



Article

Using some bio-stimulants to overcome heat stress effects on tomato plants productivity

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Abstract: Heat stress poses a major challenge to tomato plants as global warm temperatures continue to rise and its negative effects on plants performance (physiological, bio-chemical and productivity). This research assessed and evaluated the response of tomato plants “Alisa hybrid” to natural heat stress using some bio-stimulants as foliar treatments during the late summer seasons of 2024 and 2025 at a vegetables private farm in Mansoura district, Dakhlia Governorate, Egypt in clay soil conditions and using drip irrigation system. Eight treatments were applied in a complete randomized block design system with three replicates included Brassinolide at 250 ppm, Glutathione at 200 ppm, Methyl jasmonate at 100 ppm, Melatonin at 150 ppm, Glycine betaine at 2 g/L, Spirulina at 2.5%, Jasmonic acid at 100 ppm plus the control treatment (spraying only water). The findings revealed that most of studied parameters significantly reduced as a result of heat stress conditions in both seasons, while exogenous application of all mentioned substances improved plants performance. Melatonin application significantly mitigated heat stress effects and recorded maximum plant growth, chemical contents, enzymes activity, phyto-pigments and hormones as well as yield and quality followed by glycine betaine treatment in most cases. This mitigation effect by applied treatments is likely attributable to stimulate nutrients uptake and osmotic adjustment in tomato plants. Also, these results highlight the vital role of either melatonin or other foliar substances for improving productivity of tomato plants under heat stress conditions.

Key words: Tomato, heat stress, bio-stimulant, melatonin, glycine betaine.

1. Introduction

Tomato (*Solanum Lycopersicum* L.) is a vital member of the Solanaceae family. It considered one of the most important vegetable crops in Egypt and worldwide due to either cultivation in a wide range of soil and climatic conditions or its economic and nutritional values. It contributes in human health due to its high contents of vitamins, carotenoids, lycopene, minerals, and antioxidants. In Egypt, tomato is daily and widely consumed as fresh vegetables or as processed food as sauce, ketchup, soups, juices, as well as dry powder. It cultivated and grown worldwide but remains highly sensitive to heat stress

conditions, with an optimal growth temperatures of 21-24° C (Singh *et al.*, 2017). Heat shocks or stress was defined as a rise in temperature for a period of time higher than the optimum level and its effect depends on the speed of rise in temperature and the total period. Besides, the frequency of heat shocks, day or night high temperatures are considered as a main limiting factor for growth. High temperatures negatively affect phyto-synthesis, water relations, nutrients uptake as well as respiration and membrane stability ended by low quality and quantity of tomato fruits yield (Singh *et al.*, 2017). Temperature above 35° C severely restricts growth, flowering, bio-constituent activity, fruit set and development as well as yield and quality of tomato plants (Alsamir *et al.*, 2021 and Sellami and Kooli, 2026). Heat exposure is characterized by oxidative stress, which arises from an overproduction of reactive oxygen species (ROS) that can impair cellular functions (Khan *et al.*, 2024a). Plants enhance their antioxidant defenses by boosting the activity of enzymes such as peroxidase, catalase superoxide dismutase, to counteract the damage caused by ROS. These enzymatic actions are vital to preserve cellular balance (Bhattarai *et al.*, 2021). Also, plants initiate non-enzymatic strategies, such as accumulating antioxidants and producing heat shock proteins (Fig. 1).

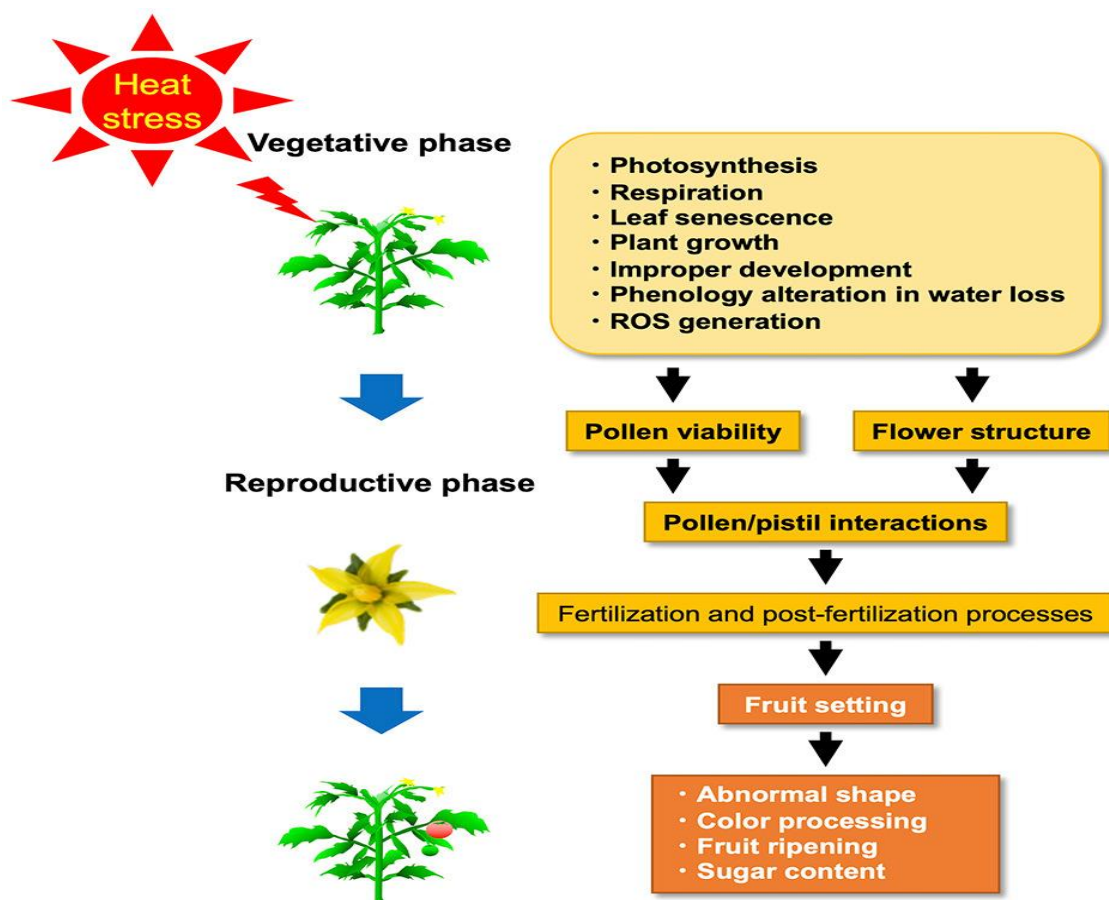


Figure (1). Adverse effects of heat stress on tomato vegetative and reproductive stages (Hoshikawa *et al.*, 2021)

Therefore, alternative strategies such as bio-stimulants are attracting attention to enhance tomato plants resilience under heat stress conditions. Applying certain stimulants such as brassinolide, melatonin, glycine betaine, jasmonic acid, glutathione, methyl jasmonate and spirulina extract, can improve plant development and enhance fruit set, yield as well as quality characteristics of tomatoes under high temperature. Bio stimulants are either natural or synthetic substances that can be applied to seeds, foliar or soil application to influence changes in vital and structural processes in plants to order to enhance plant growth through improved tolerance to abiotic stresses and increase yield and quality.

