dried at 40°C until a constant weight was achieved.

For the preparation of Tagacanth–*Urtica dioica* functionalized hydrogels, 2 mL of the tragacanth solution was mixed with 1 mL of *U. dioica* extract and homogenized at 2500 rpm. Subsequently, 300 µL of EGDMA was added as the crosslinker. Polymerization was initiated by sequential addition of 20 µL TEMED and 0.03 g APS dissolved in 100 µL deionized water. The resulting mixture was cast into Petri dishes and allowed to polymerize under ambient conditions for 4 h. The obtained hydrogels were cut into 6 mm discs, repeatedly washed with deionized water to remove residual unreacted compounds, and dried at 40°C to constant weight.

The APS concentration (0.03 g) was kept constant in both hydrogels. However, incorporation of the aqueous Urtica dioica extract affected gel formation, and a stable hydrogel could not be obtained using the original crosslinker concentration. Therefore, the EGDMA amount was increased from 200 µL to 300 µL to ensure successful hydrogel formation and structural stability. This adjustment was made for optimization rather than to modify the biological activity of the hydrogel.

The synthesized poly(Tragacanth) and poly(Tragacanth-co-*Urtica dioica*) hydrogels were designated as p(Tragacanth) and p(Tragacanth -co-Urtica), respectively.

Swelling Analysis

The swelling behavior of *U. dioica* extract and tragacanth-based hydrogels was evaluated according to Alpaslan et al. [34] Hydrogel samples were immersed in deionized (DI) water, tap

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water, sodium chloride (5%, NaCl) solution, and buffer solutions with pH values ranging from 1 to 12. All experiments were performed at ambient temperature (25 ± 1 °C).

Deionized water was used as an ion-free reference medium, whereas tap water represented natural aqueous conditions and 5% NaCl solution simulated saline environments. Buffer solutions covering a broad pH range were employed to investigate the pH responsiveness and structural stability of the hydrogel matrices under strongly acidic and alkaline conditions.

The swelling ratio (S) was determined gravimetrically after immersion periods of 1, 30 and 60 days to evaluate the effects of medium composition and exposure duration on the hydrogel network. All measurements were conducted in triplicate. Swelling and degradation equations;

$$Swelling(\%) = \frac{W_t - W_d}{W_d} \times 100$$

$$Degradation(\%) = \frac{W_0 - W_t}{W_0} \times 100$$

The point of zero charge (pH_pzc) was determined using the pH drift method. Solutions with different initial pH values were prepared and brought into contact with the hydrogel samples. After equilibrium was reached, the final pH values were measured. The pH_pzc was identified as the pH at which the net surface charge became zero, corresponding to the intersection point of the pH_initial and pH_final curves (or ΔpH = 0).

Fourier Transform Infrared (FTIR) Spectroscopy

Fourier Transform Infrared (FTIR) spectroscopy was used to characterize the chemical structure and functional groups of the synthesized tragacanth-based hydrogels. The analyses were performed using a Thermo Scientific Nicolet iS10 spectrometer (USA) equipped with an attenuated total reflectance (ATR) accessory. The main objectives of the analysis were to identify characteristic functional groups, assess evidence for the formation of a crosslinked structure, and investigate potential molecular interactions between tragacanth and the incorporated components. Before analysis, the hydrogel samples were dried and ground into a fine powder. The powdered samples were placed directly onto the ATR crystal to ensure adequate contact during measurement. Spectra were recorded over the range of 4000–650 cm^{-1} with a spectral resolution of 4 cm^{-1}. The obtained spectra were analyzed to identify functional groups associated with tragacanth, the crosslinking agents, and plant-derived components, and to evaluate structural changes associated with polymerization and hydrogel network formation.

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Application

The experiment was conducted in 2025 in the climate chamber of the Faculty of Engineering at Van Yuzuncu Yıl University under controlled environmental conditions, including a 12 h light/12 h dark photoperiod, a temperature of 23°C, and a relative humidity of 65 ± 2%.

The experiment was arranged as a 3 × 3 factorial design consisting of three hydrogel treatments and three irrigation levels, resulting in nine treatment combinations. Each treatment combination was represented by three replicate pots, giving a total of 27 experimental pots. (The experiment **three independent pots were used per treatment combination (n = 3), with three leaf samples analyzed per pot (sub-n = 9)) and the reported values represent the mean ± SD of these replicates.**). Statistical analyses were performed using the pot as the experimental unit.) Each pot contained three basil plants. The irrigation treatments consisted of full irrigation (100%) and two deficit irrigation levels corresponding to 60% and 40% of full irrigation. Each pot was considered an independent experimental unit. Three replicate pots were used for each treatment combination, and each pot contained three basil plants. Unless otherwise stated, measurements were performed using plants collected from the replicate pots, and the reported values represent the mean of the biological replicates.

Basil seeds of the BR Bu-ROK cultivar, a standard variety produced by Bursa Seed Production Company (Türkiye), were used in the experiment. Hydrogels (0.70 g per pot) were thoroughly mixed with a 2:1 peat-perlite growth medium before filling the pots with equal volumes of substrate. The pots used in the experiment had a top diameter of 15 cm, a depth of 12 cm, and a volume of 1.5 L.

The peat used as the growth substrate had the following properties: particle size of 0–8 mm, pH 5.5, electrical conductivity (EC) of 1.5 mS cm^{-1}, and nutrient content of 0.5 kg m^{-3} N-P_2O_5-K_2O (14-4-20 + TE), supplemented with dolomitic limestone. The experimental design is presented in Table 1.

Before the initiation of irrigation treatments, the pot capacity (field capacity of the substrate) was determined by saturating the pots with water and allowing excess water to drain until no further drainage occurred. The weight of each pot at pot capacity was then recorded and used as the reference value for irrigation management. Irrigation treatments consisted of maintaining the pots at 100% (full irrigation), 60%, or 40% of the water applied to the fully irrigated treatment. Pots

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were weighed every three days, and the amount of irrigation water was calculated using the following equation:

$I = (Wi\text{-}1 - Wi) \times IR$

where I is the amount of irrigation water applied (mL), Wi−1 is the pot weight at the previous irrigation (kg), Wi is the pot weight at the time of irrigation (kg), and IR is the irrigation level coefficient (1.0, 0.6, or 0.4 for the 100%, 60%, and 40% irrigation treatments, respectively).

Tap water was used for irrigation throughout the experiment. Deficit irrigation treatments were initiated 25 days after sowing. The experiment was terminated on day 43 due to visible chlorosis and marked growth differences observed in drought-stressed plants.

Following harvest, plant samples were washed first with tap water and subsequently with distilled water. The samples were then dried in an oven at 70°C for 48 h until constant weight was achieved. Dried samples were ground into powder and prepared for further analyses.

Morphological Growth Parameters

Morphological parameters, including shoot length, root length, stem diameter, and shoot and root fresh and dry weights, were measured at the end of the experiment.

Leaf Color Measurements

Leaf color parameters were measured weekly using a Minolta CR-400 colorimeter according to the CIE L*a*b* color system. Ten measurements were obtained from different regions of the leaf surface for each seedling, while avoiding the major veins.

Chroma (C*) and hue angle (h°) were calculated from the measured a* and b* values according to the following equations: $C = (a^2 + b^2)^{1/2}$ and $h° = \tan^{-1}(b/a)$. Hue angle values were expressed in degrees after appropriate quadrant correction.

At the final harvest, leaf tissues were collected from the three plants within each pot and pooled to obtain one composite sample representing a single experimental unit. Immediately after sampling, the leaves were stored at −20 °C until biochemical analyses. Frozen leaf tissues were homogenized in the appropriate extraction buffer for CAT, SOD, APX, MDA, and proline analyses, whereas methanolic extracts were prepared for FRAP and total phenolic content determinations. Plant materials intended for biomass measurements were processed separately by oven drying at 70 °C for 48 h.

Total Antioxidant Capacity

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Ferric reducing antioxidant power (FRAP) and total phenolic content (FC) were determined from total phenolic content was determined using methanolic extracts prepared from frozen leaf tissue. FRAP was measured at 593 nm using Trolox as the standard and expressed as μmol TE g^{-1} FW. Total phenolic content was measured at 765 nm using gallic acid as the standard and expressed as μg GAE g^{-1} FW.

Antioxidant enzyme activity

CAT, APX and SOD activity was determined from frozen leaf tissue homogenized in extraction buffer. To determine the enzyme activities of catalase (CAT), superoxide dismutase (SOD), and ascorbate peroxidase (APX), 1 g of frozen leaf sample was homogenized with 5 mL of cold 50 mM potassium phosphate buffer (pH 7.6) and 0.1 mM Na-EDTA extraction solution. The homogenates were centrifuged at 709 × g at 4 °C for 10 minutes, and the resulting supernatant was used in enzyme analyses. All steps of enzyme extraction were performed at 4 °C. SOD activity was determined based on the inhibition of nitro blue tetrazolium (NBT) photoreduction at 560 nm. CAT activity was measured by monitoring the decrease in absorbance due to the breakdown of H_2O_2 at 240 nm. APX activity was determined by measuring the change in absorbance due to the oxidation of ascorbate in the presence of H_2O_2 at 290 nm [35, 36].

