



Determination of Yield and Quality Parameters in Pickling Hot Peppers Grown under Different Water Stress Conditions

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Plants are exposed to various adverse environmental conditions throughout their growth period. In recent years, drought, which has occurred and necessitated different measures, ranks among these adverse conditions. At the same time, plants synthesize certain biochemical compounds in response to the adverse conditions they will encounter. These compounds not only strengthen the immune system but also provide resistance against various diseases, and they tend to increase under adverse environmental conditions that plants will face during cultivation. This study was conducted to determine the changes in yield and some biochemical components in pickling hot peppers (*Capsicum Annuum* L.) grown under different water stress conditions. Two different levels of water stress (%70 and %30 irrigation) were applied in addition to full irrigation (%100 irrigation). At the end of the research, while a yield of 269.42 g per plant was obtained in the control group (%100 irrigation), 150.14 g and 93.33 g of pickling hot peppers were harvested in each water stress treatment, respectively. Total phenolic compound levels increased with water stress; it was determined to be 0.827 mg⁻¹g in the trial irrigated with full irrigation water, 1.170 mg⁻¹g in plants exposed to mild water stress, and up to 1.536 mg⁻¹g in the trial subjected to severe water stress. In addition, total flavonoids and antioxidant compound levels also increased with increasing water stress. The amounts of flavonoid compounds obtained from the trial groups were 0.146, 0.373, and 0.412 mg⁻¹g, respectively, while the antioxidant levels determined by the DPPH method increased in quantity with increasing water stress, similar to other biochemical compounds. According to these results, it was determined that the yield of pickling hot peppers decreased in the case of water shortage that the plants would face in cultivation, but there was an increase in some biochemical compounds.

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Introduction

Rising temperatures and reduced rainfall in recent decades have increased drought in regions within tropical and subtropical climate zones, negatively impacting food production in many areas (Erken, 2022). The increasing frequency and duration of drought worldwide can lead to the death of drought-sensitive plants causing a threat to future global food security (Naing and Kim, 2021). Considering your countries per capita annual water availability, Turkey is facing water scarcity. Given the adverse effects of factors such as climate change, environmental pollution, industrial development, and rapid population growth on water resources, it can be said that Turkey is expected to face significant water shortages in the future. Finding new water sources alone may not be sufficient to solve the problem; rather, ensuring the efficient use of water is essential. Efficient water use depends on changing irrigation practices and adopting new irrigation methods (Kanber et al., 2005).

Plants are the organisms most exposed to abiotic and biotic stress factors. Abiotic stress conditions such as drought, salinity, excessive rainfall, temperature, and cold directly affect plant growth and development. Depending on the intensity and duration of water stress, they can exhibit significant metabolic changes when confronted with drought conditions, which can regulate their life cycles significantly (Öztürk, 2015).

Pepper is a crop of significant importance for consumers, producers, and the processing industry, cultivated in various countries both in open fields and undercover (Duman et al., 2002). Taxonomically known as "capsicum" and commercially and scientifically referred to as "pepper," it is described as hot or chili pepper. Peppers belong to the *Solanaceae* family and have a sharp taste in food with a natural plant color. Moreover, due to their pharmaceutical content, they are also used in organic pest control by applying them in spray form on plants

(Sanatombi and Sharma, 2008). Hot peppers contain various phytochemicals, as well as vitamins A and C, phenolic compounds, flavonoids, and carotenoids. They are particularly known for their high vitamin C content and strong antioxidant levels (Yaldız and Özgüven, 2011).

In a field trial conducted in Bursa Province in 2019, the effects of four different drip irrigation water levels (S_{100} , S_{75} , S_{50} , S_{25}) on some yield components and water use efficiency of the Burkalem pepper variety were examined. The highest pepper yield was reported to be obtained from the irrigation levels where the crop coefficient was S_{100} ($k_c=1.00$) and S_{75} ($k_c=0.75$) (Yılmaz, 2022). In another study conducted under Çanakkale conditions, the effects of five different irrigation levels on plant fruit yield and some quality parameters were examined in the California Wonder pepper variety. It was stated that the highest yield was obtained when the crop coefficient was applied as $k=0.75$ and $k=1.00$ in the irrigation levels (Erken, 2004). According to the results of another study conducted on Jalapeno pepper (*Capsicum annuum* L.) with three different irrigation water levels (W1: % 100, W2: % 75, W3: % 50), it was reported that with increasing water stress, stem diameter, fruit diameter, shoot length, shoot fresh weight, shoot dry weight, fruit length, fruit weight, leaf count, leaf thickness, and fruit count decreased, but root length, root fresh weight, root dry weight, and water-soluble dry matter content increased (Bilgin, 2019).

