Al Abboud MA *et al.* (2024)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 52, Issue 1, Article number 13464
DOI:10.15835/nbha52113464
Research Article

A novel approach for reducing water stress on sunflower plants by using medicinal plant extracts rather than artificial growth regulators

Mohamed A. AL ABBOUD[1,2], Mohamed A. AMIN[3], Khatib S. ISMAIL[1], Syed K. ALI[4], M. REMESH[1], Abdelatti I. NOWWAR[3]

[1]Jazan University, College of Science, Department of Biology, Jazan 82817, Kingdom of Saudi Arabia; malabboud@jazanu.edu.sa (M.A.A); kismail@jazanu.eu.sa (K.S.I.); remeshram@jazanu.edu.sa (M.R.)
[2]Jazan University, Center for Environmental Research and Studies, Kingdom of Saudi Arabia
[3]Al-Azhar University, Faculty of Science, Department of Botany and Microbiology, Cairo 11884, Egypt; mamin7780@azhar.edu.eg (M.A.A); abdelatti.ibrahim@azhar.edu.eg (A.I.N.)
[4]Jazan University, College of Science, Department of Chemistry, Jazan, PO Box 114, Kingdom of Saudi Arabia; skali@jazanu.edu.sa (S.K.A)

Abstract

One of the most significant physiological stressors that negatively impact plants in numerous vital areas of their growth and metabolism is water stress. This study estimates the effects of natural bio-stimulants *(Origanum majorana*, *Nigella sativa* and *Curcurma longa*) extracts for the first time, on water stress of sunflower plant in comparison with synthetic growth regulators (glycein betaine, proline, indole acetic acid, benzyl adenine and ascorbic acid). Water stress appeared to decrease of lengths, fresh and dry weights of shoot and root. Also caused a significant drop in chlorophylls and carotenoids. Foliar application of these synthetic and natural growth regulators significantly decreased the negative effects of drought stress on all studied morphological parameters and pigment contents of sunflower. HPLC analysis of bio-stimulants appeared six phenolic acids, one phenol and two flavonoids were found in all natural extracts. Gallic, rosmarinic, caffeic, syringic acids and kaempferol are the major phenolic compounds (more than 1000 μg/g) detected in *Origanum majorana*. While gallic acid, hesperetin and ferulic acid are the major phenolic compounds (more than 1000 μg/g) present in *Curcurma longa*. Chlorogenic acid, gallic acid, catechin, pyro catechol, coffeic acid and rutin are the dominant phenolic compounds present in *Nigella sativa*. The *Nigella sativa* treatment showed the highest decrease in proline levels. This may be due to the high concentration of caffeic acid (2406.97 g/g). The *Origanum majorana* treatment had the highest levels of chl. a and carotenoid contents, which rose by about 32% and 72%, respectively, compared to the stress-treated plant. The highly decrease in catalase activity, which is considered as most important indicator to water stress, appeared in *Origanum majorana* treatment. We suggest the use of these natural extracts as an alternative way, which appeared a significant increase in growth and biochemical near to synthetic regulators in the treatment of water stress due to the fact that these extracts contain many important phenolic compounds that have a role in the treatment of water stress.

Keywords: biostimulants; drought stress; *Helianthus annuus*; plant extract

Received: 16 Oct 2023. Received in revised form: 21 Nov 2023. Accepted: 07 Mar 2024. Published online: 12 Mar 2024.
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Introduction

Water stress is detrimental to a plant's growth and productivity. Consequently, Crop breeding aims to create plants that can withstand and flourish in arid settings (Osakabe *et al*., 2014). Water stress is a damaging non biological element that slows down plant evolution and growth and reduces crop yields (Rasheed *et al*. 2020). Plants under stress from drought undergo a range of morpho-anatomical, physiological, and biochemical changes. These modifications are primarily targeted at reducing transpiration-induced water loss in an effort to improve plant water use efficiency. One of the earliest regular responses to drought is stomata closure, which sets off a chain reaction of physiological and biochemical changes intended to balance photosynthetic processes and strengthen plant defenses against drought-induced stress (such as osmolyte buildup, antioxidant system stimulation, and aquaporin synthesis stimulation), all of which are attempts by the plant to get through the adverse time of restricted water availability (Kapoor *et al*., 2020). Plants involve antioxidant enzymes, e.g. superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and polyphenol oxidase (PPO) that eliminate reactive oxygen species (ROS) due to water stress in addition to antioxidant molecules, such as ascorbic acid, glutathione, salicylic acid, and tocopherols (Mohammadi *et al*., 2011)

Biostimulants, which can be natural substances achieved from plants and/or microorganisms, may support the physiological procedures of plants that gain from translocation and nutrient capture along with greater tolerance to a number of abiotic stimuli. These items are designed to help plants grow and produce more while dealing with environmental issues in a sustainable way. They can be added to crops' rhizosphere or directly treated to seeds, seedlings, and plants (Maharshi *et al*., 2021). The use of biostimulants in agriculture has shown tremendous potential in combating climate change-induced stresses such as drought, temperature stress, etc. They could be a promising tool in the current crop production scenario. Biostimulants are substances that are organic, microbiological, or a combination of the two that can modify molecules and physiological, biochemical, and anatomical processes to control the growth behavior of plants. Their special benefit lies in their ability to stimulate plant development and resilience to a variety of environmental challenges (Horoszkiewicz *et al*., 2023), So it is advised to use low-risk chemicals and non-chemical methods, which involve the least risk to health and the environment while also ensuring effective and efficient crop protection. This is due to the ecotoxicity of chemical plant protection products, the negative effects of their use, and the changes in regulations. Eco-friendly agriculture appears to hold promise when employing biofertilizers instead of chemical-based fertilizers to increase the quantity and quality of food produced by certain commercial crops, like oilseed crops and sunflowers. One of the most significant oilseed crops worldwide is sunflower. The importance of the sunflower is typically attributed to its nutritional and therapeutic benefits (Adeleke and Babalola, 2020). A study on of extracts of *Senna alatain* plant, Ikechukwu (2014) found improvement of seedling height, leaf area, dry weight and leaf area ratio and reproductive growth in *Celosia aregentea* by presoaking seeds in the extract.