Analysis of the reactive oxygen species (ROS)

Malondialdehyde (MDA) content was determined from frozen leaf tissue stored at −20 °C according to Shams et al [37]. Lipid peroxidation was evaluated by determining malondialdehyde (MDA) content using the thiobarbituric acid (TBA) assay. Frozen leaf samples (0.5 g) were homogenized in 0.1% trichloroacetic acid (TCA) and centrifuged at 15,000 rpm for 5 min at 4°C. The obtained supernatant was reacted with 20% TCA containing 0.5% TBA. The reaction mixtures were incubated at 95°C for 30 min and then rapidly cooled on ice. Absorbance values were measured at 532 and 600 nm *Ocimum basilicum* L.) [37].

Proline content was determined using frozen leaf tissue stored at −20 °C according to the method to Shams et al [37]. Subsequently, 5 mL of 40% methanol was added, and the samples were homogenized on ice. The homogenates were centrifuged at 12,000 rpm for 15 min at 4°C [37]. Thereafter, 1 mL of the supernatant was transferred into glass tubes and mixed with 1 mL of ninhydrin reagent. The reaction mixtures were incubated at 100°C for 1 h and immediately cooled in ice water. After cooling to room temperature, 3 mL of toluene was added, and absorbance was measured at 520 nm.

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Statistical analysis

All data are presented as mean ± standard deviation (SD). The experiment was conducted as a factorial design consisting of three hydrogel treatments and three irrigation levels. Individual pots were considered the experimental units for statistical analyses. Each treatment combination included nine biological replicates (plants), and the reported values represent the mean ± SD of these replicates.

A two-way analysis of variance (ANOVA) was performed to evaluate the main effects of hydrogel treatment, irrigation level, and their interaction. When a significant interaction was detected, treatment means were compared using Tukey's multiple comparison test at $p < 0.05$. Different uppercase letters within the same irrigation level indicate significant differences among hydrogel treatments, whereas different lowercase letters within the same hydrogel treatment indicate significant differences among irrigation levels. **Three independent pots were used per treatment combination (n = 3), with three leaf samples analyzed per pot (sub-n = 9)) and the reported values represent the mean ± SD of these replicates.**

Principal component analysis (PCA) was performed using the complete dataset obtained from biological replicates of all measured parameters (CAT, SOD, APX, FRAP, FC, and proline). Individual replicate values, rather than treatment means, were used in the analysis to evaluate relationships among treatments and physiological variables. Prior to PCA, the data were standardized using z-score normalization to eliminate scale effects among variables. PCA was performed based on the correlation matrix, and components with eigenvalues greater than 1 were retained. The first two principal components were used for graphical visualization of treatment clustering and variable associations. All statistical analyses were conducted using SPSS Statistics.

Results and Discussion

Swelling and Degradation Behavior

The swelling and degradation behaviors of p(Tragacanth) and p(Tragacanth-co-Urtica) hydrogels were evaluated in deionized (DI) water, tap water, and **5%** NaCl solution after 1, 30, and 60 days of immersion (Figure 1). These time intervals were selected to assess both the initial water

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absorption capacity and the long-term structural stability of the hydrogel systems.

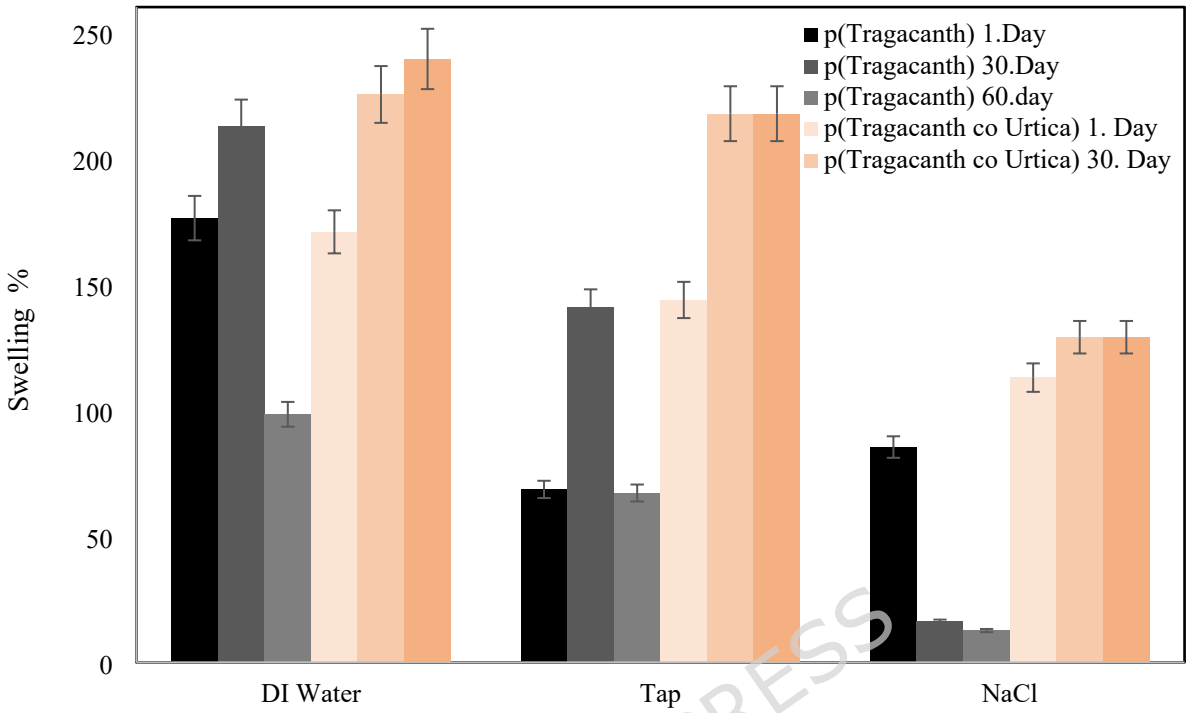


Figure 1. Swelling behavior of p(Tragacanth) and p(Tragacanth-co-Urtica) hydrogels after 1, 30, and 60 days in water (DI), tap water, and 5% NaCl solution.

Both hydrogels exhibited their highest swelling capacities in DI water during the initial immersion period. However, distinct differences emerged during prolonged incubation. While the swelling capacity of p(Tragacanth) gradually decreased over time, p(Tragacanth-co-Urtica) maintained higher swelling ratios under all tested conditions, indicating improved water-retention performance and structural stability. The effect of the surrounding medium was also evident. Reduced swelling was observed in tap water and NaCl solution compared with DI water, which may be attributed to the presence of dissolved ions that limit hydrogel expansion through charge-screening effects. Nevertheless, p(Tragacanth-co-Urtica) consistently exhibited greater swelling capacity than p(Tragacanth), suggesting that the incorporation of Urtica-derived components contributed to a more stable and hydrophilic polymer network.

Degradation analyses further supported these observations. p(Tragacanth) exhibited substantially higher degradation rates after prolonged immersion, whereas p(Tragacanth-co-Urtica) retained its structural integrity to a greater extent. In NaCl solution, the calculated degradation value of −9.16% for p(Tragacanth-co-Urtica) reflects a net increase in sample mass relative to its initial

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weight, most likely due to retained water and residual absorbed constituents within the hydrogel matrix rather than actual material degradation.

Overall, the incorporation of *Urtica dioica* extract improved the swelling stability and durability of the tragacanth-based hydrogel system under different aqueous environments, highlighting its potential for applications requiring prolonged water retention.

pH-Dependent Swelling Behavior of p(Tragacanth) and p(Tragacanth-co-*Urtica dioica*) Hydrogels

The pH-responsive swelling behavior of p(Tragacanth) and p(Tragacanth-co-Urtica) hydrogels was investigated after 24 h of immersion (Figure 2 and 3). The point of zero charge (pHpzc) values were approximately 2.0 for p(Tragacanth) and 2.7 for p(Tragacanth-co-Urtica), indicating differences in the surface characteristics of the two hydrogel systems.

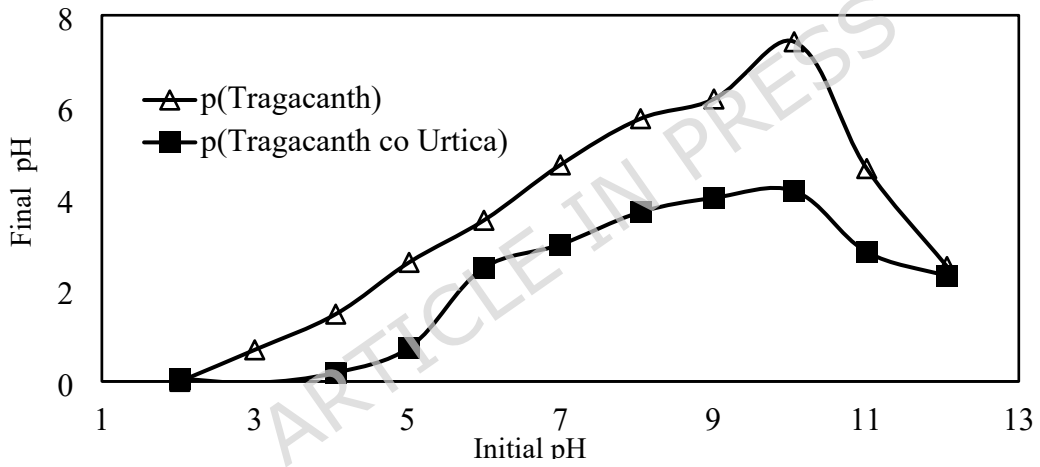


Figure 2. Determination of the point of zero charge (pHpzc) of p(Tragacanth) and p(Tragacanth-co-Urtica) hydrogels using the ΔpH (pHfinal − pHinitial) method.