This study was conducted to determine the morphological differences and changes in the synthesis of antioxidant substances, phenolic compounds, flavonoids and proline levels in hot peppers exposed to drought throughout the cultivation period.

Materials and Methods

The research was conducted in the greenhouse of Çanakkale Onsekiz Mart University, located at 40° 08' north latitude and 28° 20' east longitude. The trials were carried out using short and thin peppers known as Hungarian hot peppers. Seedlings were planted on June 1, 2021, in 10-liter pots containing clayey loam soil with a pH of 7.25, EC of 0.50 $mS^{-1}cm$, 10.35% calcium content, and 2.88% organic matter content. In the experiment, which was designed according to a randomized complete block design, cultural practices were applied as necessary, and pickling hot peppers (*Capsicum annuum* L.) were grown until 27th of September. During the cultivation period, in addition to the control pots which were irrigated normally, two different water stress levels were applied (%70 irrigation and %30 irrigation). Each treatment, including the control, was conducted with four replications.

The pots used in the research were filled with a prepared soil mixture in equal amounts, and to determine the water-holding capacity of the pots, they were initially saturated with water. The pots were left to drain under gravity for 24 hours, and their water-holding capacities were determined by weighing. After saturating the pots, seedlings were planted. Following the adaptation of the seedlings to the pots (10 days after planting), water stress applications began. The control pots were weighed every 3 days to determine the amount of evaporation and water used by the plant in terms of weight, and the amount of water lost was replenished with irrigation water. The

reduced water amounts were applied through the use of a measuring cup for irrigation purposes. The water stress treatments of 70% irrigation and 30% irrigation were determined based on the amount of irrigation water applied to the control pots in each irrigation. Çanakkale Municipality tap water was used as irrigation water. The quality of the irrigation water was determined as T2A1.

At the end of the cultivation period, the yield per plant (g per plant), yield per decare (kg per da), fruit length and diameter (mm) were determined by measuring the harvested fruits.

For the determination of internal proline content, pepper leaves were frozen in liquid nitrogen, and 0.5 g samples were weighed and homogenized in 10 ml of 3% 5-Sulfosalicylic acid using a homogenizer for 2 minutes at maximum speed at $-18^{\circ}C$ until analysis. Then, the homogenate was filtered through Whatman No. 2 filter paper and transferred to tubes. Two millilitres of the filtrate were mixed in a sealed test tube with 2 ml ninhydrin and 2 ml glacial acetic acid, and the reaction was carried out at $100^{\circ}C$ for 1 hour in a water bath set at $100^{\circ}C$. The tubes were then placed in an ice bath to complete the reaction. 4 ml of toluene were added, and the reaction mixture was mixed for 15-20 seconds using a tube mixer. The chromophore-containing phase was carefully aspirated with a fine-tipped pipette and transferred to spectrophotometer tubes. Absorbance readings at 520 nanometers were taken when the spectrophotometer tubes reached room temperature. Toluene was used as a control (Bates et al., 1973).

The Folin-Ciocalteu method, as described by Singleton et al. (1999) was used to determine the total phenolic content ($mg^{-1}g$) in peppers. Initially, 100 μl of pepper extract was vortexed with 900 μl of distilled water, 5 ml of 0.2 N Folin-Ciocalteu reagent, and 4 ml of 7.5% sodium carbonate solution in test tubes. The resulting solution was allowed to stand at room temperature for 2 hours, and then absorbance values at 765 nanometers were measured using a spectrophotometer. The total phenol content of the pepper samples was expressed as "mg of gallic acid equivalents per 100g of fresh weight" ($mg GA^{-1}100g FW$).

The total flavonoid concentrations ($mg^{-1}g$) of pepper extracts were measured using aluminium-based colorimetric analysis (Shraim et al., 2021). Test tubes were sequentially filled with 100 μl of pepper extract, 100 μl of 1 M potassium acetate, 100 μl of 10% aluminium nitrate, and 4.4 ml of 96% ethanol. After incubating the samples in the dark at room temperature for 40 minutes, absorbance values at 415 nanometers were measured using a spectrophotometer. The total flavonoid content of the pepper samples was calculated as "mg of quercetin equivalents per 100g of fresh weight" ($mg quercetin^{-1}100g FW$).

