Alleviating the Effects of High-Temperature Stress on Parsley Plants by Foliar Application of Proline, Glycine Betaine, and Salicylic Acid

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ABSTRACT

Low temperatures and high relative humidity are ideal for parsley's growth and high quality, making it a distinctive herbaceous vegetable with medicinal properties. Despite the growing local and export demands, meeting these conditions, during the summer season, in Egypt is challenging. Therefore, this study aimed to alleviate the harmful effects of high temperatures stress on the growth and quality of Balady variety of parsley by foliar application of proline, glycine betaine, and salicylic acid. Two field experiments were conducted under a sprinkler irrigation system, in Wadi El-Natroun-Beheira Governorate, during the summer seasons of 2020 and 2021. The following seven treatments were studied: proline (Pr1;2.5 mM l^{-1}),(Pr2; 5 mM l^{-1}), glycine betaine (GB1;40 mM l^{-1}), (GB2; 60 mM l^{-1}), salicylic acid (SA1;5 µM l^{-1}), (SA2;10 µM l^{-1}), and control (distilled water) as a foliar spray. The findings indicated that the optimal treatments for enhancing vegetative growth (leaf fresh weight every cut, leaf dry weight every cut, total plant fresh and dry weight per season, and the total yield of plant fresh and dry weight per M^2) were Pr2, GB2, and SA1, in progressive order. Conversely, the least effective treatments were the control, followed by SA2 treatment, in both seasons. The application of SA and GB treatments resulted in enhancements in the content of ascorbic acid, chlorophyll, nitrogen, phosphorus, potassium, protein, and total oil in the parsley leaves. Where, SA1 and the GB2 treatments exhibited the most pronounced impact. At the same time, the Pr2 treatment showed the most significant effect on the proline and oil contents compared to the control, in both seasons. The phenols and fibers exhibited contrasting characteristics, with the control treatment giving the highest values, followed by SA2. Generally, the results indicate that the most effective treatments for enhancing the growth and quality of parsley plants grown under high-temperature stress conditions were with the foliar application of glycine betaine at a concentration of 60 mM l^{-1} and salicylic acid at a concentration of 5 µM l^{-1}.

Keywords: Parsley, High temperature stress, Glycine betaine, Salicylic acid, Proline, Vegetative growth, Chemical composition, Leaf quality.

INTRODUCTION

Parsley (*Petroselinum crispum* L.) is considered one of the most important culinary herbs and belongs to the *Apiaceae* family (Toaima *et al*., 2014). It is a biennial herbaceous plant; however, it is commonly planted annually. Its leaves are rich in antioxidant substances such as vitamins A, C, K, E, and B, β-carotene, minerals (especially potassium, calcium, phosphorus, and iron), crude fibres and protein (Alibas *et al.*, 2019; Barroso *et al.*, 2019 and Marthe, 2020), and volatile oils that give it its distinctive flavour (Simon and Quinn, 1988). The leaves of parsley plants are commonly used in folk herbal medicine, pharmaceutical industries, and cosmetics (Lopez *et al.*, 1999). Also, it is an essential ingredient in many salads, soups, and cooked vegetables because of its distinctive flavour and high nutritional value. Parsley plants need a cool climate to grow well; the optimal temperature is 7–16 °C for the best growth and highest quality. However, as the temperature rises, growth parsley plants is negatively affected and the quality of the plant decreases.

Heat stress is one of the most critical environmental factors that limit the growth and productivity of various plants. Its effects depend on plant growth, temperature, and the growth stage that the plant is exposed to heat stress (Ruelland & Zachowski, 2010; Hasanuzzaman *et al.,* 2013; Raza *et al.,* 2019 and Faiz *et al.,* 2020). One of the most important physiological processes that are negatively affected is the process of photosynthesis (Berry and Bjorkman, 1980). High temperatures severely affect vegetables, especially leafy ones, due to their short life cycle and the high water content of their leaves. In Chinese cabbage (*Brassica campestris* subsp. Napus var. pekinensis) and radish (*Raphanus sativus*) plants, their photosynthetic activity decreased when exposed to temperatures exceeding 25 °C. However, the effect of heat stress depended on the cultivar grown (Choi *et al.*, 2011 and Oh *et al.*, 2015). Also, exposing cabbage (*Brassica oleracea* var capitata group) and cabbage (*Brassica oleracea* acephala group) plants to

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high temperatures (32 °C) led to a change in weight and photosynthetic activity (Rodríguez *et al.*, 2015).

Proline has been shown to help reduce damage from oxidative stress and injury to membranes. It also raises the activity of antioxidant enzymes, the amount of water in leaves, and the efficiency of photosynthesis when plants are under stress (Delauney & Verma, 1993; Dar *et al.*, 2016; Priya *et al.*, 2019 and Ghosh *et al.*, 2022). Proline plays a significant role in the scavenging of hydroxyl radicals, osmotic regulation, interactions with stress tolerance enzymes, the protection of the protein structure, enzymatic and photosynthetic activities, the maintenance of the pH balance, and the supplementation of carbon, nitrogen, and energy (Szabados & Savouré, 2010 and Zouari *et al.*, 2019). It has been demonstrated that exogenous proline application increases plant tolerance to abiotic stress (Shane, 1986; Zouari *et al.*, 2019; Hussain *et al.*, 2021 and Gruszecki *et al.*, 2022).

Glycine betaine is a compatible osmolyte that plays a vital role in plant tolerance to environmental stresses, including high temperatures (Ashraf & Foolad, 2007; Annunziata *et al.*, 2019 and Al-Huqail *et al.*, 2020). Under these conditions, glycine betaine enhances the activities of enzymatic antioxidants such as catalase, superoxide dismutase, and peroxidase, which may mitigate the negative effect of uncontrolled reactive oxygen species causing oxidation (Zulfiqar and Ashraf, 2021).