Brassinolide, a growth regulator which is known as a raw fatty extract extracted from *Brassica napus*, is also presents in plants in their unprocessed form or in combination with sugars or fatty acids. Spraying brassinolide enhances yield and quality of tomato (Rahman and Ahmad, 2025 and Ullah *et al.*, 2025).

Melatonin is a hormone or molecule induced naturally in both animals and plants (Siddiqui *et al.*, 2020), plays vital biological functions in plants against stresses by prompting strong defensive mechanisms as a master regulator in plants (Kaya *et al.*, 2020). Numerous physiological systems, such as stomatal conductance, photosynthesis, root dynamics, carbohydrate absorption, and water relations, are assisted by melatonin in withstanding biotic stressors. Additionally, melatonin increases antioxidant enzyme activity and respiration, which together lessen oxidative damage to proteins, lipids, and nucleic acids (Moustafa *et al.*, 2020). In this regard, spraying melatonin enhanced the growth, yield and quality of tomato plants (Mumithrakamatchi *et al.*, 2024 and Ibrahiem and Ahmed, 2025).

Glycine betaine, an amino acid derivative builds up in a number of plant species in reaction to environmental stressors including high temperatures. Therefore, exogenous glycine betaine application could be an alternate strategy to withstand environmental stress (Chang *et al.*, 2026). Foliar application of glycine betaine enhanced vegetative growth, antioxidant enzyme activities and economic yield (Ragab, *et al.*, 2015 and Ramadan and Shalaby, 2024) of tomato-treated plants. Additionally, Jasmonates are well characterized as defense phyto-hormones involved in numerous physiological and biochemical processes related to plant growth and development, as well as resistance to both biotic and abiotic stress (Kaushik *et al.*, 2022). When tomato plants are sprayed with jasmonates, their tissues develop defense proteins, polyphenol oxidases and protease inhibitors, among other phytochemical changes (Koiwa *et al.*, 1998). Exogenous applications of jasmonates on tomato plants grown under high-temperature conditions significantly improved plants performance and defense against stress (Okasha *et al.*, 2023 and Ramadan and Shalaby, 2024).

Marine algae extract also stimulates plant growth because it contains numerous macro and micronutrients, amino acids, vitamins, phyto-hormones (auxins, gibberellins, and cytokines), polypeptides, antioxidants, and compounds with antifungal and antibacterial properties (Godlewska *et al.*, 2019). Among these algae is *Spirulina platensis*, a blue green, spiral-shaped organism that scientists categorize as algae due to its chlorophyll content, which is akin to that found in higher plants. It was used in the past as a human food because it contains carbohydrates, vitamins, proteins and pigments such as carotenoids and chlorophyll A (ALzarie and El-Gamal, 2025). Spraying tomato plants with *Spirulina platensis* extract enhanced plant growth, leaf chlorophyll, flower number, enzyme activity, such as peroxidase as well as economic yield (Khaled *et al.*, 2023). Glutathione is a crucial tri-peptid-ethiol molecule that increases plants' resilience to abiotic stressors (Banerjee and Roychoudhury, 2019). According to Hossain *et al.* (2022), glutathione is essential for detoxifying reactive oxygen species and shielding cells from oxidative damage. Additionally, glutathione is involved in other processes such protein synthesis, enzyme control, and DNA creation and repair (Pompella *et al.*, 2003). In this regard, foliar application of glutathione significantly improved cucumber plants performance (Al-shammari *et al.*, 2020). Therefore, this study aims to investigate the effects of some stimulant materials to reduce the negative effect of high temperature on tomatoes under late summer plantation and to achieve higher productivity and better quality of tomato fruits under the experiment conditions.

2. Materials and Methods

Experimental site description: At a private farm in Mansoura district, Dakahlia Governorate, Egypt, a field experiment was conducted during the two late summer seasons in 2024 and 2025 to evaluate the effects of some bio-stimulators on growth, yield, and fruit quality of tomato “Alisa hybrid” grown in clay soil conditions using a drip irrigation system to mitigate the detrimental effects of high temperature on tomatoes in late summer. Some physical and chemical properties of the experimental soil of cultivated area listed in Table 1. The meteorological data at Dakahlia Government during the experimental seasons are presented in Table 2.

Table (1). Some physical and chemical properties of the experimental soil profile of cultivated area before planting during the two seasons 2024-2025

Soil properties		2024	2025
Physical analysis	Fine sand %	18.14	18.34
	Coarse sand %	7.71	7.91
	Silt %	33.65	33.45
	Clay %	40.50	40.30
	Texture	Clay-loam	Clay-loam
Chemical analysis	pH	7.91	7.99
	E.C. (dSm ⁻¹)	1.12	1.13
	Organic matter %	1.45	1.45
	Available P (ppm)	11.72	11.70
	CaCO ₃	4.55	4.58
	Total N %	0.2	0.2
Soluble anions (meq/L)	Cl ⁻	3.56	3.46
	HCO ₃ ⁻	3.20	3.40
	CO ₃ ⁻	0.00	0.00
	SO ₄ ⁻	5.16	5.26
Soluble cations (meq/L)	Ca ⁺⁺	4.03	4.03
	Mg ⁺⁺	1.35	1.35
	Na ⁺	1.21	1.21
	K ⁺	5.33	5.33
Available micronutrients (ppm)	Fe	3.62	3.62
	Mn	1.51	1.51
	Zn	1.35	1.35
	Cu	0.52	0.52

Table (2). Meteorological data at Dakahlia Governorate during the two growing seasons, 2024 and 2025

Month	Relative humidity (%)		Max. temperature (°C)		Min. temperature (°C)	
	2024	2025	2024	2025	2024	2025
April	49.81	44.58	37.6	38.8	17.1	18.8
May	39.27	37.84	40.5	39.6	19.7	19.4
June	37.68	43.01	41.0	41.4	20.3	20.5
July	40.68	40.17	43.5	43.0	21.2	19.5
August	43.54	44.59	40.8	40.52	21.7	20.4