Both hydrogels exhibited pH-dependent swelling behavior. At pH values below their respective pHpzc, swelling was relatively limited due to protonation of functional groups and reduced electrostatic repulsion within the polymer network. In contrast, swelling increased under neutral and alkaline conditions as ionization of the functional groups promoted electrostatic repulsion and expansion of the hydrogel matrix. The enhanced swelling observed at higher pH values is consistent with the presence of ionizable groups capable of responding to changes in environmental pH[38]. The incorporation of Urtica dioica extract significantly influenced the swelling performance of the hydrogel system. Across nearly the entire pH range, p(Tragacanth-

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co-Urtica) exhibited higher swelling ratios than the unmodified p(Tragacanth) hydrogel, suggesting that the introduced bioactive components contributed to a more hydrophilic and responsive polymer network. Although both hydrogels displayed pH-sensitive behavior, the modified hydrogel maintained superior water uptake capacity over a broader pH interval. Overall, the results demonstrate that p(Tragacanth-co-Urtica) possesses enhanced pH-responsive swelling characteristics compared with the pristine hydrogel, indicating its greater potential for applications requiring efficient water absorption under varying environmental conditions.

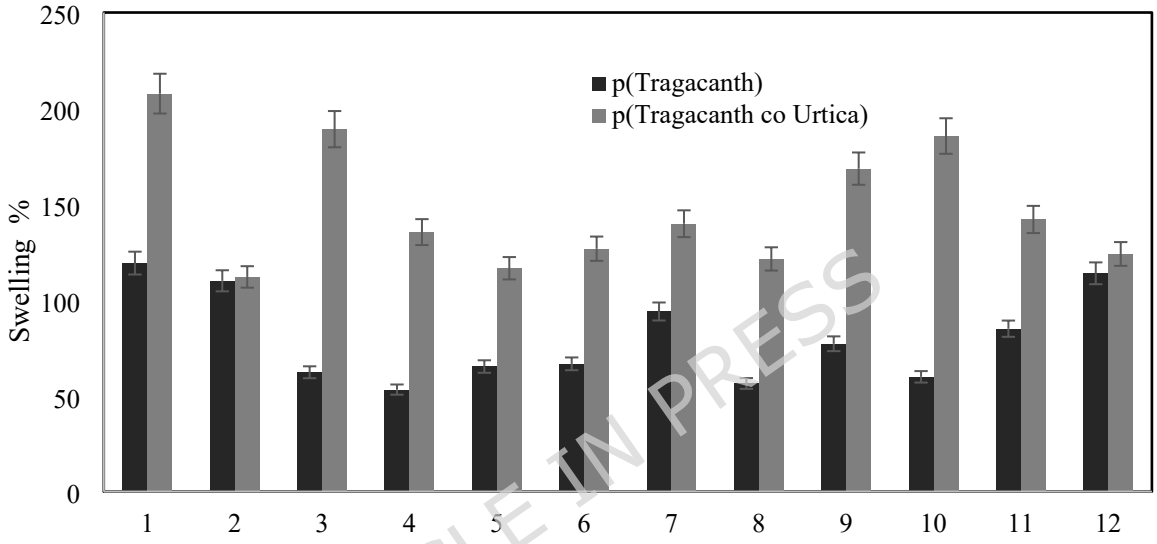


Figure 3. Swelling profiles of different hydrogel samples as a function of pH.

Fourier transform infrared spectroscopy analysis

The FTIR spectra of p(Tragacanth) and p(Tragacanth-co-Urtica) hydrogels are shown in Figure 4. In p(Tragacanth), a broad band at 3200–3600 cm^{-1} corresponds to O–H stretching vibrations. The peak at 2978 cm^{-1} is assigned to C–H stretching vibrations. The absorption band at 1700–1720 cm^{-1} is attributed to C=O stretching, while bands at 1600–1685 cm^{-1} and ~1500 cm^{-1} correspond to COO^- asymmetric and symmetric vibrations. Peaks in the 1000–1200 cm^{-1} region are associated with C–O–C and C–O stretching of polysaccharide structures.

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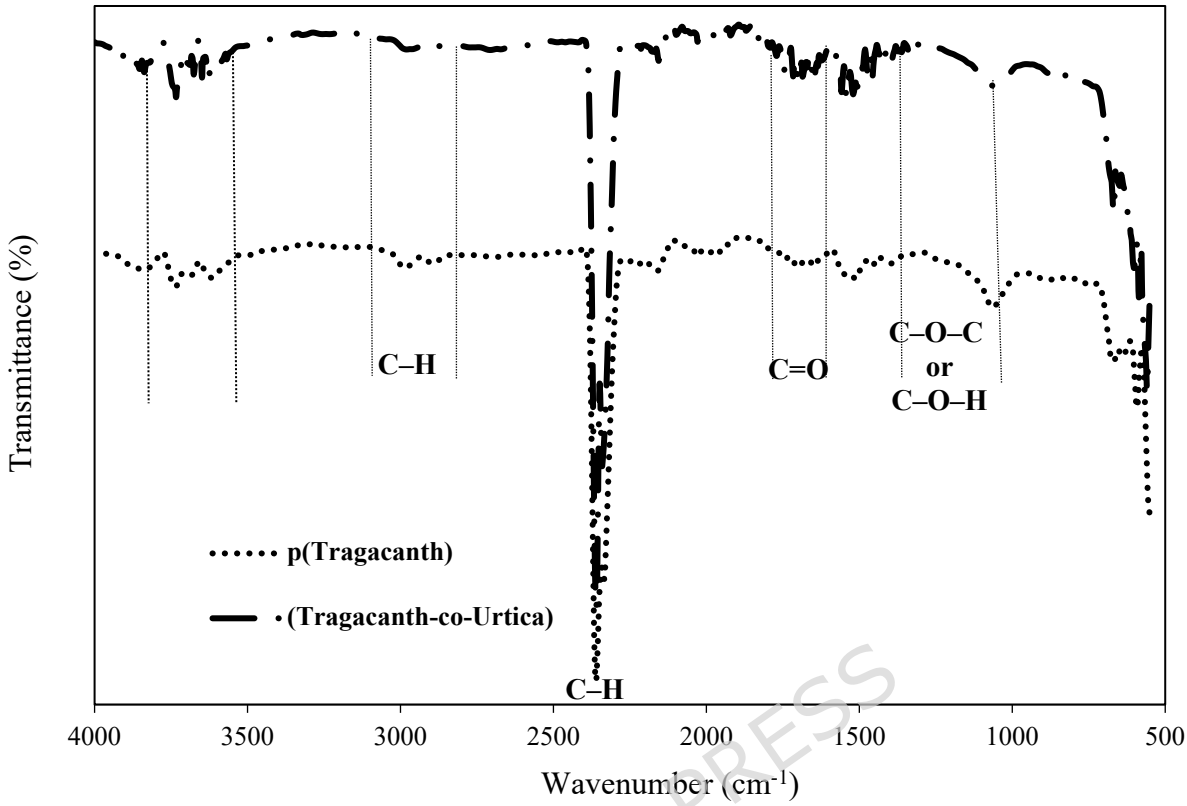


Figure 4. The hydrogels FT-IR graph.

The FTIR spectrum of p(Tragacanth co Urtica) showed a similar profile, indicating preservation of the polysaccharide backbone after incorporation of Urtica dioica extract. However, a slight shift and broadening of the O–H stretching band (~3310 cm^{-1}) was observed. Changes in the intensity and position of the C=O band (~1715 cm^{-1}) and variations in the 1200–1250 cm^{-1} region were also detected.

Morphological Growth Parameters

Plant growth responses of basil under different irrigation regimes (100%, 60%, and 40%) treated with p(Tragacanth) and p(Tragacanth co Urtica) hydrogels are presented in Table 1. Significant differences ($p < 0.05$) were observed among treatments for all measured parameters. Hydrogel treatment significantly affected root length ($F = 15.82$, $p < 0.001$), as did irrigation level ($F = 48.37$, $p < 0.001$). A significant hydrogel × irrigation interaction was also detected ($F = 3.91$, $p = 0.012$).

Table 1. Effects of p(Tragacanth co Urtica) based hydrogel on some plant growth characteristics in basil plants.

