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IMPROVING YIELD AND QUALITY OF RED PEPPER (*Capsicum annum* L.) USING CHITOSAN ELICITATION UNDER DEFICIT IRRIGATION

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ABSTRACT

In semiarid and arid climates in the Middle East, environmental stresses such as drought and water deficit result in significant reductions in the growth and productivity of horticultural and agronomic crops. To improve crop tolerance to water-deficient conditions, applying chitosan can be a practical approach. The foliar-spraying of medium molecular weight chitosan (450 kDa, 95–98% degree of deacetylation, and 30 mPa.s viscosity) under different irrigation regimes on the yield and phytochemicals of the Iranian landrace red pepper were studied. Experimental factors were irrigation system included drip and flood irrigation; irrigation frequencies included optimal irrigation (irrigation every 5 to 7 days based on irrigation at 85–90% field capacity or F.C.), deficit irrigation or 50% optimum irrigation (irrigation every 13 to 15 days based on irrigation at 45–50% F.C.); the foliar applications included negative control (no spraying), positive control (the foliar spraying by water as solvent), and foliar spraying by chitosan at 2 and 4 g L^{-1}. The highest yields of the fruit were obtained from the pepper plants treated with foliar-sprayed chitosan under a drip irrigation system and optimal irrigation conditions. However, the maximum values of capsaicin (11.49 mg g^{-1} DW), dihydrocapsaicin (4.99 mg g^{-1} DW), capsaicinoids (16.48 mg g^{-1} DW), vitamin C (1.26 mg g^{-1} DW), total phenolic content (2.15 mg GAE 100 g^{-1} DW), and antioxidant capacity (55%) were achieved in the plants sprayed by chitosan at 2 g L^{-1} under 50% optimum irrigation (deficit irrigation) and drip irrigation system. The use of chitosan under water-deficit conditions (471 g capsaicin m^{-2}) resulted in the highest capsaicin yield. In conclusion, chitosan foliar application under deficit irrigation is recommended to maintain and stabilize red pepper's quantitative and qualitative performance.

Keywords: capsaicin, dihydro-capsaicin, deficit irrigation, drip irrigation, total phenol, vitamin C

INTRODUCTION

Red pepper (*Capsicum* spp.), a member of the Solanaceae family, is a globally grown and consumed vegetable crop. Besides their use as food and in the food additive industries, pepper species are also exploited in the pharmaceutical and medicinal industries [Liu et al. 2022]. Capsaicinoids, carotenoids (capsanthin,

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α-carotene, and fatty acid esters), flavonoids, steroidal saponins, and essential oils are the main components of the red pepper fruits [Hernández–Pérez et al. 2020]. The spicy taste of the fruit of this plant is related to capsaicin [Sanati et al. 2018]. The high consumption of this horticultural crop is related to its spicy properties, conferred by capsaicinoids. Capsaicin and dihydrocapsaicin, as two main constituents, make up more than 80% of capsaicinoids [Arce-Rodriguez and Ochoa-Alejo 2019]. In addition, pepper fruits contain biologically active compounds, including vitamins A, C, B_2, and B_{11}, as well as potent antioxidants such as carotenoids [Khamoushi et al. 2021, Olatunji and Afolayan 2018].

Water is one of the environmental factors that significantly affect plant growth and the biosynthesis of secondary metabolites [Mosaedi et al. 2024]. The decrease in soil moisture is a limiting factor for biomass and dry matter yields of the herbs; however, it is possible that increasing some secondary metabolites under deficit water stress [Danesh-Shahraki et al. 2023]. This condition can depend on the duration, type, time, and growth stage under stress, genotype, agronomic management, etc. [Shaykh-Samani et al. 2023, Rezaei-Adl et al. 2025]. Reactions to water deficit stress differ among plant species. This environmental stress causes reduced available water, decreased photosynthesis rate, and increased reactive oxygen species (ROS), as well as degradation of the plasma membrane (lipid peroxidation), pigments, proteins, and DNA [Rabêlo et al. 2019, Rezaei-Adl et al. 2025].

The biosynthesis of secondary metabolites in some plants is influenced by genetic and agronomic practices and ecological factors, and their interaction impacts [Danesh-Shahraki et al. 2023]. Environmental stresses, particularly abiotic stresses, have significant impacts on the biosynthesis of biologically active substances [Alavi Samany et al. 2022]. To achieve stable quantitative and qualitative plant functions under environmental stress, such as water deficit, and to enhance secondary metabolite production, it is necessary to adopt novel horticultural management practices. One solution to this challenge could be to increase plant tolerance by utilizing elicitors such as chitosan [Ghasemi Pirbalouti et al. 2017]. Chitosan is a glucosamine polysaccharide [Hafez et al. 2020, Darani et al. 2025, Rezaei-Adl et al. 2025] which, through the induction of the defense system, will cause the secondary metabolites biosynthesis, the activity of antioxidant enzymes, and cell-compatible substances, so that it sends a series of chemical messages to the plant, which will result in increased growth and yield, as well as improving the physiological and morphological processes of many plants under stress or non-stress conditions [Hidangmayum et al. 2019, Alavi Samany et al. 2022]. Previous studies have shown that chitosan stimulates the biosynthesis of secondary metabolites in various herb species. Khodadadi et al. [2023] found that the application of chitosan could enchain the biosynthesis and accumulation of phenolic compounds via the phenylpropanoid pathway, as well as reduce the effectiveness of drought stress in sage.

It seems that the use of chitosan in plants under water-deficit conditions, which induces the production of free radicals, can reduce the adverse effects of water deficit stress on some yield traits by boosting the biosynthesis of phenolic compounds and antioxidant substances such as vitamin C and capsaicinoids. Some investigations have been conducted on the impacts of chitosan use on the growth and phytochemical properties of some herbs under varying moisture conditions. However, the interaction effects of chitosan application under different irrigation regimes on red pepper performance are not well documented. Therefore, this study was conducted to determine the effects of foliar-spraying with chitosan under different irrigation conditions on yield and selected phytochemical traits of red pepper cultivated in arid and semiarid climates.

MATERIALS AND METHODS

Experimental site description. This research was done at the research farm of Goldaru Herbal Pharmaceutical Company of Isfahan, Iran (latitude 32° 51' N, longitude 51° 52' E, altitude 1600 m) during 2018–2019. According to Koppen–Geiger's climate classification, Isfahan has a characteristic dry climate [Kottek et al. 2006]. Dry weather and very low rainfall are the prominent features of this classification, with minimum and maximum temperatures of −10.6 °C and 40.6 °C, respectively. The average annual precipitation over the city is 116.9 mm [Karimi et al. 2021]. Some meteorological parameters of the study area during the growth season are presented in Table 1.

Plant material and soil analysis. Seeds of the landrace of red chili pepper (*Capsicum annum* L.) were provided from the Research & Development Section, Goldaru Herbal Pharmaceutical Laboratories, Isfahan, Iran. Firstly, the seeds were sterilized by 1% sodium hypochlorite for 10 min and sown in 8 cm plastic pots on 8 March 2018. The pots were maintained in a glass greenhouse under controlled conditions: 25 °C ±2 / 15 ±1 °C (day/night) air temperatures, 65–70% humidity, and 12/12 hours (light/light less). The pots were filled with the same combination of farm soil (Table 2), sand, and peat moss. Soil samples of the experimental field were taken before the experiment from three random parts of each plot from 0 to 30 cm depth (Table 2). Organic carbon, soil texture, pH, and electrical conductivity (EC) were determined using the sulfuric acid method, hydrometer assay, pH meter, and EC meter [AACC 2000]. Total nitrogen was measured using micro-Kjeldahl digestion and distillation techniques [Page 1982]. Available phosphorus content (in extraction) was measured using a spectrophotometric method with a spectrophotometer Perkin-Elmer Inc. (Waltham, MA) [Olsen et al. 1954]. Potassium (extracted with ammonium acetate) was determined using a flame photometer [Black 1965]. The main micronutrients, including manganese (Mn), iron (Fe), copper (Cu), and zinc (Zn), were measured using an atomic absorption spectrophotometer (PerkinElmer Analyst 400, Waltham, United States of America) [AACC 2000].

About 45–50 days after sowing on 22–28 May 2018, when the seedlings had 4–6 true leaves and were 10–15 cm tall, they were transferred to the experimental field. The planting density was 25 cm between plants and 50 cm between the rows (~8 plant m^{-2}). The thoroughly reddened peppers were hand-harvested between 9 and 15 September 2018.