Experimental setup: This experiment included 8 treatments as foliar treatments were applied in a complete randomized block design system in three replications and arranged as follows:

1. Control treatment (spraying with water),
2. Brassinolide (C₂₈H₄₈O₆) at 250 ppm,
3. Glutathione (C₁₀H₁₇N₃O₆S) at 200 ppm,
4. Methyl jasmonate (C₁₃H₂₀O₃) at 100 ppm,
5. Melatonin (C₁₃H₁₆N₂O₂) at 150 ppm,

6. Glycine betaine ($C_5H_{11}NO_2$) at 2 g/L,
7. Spirulina (2.5%) as blue-green algae and
8. Jasmonic acid ($C_{12}H_{18}O_3$) at 100 ppm, all applied foliar substances were obtained by Sigma-Aldrich (St. Louis, MO, USA) except for spirulina-a blue-green algae were acquired from an agricultural research Center.

In order to get complete and consistent coverage of the plants' foliage, the plants were sprayed three times at 30, 45, and 60 days from transplanting using a manual atomizer. At the same time, the untreated plants (control) were sprayed with only water. In order to prevent the foliar spray from overlapping, a guard area of one line was placed between each pair of experimental units. Seedlings of the Alisa hybrid were transplanted 50 cm apart on one side of the ridge on May 24 and 26 of the first and second seasons, 30 days after the seeds were sown in the nursery. The plot measured 64 square meters. It has two dripper lines, each 1.6 meters wide and 20 meters long. Two drippers on the line were separated by fifty centimeters. During the two growing seasons preparation of the soil (organic and chemical fertilization), fertigation and pest control were applied as recommended by Egyptian Ministry of Agriculture and land reclamation.

Measurements: A random sample of three plants from each plot was taken at 70 days after transplanting, and the following parameters were determined:

1. Plant vegetative growth: Plant height (cm), number of leaves and branches /plant, leaf area, fresh weight and dry weight of shoot (foliage) were recorded after drying at 70°C till constant weight.

2-Leaf pigments: A disc sample from the fourth outer leaf of the plant was randomly taken from every experimental unit at 70 days after planting in both growing seasons to determine chlorophyll a, b and total chlorophyll (a+b) as well as carotenoid pigments according to the method described by Wettstein (1957).

3. Macro and micro elements content: Samples of dried shoots at 70 days were wet digested using sulphuric acid mixture to analyze N, P, K, Ca, Mg, Fe, Zn and Mn. Total nitrogen was determined using micro Kjeldahl apparatus (Chapman and Pratt, 1961). Potassium was evaluated using flame photometer, while Ca, Mg, Fe, Zn and Mn were determined by atomic absorption. Spectrophotometry (AOAC, 2000).

4. Enzymes activity: Assays of catalase, Peroxidase and super oxide dismutase were determined in leaf tissues at 70 days after transplanting in both seasons according to the methods described by Cao *et al.*, (2005) and calculated according to the method of Kong *et al.*, (1999).

5. Endogenous phyto-hormones: such as cytokinins, Auxins, Gibberellins as promoting hormones, and abscisic acid as an inhibitor hormone ($\mu\text{g/g}$ F.W.) in leaf tissues at the same time in the two growing seasons, were achieved by the method of (Sadeghian, 1971) and determined by using gas-liquid chromatography according to (Srinivasan *et al.*, 1996).

6. Yield and its component: Early yield of tomato fruits expressed as weight of the first three pickings. Total yield as the weight of fruits of all pickings as kg/ plot, then were summated and calculated as ton/fed. Average fruit fresh weight (g) was determined theoretically by dividing total fruit weight by the total fruit number.

7. Fruit quality: Five fruits from every plot in both experiments were taken at the full-ripe maturity stage from the second harvest to determine the following parameters: Vitamin C it was assayed in juice (mg/L juice) using 2, 6 dichlorophenol indophenol dye (AOAC, 2000). **Titrateable acidity %:** It was determined by the titration method with 0.1 sodium hydroxide using phenolphthalein indicator (AOAC, 2000). Total soluble solids (TSS) were determined in juice by Carle Zeis refractometer. Lycopene concentrations of tomato fruits were measured Spectrophotometrically using a modified method based on Fish *et al.* (2002). Reducing, non-reducing and total sugars were determined according to the method described by Forsee (1938).

Statistical analysis: Recorded data in both experiments were subjected to the analysis of variance to Snedecor and Cochran (1980). Mean separation was done by Duncan (1958).

3. Results

Vegetative growth: Data in Table 3 indicated that spraying tomato plants Alisa hybrid during late summer plantation with brassinolide, melatonin, glycine betaine, jasmonic acid, glutathione, methyl jasmonate and spirulina extract increased plant height, number of leaves/plant, number of branches/plant, leaf area, and fresh and dry weight of foliage/plant values compared to control (spraying with water) in both seasons. Exogenous application of melatonin achieved the superior results in all mentioned parameters followed by spraying glycine betaine with significant differences in all studied parameters except for number of branches in the first season. Plants in the absence of foliar substances recorded the lowest values of vegetative growth traits as a result of heat stress.

Table (3). Effect of some bio stimulants on vegetative growth of tomato plants at 70 days after transplanting during 2024 and 2025 late summer seasons