Salicylic acid is classified as a hormone-like substance that acts as a crucial single molecule involved in plant responses to environmental stresses and plays an essential role in ameliorating plants' abiotic stress (Hayat *et al.*, 2010; Wang *et al.*, 2010; Fragnière *et al.*, 2011; Wassie *et al.*, 2020 and Serag *et al.*, 2021). Salicylic acid increased marketable yield, ascorbic acid, and total free amino acid contents in spinach and parsley plants (Elhamahmy, 2015).

Most previous studies were conducted under controlled or semi-controlled conditions. However, in this study, we tried to study the effect of these materials under field conditions during sammer season (from June to September). The prevailing weather conditions, in Egypt, are unsuitable for producing high-quality parsley, whether in the open fields or the non-air-conditioned greenhouses. Consequently, the market price of parsley is experiencing a significant surge. Therefore, this study aims to test several treatments to enhance parsley growth, productivity, and quality under open-field summer season conditions.

MATERIALS AND METHODS

1. Experimental Site and Weather Conditions:

Two field experiments were conducted during the summer seasons of 2020 and 2021, in Wadi El Natrun Reigon, El-Behera Governorate, Egypt (location: latitude 30.42, longitude 30.34). To find out about the physical and chemical properties of the soil at the test site before planting, 25-cm samples were taken from different parts of the field and analyzed using the methods described by Page *et al.* (1982). The results of soil physio-chemical analyses are shown in Table (1), and the weather conditions that occurred at the experiment site during the experiment are shown in Table (2).

2. Soil Preparation and Seed Sowing:

The experimental site was ploughed and properly leveled, and then plots were prepared according to the layout plan. Parsley seeds (*Petroselinum crispum* L.) cv. Balady were planted automatically on one meter-wide terrace, and seeding was done at a rate of eight lines per terrace on the 10^{th} and 15^{th} of June in 2020 and 2021 seasons, respectively, and a sprinkler irrigation system was used. The distance between one plant and another in the line was 7 cm, and the distance between one terrace and another was 60 cm. Adjacent treatments were isolated by one bare row without planting to avoid spray drift.

3. Treatments application and agro-management practices:

The following seven treatments were tested in this study: proline (Pr 1;2.5 mM l^{-1}) ,(Pr 2;5 mM l^{-1}), glycine betaine (GB 1;40 mM l^{-1}) , (GB 2; 60 mM l^{-1}), salicylic acid (SA 1;5 µM l^{-1}), (SA 2;10 µM l^{-1}), and control (distilled water). Treatments were applied as a foliar application at a suitable wind speed to minimize spray drift until run-off three times: 25, 65, and 90 days after seed sowing. All the other agro-management practices, such as cultivation, irrigation, nutrition, and pest control, were performed whenever necessary and as recommended for the commercial production of parsley, taking into account soil analysis in Table (1) and weather conditions in Table (2).

4. Measurement of Yield Components and Calculations:

A representative sample of six random plants were random chosen after each harvested cut from each experimental unit to measure morphological characters; leaf length, total fresh weight, and dry weight. The first cut was done after 55 days, the second cut was done 25 days after the first, and the third cut was done 25 days after the second.

5. Chemical constituents:

Leaves samples from six plants were collected and washed with tap water, then washed with distilled water three times before drying in a forced-air oven at 70 °C till constant weight,

Table 1. Some physical and chemical characteristics of the experimental sites during the summer seasons of 2020 and 2021.

Characteristics	2020 Season	2021 Season
Sand (%)	87.1	89.5
Silt (%)	7.3	5.4
Caly (%)	5.6	5.1
Textural class	Sandy	Sandy
pH	7.79	7.84
EC (ds m^{-1})	1.64	1.62
Organic matter%	0.21	0.24
CaCO3 %	1.86	1.89
Available N (mg kg^{-1})	23.2	25.6
Available K (mg kg^{-1})	2.84	2.64
Available P (mg kg^{-1})	49.62	48.51
Soluble Na (mmol l^{-1})	4.56	4.76
Soluble Ca (mmol l^{-1})	7.21	6.89
Soluble Mg (mmol l^{-1})	2.74	2.67
Soluble Cl (mmol l^{-1})	3.61	3.32
Soluble SO_4 (mmol l^{-1})	7.12	7.05

Table 2. Weather conditions prevailing in a location during the 2020 and 2021 seasons at two meters Data is retrieved from: power.larc.nasa.gov/data-access-viewer.

Month	Maximum Temperature (°C)		Minimum Temperature (°C)		Relative Humidity (%)		Specific Humidity (g kg^{-1})		Dew/Frost Point (°C)		Wet Bulb Temperature (°C)	
	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
JUN	42.65	41.53	15.12	16.26	48.31	47.62	9.34	9.64	12.67	13.08	19.94	20.41
JUL	41.58	44.12	18.76	20.32	50.75	48.19	11.29	11.41	15.43	15.66	22.48	23.12
AUG	43.22	43.97	20.54	20.94	52.62	50.5	12.15	12.08	16.66	16.38	23.3	23.67
SEP	42.45	41.07	20.73	18.85	55.19	53.56	12.45	11.54	17.11	15.99	23.26	22.06

NASA/POWER CERES/MERRA2 Native Resolution Monthly and Annual (Location: Latitude 30.42 Longitude 30.34). Parameter(s) at 2 meters.

then samples were ground in a Willy mill to pass a 30-mesh screen. Weights of 0.2 g of dried fine powder from leaves samples were digested using a mixture of hydrogen peroxide and sulfuric acid (Cottenie, 1980), and the following determinations were achieved: Total nitrogen (%) was determined using the MicroKjeldahel apparatus as defined by Chapman and Pratt (1962). The other minerals were analyzed after drying at 550 °C in a Muffle furnace and dissolved in deionized water to a standard volume. Potassium was determined by flame photometry, while phosphorus was determined by the vanadomolybdate method (A.O.A.C., 1990).