There have been numerous studies demonstrating the vital role of synthetic growth regulators in reducing the harmful effects of water stress. On this regards, Mangena (2022) explain the vital role of synthetic 6-benzyl adenine to increasing drought stress tolerance of soybean, Zhang *et al*. (2020) revealed that white clover's resistance to drought is improved by indole acetic acid, (Khazaei and Estaji, 2020) on ascorbic acid and how it affects plant growth and resistance to water stress, Chun *et al*. (2018) on the influence of osmotic stress on proline accumulation in arbuscular mycorrhizal symbiotic plants and Annunziata *et al*. (2019) on glycine betaine and its connections to increased plant growth and survival, ROS scavenging, membrane stability, activation of stress-responsive genes, and buffering of redox potential, but there have not been any studies examining the impact of these natural products (*Origanum majorana*, *Nigella sativa*, and *Curcurma longa* extracts) on drought stress. Accordingly, this study carried out to examine the impact of vivo application of synthetic growth regulators (IAA, BA, ASA, proline, and glycine betaine) on the growth, yield traits and biochemical analysis of sunflower (*Helianthus annuus* L.) plant, in compared of new biostimulants with the goal to choose a treatment that will be applied fairly to promote sunflower development under water stress.

Materials and Methods

Materials

Sunflower seeds *Helianthus annuus* L. ('Giza 102') plants were obtained from Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt. IAA, BA, ASA, glycerin betaine and proline were supplied from Sigma-Aldrich Co. and used as received. *Origanum majorana*, *Nigella sativa* and *Curcurma domestica* powder were supplied from the local marketing center.

Treatments

Comparative study established in the 2019-2020 season in Nasr City, Cairo, Egypt, in the Botanical Garden, Botany and Microbiology Department, Faculty of Science to investigate the impact of synthetic growth regulators (IAA, BA, ASA, Glycein betaine and proline) and natural extracts of *Origanum majorana*, *Nigella sativa* and *Curcurma domestica*, for enhancing the metabolic components and growth of sunflower plants under water stress. This is how the experiment was set up:

Control: irrigation every 18 days (which is normal irrigation every 12 days according to Agriculture Ministry Recommendation) without any treatment, IAA (100 ppm), BA (100 ppm), ASA (200 ppm), Glycein betaine (100 ppm), proline (300 ppm), *Origanum majorana*, *Nigella sativa* and *Curcurma domestica* extracts (4 g/L). These concentrations were selected under a preliminary experiment, which resulted the best growth of sunflower plant.

Preparation of plant extracts for foliar application

Powder of plant heated with sterile distilled water for 45 min at 60 °C. The extracts were then placed in storage at 4 °C for upcoming experimental investigations after being filtered with filter paper (Anisimov *et al*., 2013). Twice, at intervals of 30 and 50 days, the extracts were applied topically at a rate of 4 grams of powdered extract per liter. After 60 days of seeding, six plant samples were chosen at random to assess the morphological characteristics of the plants (lengths, fresh and dried weight of shoot, and number of leaves). Biochemical analysis was done on the antioxidant enzyme content of apical buds, fresh leaf pigments, dry shoot carbohydrates, protein, phenol, and proline.

Preparation of Origanum majorana, Nigella sativa and Curcurma longa powder for HPLC analysis

One g of powdered dried *Nigella sativa* seeds was soaked with 50 ml of 85% methanol in a stoppered container at room temperature for 24 hours. The resultant extract was put in a sonicator at 4 °C for thirty minutes for traditional extraction. Then, to produce a crude extract, this extract was filtered and heated to 40 degrees Celsius while being concentrated under vacuum. An Agilent 1260 series was used for the HPLC analysis. A 5 m, 4.6 mm x 250 mm i.d., Eclipse C18 column was utilized for the separation. Water (A) and 0.05% trifluoroacetic acid in acetonitrile (B), with a flow rate of 0.9 ml/min, made up the mobile phase. Eight minutes (12%), twelve minutes (15%), fifteen minutes (16%), sixteen minutes (20%), and zero minutes (82% A), five minutes (80% A), and five minutes (60%) were the sequence in which the mobile phase was subjected to a linear gradient. At 280 nm, the multi-wavelength detector was visible. A volume of 5 l was used for each of the sample solutions. The temperature of the column was maintained at 40 °C consistently.

Biochemical analysis of treatment plants

Photosynthetic pigments

Freshly picked leaves weighing one gram were precisely ground and dissolved in 100 mL of 80 percent acetone. The liquid was then filtered using Whatman filter paper (No. 1) after that. To determine the extract's potency, optical densities at wavelengths of 470 nm, 649 nm, and 665 nm were determined (Vernon and Seely, 2014). The concentration of chlorophyll a, b, a + b, and carotenoid was determined by (Lichtentahler, 1989).

Estimation of total soluble carbohydrates and protein

Carbohydrates were assessed by the method of (Umbreit *et al*., 1957), and proteins were assessed by (Lowry *et al*., 1951).

Estimation of total phenols

The Folin-Ciocalteu technique was used to calculate the amount of phenolic components in plant samples (Daniel and George, 1972).

Calculation of free proline, free proline estimated by method of (Bates *et al*., 1973).

Extraction of superoxide dismutase, catalase, peroxidase and polyphenol oxidase enzymes according to (Mukherjee and Choudhuri, 1983) and assessed as follows.