Hydrogels and Irrigation %	Stem fresh weight (g)	Stem dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Root length (cm)

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	X ± **SD**	X ± **SD**	X ± **SD**	X ± **SD**	X ± **SD**
Control 100%	9.07 ±0.7 bC	1.00 ±0.1 bB	0.8 ±0.01 aC	0.056 ±0.01 abAB	6.5 ±0.8 aB
Control 60%	12.58 ±5.8 cC	0.58 ±0.01 a	0.97 ±0.01 cC	0.075 ±0.03 aA	7.5 ±0.5 bC
Control 40%	5.29 ±1.9 aC	0.66 ±0.01 bB	0.55 ±0.01 bC	0.041 ±0.01 bA	5.5 ±0.1 bA
p(Tragacanth)_100%	18.19 ±5.2 bB	1.08 ±0.2 C	2.16 ±0.9 bB	0.148 ±0.01 aC	10 ±1.1 bC
p(Tragacanth)-60%	12.52 ±0.7 cB	0.50 ±0.07 b	1.35 ±0.3 aB	0.149 ±0.01 aC	6.8 ±0.2 aA
p(Tragacanth)-40%	12.07 ±2.3 aB	0.39 ±0.03 aC	1.1 ±0.3 cB	0.076 ±0.03 bC	9 .0±0.7 cC
p(Tragacanth co Urtica)_100%	28.23 ±0.3 aA	0.69 ±0.3 aA	1.8 ±0.4 aA	0.141 ±0.04 aBC	7.2 ±0.2 aA
p(Tragacanth co Urtica)-60%	23.03 ±2.2 bA	0.72 ±0.2 b	1.24 ±0.4 bA	0.122 ±0.05 aB	8.0±0.8 bB
p(Tragacanth co Urtica)-40%	11.22 ±7.1 cA	0.79 ±0.1 cA	0.81 ±0.1 cA	0.078 ±0.01 bB	7.0±0.1 cA

*** V** Values are presented as mean ± standard deviation **(Three independent pots were used per treatment combination (n = 3), with three leaf samples analyzed per pot (sub-n = 9)).** Different uppercase letters within the same irrigation level indicate significant differences among hydrogel treatments, whereas different lowercase letters within the same hydrogel treatment indicate significant differences among irrigation levels ($p < 0.05$, Tukey's test).

The effects of hydrogel application and irrigation level on basil growth are presented in Table 1. Water deficit significantly reduced stem and root growth compared with the fully irrigated treatment. However, both hydrogel formulations alleviated the negative effects of drought, although their effectiveness depended on the evaluated growth parameter and irrigation level. Under full irrigation (100%), p(Tragacanth co Urtica) produced the greatest stem fresh weight, whereas p(Tragacanth) resulted in the highest root fresh and root dry weights. Under moderate water deficit (60%), both hydrogel formulations improved biomass production compared with the untreated control, with the Urtica-functionalized hydrogel showing higher stem biomass and the unmodified hydrogel maintaining greater root biomass. Under severe drought (40%), hydrogel-treated plants generally maintained greater stem and root growth than untreated plants, indicating that hydrogel incorporation mitigated the adverse effects of limited water availability. Nevertheless, the relative performance of the two hydrogels differed among the measured traits, suggesting that no single hydrogel was consistently superior for all morphological variables. Two-way ANOVA further demonstrated that irrigation level and hydrogel treatment significantly influenced plant growth. A significant hydrogel × irrigation interaction indicates that the response to hydrogel application depended on the severity of water deficit rather than remaining constant across irrigation regimes.

Similar improvements in plant growth under water-limited conditions have been reported for hydrogel-based soil amendments and other drought-mitigating agricultural materials [35-37, 39]. Enhanced soil water availability has been associated with improved biomass accumulation, maintenance of physiological activity, and greater drought adaptation in several crop species[40-

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43] .The present findings are consistent with these observations and further demonstrate that functionalization of tragacanth-based hydrogels with *Urtica dioica* extract can enhance the effectiveness of hydrogel systems in supporting basil growth under deficit-irrigation conditions[44-50].

Table 2. Effects of hydrogels on color and SPAD in basil plant.

Hydrogels and Irrigation %	L*	a*	b*	Chroma	Hue	SPAD
	X ± SD	X ± SD	X ± SD	X ± SD	X ± SD	X ± SD
Control 100%	37.87±6.96 ^{aA}	-5.6±0.60 ^{aA}	13.20±2.21 ^{aA}	15.57±1.39 ^{aA}	148.90±11.24 ^{aA}	30.18±6.01 ^{aA}
Control 60%	37.62±7.30 ^{aA}	-4.09±0.57 ^{aA}	13.25±1.34 ^{aA}	15.41±1.84 ^{aA}	73.38±7.75 ^{aA}	27.60±4.17 ^{aA}
Control 40%	33.98±6.06 ^{aA}	-0.49±0.62 ^{aA}	6.74±1.72 ^{aA}	14.75±1.69 ^{aA}	82.50±2.13 ^{aA}	28.88±1.80 ^{aA}
p(Tragacanth)_100%	36.89±5.96 ^{aA}	-3.66±0.22 ^{aA}	10.57±2.87 ^{aA}	13.39±1.80 ^{aA}	179.82±11.51 ^{aA}	30.92±8.07 ^{aA}
p(Tragacanth)-60%	37.55±5.21 ^{aA}	-3.65±0.17 ^{aA}	10.28±1.57 ^{aA}	11.99±1.97^{aA}	135.30±16.10 ^{aA}	31.92±4.13 ^{aA}
p(Tragacanth)-40%	35.90±4.98 ^{aA}	-1.32±0.69 ^{aA}	7.62±1.88 ^{aA}	10.25±1.53 ^{aA}	120.12±19.68 ^{aA}	36.30±8.08 ^{aA}
p(Tragacanth co Urtica)_100%	37.12±7.42 ^{aA}	-3.45±0.47 ^{aA}	9.63±1.43 ^{aA}	12.90±1.69 ^{aA}	180.17±11.35 ^{aA}	33.00±7.63 ^{aA}
p(Tragacanth co Urtica)-60%	36.83±7.91^{aA}	-2.33±0.96 ^{aA}	7.89±1.04 ^{aA}	9.29±1.17 ^{aA}	70.67±5.77 ^{aA}	33.48±6.13 ^{aA}
p(Tragacanth co Urtica)-40%	36.01±6.15^{aA}	-1.93±0.99 ^{aA}	7.79±0.86 ^{aA}	9.95±1.59 ^{aA}	180.23±16.17 ^{aA}	32.97±4.43 ^{aA}

* Values are presented as mean ± standard deviation **(Three independent pots were used per treatment combination (n = 3), with three leaf samples analyzed per pot (sub-n = 9)).** Different uppercase letters within the same irrigation level indicate significant differences among hydrogel treatments, whereas different lowercase letters within the same hydrogel treatment indicate significant differences among irrigation levels ($p < 0.05$, Tukey's test). L*, a*, b*, C, and H values were obtained directly from the color measurement instrument. Values represent the means of the corresponding instrumental measurements; therefore, C and H values were not recalculated from the reported mean a* and b* values.

The leaf color parameters (L*, a*, b*, Chroma, and Hue) and SPAD values are presented in Table 2. Although numerical differences were observed among treatments, most color parameters and SPAD values did not differ significantly according to Tukey's test ($p < 0.05$). Therefore, no consistent treatment effect on leaf color characteristics or SPAD was detected under the experimental conditions. Although numerical differences in SPAD values were observed among treatments, no statistically significant differences were detected. Therefore, the present data do not support the conclusion that either hydrogel formulation improved leaf chlorophyll status under the experimental conditions. The hydrogel × irrigation interaction did not significantly affect the CIELAB color parameters (L: $F = 0.162$, $p = 0.974$; a: $F = 0.130$, $p = 0.984$; b*: $F = 0.111$, $p = 0.989$; C*: $F = 0.386$, $p = 0.306$; SPAD: $F = 0.659$, $p = 0.657$).**

Antioxidant Enzyme Activities and Oxidative Stress Indicators in Basil under Deficit Irrigation and Hydrogel Treatments

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Antioxidant enzyme activities (CAT, SOD, and APX), lipid peroxidation (MDA), and proline content of basil plants subjected to different irrigation regimes are presented in Table 3, whereas antioxidant capacity parameters (FRAP and total phenolic content) are shown in Table 4. The hydrogel × irrigation interaction significantly affected the antioxidant enzyme activities (CAT: $F = 4.062$, $p = 0.014$; SOD: $F = 816.60$, $p < 0.001$; APX: $F = 191.25$, $p < 0.001$), as well as MDA ($F = 459.46$, $p < 0.001$) and proline content ($F = 603.83$, $p < 0.001$).