Treatments	Plant height (cm)		No. of leaves/plant		No. of branches/plant		Total leaf area (m ²)		Total fresh weight (g)		Total dry weight (g)	
	2024	2025	2024	2025	2024	2025	2024	2025	2024	2025	2024	2025
Brassinolide (250 ppm)	59.0 ^d	66.9 ^d	81.9 ^d	87.1 ^{de}	14.4 ^c	16.3 ^{cd}	16.1 ^{ef}	18.3 ^e	776.0 ^d	826.5 ^d	80.8 ^e	85.8 ^e
Glutathione (200 ppm)	71.4 ^c	79.3 ^c	84.1 ^{cd}	98.8 ^b	15.2 ^b	17.1 ^c	26.3 ^c	28.6 ^c	821.0 ^c	871.5 ^c	102.0 ^b	107.0 ^b
Methyl jasmonate (100 ppm)	69.3 ^c	77.2 ^c	94.1 ^b	89.3 ^{cde}	12.2 ^e	13.5 ^e	25.0 ^c	27.9 ^c	816.3 ^{cd}	856.9 ^{cd}	89.7 ^c	89.8 ^d
Melatonin (150 ppm)	80.0 ^a	91.2 ^a	106.4 ^a	115.0 ^a	20.2 ^a	22.8 ^a	35.5 ^a	36.4 ^a	982.3 ^a	1049.6 ^a	118.1 ^a	124.8 ^a
Glycine betaine (2 g/L)	75.9 ^b	83.6 ^b	95.9 ^b	99.3 ^b	19.8 ^a	21.0 ^b	28.4 ^b	30.6 ^b	882.4 ^b	933.0 ^b	101.6 ^b	106.6 ^b
Spirulina (2.5 %)	57.4 ^d	65.3 ^{de}	91.7 ^{bc}	96.9 ^{bc}	12.2 ^e	14.1 ^e	16.7 ^e	18.3 ^e	818.6 ^c	869.1 ^c	78.8 ^f	83.7 ^f
Jasmonic acid (100 ppm)	58.3 ^d	66.2 ^d	84.3 ^{cd}	89.5 ^{cd}	13.7 ^d	15.6 ^d	23.4 ^d	25.7 ^d	806.8 ^{cd}	857.4 ^{cd}	85.5 ^d	94.6 ^c
Control	55.7 ^d	62.4 ^e	76.2 ^d	80.7 ^e	11.6 ^e	13.5 ^e	15.1 ^f	17.4 ^e	673.6 ^e	714.1 ^e	72.6 ^g	77.6 ^g

Leaf pigments: Data in table 4 showed that plants in the presence of all applied foliar substances achieved an increment in chlorophylls a, b and total chlorophyll as well as carotenoids in both seasons. Foliar application of either melatonin or glycine betaine recorded the best values of chlorophyll b in both seasons without significant differences. Exogenous application of melatonin came in the first order in boosting both chlorophyll a and total chlorophyll, while glycine betaine recorded the best values of carotenoids in both seasons. On the other hand, a reduction in the mention studied leaf pigments was recorded in the control plants which exhibited to heat stress in both seasons.

Macro and micro elements content in leaves: Data in tables 5 and 6 illustrated that N, P, K, Ca and Mg as well as Fe, Zn, and Mn contents in tomato leaves responded positively to all applied foliar bio-stimulators in both seasons. The highest records of N, P, K, Mg, Fe, and Zn contents were obtained when plants sprayed with melatonin followed by glycine betaine. On the other hand, the best values of both Ca and Mn contents were obtained when plants sprayed with glycine betaine followed by melatonin treatment in both seasons. In contrast, as a result of heat stress, the lowest records of both macro and micro elements were obtained when plants sprayed with water only (the control treatment).

Table (4). Effect of some bio stimulants on photosynthetic pigments concentration in tomato leaves at 70 days after transplanting during 2024 and 2025 late summer seasons

Treatments	Chlorophyll a (mg/gm F.Wt.)		Chlorophyll b (mg/gm F.Wt.)		Total chlorophylls (mg/gm F.Wt.)		Carotenoides (mg/gm F.Wt.)	
	2024	2025	2024	2025	2024	2025	2024	2025
Brassinolide (250 ppm)	1.145 ^b	1.234 ^{bc}	0.724 ^{cd}	0.670 ^{cd}	1.869 ^d	1.904 ^d	0.637 ^d	0.625 ^d
Glutathione (200 ppm)	1.165 ^b	1.248 ^b	0.833 ^b	0.750 ^b	1.998 ^c	1.998 ^c	0.687 ^c	0.700 ^c
Methyl jasmonate (100 ppm)	1.065 ^c	1.148 ^d	0.758 ^c	0.700 ^c	1.823 ^{de}	1.848 ^{de}	0.620 ^d	0.630 ^d
Melatonin (150 ppm)	1.485 ^a	1.577 ^a	0.955 ^a	0.885 ^a	2.440 ^a	2.642 ^a	0.773 ^b	0.785 ^b
Glycine betaine (2 g/L)	1.170 ^b	1.251 ^b	0.940 ^a	0.880 ^a	2.110 ^b	2.131 ^b	0.848 ^a	0.837 ^a
Spirulina (2.5 %)	1.070 ^c	1.163 ^d	0.706 ^{cd}	0.655 ^{de}	1.776 ^{ef}	1.818 ^{ef}	0.616 ^d	0.628 ^d
Jasmonic acid (100 ppm)	1.075 ^c	1.185 ^{cd}	0.704 ^{cd}	0.650 ^{de}	1.779 ^{ef}	1.835 ^{def}	0.637 ^d	0.648 ^d
Control	1.056 ^c	1.136 ^d	0.671 ^d	0.620 ^e	1.727 ^f	1.756 ^f	0.571 ^e	0.559 ^e

Table (5). Effect of some bio stimulants on N, P, K, and Ca concentrations in tomato plants at 70 days after transplanting during 2024 and 2025 late summer season

Treatments	N (%)		P (%)		K (%)		Ca (%)	
	2024	2025	2024	2025	2024	2025	2024	2025
Brassinolide (250 ppm)	3.43 ^{cd}	3.67 ^b	0.44 ^d	0.48 ^d	2.55 ^d	2.04 ^d	0.19 ^c	0.20 ^c
Glutathione (200 ppm)	3.82 ^{bc}	3.65 ^b	0.54 ^c	0.58 ^c	3.37 ^b	2.86 ^b	0.21 ^b	0.22 ^b
Methyl jasmonate (100 ppm)	3.24 ^d	3.65 ^b	0.48 ^{cd}	0.52 ^{cd}	3.29 ^b	2.78 ^b	0.20 ^b	0.21 ^b
Melatonin (150 ppm)	4.66 ^a	4.40 ^a	0.96 ^a	0.96 ^a	3.85 ^a	3.34 ^a	0.21 ^b	0.22 ^b
Glycine betaine (2 g/L)	3.94 ^b	3.70 ^b	0.69 ^b	0.74 ^b	3.42 ^b	2.91 ^b	0.27 ^a	0.28 ^a
Spirulina (2.5 %)	3.23 ^d	3.64 ^b	0.46 ^{cd}	0.50 ^{cd}	2.70 ^{cd}	2.19 ^{cd}	0.20 ^{bc}	0.21 ^{bc}
Jasmonic acid (100 ppm)	3.02 ^d	3.43 ^{bc}	0.40 ^d	0.45 ^d	3.12 ^{bc}	2.61 ^{bc}	0.20 ^{bc}	0.21 ^{bc}
Control	2.99 ^d	2.87 ^c	0.30 ^e	0.34 ^e	2.07 ^e	1.56 ^e	0.16 ^d	0.17 ^d

Enzymes activity: Exogenous application of some substances as brassinolide, melatonin, glycine betaine, jasmonic acid, glutathione, methyl jasmonate and spirulina extract boosted antioxidant enzymes activity, *i.e.*, peroxidase, catalase as well as superoxide dismutase in leaves compared to the control treatment (Table 7). Spraying melatonin increased the studied enzymes in leaves in both seasons, followed by spraying glycine betaine. High temperature significantly decreased the studied enzymes activity in the control plants in both seasons.