5.1. Determination of chlorophyll, total phenolic components, proline and ascorbic acid content in leaves:

Leaf chlorophyll content was measured using a chlorophyll content meter (SPAD-502-meter, Konica Minolta, Japan). Samples were prepared, and phenols were extracted according to the method described by Bouhlali *et al.* (2017). The Folin-Ciocalteu's reagent was used to colourimetrically quantify phenolic components in plant extracts, according to the method described by Singleton *et al.* (1999). Proline content in leaves was determined according to the method of Bates *et al.* (1973).

Ascorbic acid in the supernatants was detected at 525 nm. Ascorbic acid (Analytical Reagent, Solarbio) was used to create a standard curve. The amount of ascorbic acid is stated as mg/100 g of fresh weight. The crude leaf fibers were measured using Pearson's (1976) methods.

5.2. Essential oil extraction:

Essential oils were extracted according to the method of El-Massry *et al.* (2008). Fresh parsley leaves were cut into small pieces and hydro-distilled for 3 hours using a Clevenger-type device. The essential oils were extracted, dried with anhydrous sodium sulfate, and stored in airtight glass vials with aluminium foil until analysis at 20°C.

5.3. Determination of antioxidant enzyme activities and total protein content:

Extract the total enzymes according to Agarwal *et al.* (2005); 100 mg of fresh parsley leaves were ground with an ice-chilled extraction buffer. Bovine serum albumin was employed as a standard to determine the total protein content of the crude extract (Bradford, 1976). Activity of the enzyme superoxide dismutase (EC 1.15.1.1) (SOD) was assessed, as demonstrated by Gao *et al.* (2019). One unit of SOD activity was determined to be the appropriate amount of enzyme extract to produce 50% inhibition in the photochemical degradation of NBT. According to Aebi (1984), catalase (EC 1.11.1.6) (CAT) activity was detected by the elimination of H_2O_2 in the crude extract samples. Guaiacol and H_2O_2 were used as substrates to measure the peroxidase (EC 1.11.1.7) (POD) activity (Hemeda and Klein, 1990).

6. Experimental Design and Statistical Analysis:

The experimental design was a randomized complet block design consisting of four replicates, each replicate containing seven treatments that were randomly distributed within each replicate. Each experimental unit was planned to cover an area of 192 m^2, including 24 rows in three terraces 40 m long and 4.80 m wide. Data were, statistically, analyzed according to the design used by the COSTAT computer software program. When comparing means, Duncan's multiple range test was used with a significance level of $P < 0.05$ (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSIONS

1- Vegetative growth characteristics of parsley plants:

The experiment was conducted under severe environmental conditions in the study area and during the duration of the experiment, as shown in Table (2). Heat stress is defined as an increase of 10-15°C above the optimum temperature for growth, which negatively affects the plant's growth and development, reflected in the productivity (Wahid *et al.,* 2007). It turns out that growing parsley plants were exposed to high-temperature stress. The effects of foliar application of proline (Pr), glycine betaine (GB), and salicylic acid (SA) on controlling parsley growth traits are shown in Tables (3 and 4). The treatments had a positive effect on the growth of the parsley plant, as they improved the studied traits, as shown in Table (3). The best treatments were GB 2 and SA 1, and there was insignificant difference between them. As for the effect of the other treatments, their effect varied, but they were better than the control treatment, except for SA2. There was insignificant difference between it and the control treatment in each cut, total cuts, and production per square meter, as shown in Table (4). This difference varied between the two seasons, as there was insignificant difference between SA 2 and the control in the first season. However, there was a significant difference in the second season. Applying all treatments resulted in a significant increase in plant height compared to the control in both seasons. Compared with the control, SA1 and GB2 treatments produced higher plants in the first and second seasons. In addition, the treatments applied significantly improved the fresh and dry weight of the parsley plants compared to the control. SA1 and GB2, in both seasons and pr1, pr2, and SA2 treatments, in the second season, led to an increase in the weight of fresh and dry leaves per plant and per square meter compared to the control treatment. On the other hand, there were insignificant differences between Pr2, SA1, and GB2 treatments, in both seasons compared to the control. The number of leaves per plant increased significantly as a result of SA1 and GB2 treatments, in both seasons. The results indicate that all other applied treatments led to a significant increase in the three cuts compared to the control. The study found that parsley plants showed a significant increase in organic matter content with increasing harvest times during the summer growing season (Alan *et al.*, 2017), could be due to reduced net photosynthesis, stomatal conductance, and carboxylation efficiency (Choi *et al.,* 2011 and Oh *et al.,* 2015). However, with the application of these treatments, growth traits improved. Salicylic acid treatment significantly enhanced plant growth (Elhamahmy, 2015), Zinnia elegans (Mahroof *et al.,* 2017), pansy (El-Kinany, 2020), and Chinese flowering cabbage (Zhang *et al.*, 2022), in comparison with untreated plants. They found that treating plants with salicylic acid significantly increased their vegetative development properties, reduced respiration rate, ethylene production, reactive oxygen species (ROS), and increased superoxide dismutase, catalase, and peroxidase activities, delaying leaf senescence.

The effects of salicylic acid on plants are dose-dependent, with factors such as treatment period, plant type, age, and treated part determining the treatment's effectiveness. Glycine betaine, a plant treatment, has been found to significantly increase the weight of plants, reduce respiration rate and ethylene production, and decrease ROS. El-Kinany *et al.* (2019) found that foliar spraying with glycine betaine increased the weight of coriander plants and controlled the relative water content in *Brassica rapa* L. ssp. Pekineses compared to a heat-stressed control. Quan *et al.* (2023) found that transcription factors for the hormones cytokinin, auxin, salicylic acid, and abscisic acid were up- or down-regulated in *Brassica rapa* L. ssp. Pekinensis plants given glycine betaine under heat stress. Kaushal *et al.* (2011) observed that foliar application of proline improved vegetative growth and dry matter parameters in chickpea plants exposed to heat stress. In comparison, Rizwan *et al.* (2011) found that proline and glycine betaine improved the heat tolerance of sprouting sugarcane. These treatments may have a significant effect because they increase the activity of plant-based enzymes that fight free radicals, absorb more elements, or make photosynthetic pigments work better.