Table 3. Effects of hydrogels on SOD, CAT, MDA, APX, and Proline in basil plants

Hydrogels and Irrigation dose	CAT mmol g^{-1} FW	SOD mg^{-1} FW	MDA µmol g^{-1} FW	APX mmol g^{-1} FW	Proline µmol g^{-1} FW
	X ± SD	X ± SD	X ± SD	X ± SD	X ± SD
Control 100%	0.82±0.05 ^{aA}	0.67±0.32 ^{cC}	0.052±0.001 ^{bAB}	3.15±0.47 ^{aC}	13.89±0.89 ^{cA}
Control 60%	0.61±0.01 ^{bC}	0.82±0.16 ^{aC}	0.11±0.0026 ^{aA}	2.60±0.41 ^{bA}	28.87±0.42 ^{bA}
Control 40%	0.61±0.04 ^{bA}	0.78±0.077 ^{bC}	0.065±0.006 ^{bA}	2.46±0.53 ^{cA}	46.19±0.88 ^{aA}
p(Tragacanth)_100%	0.66±0.85 ^{aB}	1.32±0.01 ^{bA}	0.03±0.002 ^{aBC}	1.12±0.16 ^{bB}	17.82±0.16 ^{cB}
p(Tragacanth)-60%	0.66±0.75 ^{aB}	0.64±0.3 ^{cB}	0.03±0.003 ^{aB}	0.21±0.08 ^{cC}	20.32±0.16 ^{bC}
p(Tragacanth)-40%	0.61±0.05 ^{bA}	1.47±0.02 ^{aA}	0.04±0.003 ^{aC}	1.31±0.09 ^{aC}	22.15±0.49 ^{cB}
p(Tragacanth co Urtica)_100%	0.44±0.01 ^{cC}	0.83±0.04 ^{cB}	0.04±0.0035 ^{aBC}	0.80±0.035 ^{cA}	24.63±0.45 ^{bC}
p(Tragacanth co Urtica)-60%	0.88±0.01 ^{aA}	1.99±0.31 ^{aA}	0.06±0.01 ^{bB}	1.04±0.16 ^{bB}	22.15±0.49 ^{cB}
p(Tragacanth co Urtica)-40%	0.55±0.01 ^{bA}	0.86±0.36 ^{bB}	0.024±0.0066 ^{aC}	1.20±0.53 ^{aB}	31.80±0.79 ^{aC}

*Values are presented as mean ± standard deviation **(Three independent pots were used per treatment combination (n = 3), with three leaf samples analyzed per pot (sub-n = 9)).**Different uppercase letters within the same irrigation level indicate significant differences among hydrogel treatments, whereas different lowercase letters within the same hydrogel treatment indicate significant differences among irrigation levels ($p < 0.05$, Tukey's test). L*, a*, b*, C, and H values were obtained directly from the color measurement instrument. Values represent the means of the corresponding instrumental measurements; therefore, C and H values were not recalculated from the reported mean a* and b* values. CAT: catalase; SOD: superoxide dismutase; APX: ascorbate peroxidase; MDA: malondialdehyde; FW: fresh weight.

Water deficit significantly influenced the biochemical responses of basil plants. In general, drought stress increased antioxidant enzyme activities, MDA accumulation, and proline content, reflecting enhanced oxidative stress and activation of plant defense mechanisms. The highest MDA and proline levels were consistently observed in untreated drought-stressed plants, indicating greater membrane damage and osmotic stress under limited water availability.

Hydrogel application moderated these stress responses under both moderate and severe drought conditions. Compared with the untreated control, hydrogel-treated plants generally exhibited lower MDA accumulation and reduced proline concentrations than untreated plants, indicating mitigation of drought-induced oxidative damage. In contrast, antioxidant enzyme activities varied depending on the treatment and irrigation level, suggesting that the hydrogel formulations differentially influenced enzymatic antioxidant responses rather than producing a uniform

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reduction. This response may indicate that hydrogel application reduced the generation of reactive oxygen species by improving water availability in the root zone, thereby decreasing the need for strong antioxidant activation.

Among the tested materials, p(Tragacanth co Urtica) exhibited the most balanced biochemical response under drought conditions. The modified hydrogel effectively limited oxidative damage while maintaining antioxidant defense capacity, which is consistent with the improved growth performance observed in hydrogel-treated plants. These findings suggest that the incorporation of *Urtica dioica* extract enhanced the protective effects of the hydrogel system and contributed to the maintenance of cellular homeostasis under water-deficit conditions.[51]

The antioxidant capacity results further supported these observations. Variations in FRAP and total phenolic content among treatments indicate that hydrogel application influenced the non-enzymatic antioxidant system in addition to enzymatic defense mechanisms. Collectively, the results demonstrate that hydrogel treatments, particularly p(Tragacanth co Urtica), mitigated drought-induced oxidative stress and promoted a more favorable physiological status in basil plants.

Similar findings have been reported for advanced agricultural materials and bioactive formulations designed to improve plant adaptation to environmental stress. Recent studies have shown that such approaches can enhance stress tolerance by improving physiological performance, regulating antioxidant responses, and reducing oxidative damage under adverse growing conditions [50-53].

Table 4. Effects of hydrogels on Frap and FC in basil plants

Hydrogels and Irrigation dose	Frap µmol TE g^{-1}	FC µg GAE g^{-1}
	X ± SD	X ± SD
Control 100%	291.32±19.4 ^{bB}	1846.13±49.2 ^{aA}
Control 60%	289.93±55.5 ^{cA}	1724.26±69.9 ^{bA}
Control 40%	458.33±30.3 ^{aA}	1666.02±49.3 ^{cA}
p(Tragacanth)_100%	152.43±15.5 ^{cC}	1092.90±42.3 ^{bB}
p(Tragacanth)-60%	170.48±61.4 ^{bC}	927.96±70.4 ^{cC}
p(Tragacanth)-40%	194.10±97.4 ^{aC}	1442.85±45.4 ^{aB}
p(Tragacanth co Urtica)_100%	337.85±46.9 ^{bA}	1070.88±96.8 ^{bC}
p(Tragacanth co Urtica)-60%	238.88±81.2 ^{cB}	1059.13±182.7 ^{cB}
p(Tragacanth co Urtica)-40%	359.38±74.8 ^{aB}	1368.94±197.6 ^{aC}

***Values are presented as mean ± standard deviation (Three independent pots were used per treatment combination (n = 3), with three leaf samples analyzed per pot (sub-n = 9)).**Different uppercase letters within the same irrigation level indicate significant differences among hydrogel treatments, whereas different lowercase letters within the same hydrogel treatment indicate

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significant differences among irrigation levels ($p < 0.05$, Tukey's test). ferric reducing antioxidant power (FRAP) and total phenolic content (FC)

Total Antioxidant Capacity (FRAP) and Total Phenolic Content

The total antioxidant capacity (FRAP) and total phenolic content (FC) of basil plants subjected to different irrigation regimes and hydrogel treatments are presented in Table 4. Hydrogel application significantly affected FRAP ($F = 11.701$, $p < 0.001$), and FC similarly affected it ($F = 18.292$, $p < 0.001$). A significant hydrogel × irrigation interaction was also detected ($F = 86.95$, $p = 0.001$). Both irrigation level and hydrogel application influenced the non-enzymatic antioxidant responses of basil plants. In general, drought stress altered FRAP and FC values, reflecting changes in the antioxidant status of the plants under water-deficit conditions. The highest FRAP values were generally observed under severe drought stress, suggesting an increased antioxidant response to enhanced oxidative pressure. Similar increases in antioxidant capacity under drought conditions have been reported in previous studies and are commonly associated with the activation of protective mechanisms against reactive oxygen species[54, 55].

Hydrogel treatments modified these responses depending on irrigation level and hydrogel type. Compared with untreated drought-stressed plants, hydrogel-treated plants generally exhibited a more balanced antioxidant profile, indicating that improved water availability may have reduced the intensity of oxidative stress. Variations in total phenolic content further suggest that hydrogel application influenced the accumulation of secondary metabolites involved in plant defense and stress adaptation. Among the tested materials, p(Tragacanth co Urtica) produced distinct effects on antioxidant capacity, which may be related to the presence of bioactive constituents derived from *Urtica dioica* extract. The combined effects of improved water retention and potential bioactive characteristics may have contributed to the regulation of antioxidant responses observed in hydrogel-treated plants[56].

Overall, the FRAP and FC results support the findings obtained from antioxidant enzyme activities, MDA accumulation, and proline content, indicating that hydrogel application alleviated drought-induced oxidative stress and contributed to improved physiological performance in basil plants**.**

Correlation Analysis and Antioxidant Responses

The Pearson correlation analysis between FRAP and total phenolic content (FC) is presented in Figure 5. A positive association was observed between the two parameters; however, this relationship was not statistically significant ($p = 0.167$). Therefore, the observed trend should be

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interpreted with caution, as it does not provide sufficient evidence for a significant linear relationship between antioxidant capacity and total phenolic content under the tested experimental conditions.

The FRAP and FC results indicate that both irrigation level and hydrogel application influenced the non-enzymatic antioxidant system of basil plants. In contrast to FC, which tended to be higher under well-watered conditions, FRAP values generally increased under water-deficit treatments, suggesting differential regulation of antioxidant responses under drought stress. These findings imply that antioxidant capacity was influenced not only by phenolic compounds but also by other antioxidant constituents and physiological defense mechanisms.