In winter 2017, the field soil was ploughed with a moldboard plow (up to a depth of 30–35 cm). Cow dung manure (10 tons ha^{-1}) and chemical fertilizers (20–20–20 kg ha^{-1} N, P, and K, respectively) were applied to the soil before transplanting as urea, triple superphosphate, and potassium sulphate, respectively. The ratio of cow manure and chemical fertilizer was determined based on the optimal plant nutrient requirements, soil characteristics, and cow manure

Table 1. Some of the meteorological parameters of the study area during the growth season

Parameters	April	May	June	July	August	September
Minimum temperature (°C)	10.7	12.9	19.7	21.4	38.2	36.5
Maximum temperature (°C)	23.7	25.0	33.9	37.1	30.9	29.8
Average temperature (°C)	17.2	18.9	26.8	29.2	34.5	33.2
Average precipitation (mm)	5.4	35.9	7.0	0.0	0.0	0.0
Relative humidity (%)	37.2	45.5	27.4	16.3	15.0	15.7
Potential evapotranspiration PET (mm/day)	5.17	5.57	7.64	8.91	7.42	6.47

Table 2. Some physicochemical properties of the soil of the study area

Depth (cm)	Soil texture	pH	EC (dS/m)	Organic carbon (%)	N (%)	P	K	Zn	Mn	Fe	Cu
						(mg/kg)					
0–30	silt loam	7.42	2.06	0.58	0.05	7.52	219.0	1.08	0.86	1.28	1.20

element concentrations. No systemic pesticides or herbicides were used during the research. Weeds were controlled manually.

Treatments and experimental design. The experimental factors were conducted using a split-split plot design based on a randomized complete block design (RCBD) in three replications. Three factors were irrigation system included drip using a seam drip irrigation tape 20 cm with a flow rate of 1.5 L ha^{-1} and flood irrigation; irrigation frequencies included optimal irrigation (irrigation every 5 to 7 days based on irrigation at 85–90% field capacity or F.C.), deficit irrigation (irrigation every 13 to 15 days based on irrigation at 45–50% F.C.); the foliar applications included negative control (no spraying), positive control (the foliar spraying by water as solvent), and foliar-spraying of chitosan at 2 and 4 g L^{-1} according to results of previous investigations by our and other researchers [Valletta et al. 2016, Alavi-Samany et al. 2022].

Deficit irrigation by changing the irrigation intervals to about 13 to 15 days, once based on moisture discharge from F.C. conditions and evaporation rate from Class A pan, calculated after 45 days of the establishment of seedlings. Irrigation time and volume were determined based on the soil moisture curve. For this purpose, soil moisture was determined daily using a TDR device (PMS-714, Lutron, Taiwan) according to the manufacturer's guidelines. Field capacity and permanent wilting point (PWP) were –33 and –1500 kPa, respectively. In semiarid and arid regions, a soil matric potential threshold range of –30 to –40 kPa at a 20 cm depth was recommended for chili pepper irrigation under a drip irrigation system [Liu et al. 2012]. To prevent water leakage between irrigation treatments, a distance of 2 m was maintained between the moisture levels. Medium molecular weight chitosan with 450 kDa molecular weight, 95–98% degree of deacetylation, and 30 mPas viscosity (Sigma–Aldrich Co., Steinheim, Germany) was dissolved in acetic acid (5%) diluted with water. The solutions were sprayed onto the entire pepper plants in three growth stages (before flowering, early flowering, and 25% flowering) at dew point (150–160 mL per plant) using a handheld garden pump sprayer.

Morpho-physiological measurements. When the pepper fruits were fully ripe and red, three plants were randomly selected from the center of each plot, after removing the effects of the margins, to evaluate their morphological characteristics. The number of fruits in each plant and the primary branches in each experimental unit were counted and recorded. The plant height and fruit length were measured using a ruler and a digital vernier caliper (0–150 mm, accuracy 0.01 mm), respectively. The harvested fruits were weighed using a sensitive digital scale (Sartorius, Germany) with an accuracy of 1 mg. Then, the fruits were dried using an oven at 55 °C for 48 hours and weighed again.

Phytochemical measurements. The total phenolic content of the fruits was determined using the Folin-Ciocâlteu colorimetric method [Menichini et al. 2009]. For this purpose, the standard curve was prepared using gallic acid. Then, 1.5 g of the extract was diluted with 1 mL of methanol, mixed with 0.2 mL of Folin-Ciocâlteu reagent, 2 mL of distilled water, and 1 mL of 15% Na_2CO_3. The blue solution formed was kept at room temperature for 2 hours. The absorbance was then measured at 765 nm using a UV–Vis spectrophotometer (Perkin-Elmer Inc., Waltham, MA). The absorption values were measured using the standard curve. Afterward, total phenolic content was determined based on mg of gallic acid as a standard in dry weight (DW). To measure the content of C in the red pepper fruits, 0.5 g of the dried samples was pounded in a mortar, and 5 mL of 4% oxalic acid was added to it and homogenized. The homogenates were centrifuged at 5000 rpm for 10 min, and then the supernatants were filtered with 541 Whatmann filter paper the obtained residues were made up to 25 mL with 4% oxalic acid. The amount of vitamin C was determined using the 2,4-dinitrophenylhydrazine reagent on a spectrophotometer (Perkin-Elmer Inc., Waltham, MA) at 540 nm [Aniel Kumar and Subba Tata 2009]. The antioxidant activity of the extracts was measured using the DPPH assay [Brand-Williams et al. 1995]. To determine the amount of capsaicin in red pepper fruit, 2.5 g of dry pepper powder was extracted with 80 mL of acetonitrile using the Soxhlet extraction technique for 6 h, until the solution became colorless. The extract was concentrated to 40 mL under vacuum and transferred to a 50 mL volumetric flask. Then, 10 mL of acetonitrile (CH_3CN) was added. Subsequently, 20 µL of supernatant was injected into the HPLC system in triplicate. In this study, an HPLC (Knauer Corp., Berlin, Germa-

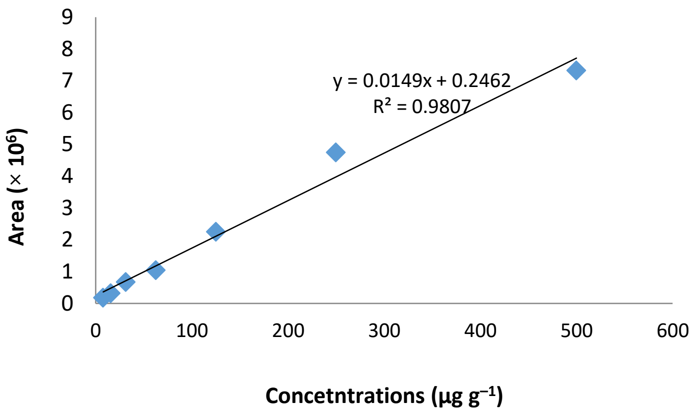

Fig. 1. Calibration curve for capsaicin

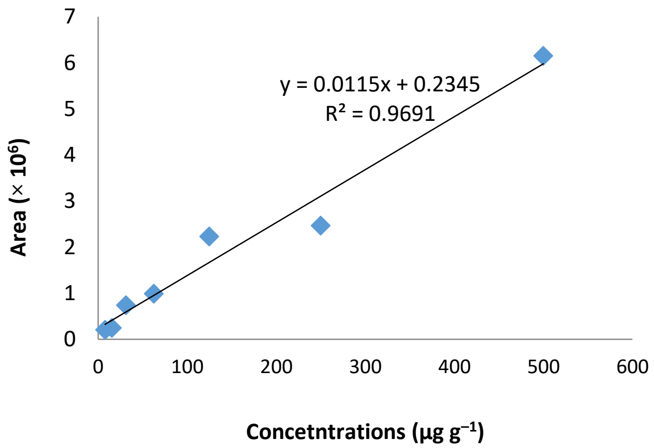

Fig. 2. Calibration curve for dihydrocapsaicin

ny) equipped with a K-1001 pump, a vacuum degasser, a tunable UV/Visible detector, and a ProntoSIL 120-5 C18 H (25 × 0.4 cm ID) column was used. Water and 0.1% phosphoric acid (A) and methanol (B) at a 30:70 (v/v) ratio were used as mobile phases at a flow rate of 1.0 mL min^{-1}. Capsaicin was monitored at 290 nm. The column and sample temperatures were 60 °C and 20 °C, respectively [Dang et al. 2014]. During HPLC sample analyses, a standard solution was injected to evaluate retention time reproducibility and instrument calibration [Othman et al. 2011]. Capsaicin and dihydrocapsaicin in red pepper were measured by comparison to external reference standards injected under the same conditions [Othman et al. 2011]. Standard solutions were prepared from stock solutions of capsaicin and dihydrocapsaicin with various concentrations (500, 250, 125, 62, 31, 15.5, and 8 µg g^{-1}). After injection of the standard solutions into the HPLC, standard curves of peak area versus concentration were plotted (Figures 1 and 2). The capsaicinoid concentrations in the samples are expressed as mg g^{-1} dry matter or dry weight.