Table (6). Effect of some bio stimulants on magnesium, iron, zinc and manganese in tomato plants at 70 days after transplanting during 2024 and 2025 late summer season

Treatments	Mg (%)		Fe (ppm)		Zn (ppm)		Mn (ppm)	
	2024	2025	2024	2025	2024	2025	2024	2025
Brassinolide (250 ppm)	0.516 ^c	0.504 ^c	194.10 ^{ab}	184.10 ^b	11.31 ^c	12.65 ^c	187.63 ^c	177.53 ^c
Glutathione (200 ppm)	0.604 ^{bc}	0.592 ^{bc}	208.76 ^a	200.86 ^{ab}	11.49 ^c	12.83 ^c	214.63 ^c	204.53 ^c
Methyl jasmonate (100 ppm)	0.533 ^{bc}	0.521 ^{bc}	167.73 ^b	199.13 ^{ab}	10.72 ^c	12.06 ^c	194.20 ^d	184.10 ^d
Melatonin (150 ppm)	0.794 ^a	0.782 ^a	210.86 ^a	204.76 ^{ab}	17.19 ^a	18.53 ^a	231.60 ^b	221.50 ^b
Glycine betaine (2 g/L)	0.616 ^b	0.604 ^b	214.76 ^a	237.35 ^a	13.27 ^b	14.61 ^b	244.20 ^a	234.10 ^a
Spirulina (2.5 %)	0.572 ^{bc}	0.560 ^{bc}	199.80 ^{ab}	189.80 ^b	11.08 ^c	12.42 ^c	166.23 ^f	156.23 ^f
Jasmonic acid (100 ppm)	0.565 ^{bc}	0.553 ^{bc}	182.20 ^{ab}	172.20 ^b	11.28 ^c	12.62 ^c	166.23 ^f	156.13 ^f
Control	0.247 ^d	0.235 ^d	78.40 ^c	68.40 ^c	9.86 ^c	11.20 ^c	146.23 ^g	136.13 ^g

Table (7). Effect of some bio stimulants on antioxidant enzymatic activity in tomato plant leaves at 70 days after transplanting during the 2024 and 2025 late summer season

Treatments	Peroxidase (mg/g F.Wt./h)		Catalase (CAT) (mg/g F.Wt./h)		Superoxide dismutase (SOD) (mg/g F.Wt./h)	
	2024	2025	2024	2025	2024	2025
Brassinolide (250 ppm)	59.03 ^d	66.93 ^d	82.00 ^d	87.20 ^{de}	14.46 ^c	16.36 ^{cd}
Glutathione (200 ppm)	71.46 ^c	79.36 ^c	84.15 ^{cd}	98.83 ^b	15.20 ^b	17.10 ^c
Methyl jasmonate (100 ppm)	69.30 ^c	77.20 ^c	94.11 ^b	89.35 ^{cde}	12.26 ^c	13.53 ^c
Melatonin (150 ppm)	80.00 ^a	91.23 ^a	106.50 ^a	115.03 ^a	20.23 ^a	22.80 ^a
Glycine betaine (2 g/L)	75.90 ^b	83.60 ^b	95.96 ^b	99.31 ^b	19.83 ^a	21.06 ^b
Spirulina (2.5 %)	57.46 ^d	65.36 ^{de}	91.72 ^{bc}	96.92 ^{bc}	12.20 ^c	14.10 ^c
Jasmonic acid (100 ppm)	58.36 ^d	66.26 ^d	84.34 ^{cd}	89.54 ^{cd}	13.73 ^d	15.63 ^d
Control	55.70 ^d	62.40 ^c	76.22 ^d	80.70 ^c	11.63 ^c	13.50 ^c

Endogenous phyto-hormones: Cytokinin, auxins as well as gibberellins in tomato leaves responded positively to the exogenous application of all sprayed bio-stimulators in both seasons, while abscisic acid responded negatively (Table 8). Plants sprayed with either glycine betaine or melatonin recorded the best values of cytokinin, auxins as well as gibberellins and the lowest values abscisic acid without significant differences in both growing seasons. On the other hand, the control plants (affected by heat stress) recorded higher values of abscisic acid and lower ones of cytokinins, auxins, and gibberellins both seasons.

Table (8). Effect of some bio stimulants on endogenous phyto-hormones in tomato leaves at 70 days after transplanting during 2024 and 2025 late summer season

Treatments	Cytokinins (CYT) (µg/g F.W.)		Auxins (IAA) (µg/g F.W.)		Gibberellins (GA ³) (µg/g F.W.)		Absciscic acid (ABA) (µg/g F.W.)	
	2024	2025	2024	2025	2024	2025	2024	2025
Brassinolide (250 ppm)	90.08 ^c	91.06 ^c	33.90 ^d	34.02 ^d	63.40 ^c	64.37 ^c	3.01 ^a	3.98 ^a
Glutathione (200 ppm)	97.88 ^b	98.86 ^b	58.41 ^a	58.25 ^a	69.77 ^b	70.95 ^b	2.86 ^a	3.82 ^a
Methyl jasmonate (100 ppm)	96.26 ^b	97.24 ^b	51.41 ^b	51.70 ^b	50.82 ^c	51.81 ^c	3.31 ^a	4.09 ^a
Melatonin (150 ppm)	104.79 ^a	106.15 ^a	59.80 ^a	59.87 ^a	77.38 ^a	76.57 ^a	1.46 ^b	2.05 ^b
Glycine betaine (2 g/L)	106.73 ^a	107.87 ^a	60.19 ^a	60.12 ^a	75.26 ^a	78.67 ^a	0.70 ^b	1.68 ^b
Spirulina (2.5 %)	96.12 ^b	97.10 ^b	43.98 ^c	44.11 ^c	48.41 ^c	49.40 ^c	2.88 ^a	2.29 ^a
Jasmonic acid (100 ppm)	83.81 ^d	84.79 ^d	34.35 ^d	34.48 ^d	53.51 ^d	54.50 ^d	3.05 ^a	3.69 ^a
Control	81.12 ^d	82.15 ^d	32.72 ^d	33.05 ^d	41.78 ^f	42.40 ^f	3.35 ^a	4.25 ^a

Yield and its components: Spraying tomato plants with brassinolide, melatonin, glycine betaine, jasmonic acid, glutathione, methyl jasmonate and spirulina extract promoted average fruit weight, number of fruits/plant, early yield and total yield/fed. in both seasons compared to control treatment (sprayed with water), as shown in Table 9. Foliar application of melatonin increased all the above mentioned parameters followed by spraying glutathione in both seasons. As a result of heat stress, plants in the absence of foliar substances recorded the lowest values in both seasons.