Superoxide dismutase (SOD) was calculated using the techniques outlined by (Marklund and Marklund, 1974), catalase (CAT) was determined by (Aebi, 1984), peroxidase (POX) according to (Castillo *et al*., 1984), polyphenoloxidase (PPO) was assessed of a method discovered by (Matta and Diamoned, 1963).

Statistical analysis

Statistical calculations were performed using SPSS version 25 at the 0.05 threshold of probability (Snedecor and Cochran, 1980). One-way ANOVA and post hoc-test Tukey's were used to analyze the variance of data that was quantitatively having a parametric distribution.

Results

Phenolic and flavonoid substances in plant extracts

To identify and measure phenolic components, nine phenolic acid, three phenolics, and seven flavonoid standards were utilized. Six phenolic acids, one phenol, and two flavonoids were found in all natural extracts, but only Methyl gallate and elagic acid were found only in *Origanum majorana*. Pyro-catechol appeared only in *Nigella sativa* Catechin, methyl gallate, Pyro catechol, rutin, elagic, cinnamic acid, and naringenin were absent in *Curcurma longa* (Table 1 and Figures 1 and 2). Gallic acid, rosmarinic acid, caffeic acid, Syringic acid and Kaempferol are the major phenolic compounds (more than 1000 μg/g) detected in *Origanum majorana*. Table 1 also, showed that gallic acid, hesperetin and ferulic acid are the major phenolic compounds (more than 1000 μg/g) detected in *Curcurma longa*. Gallic acid, chlorogenic acid, catechin, pyro catechol, rutin and coffeic acid are the dominant phenolic compounds present in *Nigella sativa.*

Table 1. Phenolic substances in natural plant treatments by HPLC

	Origanum majorana	*Nigella sativa*	*Curcurma longa*
	Conc. μg/g		
Gallic acid	2757.21	506.28	1656.10
Chlorogenic acid	540.11	1169.61	326.55
Catechin	188.44	1884.47	0.00
Methyl gallate	633.86	0.00	0.00
Caffeic acid	1553.11	2501.97	127.25
Syringic acid	1006.17	26.24	221.52
Pyro catechol	0.00	3206.57	0.00
Rutin	430.65	313.48	0.00
Ellagic acid	77.96	0.00	0.00
Coumaric acid	111.12	0.00	661.38
Vanillin	843.59	32.08	603.60
Ferulic acid	109.48	7.35	1038.43
Naringenin	197.72	69.86	0.00
Rosmarinic acid	50001.88	385	363.25
Daidzein	30.96	104.69	85.39
Querectin	729.45	0.00	200.85
Cinnamic acid	136.03	9.35	0.00
Kaempferol	1243.34	0.00	392.26
Hesperetin	84.53	59.91	2672.15