Statistical analysis. Firstly, the experimental data were tested for normality (Kolmogorov–Smirnov test) and homogeneity of variance (Levene's test). Then, the data were statistically analyzed using the GLM procedure in SAS (SAS/STAT® v.9.2; SAS Institute Inc., Cary, NC). The comparison of means was done using Duncan's multiple range test ($p \leq 0.05$). All tests were done in triplicate. Excel software was used to draw the graphs.

RESULTS

Morpho-physiological traits. Based on the ANOVA results, the simple effects of treatments – foliar spraying, irrigation frequency, and irrigation system – did not significantly affect plant height (Table 3) or the number of primary branches (data not shown). However, the interaction influence of the foliar spraying × irrigation frequency × irrigation system on the plant height was highly significant ($p \leq 0.01$), see Table 3. The highest plant height was observed for plants treated with chitosan (4 g L^{-1}) under a flood irrigation system and optimal moisture conditions. In contrast, the lowest plant height was observed in plants grown under the no-spraying (control) × optimal irrigation condition (control) and the drip irrigation system (Table 3). On the other hand, plant height increased by 6% and 15% under chitosan spraying at 2 and 4 g L^{-1}, respectively, in deficit irrigation conditions (Table 3). The results indicated that foliar spraying significantly influenced ($p \leq 0.05$) the number of fruits per plant (Table 3). The application of chitosan at 4 g L^{-1} could produce the highest number of fruits on the pepper plant. In addition, the interaction effect of foliar spraying × irrigation frequency × irrigation system significantly ($p \leq 0.05$) affected the number of fruits per plant (Table 3). The highest value for the number of fruits per plant was observed with chitosan (4 g L^{-1}) under optimal irrigation conditions and flood irrigation (Table 3).

As shown in Table 3, the simple effect of irrigation frequency was significant ($p \leq 0.05$) on the fresh and dry weights of the pepper fruits. However, the use of chitosan did not have a significant effect on the fresh and dry weights of the pepper fruit (Table 3). Increasing the irrigation interval or applying deficit irrigation significantly reduced both traits (Table 3). Our results indicate that the simple effect of the irrigation system had a significant ($p \leq 0.05$) impact on fresh and dry fruit weights (Table 3). The drip irrigation system increased fresh and dry fruit weights by 11% and 38%, respectively, compared with the flood irrigation system (Table 3).

According to the results shown in Table 3, the interaction effect of irrigation system × irrigation frequency × foliar spraying ($p \leq 0.01$) on the fresh and dry weights of fruit was significant. The maximum fresh fruit weight (5.50 kg m^{-2}) and dry fruit weight (1.92 kg m^{-2}) were observed for plants treated with chitosan (4 g L^{-1}) under a drip irrigation system in both moisture conditions (Table 3). The minimum amounts of the fresh fruit weight (2.91 kg m^{-2}) and dry fruit weight (0.61 kg m^{-2}) were obtained in the plants treated by flood irrigation system × deficit irrigation × no foliar spraying (Table 3). In deficit irrigation conditions, the use of chitosan at 2 and 4 g L^{-1} could improve dry fruit weight by 85% and 97%, respectively, compared with the optimum irrigation condition or the control.

Phytochemical traits. The chromatograms of the standard and extracted solutions are shown in Figures 3 and 4, respectively. Results of HPLC analyses showed that the capsaicin content ranged from 4.46 to 11.49 mg g^{-1} dry weight, and the dihydrocapsaicin content ranged from 1.84 to 4.99 mg g^{-1} dry weight (Table 3). According to the results shown in Table 3, the simple influences of the foliar spraying, irrigation frequency, and irrigation system on the concentrations of capsaicin and dihydrocapsaicin were significant (Table 3). The highest levels of capsaicin and dihydrocapsaicin were observed in plants treated with chitosan spray at 2 g L^{-1}, under deficit irrigation, and in the drip irrigation system (Table 3). The foliar spraying × irrigation frequency × irrigation system had significant influences ($p \leq 0.01$) on the capsaicin and dihydrocapsaicin concentrations (Table 4). The maximum values of capsaicin (11.49 mg g^{-1} DW) and dihydrocapsaicin (4.99 mg g^{-1} DW) were obtained from pepper plants sprayed with chitosan at 2 g L^{-1} under deficit irrigation conditions and a drip irrigation system (Table 3). Moreover, the highest concentration of capsaicinoids (16.48 mg g^{-1} DW) as unique spicy characteristic, which two main constituents i.e. capsaicin and dihydrocapsaicin was detected in the plants treated by 2 g L^{-1} chitosan and deficit irrigation condition and drip irrigation system (Fig. 5). The interaction impact of irrigation system × irrigation frequency ($p \leq 0.05$) demonstrated that this treatment signifi-

Table 3. The main and interaction effects of foliar spraying × irrigation frequency × irrigation system on some studied characteristics

Experimental factors	Plant height (cm)	Fruit fresh eight (kg m^{-2})	Fruit dry weight (kg m^{-2})	Number of fruits per plant	Total phenol (mg GAE per 100 g DW)	Antioxidant capacity (%)	Capsaicin (mg g^{-1} DW)	Dihydrocapsaicin (mg g^{-1} DW)
Foliar spraying:								
control – (no foliar): NOF	50.5	5	1.3	41.2 b*	1.3 b	48.9	5.8 b	2.5 b
control + (water). WAF	52.5	4.9	1.2	37.2 b	1.1 b	48.4	5.2 b	2.5 b
chitosan 2 g/L: CHF1	54.7	4.7	1.4	47.2 ab	1.2 b	49.8	8.1 a	4.0 a
chitosan 4 g/L: CHF2	58	5.2	1.6	55.6 a	1.8 a	47.5	6.0 b	2.8 b
ANOVA	n.s¶	n.s	n.s	$p \leq 0.05$	$p \leq 0.05$	n.s	$p \leq 0.05$	$p \leq 0.05$
Irrigation frequency:								
optimal irrigation: OPI	54.2	5.1 a	1.61 a	47.3	1.34 b	48.64	5.74 b	2.5 b
deficit irrigation: DEI	52.3	4.3 b	1.26 b	51.4	1.56 a	46.80	8.47 a	3.9 a
ANOVA	n.s	$p \leq 0.05$	$p \leq 0.05$	n.s	$p \leq 0.05$	n.s	$p \leq 0.05$	$p \leq 0.05$
Irrigation system:								
drip irrigation: DRI	54.6	5.0 a	1.54 a	51.4	1.4	48.5	7.7 a	3.4 a
flood irrigation: FLI	51.9	4.2 b	1.20 b	46.5	1.3	46.9	6.6 b	2.8 b
ANOVA	n.s	$p \leq 0.05$	$p \leq 0.05$	n.s	n.s	n.s	$p \leq 0.05$	$p \leq 0.05$
Interaction of experimental factors:								
FLI × OPI × NOF	52.3 g	4.6 d	1.3 d	45.3 f	1.2 e	48 b	5.5 f	2.3 f
FLI × OPI × WAF	52.3 g	4.5 e	1.2 d	37.7 gh	1.5 cd	48 b	5.1 f	2.2 f
FLI × OPI × CHF1	53.7 f	4.7 cd	1.3 d	47.7 e	1.7 c	50 b	7.5 cd	3.1 d
FLI × OPI × CHF2	61.3 a	5.0 c	1.4 c	67.7 a	1.9 b	47 bc	4.5 g	1.8 f
FLI × DEI × NOF	52.3 g	2.9 h	0.6 f	53.3 cd	1.2 e	49 b	8.1 c	3.3 cd
FLI × DEI × WAF	56.3 d	4.2 f	1.1 e	43.3 fg	1.5 cd	45 c	6.7 d	2.8 d
FLI × DEI × CHF1	60.3 ab	3.6 gh	1.3 d	54.7 c	0.8 f	40 d	8.0 c	3.4 c
FLI × DEI × CHF2	56.3 d	4.3 ef	1.5 c	61.3 b	1.3 d	45 c	8.7 c	3.8 c
DRI × OPI × NOF	48.7 h	5.3 b	1.3 d	37.0 gh	1.4 d	50 b	6.0 e	2.7 e
DRI × OPI × WAF	52.7 g	5.3 ab	1.5 c	36.7 h	0.8 f	48 b	6.3 e	2.7 e
DRI × OPI × CHF1	55.7 e	4.6 d	1.5 c	46.7 ef	0.8 f	49 b	7.0 d	3.0 d
DRI × OPI × CHF2	56.7 d	5.5 a	1.92 a	43.77 fg	1.3 d	49 b	6.1 e	2.7 e
DRI × DEI × NOF	52.70 g	3.7 g	1.0 e	47.3 e	1.4 d	46 bc	7.2 d	3.0 d
DRI × DEI × WAF	57.00 c	4.8 cd	1.4 c	42.30 g	1.1 e	40 d	7.1 d	2.9 d
DRI × DEI × CHF1	56.00 d	5.5 ab	1.8 b	65.7 ab	2.2 a	55 a	11.5 a	5.0 a
DRI × DEI × CHF2	56.70 d	5.4 ab	1.9 a	52.3 d	1.5 cd	44 c	10.0 b	4.6 b
ANOVA	$p \leq 0.01$	$p \leq 0.01$	$p \leq 0.01$	$p \leq 0.05$	$p \leq 0.01$	$p \leq 0.01$	$p \leq 0.01$	$p \leq 0.01$