Table 3. Effect of foliar application of proline, salicylic acid, and glycine betaine on plant height, number of leaves per plant, leaf weight, leaves weight per plant, and dry weight of parsley during summer seasons of 2020 and 2021.

Treatments	Season of 2020			Season of 2021		
	First cut	Second cut	Third cut	First cut	Second cut	Third cut
			Plant height (cm)			
Control	24.73 C*	31.17 D	22.80 D	23.60 C	27.50 C	25.27 D
Pr 1	25.33 BC	32.97 C	24.67 C	24.73 B	30.93 B	27.83 C
Pr 2	26.43 AB	33.93 B	25.37 BC	26.23 A	31.63 AB	27.30 C
SA1	27.10 A	34.53 AB	26.37 B	26.73 A	32.90 A	31.30 A
SA2	25.40 BC	32.80 C	24.00 CD	25.10 B	28.77 C	27.23 C
GB 1	25.23 BC	34.33 AB	24.27 C	26.23 A	31.53 AB	27.93 C
GB 2	26.90 A	35.10 A	28.33 A	26.83 A	32.33 AB	29.63 B
			Number of leaves per plant			
Control	12.50 D	13.33 CD	13.33 CD	13.67 D	14.00 B	14.22 B
Pr 1	13.00 C	13.50 C	13.50 C	14.67 BCD	14.67 B	14.67 B
Pr 2	14.11 AB	14.44 A	14.56 A	15.33 ABC	15.67 A	15.89 A
SA1	14.45 A	14.67 A	14.67 A	15.89 A	16.00 A	16.00 A
SA2	13.22 C	13.67 C	13.78 C	14.33 CD	14.78 B	15.00 B
GB 1	13.67 B	14.00 B	14.00 B	15.33 ABC	16.11 A	16.33 A
GB 2	14.22 A	14.56 A	14.67 A	15.44 AB	16.00 A	16.44 A
			Leaf weight (g)			
Control	0.84 C	0.86 C	0.84 D	0.83 E	0.86 C	0.84 C
Pr 1	0.86 B	0.87 B	0.85 C	0.86 CD	0.87 BC	0.84 C
Pr 2	0.87 AB	0.87 B	0.86 B	0.87 BC	0.87 BC	0.85 BC
SA1	0.88 A	0.88 A	0.86 B	0.88 AB	0.88 AB	0.86 AB
SA2	0.86 B	0.87 B	0.84 D	0.85 D	0.86 C	0.84 C
GB 1	0.86 B	0.87 B	0.86 B	0.87 BC	0.88 AB	0.87 A
GB 2	0.88 A	0.88 A	0.87 A	0.89 A	0.89 A	0.87 A
			leaves weight per plant (g)			
Control	10.59 D	11.47C	11.20 C	11.39 D	11.99 B	11.95 C
Pr 1	11.56 C	11.74 BC	11.66 BC	12.57 BC	12.76 B	12.37 C
Pr 2	12.18 BC	12.61 AB	12.47 AB	13.34 AB	13.63 A	13.51 B
SA1	12.86 A	13.05 A	12.80 A	13.98 A	14.03 A	13.76 AB
SA2	10.85 D	11.36 C	11.15 C	12.18 CD	12.76 B	12.55 C
GB 1	12.13 BC	12.71 A	12.66 A	13.39 AB	14.12 A	14.21 AB
GB 2	12.66 AB	12.86 A	12.71 A	13.60 A	14.19 A	14.31 A
			Dry weight per plant (g)			
Control	1.52 D	1.75 C	2.02 C	1.65 D	1.84 B	2.21 C
Pr 1	1.66 C	1.82 BC	2.10 BC	1.82 BC	1.95 B	2.29 C
Pr 2	1.75 BC	1.93 AB	2.24 AB	1.93 AB	2.09 A	2.50 B
SA1	1.85 A	2.00 A	2.30 A	2.03 A	2.14 A	2.55 AB
SA2	1.56 D	1.74 C	2.01 C	1.77 CD	1.95 B	2.32 C
GB 1	1.74 BC	1.94 A	2.28 A	1.94 AB	2.16 A	2.63 AB
GB 2	1.82 AB	1.97 A	2.29 A	1.97 A	2.17 A	2.65 A

*Values having the same alphabetical letter (s), do not significantly differ, using the L.S.D. test at 0.05 level.

Table 4. Effect of foliar application of proline, salicylic acid, and glycine betaine on foliage fresh weight per plant, foliage dry weight, fresh herb yield, and dry herb yield of parsley during summer seasons of 2020 and 2021.

Treatments	Season of 2020				Season of 2021			
	Foliage fresh weight / plant (g)	Foliage dry weight/plant (g)	Fresh herb yield (g/m^2)	Dry herb yield (g/m^2)	Foliage fresh weight per plant (g)	Foliage dry weight (g)	Fresh herb yield (g/m^2)	Dry herb yield (g/m^2)
Control	32.22 C*	5.29 B	4743.34 C	755.41 B	35.33 C	5.70 C	5045.60 C	813.48 C
Pr 1	35.11 B	5.58 B	5014.18 B	797.30 B	37.69 B	6.07 B	5382.61 B	866.32 B
Pr 2	37.27 A	5.93 A	5322.16 A	846.33 A	40.47 A	6.52 A	5779.59 A	931.06 A
SA1	38.71 A	6.15 A	5527.79 A	878.70 A	41.77 A	6.72 A	5964.76 A	959.62 A
SA2	33.37 BC	5.31 B	4765.24 BC	758.27 B	37.49 B	6.04 B	5353.57 B	862.04 B
GB 1	37.51 A	5.97 A	5356.43 A	852.99 A	41.72 A	6.73 A	5957.14 A	960.57 A
GB 2	38.23 A	6.08 A	5459.72 A	868.22 A	42.09 A	6.79 A	6010.93 A	969.14 A

*Values having the same alphabetical letter (s), do not significantly differ, using the L.S.D. test at 0.05 level.