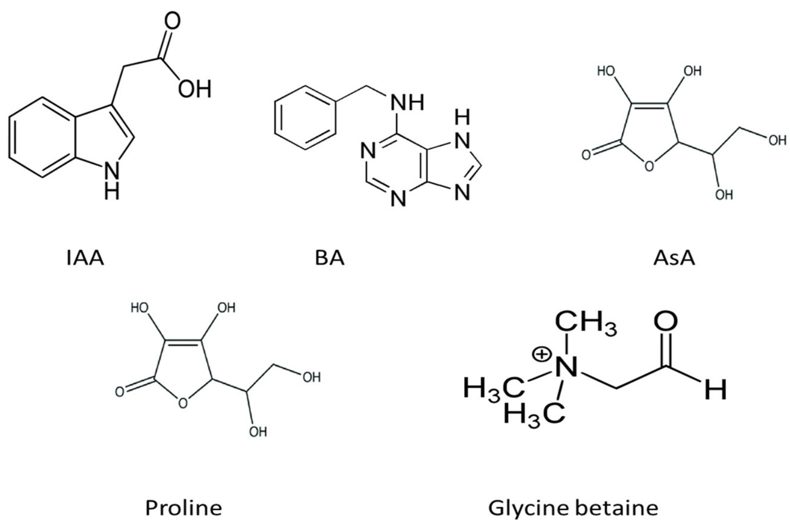

Figure 1. Show chemical structure of synthetic growth regulators

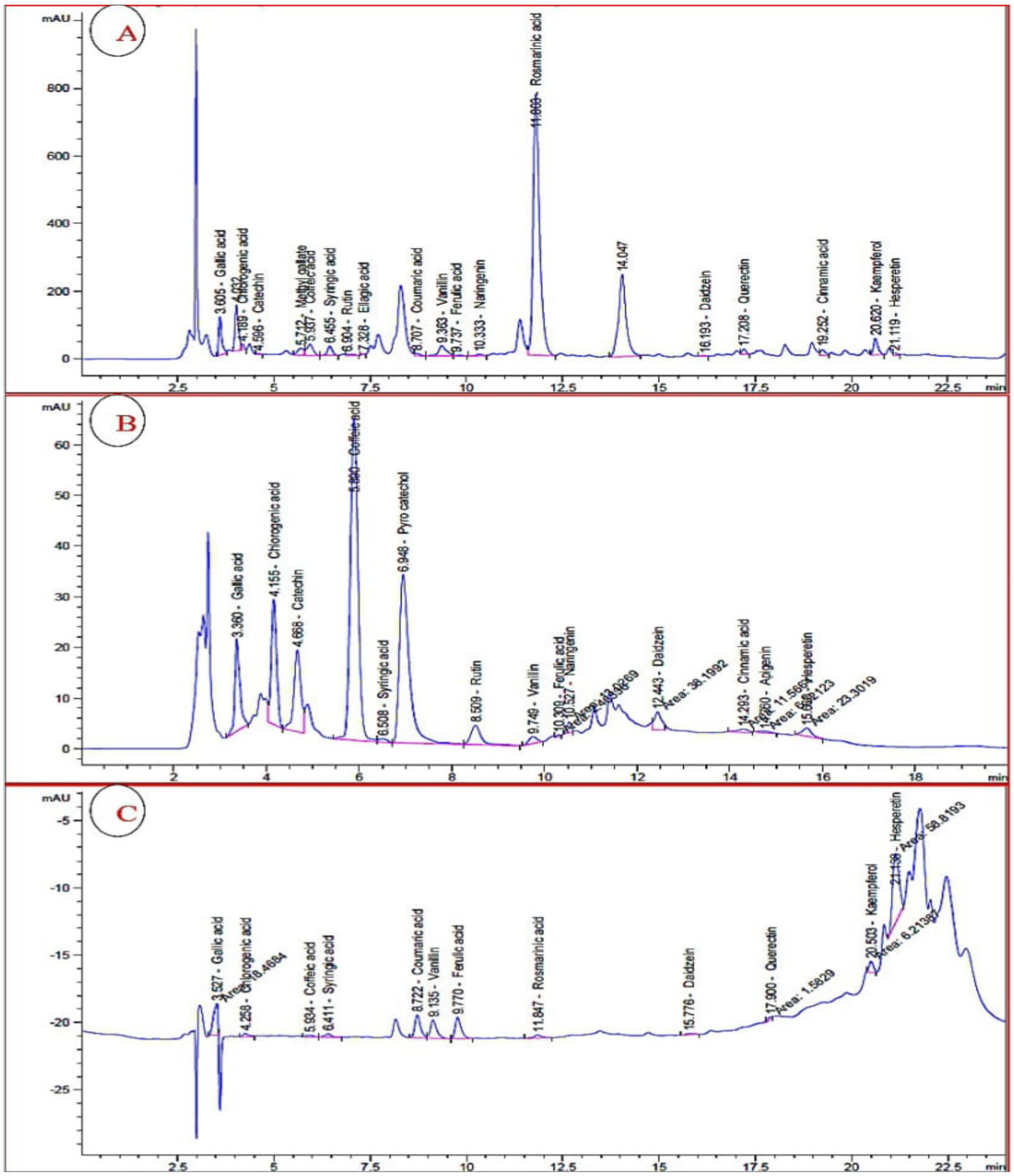

Figure 2. Chromatograms displaying the peaks found in the combination made up of all 19 of A. *Origanum majorana,* B. *Nigella sativa* and C. *Curcurma longa* phenolic components at: λ=280 nm.

Morphological characters

Data in Table 2, appeared to decrease of lengths, and fresh and dry weights of both the shoot and root of sunflower plants under water stress, in comparison to other treatments. On the other hand, foliar application of synthetic (IAA, BA, ASc, Glycein betaine and Proline) and natural (*Origanum majorana*, *Nigella sativa* and *Curcurma longa*) growth regulators significantly decreased the adverse effects of water stress on all studied morphological parameters of sunflower plant. The foliar application of proline, had the highest value of shoot length by about 10% more than the control (stress plant). In the case of root length, the maximum value was observed when treated with glycerin betaine by about 2.8%. The highest values of fresh and dry weight of shoots and fresh weight of roots were observed when the treatment occurred with IAA by about 26%, 95% and 42%, respectively, over the control. Whereas, the highest value of the dry weight of root was observed when treatment with *Nigella sativa* by about 59% more than the control (Table 2).

Table 2. Effects of synthetic (IAA, BA, ASc, Glycein betaine and Proline) and natural (*Origanum majorana*, *Nigella sativa* and *Curcurma longa*) growth regulators on morphological characters of sunflower plant under water stress

Treatments	Shoot length (cm)	Root length (cm)	Fresh weight of shoot (g)	Dry weight of shoot (g)	Fresh weight of root (g)	Dry weight of root (g)
Control	73.93± 1.36 c	12.33±0.21 ab	5.24± 0.214 b	0.43±0.02 b	0.45±0.07 a	0.022±0.006 a
IAA	77.70 ±2.36 abc	10.42±0.25 c	6.62± 0.451 a	0.84±0.05 a	0.64±0.09 a	0.022±0.004 a
BA	75.95 ±2.014 bc	12.17±0.14 ab	6.10± 0.19 ab	0.69±0.05 ab	0.59±0.07 a	0.026±0.008 a
Asc	79.67 ±1.54 ab	12.65±0.65 a	5.48± 0.25 ab	0.34±0.05 ab	0.37±0.06 a	0.025±0.004 a
Glycein betaine	80.00 ±1.65 ab	12.68±0.42 a	6.29±0.19 ab	0.71±0.04 ab	0.46±0.03 a	0.023±0.007 a
Proline	81.33 ±2.14 a	11.05±0.47 bc	6.13±0.29 ab	0.69±0.07 ab	0.35±0.09 a	0.033±0.007 a
Origanum majorana	78.97 ±0.98 abc	10.57±0.47 c	6.44±0.36 ab	0.72±0.02 ab	0.53±0.03 a	0.029±0.008 a
Nigella sativa	78.67 ±1.12 abc	11.17±0.65 bc	6.40±0.15 ab	0.72±0.08 ab	0.48±0.05 a	0.035±0.003 a
Curcurma longa	78.42 ±1.241 abc	12.38±0.48 ab	6.12±0.42 ab	0.69±0.09 ab	0.57±0.07 a	0.024±0.006 a
LSD 5%	5.62	1.71	1.32	0.05	0.04	0.001`

Data represents means ± standard error (n=5). Different lowercase letters in the same species within a column indicate significant differences (P≤0.05). Chl is represented by a chlorophyll. HSD is honestly significant difference by post hoc-Tukey's test.