* The averages with at least a standard alphabet are not statistically significant at the 5% level.

¶ Not significant.

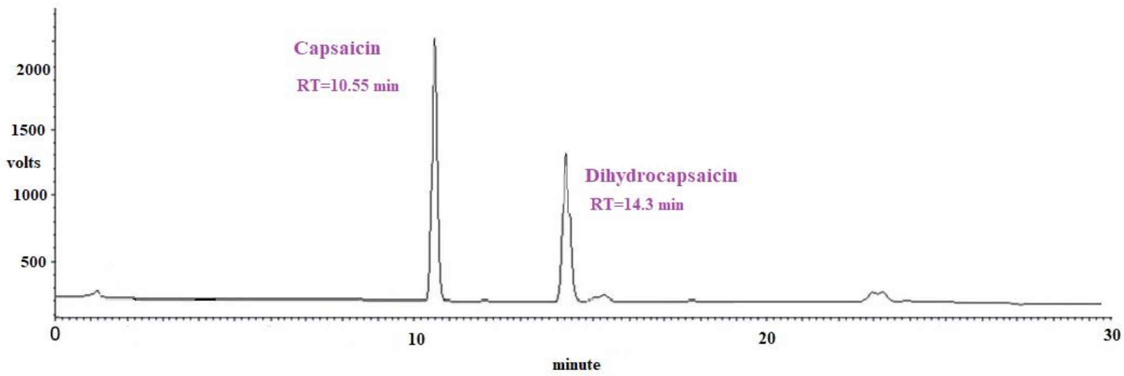

Fig. 3. Chromatogram of the standard solution of capsaicin and dihydrocapsaicin

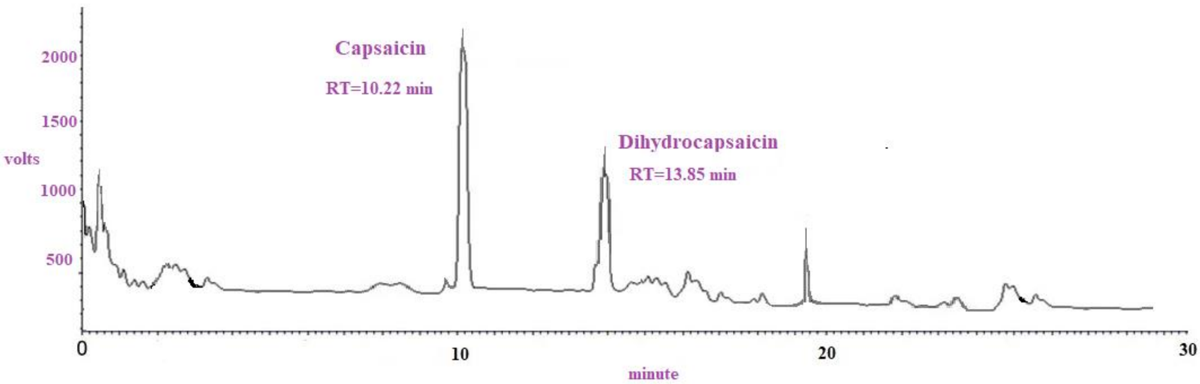

Fig. 4. Chromatogram of the extract from the red pepper fruits

cantly changed the capsaicin yield per area unit (m^2), see Figure 6. However, the interaction effect of foliar spraying × irrigation frequency × irrigation system did not significantly impact this agronomic parameter. In this investigation on irrigation system × irrigation frequency, the maximum capsaicin yield (384 g capsaicin m^{-2}) was observed in the drip irrigation system under deficit irrigation conditions (Fig. 6). In this research, the foliar spraying × irrigation frequency treatment greatly influenced capsaicin yield ($p \leq 0.01$). The maximum capsaicin yield (471 g capsaicin m^{-2}) was obtained from foliar application of chitosan × deficit irrigation (Fig. 7). Interestingly, this improvement under the applied chitosan was about 95% compared to controls. The simple effects of the foliar spraying and irrigation frequency significantly changed the amount of vitamin C in the red pepper fruit (Table 3). However, there was no significant difference between the two irrigation systems in vitamin C concentration (Table 3). The interaction effect of foliar-spraying × irrigation frequency × irrigation system ($p \leq 0.01$) on the ascorbic acid content in red pepper fruit was significant (Fig. 8). The effects of the foliar application and irrigation frequency significantly affected the total phenolic content in the red pepper fruit (Table 3); however, the irrigation system treatment had not significantly effect on the total phenolic content (Table 3). The highest content of total phenolic was recorded once plants were treated with 4 g L^{-1} chitosan, compared to the untreated plants (1.81 mg GAE 100 g^{-1} DW), see Ta-

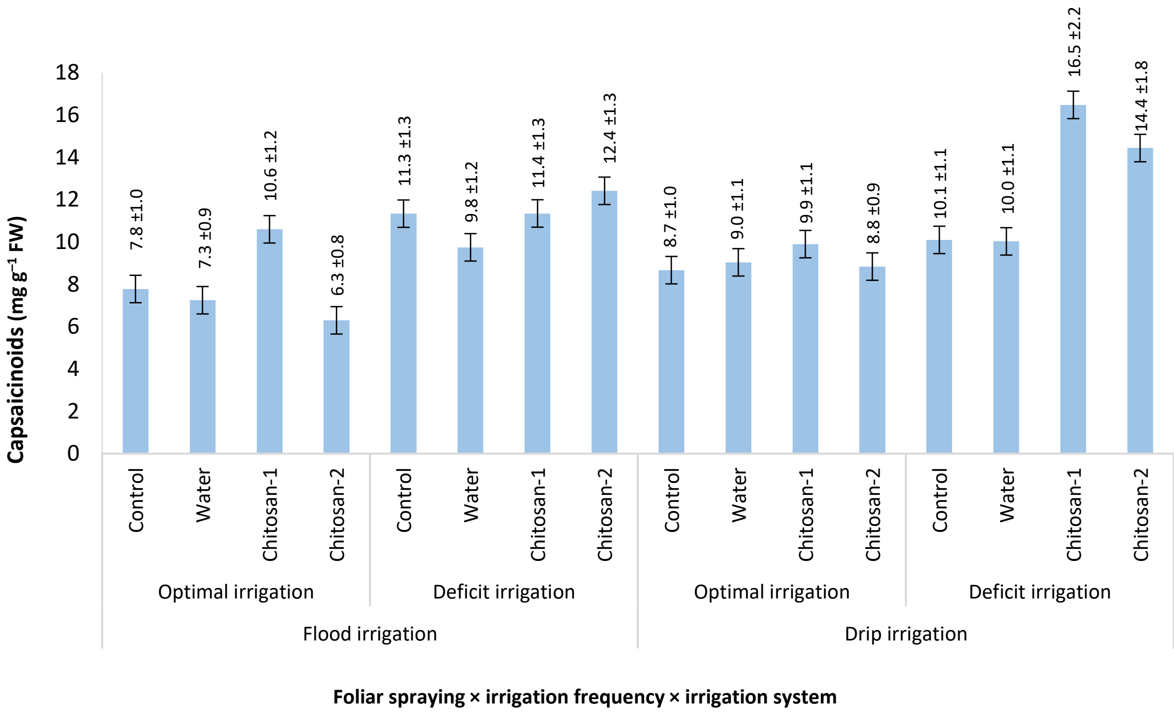

Fig. 5. Interaction effect of foliar spraying × irrigation frequency × irrigation system on the amount of capsaicinoid