The CUPRAC method, which is a copper-reducing antioxidant capacity test according to Apak et al. (2004), is based on an electron transfer method to determine the ability of plant samples to reduce copper ions (Cu^{+2}). As described by Marangoz (2016) 30 μL of plant extracts were mixed with 1 ml of 0.01 M copper(II) chloride, 1 ml of 7.5 x 10⁻³ neocuproine, 1 ml of 1 M pH 7 ammonium acetate, and 1080 μL of distilled water. After incubation at $20^{\circ}C$ for 30 minutes, absorbance values at 450 nm were determined using a spectrophotometer. Antioxidant

activity was calculated as trolox equivalents in mg per 100g of fresh sample (mg trolox⁻¹100g FW) using a standard calibration curve.

The antioxidant activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging method as described by Brand-Williams et al. (1995) and Ak and Türker (2018). Different concentrations of methanol-diluted pepper extracts were mixed with DPPH solution after being allowed to stand at room temperature for 30 minutes.

The absorbance values of the prepared samples were measured at 515 nanometers using a UV spectrophotometer. The percentage of DPPH radical scavenging activity was determined using the following equation:

$$\text{DPPH}(\%) = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

(Note: In the equation, A_{control} represents the absorbance of the control sample, and A_{sample} represents the absorbance of the sample.)

The IC₅₀ inhibition values were calculated considering the concentration at which 50% of the total DPPH radicals were captured, with lower IC₅₀ values indicating higher radical scavenging activity. In this test, butylated hydroxytoluene (BHT) was used as a positive control.

Analysis of variance (ANOVA) was used to determine the level of differences among the data obtained from the experimental subjects, and the Duncan test was applied to classify these identified differences.

Results and Discussion

The total irrigation water applied to the Hungarian hot pepper variety, which is the subject of the research, and some morphological measurement results obtained after harvest are given in Table 1. Irrigation water was applied at 3-day intervals until the end of the research, and the total water consumption for the control plants was determined to be 518.2 mm. The average yields per plant decreased with water stress by the end of the research. The highest yield was determined as 269.42 g from the trial with full irrigation water application, while the lowest yield was measured as 93.33 g from the trial with 30% irrigation water application. In the study conducted by Demirel et al. (2012), it was found that along with water scarcity, the yield and quality parameters of pepper plants decreased. They have stated that to obtain high yield and quality

products, it is essential to provide the required amount of water in a timely manner and that there will be decreases in yield in case of water scarcity.

When the yields per decare were calculated, they were determined as 1122.40 kg⁻¹da for the 100% irrigation trial, 625.49 kg⁻¹da for the 70% irrigation trial, and 388.79 kg⁻¹da for the trial with 30% irrigation water application. According to these results, it can be understood from the yield results that the pickling hot pepper plant is sensitive to water. In other words, unless necessary, the application of water restriction in pickling hot pepper cultivation is not recommended according to the research results.

When looking at the measurement values of single fruit weight, fruit count, fruit diameter, and length, statistically significant differences were detected as observed in the yield values. It was determined in the study that as water stress increased, the parameters determining the quality of pickling hot peppers also decreased. The highest fruit count was determined as an average of 85 fruits from the fully irrigated trial, while the lowest fruit count was found to be 26 fruits from the trial with severe water stress, which received 30% irrigation.

At the end of the research, the analysis of some biochemical components was conducted on samples taken from each trial, and the results obtained are presented in Table 2.

The amounts of phenolic compounds varied between 0.827-1.536 mg⁻¹g depending on the applied irrigation water levels. Phenolic compound accumulation increased with the increasing water stress. The highest accumulation of phenolic compounds occurred in the trial with 30% irrigation water application. Phenolic compounds have received significant attention as a result of in vitro and in vivo studies due to their effective antioxidant capacity. They can be divided into three groups: free, esterified, and insoluble bound forms. These are determined based on whether they exist in a free form or are covalently bound to other molecules like fatty acids (soluble esters) or macromolecules (insoluble bound phenolics). Most insoluble bound phenolic compounds form covalent bonds with cell wall components such as pectin, cellulose, arabinoxylans, and structural proteins and are relatively more abundant in foods compared to soluble phenolic compounds (20-60% in vegetables, fruits, and legume seeds) (Nayak et al., 2015; Shahidi and Yeo, 2016). Considering the analytical methods available to measure the contents of insoluble bound phenolics in natural sources, it can be assumed that the amount of insoluble bound phenolics may be higher than expected (Shahidi and Yeo, 2016).

Table 1. Irrigation water amount and some morphological measurement results.