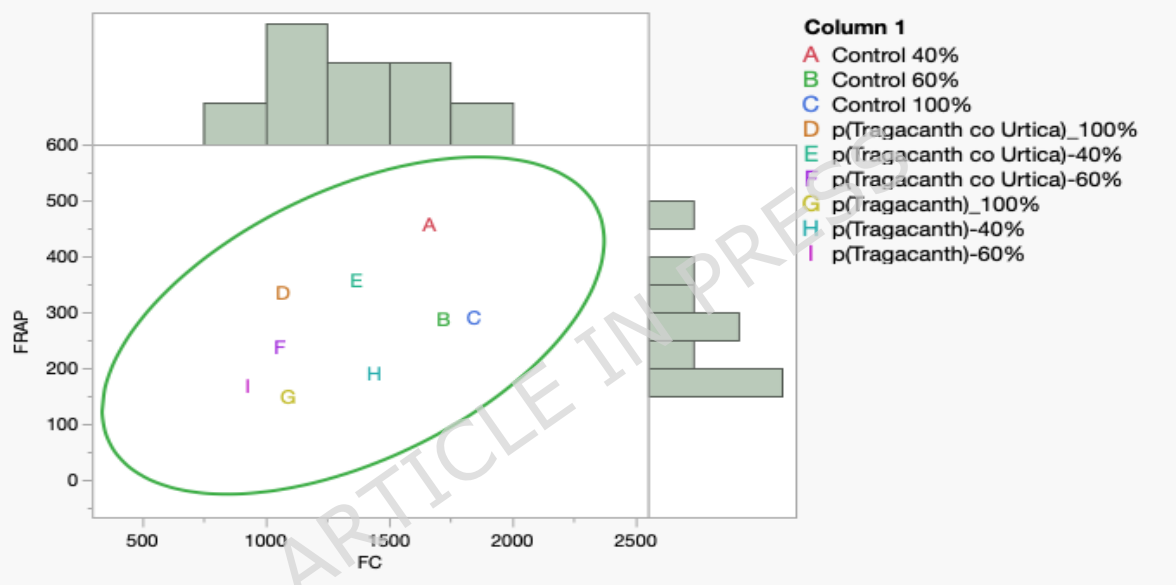


Figure 5. Pearson correlation between FRAP and total phenolic content (FC) based on pot-level means. **Three independent pots were used per treatment combination (n = 3), with three leaf samples analyzed per pot (sub-n = 9).**

Consistent with these observations, antioxidant enzyme activities (CAT, SOD, and APX), lipid peroxidation (MDA), and proline accumulation varied significantly among irrigation regimes and hydrogel treatments (Table 3). Untreated drought-stressed plants generally exhibited higher MDA and proline levels, indicating greater oxidative and osmotic stress. In contrast, hydrogel-treated plants showed moderated biochemical responses, suggesting that improved water availability reduced stress intensity and contributed to the maintenance of cellular homeostasis.

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Overall, the combined biochemical and antioxidant analyses indicate that hydrogel application influenced both enzymatic and non-enzymatic defense systems under drought conditions. The modified p(Tragacanth co Urtica) hydrogel was particularly effective in regulating stress-related responses, supporting its potential role in mitigating drought-induced physiological damage in basil plants [57, 58].

Principal Component Analysis (PCA)

Principal component analysis (PCA) was performed using the complete dataset obtained from biological replicates to evaluate relationships among the measured biochemical parameters (CAT, SOD, APX, FRAP, FC, and proline) under different irrigation regimes and hydrogel treatments (Figure 6).

The first two principal components explained 74.7% of the total variance, with Component 1 and Component 2 accounting for 45.8% and 28.9% of the variance, respectively. The PCA biplot revealed distinct clustering patterns among treatments, indicating that irrigation level and hydrogel application substantially influenced the biochemical responses of basil plants.

The loading plot showed that FRAP, FC, and proline were the major contributors to Component 1, suggesting that this axis was primarily associated with non-enzymatic antioxidant responses and osmotic adjustment. In contrast, CAT, SOD, and APX contributed mainly to Component 2, reflecting variation in enzymatic antioxidant defense mechanisms among treatments.

The observed distribution of biological replicates in the PCA space indicates that hydrogel application modified plant biochemical responses under different irrigation conditions. Treatments receiving p(Tragacanth co Urtica) showed a tendency toward separation from untreated controls under water-deficit conditions, supporting the results obtained from antioxidant enzyme activities, oxidative stress indicators, and antioxidant capacity analyses. Overall, the PCA results confirm that hydrogel treatments influenced both enzymatic and non-enzymatic defense responses and contributed to differential adaptation of basil plants to drought stress.

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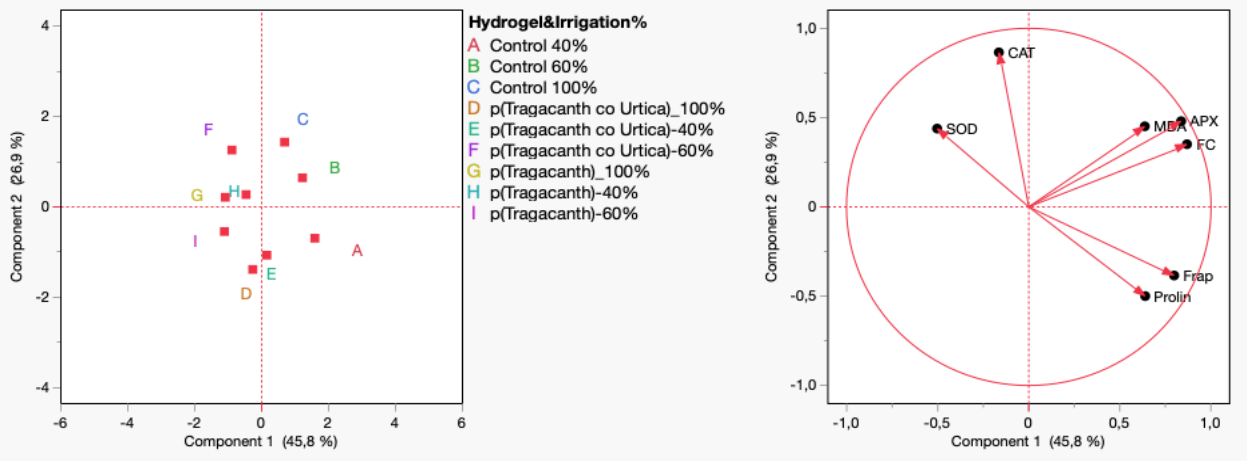


Figure 6. Principal component analysis (PCA) showing the relationships among antioxidant and oxidative stress parameters (CAT, SOD, APX, FRAP, FC, and proline) in basil plants grown under different irrigation levels and hydrogel treatments. The percentages shown on each axis represent the proportion of total variance explained by the corresponding principal component.

Drought stress significantly impaired the growth and physiological performance of basil plants, consistent with previous reports demonstrating that limited water availability restricts cell expansion, biomass accumulation, and metabolic activity in drought-sensitive aromatic species. Overall, the present results demonstrate that hydrogel application mitigated the adverse effects of water deficit on basil growth and physiological performance under greenhouse pot conditions. Improvements in several growth, biochemical, and antioxidant parameters were observed in hydrogel-treated plants compared with the untreated control, although the magnitude of these responses depended on both irrigation level and the parameter evaluated. These findings are consistent with the hydrogel characterization, which indicated favorable swelling behavior and water-retention properties that may contribute to improved water availability in the root zone.

The p(Tragacanth co Urtica) hydrogels was associated with greater improvements in several stem growth and oxidative stress-related parameters, whereas p(Tragacanth) performed similarly or better for some traits, including root length and SPAD under specific irrigation regimes. Therefore, neither hydrogel can be considered consistently superior across all measured variables. Furthermore, because the functionalized hydrogel differed not only by the incorporation of *Urtica dioica* extract but also by the increased EGDMA concentration required for successful hydrogel formation, the independent contribution of the plant extract cannot be distinguished from that of the modified hydrogels.

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The biochemical responses and PCA analysis further confirmed that both irrigation level and hydrogel application influenced plant responses to drought stress. However, these observations should be interpreted within the scope of the present experimental design. Irrigation treatments were based on equal water inputs rather than substrate-specific water-holding capacities, and the experiment was conducted under controlled greenhouse conditions using pot-level experimental units.

Taken together, the results support the potential of tragacanth-based hydrogels as sustainable soil amendments for improving basil performance under limited water availability. Nevertheless, additional studies under standardized hydrogels conditions, including appropriate hydrogels controls and long-term field experiments, are required to clarify the specific contribution of *Urtica dioica* extract and to evaluate the agronomic performance of these hydrogels under practical cultivation conditions.