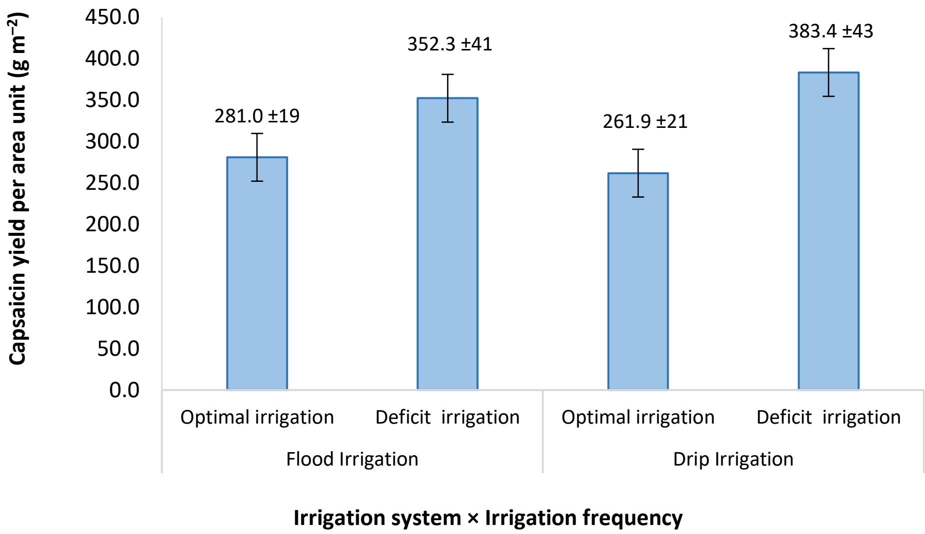

Fig. 6. Interaction effect of irrigation system × irrigation frequency on the capsaicin yield per area unit

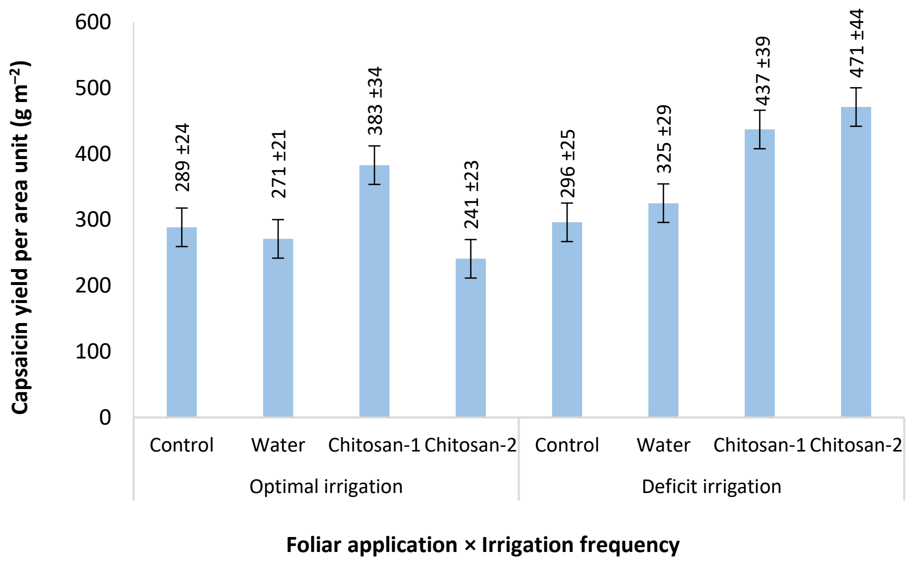

Fig. 7. Interaction effect of foliar spraying × irrigation frequency on the capsaicin yield per area unit

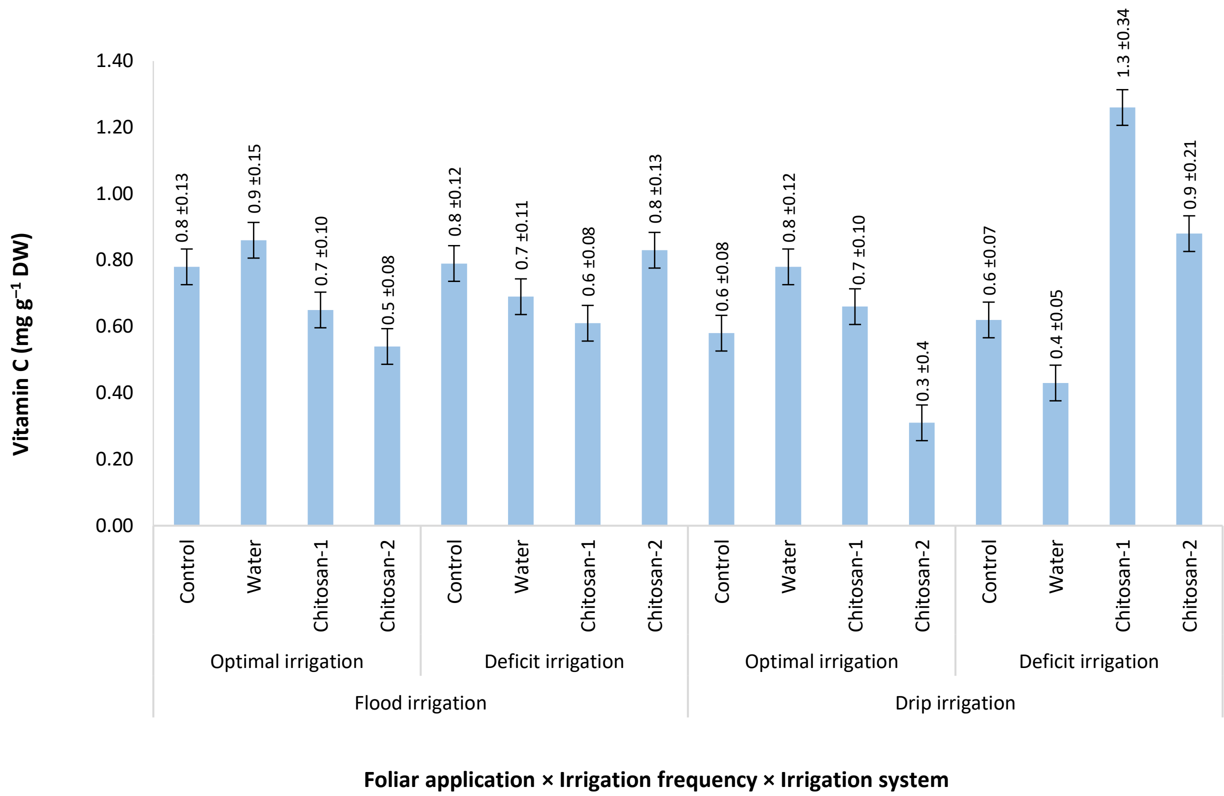

Fig. 8. Interaction effect of foliar spraying × irrigation frequency × irrigation system on the vitamin C content

ble 3. On the other hand, deficit irrigation significantly increased (1.56 mg GAE 100 g^{-1} DW) the total phenol content compared to the optimum irrigation condition (Table 3).

The results of this study show that there were no differences in the simple effects of the experimental treatments on the antioxidant activity of red pepper fruit (Table 3). At the same time, the simple effects of the experimental treatments had significant effects on antioxidant activity, with the maximum antioxidant activity observed in plants under chitosan utilization × optimal irrigation conditions × drip irrigation system (55%), see Table 3.