Table (9). Effect of some bio stimulants on yield parameters of tomato plants during 2024 and 2025 late summer seasons

Treatments	Average fruit weight (g)		Number of fruit/plant		Early yield (ton./fed.)		Total yield (ton./fed.)	
	2024	2025	2024	2025	2024	2025	2024	2025
Brassinolide (250 ppm)	185.9 ^e	196.6 ^e	38.0 ^c	39.0 ^c	8.396 ^d	7.947 ^{bc}	33.153 ^d	33.585 ^d
Glutathione (200 ppm)	200.1 ^b	210.8 ^b	43.6 ^b	44.6 ^b	9.051 ^{bc}	8.354 ^{bc}	36.775 ^b	37.208 ^b
Methyl jasmonate (100 ppm)	187.8 ^{de}	198.5 ^{de}	39.3 ^c	40.3 ^c	8.511 ^d	8.012 ^{bc}	33.611 ^d	34.044 ^d
Melatonin (150 ppm)	207.9 ^a	218.7 ^a	48.0 ^a	49.0 ^a	10.428 ^a	9.469 ^a	41.278 ^a	41.711 ^a
Glycine betaine (2 g/L)	195.8 ^c	206.6 ^c	40.0 ^c	41.0 ^c	9.302 ^b	8.462 ^b	35.773 ^{bc}	36.206 ^{bc}
Spirulina (2.5 %)	194.1 ^c	204.8 ^c	39.3 ^c	40.3 ^c	8.768 ^{cd}	8.399 ^{bc}	34.640 ^{cd}	35.072 ^{cd}
Jasmonic acid (100 ppm)	190.1 ^d	200.8 ^d	40.0 ^c	41.0 ^c	8.461 ^d	7.780 ^{bc}	33.411 ^d	33.844 ^d
Control	180.5 ^f	191.1 ^f	31.6 ^d	31.3 ^d	7.718 ^e	7.654 ^c	30.441 ^e	30.874 ^e

Fruits quality: Tomato plants responded negatively to the absence of foliar substances, so the control plants (exhibited to high temperature) recorded the lowest values of vitamin C, acidity, TSS, lycopene, lycopene, reducing sugars and non-reducing sugars in the two growing seasons (Table 10). In contrast, foliar application of brassinolide, melatonin, glycine betaine, jasmonic acid, glutathione, methyl jasmonate and spirulina extract enhanced the mentioned studied quality characteristics in both seasons. Exogenous application of melatonin significantly increased the studied quality parameters in both seasons followed by glutathione application. The lowest records were obtained when plants sprayed with water only.

Table (10). Effect of some bio stimulants on yield quality parameters of tomato plants during 2024 and 2025 late summer seasons

Treatments	Vit. C (mg/100 g FW)		Acidity (%)		TSS (Brix)		Lycopene (mg/100 g FW)		Reducing sugars (%)		Non-reducing sugars (%)	
	2024	2025	2024	2025	2024	2025	2024	2025	2024	2025	2024	2025
Brassinolide (250 ppm)	15.08 ^c	15.64 ^c	0.42 ^d	0.55 ^d	5.01 ^c	5.04 ^c	11.56 ^c	11.39 ^c	2.37 ^d	2.50 ^d	1.67 ^d	1.57 ^c
Glutathione (200 ppm)	15.58 ^b	16.18 ^b	0.45 ^{bc}	0.58 ^{bcd}	5.24 ^b	5.28 ^b	12.14 ^b	12.00 ^b	2.59 ^c	2.72 ^c	1.55 ^e	1.45 ^d
Methyl jasmonate (100 ppm)	15.11 ^c	15.67 ^c	0.44 ^{cd}	0.56 ^{cd}	5.00 ^c	5.03 ^c	11.52 ^c	11.37 ^c	2.64 ^c	2.77 ^c	1.82 ^c	1.73 ^b
Melatonin (150 ppm)	17.94 ^a	18.47 ^a	0.51 ^a	0.63 ^a	5.97 ^a	6.00 ^a	12.62 ^a	12.47 ^a	3.41 ^a	3.55 ^a	1.97 ^b	1.87 ^a
Glycine betaine (2 g/L)	15.22 ^b _c	15.77 ^b _c	0.48 ^{ab}	0.60 ^{ab}	4.83 ^c	4.86 ^c	11.27 ^c	11.14 ^c	2.92 ^b	3.05 ^b	2.02 ^a	1.92 ^a
Spirulina (2.5 %)	15.21 ^b _c	15.78 ^b _c	0.47 ^b	0.59 ^b	4.84 ^c	4.87 ^c	10.59 ^d	10.45 ^d	2.32 ^d	2.45 ^d	1.70 ^d	1.60 ^c
Jasmonic acid (100 ppm)	15.23 ^b _c	15.79 ^b _c	0.46 ^{bc}	0.59 ^{bc}	4.87 ^c	4.90 ^c	11.30 ^c	11.14 ^c	2.85 ^b	2.98 ^b	1.86 ^c	1.76 ^b
Control	12.13 ^d	12.75 ^d	0.39 ^e	0.50 ^e	4.60 ^d	4.57 ^d	10.46 ^d	10.30 ^d	2.26 ^d	2.39 ^d	1.21 ^f	1.11 ^e

4. Discussion

This study offers new perspectives to mitigate the harmful impacts of high temperature on tomato plants performance grown in the late summer seasons. Tomato vegetative and reproductive growth is highly influenced by high temperature in open fields during the late summer season. High temperature has adverse effects on plant vegetative and reproductive development and reduces crop yield. This study reveals that the ambient high temperatures (Table 2 and Fig. 1) during growth negatively affected vegetative growth and resulted in minimum values of plant height, number of leaves and branches/plant, leaf area as well as fresh and dry weights. Elevated temperatures are adversely affected vital and physiological processes such as photosynthesis, growth and development (**Bokszczanin *et al.*, 2013**). Additionally, it restricts water relations, roots growth and distribution, nutrients absorption as well as the synthesis of hormones that affect sink-source relationships between roots and shoots, which in turn decreases growth (**Giri *et al.*, 2017**).