2. Nitrogen, phosphorus, potassium, and protein contents of parsley plants:

The data presented in Table (5) shows the effect of the different treatments on the leaf content of nitrogen, phosphorus, potassium, and protein. The treatments made the leaves much higher in nitrogen, phosphorus, potassium, and protein compared to the control treatment. The SA2 treatment was the only one that changed from the first to the second season. In the study of Hayat *et al.* (2009), it was noted that the high temperature reduces the nitrogen, phosphorus, and potassium in the leaves of Indian mustard plants. According to Rasheed *et al.* (2011), soaking nodal buds of sugarcane in a solution containing proline or glycine betaine improved K^+ and Ca^{2+} levels under heat stress. Khodabakhsh and Danaee (2022) the capacity and rate of photosynthesis are enhanced by using organic osmolytes like proline and glycine-betaine, in addition to absorbing ions like magnesium and potassium as well as delaying the degradation of pigment-protein compounds. According to studies carried out (Sakhabutdinova *et al.,* 2003; Sheehy *et al.,* 2005; Teixeira *et al.,* 2010 and Akasha *et al.,* 2019), the application of salicylic acid as a foliar application resulted in elevated levels of nitrogen, phosphorus, potassium, and total soluble protein in various plants such as Indian mustard, wheat, and rice exposed to heat stress. Salicylic acid increased N assimilation in wheat plants under heat-stress conditions (Khan *et al.,* 2013) by improving the enzymatic activities of nitrite and nitrate reductase enzymes (Akasha *et al.,* 2019).

3. Proline content, Catalase, Peroxidase, and Superoxide Dismutase of parsley plants:

The data in Table (6) show the proline content and activities of catalase, peroxidase, and superoxide dismutase in the leaves in each plot. From the results, it is clear that all treatments significantly affect the proline content in the plant compared to the control. The treatments varied, and the highest estimated proline values were obtained under the Pr2 treatment. The highest catalase and peroxidase activity values were obtained in both study seasons under the GB2 treatment.

At the same time, there was insignificant difference between the SA1 and GB2 treatments in their effect on the activity of the superoxide dismutase enzyme in the leaves. Heat stress in plants leads to reactive oxidative species (ROS) forming, which damage cell membranes, lipid peroxidation, and protein degradation. This can inhibit root growth (Hasanuzzaman *et al.,* 2013 and Brengi, 2018), negatively affect chlorophyll content, and potentially lead to death (Das & Roychoudhury, 2014 and Mittler, 2017). Studies have shown that enzymatic antioxidants are higher in rice plants treated with salicylic acid, which reduces ROS levels and increases antioxidant activities in rice plants (Zhang *et al.,* 2017), reduces the respiration rate and ethylene production in Chinese flowering cabbage (Brassica rapa var. parachinensis), decreases the levels of reactive oxygen species (ROS), and increases the activities of superoxide dismutase, catalase, and peroxidase, which resulted in delaying leaf senescence (Zhang *et al.,* 2022). Proline, an exogenous agent, has also been found to have a positive effect on plants, especially under heat stress. By increasing the activity of antioxidative enzymes such as catalase (CAT), peroxidase (POX), and superoxide dismutase (SOD), exogenous administration of proline has been observed to have a positive effect in the study conducted by Hayat *et al.* (2012). Okra (*Abelmoschus esculentus* L.) leaves treated with 2.5 mM proline showed the highest amounts of proline under heat stress (Hussain *et al.,* 2021). Glycine betaine, which activates antioxidant enzymes, protects plants from oxidative damage. According to Rasheed *et *al.* (2017), glycine betaine protects plants from oxidative harm by activating various antioxidant enzymes such as peroxidase (POX), catalase (CAT), and superoxide dismutase (SOD) in plants. This activation subsequently protects the plant against oxidative damage. According to Rasheed *et al.* (2011), soaking nodal buds of sugarcane in a solution containing proline or glycine betaine increased free proline concentrations, improving the buds' ability to tolerate heat.

4. Leaf content of oil, ascorbic acid, fibers, phenols, and total chlorophyll of parsley leaves:

Table (7) shows some quality characteristics of parsley leaves that were estimated for each cut, and they included the leaf content of oil, ascorbic acid, fibre, phenols, and total chlorophyll expressed in the SPAD unit. The planting date affects the dry matter content of parsley plants and the leaves' content of phenolic substances and chlorophyll: antioxidants and ascorbic acid. In a study by Alan *et al.* (2017), significant differences were found in parsley leaves dry matter content, colour values, and total phenols between harvest times for the summer and winter growing seasons.

Table 5. Effect of foliar application of proline, salicylic acid, and glycine betaine on nitrogen, phosphorus, potassium,and protein content of parsley during summer seasons of 2020 and 2021.