DISCUSSION

According to the results of the experimental factors including the chitosan utilization, irrigation frequency and system on some traits of red pepper showed that the applied of chitosan elicited the biosynthesis and accumulation of secondary metabolites, the activity of antioxidant enzymes and cell compatible substances, the growth and productivity as well as improve the physiological and morphological processes of plants and improved the accessibility and absorb of indispensable elements and water [Farouk and El-Metwally 2019, Hidangmayum et al. 2019]. Additionally, the antiperspirant properties of chitosan reduce water loss from the plant, and the plant cools down, especially under stress [Ghasemi Pirbalouti et al. 2017]. The results showed that the maximum number of fruits was obtained from plants sprayed with chitosan under optimal irrigation conditions and under flood irrigation. In this regard, Ghanbari et al. [2021] reported that the number of bell pepper fruits significantly decreased with increasing irrigation interval. Chitosan enhances the absorption of water and nutrients, stimulates photosynthesis and CO_2 stabilization, decreases the generation of free radicals by increasing the activity of antioxidant enzymes, and improves plant performance and growth, such as fruit length [Ghasemi Pirbalouti et al. 2017]. Also, the maximum fresh and dry weights of pepper fruit in this study were observed under optimal irrigation conditions, which were attributed to the plant's higher growth rate due to the availability of water [Babaei et al. 2021]. The reason for the improvement in yield under the chitosan treatment in water-deficient conditions could probably be related to the positive effects of the chitosan utilization on enhancing photosynthesis rate, chlorophyll content, and adequate supply of nutrients by increasing the activity of enzymes involved in nitrogen metabolism (nitrate reductase, glutamine synthetase, and protease) [Bistgani et al. 2017a]. We found that the fresh and dry fruit weights in the drip irrigation system were higher than those in the flood irrigation system. More moisture available to the plant and proper soil moisture supply in the drip irrigation system improved fresh and dry fruit weights compared with flood irrigation [Peyrov et al. 2017]. Agronomic and horticultural crops are typically irrigated at close intervals with small amounts of water via drip irrigation [Zamljen et al. 2020]. In the present study, the interaction effects of the experimental factors on fruit weight indicated that plants grown under chitosan × drip irrigation system × optimal and deficit irrigation had the highest fruit yields. Similar to our results, Farouk and El-Metwally [2021] reported that deficit irrigation with a drip irrigation system, combined with chitosan, increased wheat biomass and dry matter yields compared to the control.

In contrast, deficit water stress reduces the absorption of available water and nutrients through the plant roots and also decreases the transfer of the elements from the roots to the branches [Gharakhani-Beni et al. 2021, Rezaei-Adl et al. 2025]. In this study, it appears that chitosan reduced the effects of deficit moisture stress and improved the performance of the aerial parts by enhancing plant physiological processes, such as photosynthetic tissue activity [Ghasemi Pirbalouti et al. 2017, Bistgani et al. 2017a]. In this research, a drip irrigation system could significantly increase plant growth and crop productivity, water and nutrient use efficiency, and reduce evaporation, plant stress, the occurrence of diseases and weed competition, fertilizer leaching, and soil salinity [Yang et al. 2023].

Capsaicinoids, as natural organic components, are responsible for the pungency of pepper fruits [Koleva-Gudeva et al. 2013]. In our experiment, the use of chitosan, a water-deficient condition, and a drip irrigation system could, by increasing the active substances, especially capsaicin, improve the commercial quality of red pepper [Perucka and Materska 2001]. In line with our results, Zamljen et al. [2020] reported increased capsaicin and dihydrocapsaicin concentrations in pepper plants under water-deficit conditions, attributing the increase to four key enzymes of the capsaicinoid biosynthesis pathway [Sung et al. 2005].

Based on our results, deficit irrigation increased capsaicin yield by 20% compared with optimal irrigation. The research results have shown that although soil moisture is a limiting factor for plant production, under deficit water conditions, through metabolic pathways, by stimulating the secondary metabolites biosynthesis, can increase the active biological compounds in the medicinal and aromatic plants [Danesh-Shahraki et al. 2023, Shaykh-Samani et al. 2023, Mosaedi et al. 2024, Darani et al. 2025, Rezaei-Adl et al. 2025]. In fact, the production of this metabolite group is considered a survival strategy against environmental stress [Maghsoudi et al. 2023]. The deficit in moisture triggers various metabolic reactions and the activity of specific genes [Maghsoudi et al. 2023]. The most important biologically active compound among the secondary metabolites of pepper species is capsaicin, which is derived from benzylamine [Koleva-Gudeva et al. 2013]. This active substance is a unique alkaloid that gives pepper its spicy taste. Similarly, Zamljen et al. [2020] reported that the capsaicin and dihydrocapsaicin contents or pungency of the *C. annuum* Chili-AS Rot fruits under deficit irrigation conditions were higher than optimum irrigation.

Additionally, the results of this research illustrated the positive impact of the utilization of chitosan on the levels of capsaicinoides. Probably, chitosan leads to the activation of new genes that stimulate enzymes and metabolic pathways involved in the biosynthesis of secondary metabolites [Bistgani et al. 2017a, Darani et al. 2025, Rezaei-Adl et al. 2025]. Elicitors such as chitosan are first recognized by plant receptors, which activate ion channels, GTP-binding proteins, and protein kinases.

According to the results of the experimental factors on vitamin C concentration, the highest vitamin C concentration was obtained from pepper plants sprayed with chitosan. This finding concurs with the results of a previous investigation [Metwaly et al. 2023], they found that the foliar application of chitosan (0.5 and 1 g L^{-1}) significantly improved fruit quality. Similar to our results, Zamljen et al. [2020] reported that ascorbic acid content in the *C. annuum* Chili-AS Rot fruit decreased by 20% under deficit irrigation. Vitamin C or ascorbic acid is the most abundant and strongest water-soluble antioxidant that prevents or reduces damage caused by reactive oxygen species in plants. Ascorbic acid plays a role in removing reactive species *via* the ascorbate peroxidase reaction. The increase in ascorbic acid synthesis is one of the positive aspects under water-deficit stress conditions. In this study, it seems that the application of chitosan under deficit irrigation conditions could increase ascorbic acid levels, which have a protective role against stress. Li et al. [2017] reported that the use of chitosan significantly increased chitosan content under water-deficit conditions by maintaining higher accumulation of metabolites related to osmotic regulation, antioxidant defense, stress signaling, and energy metabolism. They reported that chitosan-induced drought tolerance was associated with the accumulation of stress-protective metabolites, increased polyamine synthesis, and flavonoid metabolism [Li et al. 2017].

Regarding the interaction effects, the highest total phenolic content was observed with foliar chitosan spraying under deficit irrigation and drip irrigation conditions. Similar to the present research, Maghsoudi et al. [2023] reported that phenolic compounds accumulated in the plant under water deficiency stress. Moreover, Bistgani et al. [2017b] reported that varying levels of chitosan increased total phenolic content in thyme (*Thymus daenensis* Celak.) compared with the control under deficit irrigation conditions. Some plants have different protective mechanisms, such as enzymatic and non-enzymatic antioxidant systems (phenolic compounds and flavonoids), under environmental stress [Babaei et al. 2021].

Water deficit stress causes oxidative stress by disrupting the balance between the production of reactive oxygen species and the plant's antioxidant defense activities. Antioxidants are molecules that neutralize reactive oxygen species and help prevent damage to plant cells [Shaykh-Samani et al. 2023]. It seems that chitosan dissolution has significantly increased the activity of the antioxidant defense system and reduced lipid peroxidation. Results from several studies have shown that the main components of the cell wall of the biological elicitor chitosan may be able to scavenge free radicals and stimulate plant defense mechanisms and secondary metabolite production [Ghasemi Pirbalouti et al. 2017]. Based on our hypotheses and results, the use of medium molecular weight chitosan at 2 g L^{-1} in red pepper plants could mitigate the negative effects of water deficit stress on fruit yield traits by boosting the biosynthesis of phenolic compounds and antioxidant substances such as capsaicinoids.