Photosynthetic pigments

Data presented in Table 3 observed clearly that, drought stress caused a significant drop in the chlorophyll (Chl. a, Chl. b and total Chl.) and carotenoid contents of sunflower plant. On the other view, data of the present work (Table 3) also portrays that, the chlorophyll and carotenoid contents of sunflower plants, mostly, significantly increased following spray application of IAA, BA, ASc, glycein betaine, proline, *Origanum majorana*, *Nigella sativa* and *Curcurma longa*. The highest value of chl. a and carotenoid contents were recorded at *Origanum majorana* treatment, which increased by about 32% and 72%, respectively, compared to the stress (control) plant. Whereas, the highest value of chl. b and a + b contents were recorded at proline treatment, which increased by about 27% and 20%, respectively, compared to the stress (control) plant.

Table 3. Effects of synthetic (IAA, BA, ASc, Glycein betaine and Proline) and natural (*Origanum majorana*, *Nigella sativa* and *Curcurma longa*) growth regulators on photosynthetic pigments of sunflower plant under water stress

Treatments	Chlorophyll (a)	Chlorophyll (b)	Total chlorophyll (a + b)	Carotenoids
	(mg/g fresh weight)			
Control	11.47±1.02b	4.72±0.21b	16.18±2.32b	2.64±0.21b
IAA	8.94±1.36c	4.26±0.36b	13.20±1.74c	1.51±0.32c
BA	11.21±2.32b	4.54±0.14b	15.75±2.36b	2.88±0.45b
Asc	5.56±1.84d	3.29±0.45c	8.85±1.25d	0.54±0.65d
Glycein betaine	7.58±1.41c	3.53±0.75c	11.12±1.89cd	1.57±0.19c
Proline	13.38±1.36b	5.98±0.52a	19.35±3.25a	2.42±0.39b
Origanum majorana	15.10±2.01a	2.85±0.21c	17.95±1.45b	4.53±0.12a
Nigella sativa	10.08±2.34b	5.02±0.36a	15.09±1.03b	2.08±0.25b
Curcurma longa	6.46±1.04c	3.01±0.45c	9.46±2.32d	1.62±0.34c
LSD 5%	2.32	0.65	2.78	0.75

Data represents means ± standard error (n=5). Different lowercase letters in the same species within a column indicate significant differences (P≤0.05). Chl is represented by a chlorophyll. HSD is honestly significant difference by post hoc-Tukey's test.

Metabolic aspects

According to the results (Figure 3), water stress appeared to have a considerable impact on the metabolic components of sunflower plants. The lowest values of carbohydrate and protein contents in plant were observed under water stress. Whereas, the free proline and phenol contents were significantly promoted due to water stress. Conversely, compared to control groups, our treatments dramatically reduced the amount of free proline and total phenols present.

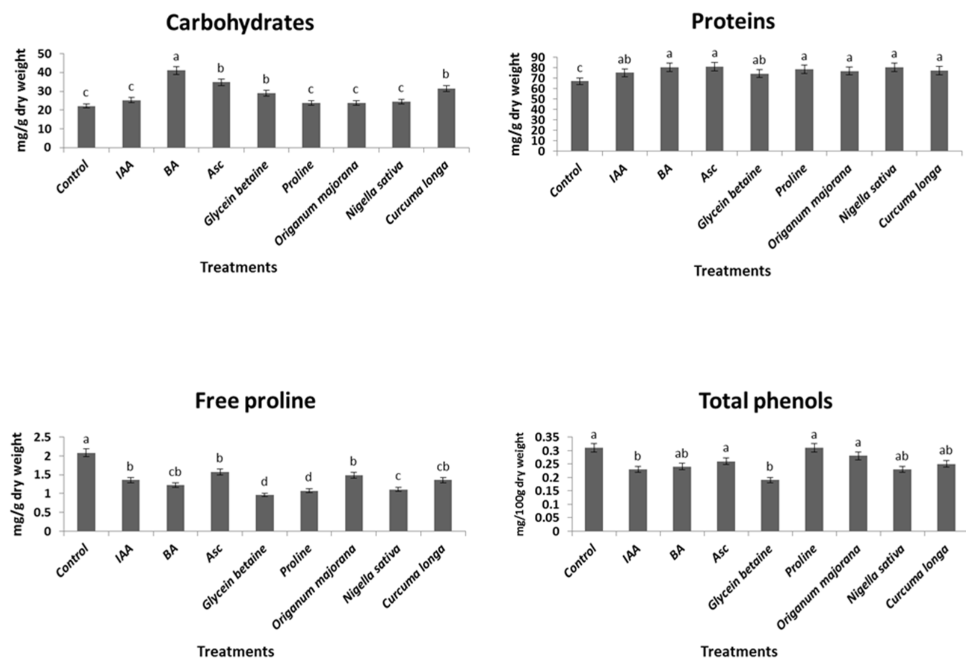

Figure 3. Effects of synthetic (IAA, BA, ASc, Glycein betaine and Proline) and natural (*Origanum majorana*, *Nigella sativa* and *Curcurma longa*) growth regulators on metabolic aspect of shoot of sunflower plant under water stress

Data represents means ± standard error (n=3). Different lowercase letters in the same species within a column indicate significant differences ($P \leq 0.05$).

Antioxidant enzymes

The illustrated data in Figure 4 demonstrate how water stress, external application of artificial regulators, and organic regulators affected antioxidant enzymes (SOD, CAT, POX, PPO) activities differently in sunflower plants. On the other hand, exogenous application of IAA, BA, Asc, glycein betaine, proline, *Origanum majorana, Nigella sativa* and *Curcurma longa* significantly reduced (in many cases) the activities of antioxidant enzymes compared to control (stress) plants.