Conclusion

In conclusion, the developed p(Tragacanth co Urtica) hydrogel exhibited favorable physicochemical characteristics, including high swelling capacity and water-retention properties, and was associated with improved plant performance under deficit irrigation compared with the untreated control under the experimental conditions of this study. Hydrogel application generally alleviated the adverse effects of water deficit by maintaining morphological, physiological, and biochemical characteristics of basil plants, although the magnitude of these responses varied among the evaluated parameters and irrigation levels. Compared with the p(Tragacanth) hydrogel, the p(Tragacanth co Urtica) hydrogel showed improved performance for several measured parameters. However, because the functionalized hydrogel differed not only by the incorporation of *Urtica dioica* extract but also by the optimized EGDMA concentration required for successful hydrogels, the individual contribution of the plant extract cannot be distinguished from that of the modified hydrogels. Therefore, the observed responses should be interpreted as the performance of the overall hydrogel rather than being attributed exclusively to *Urtica dioica.*

The present findings demonstrate the potential of bio-based tragacanth hydrogels as sustainable soil amendments for improving plant performance under limited water availability. Nevertheless, the conclusions are limited to the hydrogels evaluated under greenhouse pot conditions. Further studies using identical polymerization conditions, appropriate extract-only controls, and long-term

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field experiments are required to clarify the specific role of *Urtica dioica* extract and to evaluate biomass production, water-use efficiency, and agronomic performance under practical cultivation conditions.

Declaration

Abbreviations

Analysis of Variance (ANOVA)

Ascorbate Peroxidase (APX)

Catalase (CAT)

Deionized Water (DI)

Ferric Reducing Antioxidant Power (FRAP)

Fourier Transform Infrared Spectroscopy (FTIR)

Malondialdehyde (MDA)

Point of Zero Charge (pHpzc)

Principal Component Analysis (PCA)

Reactive Oxygen Species (ROS)

Soil Plant Analysis Development (SPAD)

Superoxide Dismutase (SOD)

Total Phenolic Content (FC)

Ethics approval and consent to participate

No human application was made in the study.

Consent for publication*

Not applicable

Availability of data and materials

All data generated or analyses performed during this study are included in this published article.

Competing Interests

The authors declare that they have no conflict of interest.

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Authors' contributions

Duygu Alpaslan: Formal analysis, Investigation; Methodology; Original draft; Writing: review & editing.

Selma Kıpçak Bitik: Formal analysis, Investigation; Methodology; Original draft;

Tuba Ersen Dudu: Formal analysis: Original draft.

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Abdullah Turan, Onur Tekin, Abdulbaki Demir: Formal analysis.

Nahit Aktas: Methodology

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References

1. Carović-Stanko K, Orlić S, Politeo O, Strikić F, Kolak I, Milos M, Satovic Z: **Composition and antibacterial activities of essential oils of seven Ocimum taxa**. *Food Chemistry* 2010, **119**(1):5.
2. Alhasan AS: **Effect of different NPK nano-fertilizer rates on agronomic traits, essential oil, and seed yield of basil (Ocimum basilicum L. cv Dolly) grown under field conditions**. *Plant Archives* 2020, **20**(2):3.
3. Alpaslan D: **Use of colorimetric hydrogel as an indicator for food packaging applications**. *Bulletin of Materials Science* 2019, **42**:11.
4. Beltrán-Medina JI, Toro-Tobón G, Mendoza-Labrador JA, Quintero-Beyoda AC, Bermudez-Cordoba MB, Estrada-Bonilla GA: **Optimizing basil production and fertilizer use efficiency with consortia of plant growth-promoting bacteria**. *Frontiers in Plant Science* 2025, **16**.
5. Tangpao i, Charoimek N, Teerakitchotikan P, Leksawasdi N, Jantanasakulwong K, Rachtanapun P, Seesuriyachan P, Phimolsiripol Y, Chaiyaso T, Ruksiriwanich W *et al*: **Volatile Organic Compounds from Basil Essential Oils: Plant Taxonomy, Biological Activities, and Their Applications in Tropical Fruit Productions**. *Horticulturae* 2022, **8**(1):14.
6. Azizah NS, Irawan B, Kusmoro J, Safriansyah W, Farabi K, Oktavia D, Doni F, Miranti M: **Sweet basil (Ocimum basilicum L.)—A review of its botany, phytochemistry, pharmacological activities, and biotechnological development**. *Plants* 2023, **12**(24):13.
7. Hirose S, Horiyama S, Morikami A, Fujiwara K, Tsukagoshi H: **Eugenol and basil essential oil as priming agents for enhancing Arabidopsis immune response**. *Bioscience, Biotechnology and Biochemistry* 2025, **89**(1):9.
8. Vassilina G, Sabitova A, Idrisheva Z, Zhumabekova A, Kanapiyeva F, Orynbassar R, Zhamanbayeva M, Kamalova M, Assilbayeva J, Turgumbayeva A *et al*: **Bio-active compounds and major biomedical properties of basil (Ocimum basilicum, lamiaceae)**. *Natural Product Research* 2025, **39**(5):18.
9. Reichling J: **Allergy to Compositae (Asteraceae, Compositae)-relevance for topically applied phytotherapy extracts**. *Forschende Komplementarmedizin* 2009, **16**(2):1.
10. Al-Huqail A, El-Dakak RM, Sanad MN, Badr RH, Ibrahim MM, Soliman D, Khan F: **Effects of climate temperature and water stress on plant growth and accumulation of antioxidant compounds in sweet basil (*Ocimum basilicum L.*) leafy vegetable**. *Scientifica* 2020, **20201**:11.
11. Kulak M, Jorrín-Novo JV, Romero-Rodriguez MC, Yildirim ED, Gul F, Karaman S: **Seed priming with salicylic acid on plant growth and essential oil composition in basil (*Ocimum basilicum L.*) plants grown under water stress conditions**. *Industrial Crops and Products* 2021, **161**:11.

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12. Farouk S, Omar MM: **Sweet basil growth, physiological and ultrastructural modification, and oxidative defense system under water deficit and silicon forms treatment**. *Journal of Plant Growth Regulation* 2020, **39**(3):24.
13. Khakdan F, Nasiri J, Ranjbar M, Alizadeh H: **Water deficit stress fluctuates expression profiles of 4Cl, C3H, COMT, CVOMT and EOMT genes involved in the biosynthetic pathway of volatile phenylpropanoids alongside accumulation of methylchavicol and methyleugenol in different Iranian cultivars of basil**. *Journal of Plant Physiology* 2017, **218**:9.
14. Mandoulakani BA, Eyvazpour E, Ghadimzadeh M: **The effect of drought stress on the expression of key genes involved in the biosynthesis of phenylpropanoids and essential oil components in basil (*Ocimum basilicum* L.)**. *Phytochemistry* 2017, **139**:7.
15. Chinnusamy V, Zhu JK: **Epigenetic regulation of stress responses in plants.** *Current Opinion in Plant Biology* 2009, **12**(2):6.
16. Janska´ A, Marsˇı´k P, Zelenkova´ S, Ovesna´ J: **Cold stress and acclimation–what is important for metabolic adjustment?** *Plant Biology* 2010, **12**(3):10.
17. Krasensky J, Jonak C: **Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks**. *Journal of Experimental Botany* 2012, **63**(4):15.
18. Chinnici F, Bendini A, Gaiani A, Riponi C: **Radical scavenging activities of peels and pulps from cv. Golden Delicious apples as related to their phenolic composition**. *Journal of Agricultural and Food Chemistry* 2004, **52**(15).
19. Rika SR, UemuraIvan OT, Arimura* G-i: **Bush Basil Companion Plants Act as Plant Defense Potentiators for Cultivated Plants**. *Journal of Agricultural and Food Chemistry* 2025, **73**(28):7.
20. Cheynier V, Comte G, Davies KM, Lattanzio V, Martens S: **Plant phenolics: recent advances on their biosynthesis, genetics, and ecophysiology**. *Plant Physiology and Biochemistry* 2013, **72**:20.
21. Yousif MA, Ali AA, Faris. MG: **Growth and productivity dynamics of Proso millet (Panicum miliaceum L.) under water stress at pre- and post-flowering stages**. *J Glob Innov Agric Sci* 2026, **14**:193-199.
22. Rivera SAG, García JIB, Aleman LSA, Cardenas AAC, Saltos AES, Sánchez-Quispe HR, Salgado MVR, Tapia MFB: **Effect of Acetylsalicylic Acid to Mitigate Biotic Stress of Grass (Panicum maximum)**. *J Glob Innov Agric Sci* 2026, **14**(1).
23. Alpaslan D: **Use of colorimetric hydrogel as an indicator for food packaging applications**. *Bulletin of Materials Science* 2019, **42**(5):247-258.
24. Alpaslan D, Dudu TE, Sahiner N, Aktas N: **Synthesis and preparation of responsive poly(Dimethyl acrylamide/gelatin and pomegranate extract) as a novel food packaging material**. *Mater Sci Eng C Mater Biol Appl* 2020, **108**:110339.
25. Lysiuk R, Darmohray R: **Pharmacology and Ethnomedicine of the Genus Astragalus**. *International Journal of Pharmacology, Phytochemistry and Ethnomedicine* 2016, **3**:7.
26. Emam-Djomeh Z, Fathi M, Askari G: **Gum Tragacanth (Astragalus gummifer Labillardiere)**. In: *Emerging Natural Hydrocolloids: Rheology and Functions.* Edited by Razavi SMA. USA: John Wiley & Sons Ltd; 2019.
27. Gonabadi NAZ, Karazhiyan H: **Investigating the rheological properties of dough and evaluating physiochemical properties of Sangak bread using whey powder and**

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hydrocolloids of guar and tragacanth. *Journal of Food Science and Technology* 2024, **21**(149):17.