Treatments	IWA	YP	YD	SFW	FC	FD	FL
100% irrigation	518.2	269.42a	1122.40a	2.37a	85a	49.66a	76.82a
70% irrigation	362.7	150.14b	625.49b	1.49b	62b	39.87b	59.45b
30% irrigation	155.5	93.33c	388.79c	0.90c	26c	32.15c	21.38c

IWA: Irrigation Water Amount (mm); YP: Yield (g⁻¹ plant); YD: Yield (kg⁻¹ decare); SFW: Single Fruit Weight (g); FC: Fruit Count (number); FD: Fruit Diameter (mm); FL: Fruit Length (mm)

Table 2. Applied irrigation water amounts and some biochemical parameter results.

Treatments	IWA	PC	TF	CUPRAC (mg ⁻¹ g)	DPPH (mg ⁻¹ g)	Proline (µg ⁻¹ g)
100% irrigation	518.2	0.827 ^c	0.146 ^b	0.717 ^c	0.763 ^a	308
70% irrigation	362.7	1.170 ^b	0.373 ^{ab}	1.541 ^b	0.630 ^b	482
30% irrigation	155.5	1.536 ^a	0.412 ^a	1.882 ^a	0.568 ^c	641

IWA: Irrigation Water Amount (mm); PC: Phenolic Compound (mg⁻¹g); TF: Total Flavonoids (mg⁻¹g)

The total flavonoid content, which is among the biochemical components, has been synthesized to a greater extent with increasing water stress, similar to the observed increase in phenolic compound levels. The highest amount, 0.412 mg⁻¹g, was measured in the trial with 30% irrigation water application, while the lowest amount was measured in the peppers harvested from the control plots with 100% irrigation water application.

Plants develop resistance strategies to adverse conditions such as osmotic adjustment, involving components like potassium, proline, glycine betaine, and soluble sugars (Turan et al., 2009; Benhassaini et al., 2012). They stimulate antioxidant enzyme activities by increasing some plant growth regulators, leading to an accumulation of antioxidant substances (Çelik and Atak, 2012). This phenomenon was also observed in pickling hot peppers when looking at the results of our study. Antioxidant contents were determined using two different methods. According to the analysis results conducted using the CUPRAC method, the lowest antioxidant content was determined as 0.717 mg⁻¹g in the peppers harvested from the control group. The highest antioxidant content was determined as 1.882 mg⁻¹g in the peppers treated with 30% irrigation water. The results of the antioxidant substance analysis using the DPPH method were similar to those obtained with the other method. In their study using different pepper varieties, Uğur and Saka (2022) have determined that the antioxidant content of hot peppers is higher than that of other varieties. Furthermore, in the results of the study conducted using only pickling hot peppers, it was found that the antioxidant levels increased in conjunction with water stress.

When plants encounter any stress during their growth period, they accumulate a range of secondary metabolites in their bodies, especially amino acids (Giordano et al., 2021). Among these substances, proline is synthesized as an amino acid that plays a beneficial role when plants are exposed to stress conditions (Hayat et al., 2012). According to the proline analysis results obtained at the end of the study (Table 2), proline in the leaves varied between 308 and 641 µg⁻¹g. Based on these findings, it was determined that as soil moisture content decreased, the amount of proline in the leaves increased to enhance the plant's resistance to these conditions, and it was observed that it was synthesized more in response to water restriction. Escalante-Magaña et al. (2019) stated that proline concentration significantly increased with increasing water stress in pepper species (*Capsicum* sp.) compared to control plants. The best characteristic of proline is its strong osmotic protective property. Additionally, previous research has identified significant increases in proline levels under conditions such as salt and drought stress (Mafakheri et al., 2010; Erken et al., 2013; Özkoku et al., 2019).

Conclusion

In today's world, both the increasing population growth and global warming have made water resources even more critical in terms of meeting food needs worldwide. When we look at the scientific studies examining the relationship between water and yield in plant production, it can be observed that these studies aim to determine the changes in the biochemical properties and economic yield values of

plants grown at different water levels. The changes in the chemical substances synthesized by plants under different water levels are crucial in terms of their effects on human health.

When the research results are evaluated together, it is determined that hot pepper plants for pickling are sensitive to water. Water shortage should be avoided during the cultivation period unless absolutely necessary. In regions with high temperatures and limited water resources, insufficient irrigation conditions can lead to significant yield losses in the cultivation of hot peppers for pickling. If water shortage is necessary, it has been found that applying the minimum amount of water restriction, preferably as little as possible, is important in terms of yield and quality parameters.

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