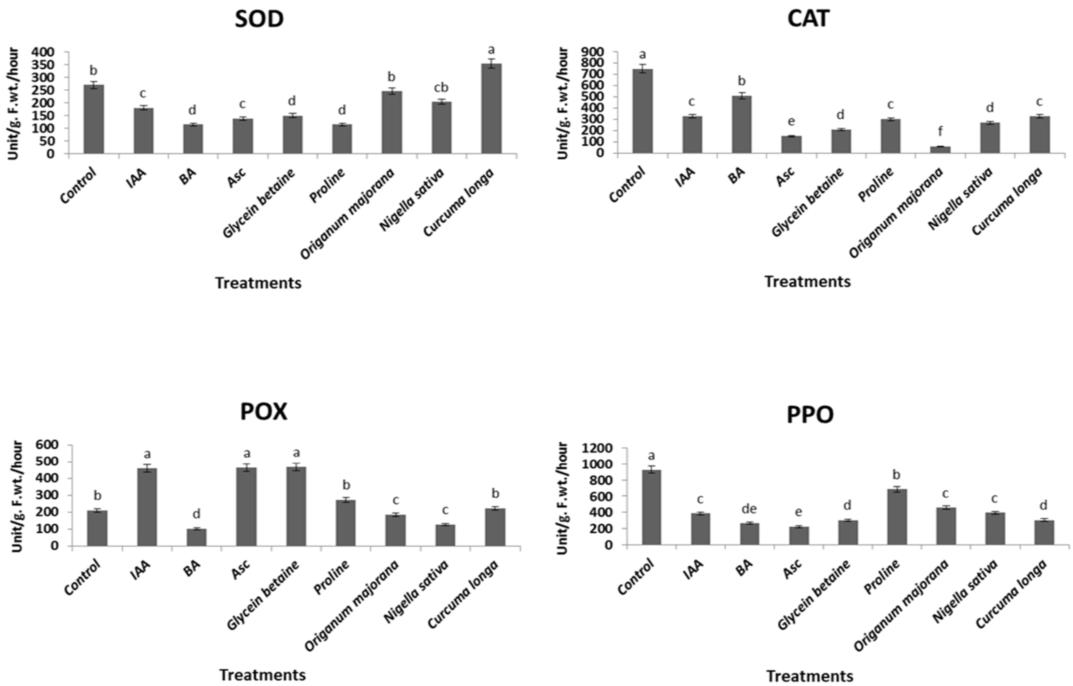

Figure 4. Effects of synthetic (IAA, BA, ASc, Glycein betaine and Proline) and natural (*Origanum majorana*, *Nigella sativa* and *Curcurma longa*) growth regulators on antioxidant enzymes of sunflower plant under water stress
Data represents means ± standard error (n=3). Different lowercase letters in the same species within a column indicate significant differences ($P \leq 0.05$).

Discussion

High performance liquid chromatography analysis of bio-stimulants appeared six phenolic acids, one phenol and two flavonoids were found in all natural extracts. Gallic, rosmarinic, caffeic, syringic acids and kaempferol are the major phenolic compounds (more than 1000 µg/g) detected in *Origanum majorana*. While gallic acid, hesperetin and ferulic acid are the major phenolic compounds (more than 1000 µg/g) present in *Curcurma longa*. Gallic acid, Chlorogenic acid, catechin, Pyro catechol, rutin and coffeic acid are the dominant phenolic compounds present in *Nigella sativa*. In this concept, Çelik *et al*. (2017) showed that *Origanum majorana* is known for its high amount of rosmarinic acid, rutin and caffeic acid. Also, Elansary and Mahmoud (2015) revealed that the main components in the leaves of *Origanum majorana* were rosmarinic acid and caffeic acid, also Bower *et al*. (2014) found that naringenin flavonoid have been found in the methanolic extract of *Origanum majorana*. Also, Mechraoui *et al*. (2018) found that, rutin and gallic acid were the main compounds of *Nigella sativa*.

Water stress events that plants experience during growth force them to develop defense mechanisms in order to make up for changes brought on by drought (Skowron and Trojak 2021). Our results revealed that, decreasing soil water content significantly reduced lengths, and fresh and dry weights of both the shoot and root of sunflower plants, in comparison to other treatments (Table 1). Many studies appeared on the crucial role of water stress on sunflowers. For example, Mantawy and El-hag (2018) reported that, drought stress affected most of the measured parameters of sunflower plants. Plant height, stem diameter and head size declined upon drought stress as compared to the control during the two growing seasons 2015 and 2016. In another oil plant, Pourghasemian *et al.* (2020) indicated that, sesame plants subjected to drought-induced stress exhibited significant reductions in the height of the plants, the area of leaves index, physiological and production of seeds, both chlorophyll an and b content, net rates of photosynthesis, stomatal conductivity, evaporation and water consumption efficiency, but promoted Malondialdehyde, proline, proteins and carotenoid pigment contents, and catalase (CAT), ascorbate peroxidase (APX), Guaiacol peroxidase (GPX) and glutathione reductase (GR) activity. While the external foliar use of licorice extract, salicylic acid, and beeswax waste extract reduced the oxidative effects of drought stress.

On the other hand, foliar application of synthetic (IAA, BA, ASc, Glycein betaine and Proline) and natural (*Origanum majorana*, *Nigella sativa* and *Curcurma longa*) growth regulators significantly decreased the adverse effects of water stress on all studied morphological parameters of sunflower plant (Table 2). These results are in agreement with El-Din (2015) found that, spraying sunflower plants with anti-oxidant and/or growth regulator (ascorbic acid and salicylic acid) seemed to be the most suitable treatments to increase the growth characters (plant height, number of leaves/plant, stem dry weight, leaves dry weight/plant, head dry weight, total plant dry weight and blades area/plant) of sunflower plants under water stress conditions. Recently, Seleiman *et al*. (2021) demonstrated that the growth and productivity of plants (including rice, maize, barley, soybean, and others) under drought stress could be improved by the exogenous application of plant growth regulators (auxin, ascorbic acid), and osmoprotectants (glycein betaine and proline). Due to the public's growing concern over the use of synthetic chemicals in agriculture, previous research have also revealed that secondary metabolites in some plant extracts have attracted significant attention, while aromatic plants have gained interest as a source of allelopathic secondary metabolites. (Duke, 2010). In plants, alkaloids, terpenoids, carbohydrates, flavonoids, phytosterols, and glycosides make up the majority of the allelochemicals. (Kong et al. 2019). They can affect plant growth, phytohormones activity, plant-water relationship and other plant processes (Elena 2014). In this regards, Bonea and Urechean (2018) examined how the extract of sweet marjoram (*Origanum majorana* L.) affected the growth of the *Zea mays* plant. They discovered that applying sweet marjoram extracts to the leaves of maize plants at concentrations of 1% and 2% considerably increased the plant's growth characteristics.