28. Babita A, Smriti A: **Effect of drying temperature and natural fermentation on the phytochemical composition of stinging nettle buds (Urtica parviflora)**. *Himalayan Journal of Science and Technology* 2020:7.
29. Dhouibi R, Affes H, Salem MB, Hammami S, Sahnoun Z, Zeghal KM, Ksouda K: **Screening of pharmacological uses of Urtica dioica and others benefits**. *Progress in Biophysics and Molecular Biology* 2020, **150**:10.
30. Taheri Y, Quispe C, Herrera-Bravo J, Sharifi-Rad J, Ezzat SM, Merghany RM, Shaheen S, Azmi L, Mishra AP, Sener B *et al*: **Urtica dioica-Derived Phytochemicals for Pharmacological and Therapeutic Applications**. *Evidence-Based Complementary and Alternative Medicine* 2022, **1**:30.
31. Alpaslan D, Turan A, Dudu TE, Aktaş N: **Evaluation of particle biosynthesis, p(Okra) particle bioactivity, and drug release properties using Abelmoschus esculentus (okra) plant extract**. *Polymer Bulletin* 2024, **81**:4557–4572.
32. Yavuzcanli M, Alpaslan D, Turan A, Dudu TE: **Biomedical Potential of p(Rt) Particles Synthesized from Rubia Tinctorum L. Extract: Characterization, Bioactivity and Controlled Drug Release**. *Journal of the Turkish Chemical Society Section B: Chemical Engineering* 2025, **8**(2):185-202185.
33. Alpaslan D, Dudu TE, Aktas N: **Synthesis of poly (Oak bark) particles from oak bark extract and its utilization as a drug carrier material**. *Vietnam Journal of Chemistry* 2023, **61**(6):719-731.
34. Alpaslan D, Dudu TE, Turan A, Aktas N: **Properties, Bioactivity, In vitro Drug Release and Functionality as a Biomaterial of Poly(Apigenin) Hydrogel**. *Adv Pharmacol Pharm* 2025, **13**(3):323-332.
35. Dhindsa RS, Plumb-Dhindsa P, Thorpe TA: **Leaf Senescence: Correlated with Increased Levels of Membrane Permeability and Lipid Peroxidation, and Decreased Levels of Superoxide Dismutase and Catalase** *Journal of Experimental Botany* 1981, **32**(1):10.
36. Sairam RK, Srivastava GC: **Water Stress Tolerance of Wheat (Triticum aestivum L.): Variations in Hydrogen Peroxide Accumulation and Antioxidant Activity in Tolerant and Susceptible Genotypes**. *Journal of Agronomy and Crop Science* 2001, **186**:9.
37. Shams M, Ekinci M, Ors S, Turan M, Agar G, Kul R, Yildirim E: **Nitric oxide mitigates salt stress effects of pepper seedlings by altering nutrient uptake, enzyme activity and osmolyte accumulation**. *Physiology and Molecular Biology of Plants* 2019, **25**:13.
38. De SK, Aluru NR, Johnso B, Crone WC, Beebe DJ: **Equilibrium swelling and kinetics of pH-responsive hydrogels: models, experiments, and simulations**. *JOURNAL OF MICROELECTROMECHANICAL SYSTEMS* 2002, **11**(5):12.
39. Afrayeem SM, Chaurasia A: **Effect of zinc oxide nanoparticles on seed germination and seed vigour in chilli (*Capsicum annuum* L.)**. *J Pharmacogn Phytochem Journal of Pharmacognosy and Phytochemistry* 2017, **6**(5):2.
40. Avila RG, Magalhães PC, Silva EMd, Souza KRDd, Campos CN, Alvarenga AAd, Souza TCd: **Application of silicon to irrigated and water deficit sorghum plants increases yield via the regulation of primary, antioxidant, and osmoregulatory metabolism** *Agricultural Water Management* 2021, **255**:10.

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41. Alzahrani Y, Kuşvuran A, Alharby HF, Kuşvuran S, Rady MM: **The defensive role of silicon in wheat against stress conditions induced by drought, salinity or cadmium**. *Ecotoxicology and Environmental Safety* 2018, **154**:9.
42. Merwad A-RMA, Desoky E-SM, Rady MM: **Response of water deficitstressed Vigna unguiculata performances to silicon, proline or methionine foliar application** *Scientia Horticulturae* 2018, **228**:12.
43. Hussain S, Hafeez MB, Azam R, Mehmood K, Aziz M, Ercisli S, Javed T, Raza A, Zahra N, Hussain S *et al*: **Deciphering the role of phytohormones and osmolytes in plant tolerance against salt stress: implications, possible cross-talk, and prospects**. *Journal of Plant Growth Regulation* 2023, **43**:11.
44. Zafar M, Ahmed S, Munir MK, Zafar N, Saqib M, Sarwar MA, Iqbal S, Ali B, Akhtar N, Ali B *et al*: **Application of Zinc, Iron and Boron Enhances Productivity and Grain Biofortification of Mungbean**. *Phyton* 2023, **92**(4):17.
45. Chakma R, Saekong P, Biswas A, Ullah H, Datta A: **Growth, fruit yield, quality, and water productivity of grape tomato as affected by seed priming and soil application of silicon under drought stress**. *Agricultural Water Management* 2021, **256**:12.
46. Hussan Mu, Saleem MF, Hafeez MB, Khan S, Hussain S, Ahmad N, Ramzan Y, Nadeem M: **Impact of soil applied humic acid, zinc and boron supplementation on the growth, yield and zinc translocation in wheat**. *Asian Journal of Agriculture and Biology* 2022, **1**:8.
47. Hameed A, Farooq T, Hameed A, Sheikh MA: **Silicon-mediated priming induces acclimation to mild water-deficit stress by altering physio-biochemical attributes in wheat plants**. *Frontiers in Plant Science* 2021, **12**:13.
48. Malik MA, Wani AH, Mir SH, Rehman IU, Tahir I, Ahmad P, Rashid I: **Elucidating the role of silicon in drought stress tolerance in plants**. *Plant Physiology and Biochemistry* 2021, **165**:8.
49. Rocha JR, Prado RdM, Piccolo MdC: **Mitigation of water deficit in two cultivars of Panicum maximum by the application of silicon**. *Water, Air Soil Pollution* 2022, **233**(3):11.
50. Sodkouieh SM, Kalantari M: **Synthesis of a New Hydrogel Based on Ethylene Glycol Crosslinked Tragacanth Gum with Emphasis on Its Agricultural Potential**. *Journal of Polymers and the Environment* 2022, **315**:11.
51. Wang C, Zhou X, Qin1 J, Wang X, Yu H, Zhu Q, Lee D, Chen L: **Comprehensive Response Mechanisms of Plants to Water Deficit: A Physiological, Biochemical, Molecular, and Ecological Review**. *Molecular Soil Biology* 2024, **15**(5):11.
52. Muhammad HMD, Naz S, Bibi Z, Ahmed MAA, Stanciu A-S, Ahmad R: **Nanoparticles and phytohormonal synergy in plants: sustainable agriculture approach**. *Phyton* 2025, **94**(9):13.
53. Abdelghafar R, Abdelfattah A, Mostafa H: **Effect of super absorbent hydrogel on hydro-physical properties of soil under deficit irrigation**. *Scientific Reports* 2024, **14**:9.
54. Popović BM, Štajner D, Ždero-Pavlović R, Tumbas-Šaponjac V, Čanadanović-Brunet J, Orlović S: **Water stress induces changes in polyphenol profile and antioxidant capacity in poplar plants (Populus spp.)**. *Plant Physiology and Biochemistry* 2016, **105**:8.

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55. Popova AV, Mihailova G, Geneva M, Peeva V, Kirova E, Sichanova M, Dobrikova A, Georgieva K: **Different Responses to Water Deficit of Two Common Winter Wheat Varieties: Physiological and Biochemical Characteristics**. *Plants* 2023, **12**(12):22.
56. El-diehy MA, Farghal II, Amin MA, Ghobashy Mm, Nowwar AI, Gayed HM: **Radiation synthesis of sodium alginate/gelatin based ultra-absorbent hydrogel for efficient water and nitrogen management in wheat under drought stress**. *Scientific Reports* 2024, **14**:19463
57. Mendonça JdS, Guimarães RdCA, Zorgetto-Pinheiro VA, Fernandes CDP, Marcelino G, Bogo D, Freitas KdC, Hiane PA, Melo ESdP, Vilela MLB *et al*: **Natural Antioxidant Evaluation: A Review of Detection Methods**. *Molecules* 2022, **27**(11):21.
58. Spiegel M, Kapusta K, Kołodziejczyk W, Saloni J, Żbikowska B, Hill GA, Sroka Z: **Antioxidant Activity of Selected Phenolic Acids–Ferric Reducing Antioxidant Power Assay and QSAR Analysis of the Structural Features**. *Molecules* 2020, **25**(13):17.

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