Additionally, the foliar application had positive impacts on pepper performance and alleviated the adverse effects of water deficit stress on phytochemical and antioxidant properties. Complex processes are activated in plants in response to drought stress, including hormonal modulation, transcription factor-mediated signaling, and biosynthesis of secondary metabolites [Arabsalehi et al. 2022]. Among drought stress resistance traits, secondary metabolites are of great importance because these compounds play an important role in regulating plant-environment interactions and subsequent adaptive responses [Babaei et al. 2021, Arabsalehi et al. 2022]. Water shortage affects secondary metabolites by altering gene expression or the activity of enzymes involved in their biosynthesis pathways [Arabsalehi et al. 2022]. The mechanism of chitosan effects on the biosynthesis pathways of capsaicin, dihydrocapsaicin, and vitamin C in pepper fruit is not currently understood. In general, molecular-level analysis, identification of secondary metabolite biosynthesis pathways, and the nutritional quality of the fruit need to be studied in red pepper under foliar-spraying with chitosan and under water-deficit stress conditions.

CONCLUSIONS

Based on the results of this investigation, the active biologically compounds, especially the capsaicin and dihydrocapsaicin concentrations in the pepper fruits under deficit irrigation, improved significantly. The foliar application of chitosan could, by stimulating biosynthetic pathways, increase the biosynthesis of secondary metabolites such as capsaicin, dihydrocapsaicin, vitamin C, and total phenols in pepper fruit. Interestingly, the quality yield of red pepper was improved by increases in the concentrations of capsaicin, dihydrocapsaicin, vitamin C, total phenolic content, and capsaicin yield, as well as antioxidant capacity, with the utilization of chitosan under water deficit stress showing the highest amounts. In conclusion, foliar spraying of medium molecular weight chitosan, particularly at 2 g L^{-1}, could mitigate the adverse effects of water deficit stress and improve active substances such as capsaicin, dihydrocapsaicin, vitamin C, and total phenolic content under water deficit stress, particularly in arid and semiarid conditions. Future studies should investigate the molecules, genes, proteins, and specific metabolic pathways underlying capsaicin and vitamin C biosynthesis in response to chitosan application under dehydration conditions in red pepper, and optimize its use in combination with deficit irrigation strategies.

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REFERENCES

AACC (2000). Approved methods of the American association of cereal chemists. 10th ed. American Association of Cereal Chemists, St. Paul.

Aniel Kumar, O., Subba Tata, S. (2009). Ascorbic acid contents in chili peppers (*Capsicum* L.). Not Sci. Biol., 11(1), 50–52.

Arabsalehi, F., Rahimmalek, M., Sabzalian, M.R., Barzegar Sadeghabad, A., Matkowski., Szumny, A. (2022). Metabolic and physiological effects of water stress on Moshgak (*Ducrosia anethifolia* Boiss) populations using GC–MS and multivariate analyses. Sci. Rep., 12, 22148. https://doi.org/10.1038/s41598-022-25195-1

Alavi Samany, S.M., Ghasemi Pirbalouti, A., Malekpoor, F. (2022) Phytochemical and morpho-physiological changes of hyssop in response to chitosan-spraying under different levels of irrigation. Ind. Crops Prod., 176, 114330. https://doi.org/10.1016/j.indcrop.2021.114330

Arce-Rodriguez, M.L., Ochoa-Alejo, N. (2019). Biochemistry and molecular biology of capsaicinoid biosynthesis. Recent advances and perspectives. Plant Cell Rep., 38, 1017–1030. https://doi.org/10.1007/s00299-019-02406-0

Babaei, K., Moghaddam, M., Farhadi, N., Ghasemi Pirbalouti, A. (2021). Morphological, physiological and phytochemical responses of Mexican marigold (*Tagetes minuta* L.) to drought stress. Sci. Hortic., 284, 110116. https://doi.org/10.1016/j.scienta.2021.110116

Bistgani, Z.E., Siadat, S.A., Bakhshandeh, A., Ghasemi Pirbalouti, A., Hashemi, M. (2017a). Interactive effects of drought stress and chitosan application on physiological characteristics and essential oil yield of *Thymus daenensis* Celak. Crop J., 5(5), 407–415.

Bistgani, Z.E., Siadat, S.A., Bakhshandeh, A., Ghasemi Pirbalouti, A., Hashemi, M. (2017b). Morpho-physiological and phytochemical traits of (*Thymus daenensis* Celak.) in response to deficit irrigation and chitosan application. Acta Physiol. Plant., 39, 231. https://doi.org/10.1007/s11738-017-2526-2

Black, C.A. (1965). Methods of soil analysis, 1965. Part I. Physical and mineralogical properties. American Society of Agronomy, Madison, Wisconsin.

Brand-Williams, W., Cuvelier, M.E., Berset, C. (1995). Use of free radical method to evaluate antioxidant activity. Food Sci. Technol., 28(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Danesh-Shahraki, H., Ghasemi Pirbalouti, A., Rajabzadeh, F., Kachouei, M.A. (2023). Foliar application of salicylic acid and proline to mitigate water deficit impact on purple coneflower (*Echinacea purpurea* (L.) Moench.). Acta Sci. Pol. Hortorum. Cultus, 22(5), 89–97. https://doi.org/10.24326/asphc.2023.4964

Dang, Y.Y., Zhang, H., Xiu, Z.L. (2014). Three-liquid-phase extraction and separation of capsanthin and capsaicin from *Capsicum annum* L. Czech J. Food Sci., 32(1), 109–114. https://doi.org/10.17221/96/2013-CJFS

Darani, M.K., Ghasemi Pirbalouti, A., Ardakani, M.R., Mozafari, H., Habibi, D. (2025). The simultaneous effect of chitosan-plus and arbuscular mycorrhizal fungi on herbage yield, quantity and quality of spearmint (*Mentha spicata* L.) essential oil under an organic farming system. J. Essent. Oil Bear. Plants., 28(3), 1–15. https://doi.org/10.1080/0972060X.2025.2496657

Farouk, S., El-Metwally, I.M. (2019). Synergistic responses of drip-irrigated wheat crop to chitosan and/or silicon under different irrigation regimes. Agric. Water Manag., 226, 105807. https://doi.org/10.1016/j.agwat.2019.105807

Ghanbari, F., Cheraghi, M., Erfani Moghadam, J. (2021). The effect of kaolin on drought stress tolerance and some physiological responses of bell pepper (*Capsicum annuum* L.). J. Veg. Sci., 5(9), 63–75. https://doi.org/10.22034/iuvs.2020.137652.1122

Gharakhani-Beni, A., Ghasemi Pirbalouti, A., Hamid Reza, J., Soleymani, A., Golparvar, A. (2021). Competition indices of intercropping of hyssop (*Hyssopus officinalis* L.) and fenugreek (*Trigonella foenum-graecum* L.) in different soil moisture conditions. Crop Sci. Res. Arid Reg., 3(2), 237–252. [In Farsi]. https://doi.org/10.22034/CSRAR.2021.298016.1114

Ghasemi Pirbalouti, A., Malekpoor, F., Salimi, A., Golparvar, A. (2017). Exogenous application of chitosan on biochemical and physiological characteristics, phenolic content and antioxidant activity of two species of basil (*Ocimum ciliatum* and *Ocimum basilicum*) under reduced irrigation. Sci. Hortic., 217, 114–122. https://doi.org/10.1016/j.scienta.2017.01.031

Hafez, Y., Attia, K., Alamery, S., Ghazy, A., Al-Doss, A., Ibrahim, E., Rashwan, E., El-Maghraby, L., Awad, A., Abdelaal, K. (2020). Beneficial effects of biochar and chitosan on antioxidative capacity, osmolytes accumulation, and anatomical characters of water-stressed barley plants. Agronomy, 10(5), 630. https://doi.org/10.3390/agronomy10050630

Hernández–Pérez, T., Gómez–García, M.D.R., Valverde, M.E., Paredes–López, O. (2020). *Capsicum annuum* (hot pepper). An ancient Latin–American crop with outstanding bioactive compounds and nutraceutical potential. Compr. Rev. Food Sci. Food Saf., 19(6), 2972–2993. https://doi.org/10.1111/1541-4337.12634

Hidangmayum, A., Dwivedi, P., Katiyar, D., Hemantaranjan, A. (2019). Application of chitosan on plant responses with special reference to abiotic stress. Physiol. Mol. Biol. Plants., 25(2), 313–326. https://doi.org/10.1007/s12298-018-0633-1

Karimi, A., Mohammad, P., Gachkar, S., Gachkar, D., García-Martínez, A., Moreno-Rangel, D., Brown, R.D. (2021). Surface urban heat island assessment of a cold desert city: a case study over the Isfahan Metropolitan Area of Iran. Atmosphere, 12(10), 1368. https://doi.org/10.3390/atmos12101368