Data presented in Table 3 showed that, drought stress caused a significant drop in the chlorophyll (Chl. a, Chl. b and total Chl.) and carotenoid contents of sunflower plant. These findings could be the result of water stress, which decreases the photosynthetic capacity and raises reactive oxygen species (ROS), which causes oxidative damage to DNA, lipids, and proteins and, therefore lower chlorophyll concentrations *(Ebrahimi et al*., 2014). Also, due to the reduced CO_2 availability caused by stomatal closure, water stress has a direct impact on photosynthetic rates (Chaves *et al*., 2009). On the other view, data of the present work (Table 3) also portrays that, the chlorophyll and carotenoid contents of sunflower plants, mostly, significantly increased following spray application of IAA, BA, ASc, glycein betaine, proline, *Origanum majorana*, *Nigella sativa* and *Curcurma longa*. These results align with those that were obtained by Kim *et a*l. (2013) on potatoes and Ashraf and Foolad (2007) on maize, rice and barley plants.

According to the results (Figure 3), the lowest values of carbohydrate and protein contents in plant were observed under water stress. Whereas, the free proline and phenol contents were significantly promoted due to water stress. These results are consistent with other findings (Hussain *et al*., 2018). As opposed to that, foliar application of IAA, BA, ASc, glycein betaine, proline, *Origanum majorana*, *Nigella sativa* and *Curcurma longa* significantly improves carbohydrate and protein contents. Conversely, compared to control groups, our treatments dramatically reduced the amount of free proline and total phenols present (Figure 3). The obtained results showed the role of such treatments in dropping the harmful effects of water stress on the studied plant and helping the plant to restore its normal condition. These findings are in a parallel with that of Muhammad *et al*. (2016), who stated that, spray application of IAA showed an increasing trend in sugar and protein contents on wheat plants under drought stress, while the proline contents significantly decreased because IAA treatment alleviated water limiting effect. Also, Nawaz and Wang (2020) concluded that, spray application of glycine betaine promoted the production and accumulation of proline, soluble proteins and sugars, and total phenols in (A-38, A-58 and A-59) accessions of *Axonopus compressus* under drought stress. Compared to other natural plant extracts, the *Nigella sativa* treatment showed the highest decrease in proline levels. This may be due to the high concentration of caffeic acid (2406.97 g/g), which is well known for its ability to scavenge reactive oxygen species under different abiotic stressors (Mehmood *et al*., 2021).

The illustrated data in Figure (4) showed that, drought stress stimulated antioxidant enzymes activities in sunflower plants in compeared to control. Our findings agreed with those that were published by Khazaei et al. (2020) reported that the activity of the enzymes SOD, CAT, POX, and PPO in the leaves of sweet pepper were dramatically elevated by drought stress. Also, Mohammadi *et al*. (2011) examined how drought stress affected the activity of antioxidant enzymes in chickpea plants. They observed that under stressful circumstances, the activity of the enzyme's superoxide dismutase, catalases, and glutathione peroxidase considerably increased.

On the other hand, exogenous application of IAA, BA, Asc, glycein betaine, proline, *Origanum majorana, Nigella sativa* and *Curcurma longa* significantly reduced (in many cases) the activities of antioxidant enzymes compared to control (stress) plants (Figure 4). Such reductions in SOD, CAT, POX and PPO activities showed how these treatments helped sunflower plants recover from the oxidative damage brought on by drought. In this concept, Shi *et al*. (2014) observed that exogenous administration of IAA reduced the negative effects of drought stress in Arabidopsis plants by lowering the levels of various ROS; as a result, the activation of antioxidant enzymes (CAT, SOD, glutathione reductase, and POX) was significantly reduced.. Recently, Yadav *et al*. (2021) stated that auxins' ability to modify ROS production and activate other stress-responsive hormones makes IAA a key player in the regulation of the drought stress response. Also, Todorova *et al*. (2022) reached the conclusion that the exogenous application of 1-[2-chloroethoxycarbonyl-methyl] auxin-type compounds in wheat and maize plants growing under drought stress, -4-naphthalenesulfonic acid calcium salt (TA-12) and 1-[2-dimethylaminoethoxicarbonylmethyl] naphthalene chlormethylate (TA-14) led to adjustments in CAT and POX activities and suggested that the auxin compounds played a crucial role in balancing H_2O_2 levels (the creation or decomposition). Additionally, it has been discovered that the use of biostimulants, such as amino acids and plant extracts, increases the beneficial effect of plant development and mitigates the negative effects of various environmental stresses, such as drought. The production of a wide range of nonproteinic nitrogenous components, such as pigments, vitamins, coenzymes, purine and pyrimidine bases, uses amino acids extensively, which contributes to their significance. Previous research has demonstrated that amino acids can have a direct or indirect impact on physiological processes involved in plant growth. (Haghighi *et al*., 2020). In this regards, Gebaly *et al*. (2013) was conducted that indicated spraying cotton plants with an organic acid, amino acids (glycine at 600 PPM and proline at 200 PPM), and combinations of them with potassium citrate under drought conditions tended to increase growth parameters, such as plant height, number of fruiting branches, number of open bolls per plant, seed index, boll weight, lint percentage, seed cotton yield per feddan, and some chemical content in cotton leaves, such as chlorophyll a, b, Recently, Lalarukh *et al*. (2022) portrays that exogenously application of moringa leaves extract decrease the harmful effects of water stress to wheat plants. As a result, the levels of proline, glycine betaine, electrolyte leakage, malondialdehyde, hydrogen peroxide, superoxide dismutase (SOD), and peroxide (POD) significantly decreased.