Treatments	Season of 2020			Season of 2021		
	First cut	Second cut	Third cut	First cut	Second cut	Third cut
			Nitrogen %			
Control	2.68 C*	2.70 B	2.72 B	2.71 D	2.77 C	2.79 B
Pr 1	2.77 B	2.79 A	2.82 A	2.78 C	2.84 B	2.87 A
Pr 2	2.83 A	2.82 A	2.83 A	2.85 AB	2.87 AB	2.88 A
SA1	2.84 A	2.82 A	2.85 A	2.85 AB	2.88 AB	2.91 A
SA2	2.70 C	2.69 B	2.72 B	2.73 D	2.76 C	2.79 B
GB 1	2.80 AB	2.79 A	2.83 A	2.83 B	2.85 AB	2.90 A
GB 2	2.84 A	2.84 A	2.87 A	2.86 A	2.89 A	2.93 A
			Phosphours %			
Control	0.63 D	0.65 D	0.66 C	0.62 C	0.63 D	0.62 C
Pr 1	0.65 C	0.67 BC	0.67 BC	0.64 B	0.66 BC	0.65 B
Pr 2	0.68 AB	0.68 AB	0.69 AB	0.67 A	0.68 AB	0.67 AB
SA1	0.70 A	0.70 A	0.70 A	0.69 A	0.69 A	0.70 A
SA2	0.65 C	0.66 CD	0.67 BC	0.65 B	0.65 CD	0.65 B
GB 1	0.67 B	0.68 AB	0.69 AB	0.67 A	0.67 AB	0.68 A
GB 2	0.69 A	0.70 A	0.70 A	0.68 A	0.69 A	0.69 A
			Potassium %			
Control	2.79 D	2.82 C	2.84 D	2.77 D	2.83 E	2.86 D
Pr 1	2.82 CD	2.87 BC	2.87 CD	2.85 B	2.86 CD	2.88 CD
Pr 2	2.84 BC	2.87 BC	2.88 BCD	2.86 B	2.89 BC	2.91 BC
SA1	2.88 B	2.90 AB	2.94 AB	2.87 B	2.90 B	2.95 A
SA2	2.81 CD	2.84 BC	2.87 CD	2.82 C	2.84 DE	2.86 D
GB 1	2.86 B	2.89 B	2.93 ABC	2.86 B	2.88 BC	2.94 AB
GB 2	2.92 A	2.95 A	2.96 A	2.89 A	2.94 A	2.97 A
			Protein %			
Control	16.77 C	16.90 B	17.02 B	16.94 D	17.32 C	17.44 B
Pr 1	17.34 B	17.42 A	17.63 A	17.40 C	17.73 B	17.96 A
Pr 2	17.71 A	17.61 A	17.67 A	17.79AB	17.94 AB	18.02 A
SA1	17.73 A	17.63 A	17.81 A	17.84 AB	17.98 AB	18.17 A
SA2	16.90 C	16.81 B	16.98 B	17.09 D	17.27 C	17.42 B
GB 1	17.52 AB	17.42 A	17.67 A	17.69 B	17.84 AB	18.11 A
GB 2	17.77 A	17.73 A	17.96 A	17.87 A	18.08 A	18.31 A

*Values having the same alphabetical letter (s), do not significantly differ, using the L.S.D. test at 0.05 level.

Table 6. Effect of foliar application of proline, salicylic acid and glycine betaine on Proline content, Catalase, Peroxidase, and Superoxide Dismutase of parsley during summer seasons of 2020 and 2021.

Treatments	Season of 2020			Season of 2021		
	First cut	Second cut	Third cut	First cut	Second cut	Third cut
			Proline (mmol g^{-1} DW)			
Control	0.253 E*	0.240 E	0.220 E	0.313 E	0.300 E	0.283 C
Pr 1	0.397 B	0.317 D	0.373 A	0.410 B	0.403 B	0.387 A
Pr 2	0.417 A	0.407 A	0.390 A	0.440 A	0.427 A	0.393 A
SA1	0.393 B	0.377 B	0.350 B	0.407 B	0.397 B	0.373 A
SA2	0.330 D	0.313 D	0.293 D	0.343 D	0.323 D	0.307 C
GB 1	0.340 D	0.383 B	0.290 D	0.353 CD	0.330 D	0.310 C
GB 2	0.370 C	0.350 C	0.327 C	0.380 BC	0.363 C	0.340 B
			Catalase (Unit g^{-1} FW min^{-1})			
Control	26.04 D	26.80 E	26.63 E	25.84 D	26.69 D	25.57 D
Pr 1	27.90 C	29.31 C	28.41 D	28.43 BC	28.95 C	28.81 B
Pr 2	30.67 B	30.93 B	30.70 C	30.57 A	31.13 B	30.71 A
SA1	30.80 B	30.99 B	32.04 B	30.77 A	31.00 B	31.07 A
SA2	27.53 C	27.69 D	27.70 DE	27.24 C	27.51 D	27.52 C
GB 1	30.27 B	31.33 B	31.17 BC	28.73 B	29.02 C	29.24 B
GB 2	32.80 A	33.57 A	33.60 A	31.43 A	32.80 A	31.84 A
			Peroxidase (Unit g^{-1} FW min^{-1})			
Control	1.94 D	1.99 D	2.00 C	1.96 C	1.99 D	2.00 B
Pr 1	2.03 C	2.05 BC	2.07 B	2.01 B	2.02 C	2.03 AB
Pr 2	2.04 BC	2.05 BC	2.07 B	2.03 B	2.04 B	2.02 AB
SA1	2.03 C	2.04 C	2.06 B	2.02 B	2.04 B	2.03AB
SA2	2.03 C	2.05 BC	2.06 B	2.01B	2.03 BC	2.04 AB
GB 1	2.06 AB	2.07 AB	2.10 A	2.06 A	2.06 A	2.08 A
GB 2	2.07 A	2.09 A	2.11 A	2.08 A	2.07 A	2.08 A
			Superoxide Dismutase (Unit g^{-1} FW min^{-1})			
Control	1.43 D	1.45 E	1.50 E	1.44 C	1.48 E	1.52 D
Pr 1	1.72 C	1.80 D	1.84 C	1.75 B	1.86 C	1.86 B
Pr 2	2.03 B	2.10 C	2.14 B	2.13 A	2.11 B	2.22 A
SA1	2.17 A	2.22 A	2.25 A	2.19 A	2.21 A	2.26 A
SA2	1.72 C	1.78 D	1.79 D	1.68 B	1.70 D	1.70 C
GB 1	2.16 A	2.17 B	2.23 A	2.16 A	2.19 A	2.24 A
GB 2	2.19 A	2.23 A	2.26 A	2.21 A	2.26 A	2.29 A

*Values having the same alphabetical letter (s), do not significantly differ, using the L.S.D. test at 0.05 level.