Khamoushi, M., Khani, M., Ghasemi Pirbalouti, A. (2021). Comparing the effects of red, black and bell pepper extracts on Aflatoxin production, peroxide value and sensory properties in pistachio. J. Food Sci. Technol. (Iran), 18(116), 183–194. [In Farsi]. https://doi.org/10.52547/fsct.18.116.183

Khodadadi, F., Ahmadi, F.S., Talebi, M., Matkowski, A., Szumny, A., Afshari, M., Rahimmalek, M. (2023). Metabolic and transcriptomic approaches of chitosan and water stress on polyphenolic and terpenoid components and gene expression in *Salvia abrotanoides* (Karl.) and *S. yangii*. Int. J. Mol. Sci., 24(20), 15426. https://doi.org/10.3390/ijms242015426

Koleva-Gudeva, L., Mitrev, S., Maksimova, V., Spasov, D. (2013). Content of capsaicin extracted from hot pepper (*Capsicum annuum* ssp. *microcarpum* L.) and its use as an ecopesticide. Hem. Industr. (Chem. Ind.)., 67(4), 671–675.

Kottek, M., Grieser, J., Beck, C., Rudolf, B., Rubel, F. (2006). World map of the Köppen-Geiger climate classification updated. Meteorolog. Z., 15(3), 259–263. Li, Z., Zhang, Y., Zhang, X., Merewitz, E., Peng, Y., Ma, X., Huang, L., Yan, Y. (2017). Metabolic pathways regulated by chitosan contributing to drought resistance in white clover. J. Proteome Res., 16(8), 3039–3052. https://doi.org/10.1021/acs.jproteome.7b00334

Liu, C., Wan, H., Yang, Y., Ye, Q., Zhou, G., Wang, X., Ahammed, G.J., Cheng, Y. (2022). Post-Harvest LED Light irradiation affects firmness, bioactive substances, and amino acid compositions in chili pepper (*Capsicum annum* L.). Foods., 11(17), 2712. https://doi.org/10.3390/foods11172712

Liu, H., Yang, H., Zheng, J., Jia, D., Wang, J., Li, Y., Huang, G. (2012). Irrigation scheduling strategies based on soil matric potential on yield and fruit quality of mulched-drip irrigated chili pepper in Northwest China. Agric. Water Manag., 115, 232–241. https://doi.org/10.1016/j.agwat.2012.09.009

Maghsoudi, E., Abbaspour, H., Ghasemi Pirbalouti, A., Saeidi-Sar, S. (2023). Influence of the foliar applications of paclobutrazol and 24-epibrassinolide on the quantitative and qualitative traits of sage (*Salvia officinalis* L.) volatile oil under different soil moisture conditions. J. Plant Growth Regul., 42, 5495–5506. https://doi.org/10.1007/s00344-023-10931-2

Mosaedi, H., Mozafari, H., Sani, B., Ghasemi Pirbalouti, A., Rajabzadeh, F. (2024). Foliar-applied silicon and zinc nanoparticles improve plant growth, biochemical attributes, and essential oil profile of fennel (*Foeniculum vulgare*) under different irrigation regimes. Funct. Plant Biol., 51(10), FP24149. https://doi.org/10.1071/FP24149

Menichini, F., Tundis, R., Bonesi, M., Loizzo, M.R., Conforti, F., Statti, G., Menichini F. (2009). The influence of fruit ripening on the phytochemical content and biological activity of *Capsicum chinense* Jacq. cv *Habanero*. Food Chem., 114(2), 553–560. https://doi.org/10.1016/j.foodchem.2008.09.086

Metwaly, E.S.E., AL-Huqail, A.A., Farouk, S., Omar, G.F. (2023). Effect of chitosan and micro-carbon-based phosphorus fertilizer on strawberry growth and productivity. Horticulturae, 9(3), 368. https://doi.org/10.3390/horticulturae9030368

Olatunji, T.L., Afolayan, A.J. (2018). The suitability of chili pepper (*Capsicum annuum* L.) for alleviating human micronutrient dietary deficiencies. A review. Food Sci. Nutr., 6(8), 2239–2251. https://doi.org/10.1002/fsn3.790

Olsen, S.R., Cole, C.V., Watanabe, F.S., Dean, L.A. (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. In: Banderis, A.D., Barter, D.H., Anderson, K. (eds), Agricultural and Advisor. U.S. Department of Agriculture Circular, 939.

Othman, Z.A.A., Ahmed, Y.B.H., Habila, M.A., Ghafar, A.A. (2011). Determination of capsaicin and dihydrocapsaicin in *Capsicum* fruit samples using high performance liquid chromatography. Molecules, 16(10), 8919–8929. https://doi.org/10.3390/molecules16108919

Page, A.L. (ed.) (1982). Methods of soil analysis. Part 2. Chemical and Microbiological Properties, 2 ed.

Perucka, I., Materska, M. (2001). Phenylalanine ammonialyase and antioxidant activities of lipophilic fraction of fresh pepper fruits *Capsicum annuum* L. Innov. Food Sci. Emerg. Technol., 2(3),189–192. https://doi.org/10.1016/S1466-8564(01)00022-4

Peyrov, S., Sosani, J., Jalilvand, H., Pirikia, M. (2017). Evaluation of growth of trees using drip and furrow irrigation systems. J. Plant Res. (Iranian J. Biol.), 30(2), 280–290. [In Farsi]. https://dor.isc.ac/dor/20.1001.1.23832592.1396.30.2.4.5

Rabêlo, V.M., Magalhães, P.C., Bressanin, L.A., Carvalho, D.T., dos Reis, C.O., Karam, D., Doriguetto, A.C., dos Santos, M.H., dos Santos Santos Filho, P.R., de Souza, T.C. (2019). The foliar application of a mixture of semisynthetic chitosan derivatives induces tolerance to water deficit in maize, improving the antioxidant system and increasing photosynthesis and grain yield. Sci. Rep., 9, 8164. https://doi.org/10.1038/s41598-019-44649-7

Rezaei-Adl, F., Ghasemi Pirbalouti, A., Rahimi, T., Rajabzadeh, F. and Mozafari, H. (2025). Soil-based biochar and foliar-spraying of chitosan enhances the phytochemical traits of yarrow (*Achillea millefolium* L.) under varying moisture levels. Sci. Rep., 15, 30881. https://doi.org/10.1038/s41598-025-04308-6

Sanati, S., Razavi, B.M., Hosseinzadeh, H. (2018). A review of the effects of *Capsicum annuum* L. and its constituent, capsaicin, in metabolic syndrome. Iran. J. Basic Med. Sci., 21(5), 439–448. https://doi.org/10.22038/IJBMS.2018.25200.6238

Shaykh-Samani, A., Ghasemi Pirbalouti, A., Yadegari, M., Rajabzadeh, F. (2023). Foliar application of salicylic acid improved the yield and quality of the essential oil from *Dracocephalum kotschyi* Boiss. under water deficit stress. J. Essent. Oil Bear. Plants., 26(3), 769–779. https://doi.org/10.1080/0972060X.2023.2236647

Sung, Y., Chang, Y.Y., Ting, N.-L. (2005). Capsaicin biosynthesis in water-stressed hot pepper fruits. Bot. Bull. Acad. Sin., 46, 35–42.

Yang, P., Wu, L., Cheng, M., Fan, J., Li, S., Wang, H., Qian, L. (2023). Review on drip irrigation. Impact on crop yield, quality, and water productivity in China. Water., 15(9), 1733. https://doi.org/10.3390/w15091733

Valletta, A., De Angelis, G., Badiali, C., Brasili, E., Miccheli, A., Di Cocco, M.E., Pasqua, G. (2016). Acetic acid acts as an elicitor exerting a chitosan-like effect on xanthone biosynthesis in *Hypericum perforatum* L. root culture. Plant Cell Rep., 35, 1009–1020. https://doi.org/10.1007/s00299-016-1934-x

Zamljen, T., Zupanc. V., Slatnar, A. (2020). Influence of irrigation on yield and primary and secondary metabolites in two chilies species, *Capsicum annuum* L. and *Capsicum chinense* Jacq. Agric. Water Manag., 234, 106104. https://doi.org/10.1016/j.agwat.2020.106104