Previous studies investigated the impacts of exogenous applications of some stimulators, *i.e.*, with brassinolide, melatonin, glycine betaine, jasmonic acid, glutathione, methyl jasmonate and spirulina extract to improve tomato plants performance and abiotic stress resistance, particularly heat stress. This study illustrated that exogenous application of the previous mentioned substances restores the negative effects of heat stress on tomato plants performance. Plants treated with melatonin showed the best improvement in plant height, number of leaves and branches/plant, leaf area as well as fresh and dry weights as compared other treatments. The positive impact of melatonin may be due it has a similar structure to auxin specifically IAA, hence it is involved in regulating root and shoot development, so it plays the role of auxins to encourage vegetative growth of plant species (**Kolář and Macháčková,**

2005), enhancing both cell division and cell elongation as well as in restricting production of reactive oxygen species (Zhang *et al.*, 2015). Also, the improvement in vegetative growth due to spraying glycine betaine may be due to the fact that it penetrates into plant leaves soon after application and translocate to roots, meristems, and expanding leaves. Thus, developing and expanding plant organs are primarily protected from stress and enhanced growth and reproduction, it acts as an osmo-protectant in the cells (Makela *et al.* 1998).

Additionally, heat stress affects photosynthesis and the efficiency of photosystems through the inhibition of chlorophyll biosynthesis (Sellami and Kooli, 2026) due to non-stomatal limitations (Zhang *et al.*, 2011), changes the membrane fluidity, limits the general stability of vital metabolic mechanisms, hence thus causes both oxidative stress and over production of reactive oxygen species (Larkindale *et al.*, 2005). The obtained results from this study indicate that high temperature caused remarkable reduction in chlorophylls, carotenoids as well as macro and microelement contents in tomato leaves (Tables 4, 5, and 6). In the same manner, heat stress affected nutrients metabolism and ammonium assimilation in tomato plants due to the decrease in root growth and nutrients-uptake per root unit (Giri *et al.*, 2017). By increasing nutrient uptake efficiency and plant tolerance to abiotic stress, particularly heat stress, the use of certain stimulants, such as brassinolide, melatonin, glycine betaine, jasmonic acid, glutathione, methyl jasmonate and spirulina extract, can improve plant development (Poberezny *et al.*, 2020) and protect cell membranes, maintaining their structure and function against the harmful and destructive effects of ROS during stress which leads to increased minerals absorption and translocation. In this case, it is possible to draw the conclusion that the increased dry matter accumulation in leaves, photosynthetic pigments, and leaf area reverses the stimulatory effects of these elements on the efficiency of the photosynthesis process, resulting in the creation of more photo-synthates and an improvement in the translocation of minerals from roots to leaves (El-Desouky *et al.*, 2009). Hence, the positive respond of chlorophylls and carotenoids contents in melatonin-treated tomato leaves may be due to the fact that it has a valuable role in controlling both physiological and biological processes in plants as root growth and photosynthetic activity through regulating the biosynthesis of several plant growth hormones (Sharif *et al.*, 2018). Results are in harmony with those obtained with El-Metwaly *et al.* (2024) on potato and Ibrahim and Ahmed (2025) on tomato.

Enzymes activity significantly declined under heat stress as heat stress may influence biochemical processes in tomato plants, which could contribute to the observed variations in antioxidant activity and endogenous phyto-hormones. Heat stress enhanced the production of ROS species which can impair cellular functions (Khan *et al.*, 2024a) and caused phototoxic reactions like lipid peroxidation, protein degradation and alteration in DNA (Pospisil and Yamamoto, 2017). To counteract the damage caused by ROS, plants enhance their antioxidant defenses by enhancing the activity peroxidase, superoxide dismutase and catalase enzymes. These enzymatic actions are vital for preserving cellular balance (Khan *et al.*, 2024b). Plants sprayed with melatonin recorded superior activities of peroxidase, superoxide dismutase and catalase enzymes in tomato leaves. The increased activity of antioxidant enzymes was due to the role of melatonin in scavenging ROS species, hence, melatonin significantly mitigated the effects of heat stress by minimizing the ROS species and lipid peroxidation with the help of antioxidant enzymes (Wang *et al.* 2016). Hence, melatonin also augmented antioxidant enzyme activity due to its quality of electron donor to ROS species leading to reduced oxidative damage. Additionally, melatonin has a valuable role in controlling both physiological and biological processes in plants as root growth, photosynthetic activity and regulating plant hormones (Arano and Hernández, 2018). In this regard, stress hormones including ethylene and abscisic acid (ABA) function as signal molecules to regulate a variety of physiological characteristics as ABA levels rise in response to many environmental stressors, such as high temperatures (Larkindale and Haung, 2005).

Tomato plants are exposed to unfavorable environmental conditions in the late summer season, especially higher day and night temperatures for successive days or even weeks during the reproductive stage, which greatly cause flower abortion and negatively impact fruit set percentage by reducing pollen viability (Pham *et al.*, 2020 and Lee *et al.*, 2022). Also, heat stress is a significant challenge for plant growth and development, leading to considerable economic losses in yield due to various changes in the plants either biochemical or physiological traits (Wahid *et al.*, 2007). Furthermore, Zhao *et al.* (2017) estimated that each 1°C elevation could decrease the total yield of important commodities by 4.1% -

6.4%, which has a negative impact on the economic and environmental levels. In addition, fruit quantities are all negatively impacted by late summer planting in open fields because it exposes plants to heat stress, which increases ROS species, reduces the amount of water in leaf cells, and slows down photosynthesis. Accordingly, heat stress significantly diminishes the production of larger fruits while encouraging the growth of smaller fruits, ultimately leading to a decline in the commercial quality of the yield (**Sellami and Kooli, 2026**). Also, increasing the day temperature over 25°C in tomato production significantly affected flower abortion leading to 80% flowers loss causing reduced fruit sit (**Rieu *et al.*, 2017**) and decreased numbers and weights of fruits (**Peet *et al.*, 1998**). Heat stress decreased tomato yield and quality by inducing ROS, reducing water contents, disrupting membrane stability and photosynthetic apparatus, hence, these traits are responsible for improving tomato yield and quality (**Hassan *et al.*, 2022**). The positive respond of sprayed-tomato plants with the different mentioned materials, *i.e.*, brassinolide, melatonin, glycine betaine, jasmonic acid, glutathione, methyl jasmonate and spirulina extract may be due to the previous mentioned improved vegetative growth (Table3), the enhanced leaves photosynthetic pigments (Table 5), mineral contents (Tables 5 and 6) as well as plant phyto-hormones (Table 8) may be the reason for the obtained higher yield and fruit quality in both growing seasons. Yield and quality attributes were improved by spraying melatonin as it is considered a master regulator and defensive player under stresses and plays its role in increasing water, proteins as well as amino acids contents and photosynthetic pigment. All these increased substances improved tomato yield and quality (**Jahan *et al.*, 2019**). Besides, foliar application of melatonin on tomato plants preserves pollen activity and lowers ROS accumulation by inducing antioxidant enzyme activities under heat stress (**Qi *et al.*, 2018**). Additionally, under heat stress, tomato plants may have less chlorophyll and a decrease in the activity of the enzyme needed for chlorophyll production; however, exogenous melatonin and glycine betaine significantly improved the fruit quality of tomato plants under heat stress (**Khan *et al.*, 2024b**).