On the other hand, antioxidant values and ascorbic acid concentration were insignificantly affected by harvest times for both growing seasons. The values of these characteristics are shown in Table (7). The highest values obtained for the percentage of oil were under the Pr2 treatment, followed by SA1. Salicylic acid had the highest values for ascorbic acid and chlorophyll. Conversely, The highest values for the percentage of fibers and phenolics were obtained under the control treatment followed by the SA2 treatment. These results are in agreement with what he found. Zhang *et al.* (2022) found that treatment of Chinese flowering cabbage (*Brassica rapa* var. parachinensis) leaves enhanced the accumulation of phenols and flavonoids in the plant leaves treated with SA compared to plants that were not treated., while the lowest values were under the GB2 treatment in the two seasons of the study. High temperatures (33°C) induced bolting and bitterness processes, reducing the quality of dark red "Lollo" Rosso (*Lactuca sativa* L.) lettuce plants grown in a growth room for 30 days, compared to plants grown at a moderate temperature (25°C) (Sublett *et al.*, 2018).

Table 7. Effect of foliar application of proline, salicylic acid and glycine betaine on essential oil percent, ascorbic acid, fiber, total chlorophyll (SPAD Unite), and total Phenolic content of parsley leaves during summer seasons of 2020 and 2021.

Treatments	Season of 2020			Season of 2021		
	First cut	Second cut	Third cut	First cut	Second cut	Third cut
			Essential oil percent (%)			
Control	0.029 A*	0.038 C	0.038 E	0.030 C	0.037 C	0.039 D
Pr 1	0.030 A	0.039 BC	0.039 DE	0.031 C	0.037 C	0.038 CD
Pr 2	0.040 A	0.041 A	0.044 A	0.037 A	0.043 A	0.043 A
SA1	0.040 A	0.042 A	0.043 AB	0.037 A	0.041B	0.043 A
SA2	0.028 A	0.039 BC	0.040 CDE	0.029 D	0.037 C	0.040 BC
GB 1	0.030 A	0.040 B	0.042 ABC	0.031 C	0.040 B	0.042 AB
GB 2	0.032 A	0.042 A	0.041 BCD	0.033 B	0.040 B	0.040 BC
			Ascorbic acid (mg100g^{-1} FW)			
Control	123.94 E	128.31 C	122.10 C	125.52 D	127.70 C	128.13 C
Pr 1	126.27 CD	129.28 BC	130.85 B	126.76 CD	127.72 C	128.30 C
Pr 2	127.80 BC	131.28 AB	131.83 AB	128.03 C	130.27 B	131.54 AB
SA1	130.70 A	132.27 A	133.54 A	131.92 A	132.64 A	133.28 A
SA2	125.83 CD	129.28 BC	130.07 B	125.37 D	128.18 C	129.86 BC
GB 1	129.80 A	130.91 AB	131.49 AB	129.95 B	130.04 B	131.40 AB
GB 2	129.25 AB	130.91 AB	132.42 AB	130.30 B	131.75 A	131.83 AB
			Fibers (g100g^{-1} D.W)			
Control	3.36 A	3.37 A	3.38 A	3.35 A	3.37 A	3.37 A
Pr 1	3.35 AB	3.35 BC	3.37 AB	3.34 AB	3.35 ABC	3.36 A
Pr 2	3.33 BC	3.35 BC	3.36 BC	3.33 ABC	3.34 BCD	3.35 A
SA1	3.32 C	3.34 CD	3.35 C D	3.27 BC	3.34 BCD	3.32 B
SA2	3.34 BC	3.36 B	3.37 AB	3.33 ABC	3.36 AB	3.36 A
GB 1	3.33 BC	3.35 BC	3.35 CD	3.32 BC	3.34 BCD	3.35 A
GB 2	3.27 D	3.33 D	3.34 D	3.31 C	3.33 D	3.36 A
			Total chlorophyll (SPAD Unite)			
Control	41.60 D	42.53 C	42.83 C	42.33 C	42.67 C	43.33 C
Pr 1	42.30 C	43.07 ABC	43.63 ABC	43.00 BCD	43.56 B	43.76 BC
Pr 2	43.30 AB	43.63 AB	43.83 AB	43.78 AB	44.00 AB	44.20 AB
SA1	43.63 A	43.97 A	44.40 A	44.09 A	44.45 A	44.66 A
SA2	42.30 C	42.73 BC	43.17 BC	42.67 CD	43.31 BC	43.62 BC
GB 1	43.30 AB	43.63 AB	44.30 A	43.11 BCD	43.76 AB	44.30 AB
GB 2	42.67 BC	43.17 ABC	43.73 ABC	43.30 ABC	43.76 AB	44.53 A
			Total Phenolic contents (mg GAE100 g^{-1} FW)			
Control	125.87 A	117.47 A	114.40 A	126.47 A	118.33 A	114.47 A
Pr 1	121.23 BC	114.83 BC	113.70 ABC	124.03 B	115.80 B	112.53 BC
Pr 2	120.83 BC	114.27 BC	112.10 BC	121.17 CD	115.00 B	112.70 ABC
SA1	118.50 CD	114.40 BC	112.23 ABC	119.63 D	114.07 B	112.93 ABC
SA2	122.80 AB	116.20 AB	113.97 AB	122.07 C	115.47 B	113.40 AB
GB 1	119.10 BCD	113.83 BC	112.30 ABC	120.00 D	115.43 B	112.97 ABC
GB 2	115.50 D	112.50 C	110.90 C	117.60 E	114.77 B	111.40 C

*Values having the same alphabetical letter (s), do not significantly differ, using the L.S.D. test at 0.05 level.

In the study of Hayat *et al*. (2009), it was noted that the high temperature reduces the chlorophyll (SPAD) value of Indian mustard leaves. These results were consistent with what was found by Hussain *et al.* (2021). A foliar spray of proline at 2.5 mM on okra (*Abelmoschus esculentus* L.) leaves showed the highest chlorophyll content under heat stress. However, applying proline twice or thrice decreased the total essential oil content and altered the essential oil content's composition in parsley with turnip root (Gruszecki *et al.,* 2022).