Generally, the favorable impact of applying brassinolide on tomato plants have been reported to accumulate more heat shock proteins as compared to non-treated plants, which resulted in induced thermo tolerance as well as enhanced photosynthetic efficiency (**Singh and Shono, 2005**). Also, brassinolide application enhances chlorophyll content, photosynthetic efficiency, sugar production, which may be manifested in higher sugar levels in fruits (**Maia *et al.*, 2022**). Additionally, compared to the control treatment, the administration of glutathione improved the growth indices, chlorophyll, photosynthesis, and antioxidant enzyme activities, indicating a rise in the abiotic stress tolerance of plants (**Koh *et al.*, 2021**). In the same manner. Spirulina extract contains growth hormones, vitamins, amino acids, and mineral elements responsible for increasing chlorophyll and thus improving plant productivity (**Mostafa *et al.*, 2024**). These results agreed with **Mumithrakamatchi *et al.* (2024)** on tomato applying melatonin; **Rezaei *et al.* (2012)** using glycine betaine; **Rahman and Ahmad (2025)** applying brassinolide; **Okasha *et al.* (2023)** and **Ramadan and Shalaby (2024)** spraying jasmonic acid; **Khaled *et al.* (2023)** applying spirulina extract and **Al-shammari *et al.* (2020)** applying glutathione.

One of the greatest challenges to sustainable agriculture is heat stress, which become more frequent and severe due to climate changes. Actually, heat shocks are considered one of the most vital issues for both growing crops and food security in the present and the future with the increased population around the world. To minimize these adverse effects, sustainable agricultural practices such as cultivating heat-tolerant crops, irrigation management, soil practices, and the application of bio-stimulants can improve maintain productivity and crop resilience. Our study focus on using some natural eco-friendly substances as one of the management strategies to sustain tomato productivity, overcome the high ambient temperature and supporting sustainable agricultural systems under changing climatic conditions.

5. Conclusion

Heat stress affects growth, yield and productivity of crops and is considered a major risk for sustainable agriculture. To mitigate the harmful effects of elevated temperature on tomato plants (Alisa hybrid) grown in the late summer season, it could be concluded that spraying melatonin at 150 ppm three times at 30, 45, and 60 days after transplanting significantly improved all vegetative growth parameters (plant height, number of leaves/plant, number of branches/plant, leaf area, and foliage fresh

weight as well as dry weight of foliage/plant), total chlorophyll, macro and micro elements (N, P, K, Mg, Fe, and Zn), enzymes activity (peroxidase, catalase and superoxide dismutase) and phyto-hormones (cytokinin, auxins and gibberellins) as well as average fruit weight, number of fruits/plant, early and total yield/fed. and fruit quality (vitamin C, acidity, TSS, and total sugars) followed by spraying glycine betaine at 2 g/l under the experiment conditions. These insights can help in improve heat-resistance to maintain tomato productivity with the rising temperatures.

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استخدام بعض منشطات النمو للتغلب على تأثير الإجهاد الحراري على إنتاجية نباتات الطماطم

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الملخص العربي

تعتبر التغيرات المناخية وخاصة الإجهاد الحراري تحديًا كبيرًا بالنسبة لنباتات الطماطم ومع الارتفاع المستمر في درجات الحرارة تتأثر النباتات سلبًا (العمليات الفسيولوجية - الكيميائية - الحيوية) مما يؤثر في النهاية على الإنتاجية. الهدف من هذه الدراسة تقدير مدى استجابة نباتات الطماطم «هجين أليسا» للإجهاد الحراري الطبيعي بالرش الورقي لبعض المنشطات الحيوية في العروة الصيفية المتأخرة لموسم الزراعة 2024 و 2025 وذلك بمزرعة خضر خاصة بمنطقة المنصورة، محافظة الدقهلية تحت ظروف الأراضي الطينية القديمة واستخدام نظام الري بالتنقيط. أشتملت التجربة ثمانية معاملات في تصميم قطاعات كاملة العشوائية مع ثلاث مكررات وهي كالتالي: البراسينوليد (250 جزء في المليون)، والجلوتاثيون (200 جزء في المليون) والميثيل جاسمونيت (100 جزء في المليون) والميلاتونين (150 جزء في المليون) والجليسين بيتاين (2 جم/لتر) والاسبيرولينا (2.5%) وحمض الجاسمونيك (100 جزء في المليون) بالإضافة إلى معاملة الكنترول (الرش بالماء فقط). أوضحت نتائج التجربة أن معظم قيم الصفات محل الدراسة انخفضت بشكل كبير نتيجة تأثير ظروف الإجهاد الحراري في كلا الموسمين، بينما أدت جميع مواد الرش المستخدمة إلى تحسين أداء النباتات شملت صفات النمو الخضري والمحتوى من العناصر والصبغات والمحصول وجودة الثمار. هذا وقد أدى رش النباتات بالميلاتونين (150 جزء في المليون) إلى تخفيف تأثير الإجهاد الحراري وتسجيل أفضل قيم لنمو للنبات، ومحتوى كيميائي بالأوراق، ونشاط الإنزيمات، والصبغات نباتية والهرمونات، بالإضافة إلى المحصول والجودة، تليها معاملة رش الجليسين بيتاين (2 جم/لتر) في معظم الحالات مقارنة بالكماترول المجهدة حرارياً. ويرجع تخفيف تأثير الحرارة العالية باستخدام مواد الرش إلى تحفيز امتصاص العناصر الغذائية وضبط الضغط الاسموزي في خلايا أنسجة نباتات الطماطم. كذلك لوحظ الدور المهم لكل من الميلاتونين وباقي معاملات الرش الورقي في تحسين إنتاجية نباتات الطماطم تحت ظروف الإجهاد الحراري.

الكلمات الدالة: الطماطم - الإجهاد حراري - المنشطات الحيوية - الميلاتونين - الجليسين بيتاين.