Salicylic acid has been found to improve photosynthesis (Ahmed *et al.,* 2020) in peas, increase ascorbic acid and total free amino acids (Elhamahmy, 2015) in spinach and parsley, control proline metabolism, and enhance nitrogen uptake, proline metabolism and photosynthesis in various plants (Khan *et al.,* 2013). It also reduces respiration rate and ethylene production in Chinese flowering cabbage and delays leaf senescence (Zhang *et al.,* 2022). Salicylic acid also increases essential oil in Salvia macrosiphon (Gruszecki *et al.,* 2022), improves seed oil content in dill plants (Ghassemi-Golezani, 2022), enhances the amount of seed oil, and reduces the adverse effects of stress on sunflowers (Noreen and Ashraf, 2010). It also increases the total phenolic content of peppermint when applied exogenously (Cappellari *et al.,* 2020). According to Kiddle *et al.* (1994), oilseed rape (*Brassica napus* L.) plants that received a salicylic acid soil drench had higher levels of glucosinolates in their leaves and increased proline and essential oil content of chamomile under normal and heat stress conditions (Ghasemi *et al.,* 2016). These findings suggest that salicylic acid can benefit various plants, including those tolerant of high temperatures.

Foliar spraying of glycine betaine increased the chlorophyll content and fluorescence of Chinese cabbage (Quan *et al.,* 2022) and eggplant (Niu *et al.,* 2023). Thus, Glycine betaine can stabilize photosynthesis in heat-stressed plants, promoting growth under heat stress. Glycine betaine can also significantly prevent photoinhibition by stabilizing the structure of the oxygen-evolving center (PSII) (Yang *et al.,* 1996 and Allakhverdiev *et al.,* 2007).

Previous results, which were presented in five Tables (3-7), it is clear that proline, glycine betaine, and salicylic acid, at low concentrations, have a significant effect on improving the growth and productivity of parsley in the study area and under the summer climate shown in Table (2). This positive effect may be due to improving the activity of enzymatic antioxidants, represented by the catalase, peroxidase and superoxide dismutase enzymes and increase the leaves' content of proline and ascorbic acid. As well as increasing the leaves' content of macroelements such as nitrogen, phosphorus, and potassium, which preserved the leaves' chlorophyll content and was then reflected in growth and quality by increasing the leaves' content of chlorophyll, the percentage of total oil and ascorbic acid, and reducing fiber and phenols.

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الملخص العربي

التخفيف من آثار الإجهاد الناتج عن درجات الحرارة المرتفعة على نباتات البقدونس بالرش الورقي بالبرولين والجلايسين بيتائين وحمض السالسيليك

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البقدونس نبات عشبي مميز ذو خصائص طبية يحتاج الي درجات حرارة منخفضة ورطوبة نسبية مرتفعة لتحقيق النمو الامثل مع جودة عالية. وعلى الرغم من الطلب المحلي والتصديري المتزايد عليه فإن تلبية هذه الظروف خلال فصل الصيف في مصر يمثل تحديًا. ولذلك هدفت هذه الدراسة إلى التخفيف من الآثار الضارة لدرجات الحرارة المرتفعة على نمو وجودة البقدونس الصنف البلدي. تم إجراء تجربتين حقليتين تحت نظام الري بالرش في محافظة البحيرة– مركز وادي النطرون، خلال الموسم الصيفي لعامي ٢٠٢٠ ، و٢٠٢١. وتمت دراسة المعاملات السبعة التالية: البرولين (Pr1:٢,٥ ملي مول للتر)، (Pr ٢: ٥ملي مول للتر)، جلايسين بيتائين (GB١: ٤٠ ملي مول للتر) ، (GB ٢: ٦٠ ملي مول للتر) ، حمض الساليسيليك (SA١: ٥ميكرو مول للتر)، (SA٢ : ١٠ميكرو مول للتر)، والكنترول (الماء المقطر) رشا ورقيا كل حشة. أشارت نتائج الدراسة إلى أن افضل النتائج لصفات النمو الخضري متمثلا في (الوزن الطازج للأوراق لكل حشة، الوزن الجاف للأوراق لكل حشة، الوزن الكلي للنبات الطازج والجاف في الموسم، محصول النبات بالوزن الطازج والجاف(لكل متر مربع) هيGB ٢ ، SA ١ ، و Pr٢ بالترتيب التدريجي. على العكس من ذلك، كانت المعاملات الأقل فعالية هي معاملة الكنترول تليها SA ٢خلال كلا موسمي الدراسة. كما أدى تطبيق المعاملات إلى تحسين محتوى حامض الاسكوربيك، والكلوروفيل، والنيتروجين، والفوسفور، والبوتاسيوم، والبروتين، والزيت الكلي في الأوراق. وقد أظهرت معاملات SA١، وGB٢ التأثير الأكثر وضوحًا. وفي الوقت نفسه أظهرت معاملة Pr٢ التأثير الأكبر على محتوى البرولين، والزيت مقارنة بالكنترول في كلا موسمي الدراسة. أظهرت الفينولات، والألياف خصائص متناقضة حيث أعطت معاملة الكنترول أعلى القيم تليها SA٢. تشير نتائج هذه الدراسة إلى أن المعاملات الأكثر فعالية لتحسين نمو، وجودة البقدونس في ظل ظروف إجهاد درجات الحرارة العالية هي تطبيق الجلايسين بيتائين من خلال الرش الورقي الورقي بتركيز ٦٠ ملي مول للتر وكذلك حمض الساليسيليك بتركيز ٥ ميكرومول للتر.

الكلمات المفتاحية: البقدونس، ارتفاع درجة الحرارة، الجلايسين بيتائين، حمض الساليسيليك، البرولين، النمو الخضري، التركيب الكيميائي، جودة الأوراق.