Conclusions

It can be concluded that drought stress had a negative effect on plant growth parameters and metabolic contents of sunflower plant. However, when drought stress was applied with synthetic (IAA, BA, ASc, glycein betaine and proline) and natural (*Origanum majorana, Nigella sativa and Curcurma longa*) growth regulators, drought tolerance was improved and all growth factors were increased. Moreover, HPLC analysis of *Origanum majorana, Curcurma longa and Nigella sativa* treatments appeared six phenolic acids. Gallic, rosmarinic, caffeic, syringic acids and kaempferol are the major phenolic compounds detected in *Origanum majorana*. While gallic acid, hesperetin and ferulic acid are the major phenolic compounds present in *Curcurma longa*. Gallic acid, chlorogenic acid, catechin, pyro catechol, rutin and caffeic acid are the dominant phenolic compounds present in *Nigella sativa*. The *Nigella sativa* treatment showed the highest decrease in proline levels. Finally, these natural medicinal plant extracts can be effectively used to protect sunflower plants from the damaging effects of drought stress.

Authors' Contributions

Conceptualization: M.A.A., Kh.S.I., S.K.A., and M.R.*;* Formal analysis; Funding acquisition; Methodology and Project administration; M.A.A., M.A.A., Kh.S.I., S.K.A., M.R. and A.I.N.*;* Resources; Software; Supervision; Validation; Visualization; Writing - original draft; Writing - review and editing.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

The authors extend their appreciation to the Deputyship for Research & Innovation, Ministry of Education in Saudi Arabia for funding this research work through project number ISP23-62

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Adeleke BS, Babalola OO (2020). Oilseed crop sunflower (Helianthus annuus) as a source of food: Nutritional and health benefits. Food Science & Nutrition 8(9):4666-4684. *https://doi.org/10.1002/fsn3.1783*

Aebi H (1984). Catalase *in vitro*. Academic Press, pp 121-126. *https://doi.org/10.1016/S0076-6879(84)05016-3*

Anisimov MM, Skriptsova AV, Chaikina EL, Klykov AG (2013). Effect of water extracts of seaweeds on the growth of seedling roots of buckwheat (*Fagopyrum esculentum* Moench). International Journal of Research and Reviews in Applied Sciences 16(2):282-287.

Annunziata MG, Ciarmiello LF, Woodrow P, Dell'Aversana E, Carillo P (2019). Spatial and temporal profile of glycine betaine accumulation in plants under abiotic stresses. Frontiers in Plant Science 10:230. *https://doi.org/10.3389/fpls.2019.00230*

Ashraf M, Foolad MR (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environmental and Experimental Botany 59(2):206-216. *https://doi.org/10.1016/j.envexpbot.2005.12.006*

Bates LS, Waldren RP, Teare ID (1973). Rapid determination of free proline for water-stress studies. Plant and Soil 39(1):205-207. *https://doi.org/10.1007/BF00018060*

Bonea D, Urechean V (2018). Effect of sweet marjoram (*Origanum majorana* L.) cogermination and aqueous extracts on maize (*Zea mays* L.). "Agriculture for Life, Life for Agriculture" Conference Proceedings 1(1):11-16. *https://doi.org/10.2478/alife-2018-0002*

Bower AM, Real Hernandez LM, Berhow MA, De Mejia EG (2014). Bioactive compounds from culinary herbs inhibit a molecular target for type 2 diabetes management, dipeptidyl peptidase IV. Journal of Agricultural and Food Chemistry 62(26):6147-6158. *https://doi.org/10.1021/jf500639f*

Castillo FJ, Penel C, Greppin H (1984). Peroxidase release induced by ozone in sedum album leaves. Plant Physiology 74(4):846-851. *https://doi.org/10.1104/pp.74.4.846*

Çelik SE, Tufan AN, Bekdeşer B, Özyürek M, Güçlü K, Apak R (2017). Identification and determination of phenolics in Lamiaceae species by UPLC-DAD-ESI-MS/MS. Journal of Chromatographic Science 55(3):291-300. *https://doi.org/10.1093/chromsci/bmw184*

Chun SC, Paramasivan M, Chandrasekaran M (2018). Proline accumulation influenced by osmotic stress in arbuscular mycorrhizal symbiotic plants. Frontiers in Microbiology 9:2525. *https://doi.org/10.3389/fmicb.2018.02525*

Daniel HD, George C (1972). Peach seed dormancy in relation to endogenous inhibitors and applied growth substances. Journal of the American Society for Horticultural Science 97(5):651-654. *https://doi.org/10.21273/JASHS.97.5.651*

Duke SO (2010). Allelopathy: Current status of research and future of the discipline: A commentary. Allelo Journal 25:17-30.

Ebrahimi M, Khajehpour M R, Naderi A, Nassiri B M (2014). Physiological responses of sunflower to water stress under different levels of zinc fertilizer. International Journal of Plant Production 8(4).

El Mantawy R, El-Hag D (2018). Effect of skipping irrigation at different growth stages and zinc foliar application on yield and water productivity of sunflower. Journal of Plant Production 9(3):273-279. *https://doi.org/10.21608/jpp.2018.35488*

Elansary HO, Mahmoud EA (2015). Egyptian herbal tea infusions' antioxidants and their antiproliferative and cytotoxic activities against cancer cells. Natural Product Research 29(5):474-479. *https://doi.org/10.1080/14786419.2014.951354*

El-Din G (2015). Alleviation of drought effect on sunflower (*Helianthus annus* L.) cv Sakha-53 cultivar by foliar spraying with antioxidant. Middle East Journal 4(4):794-801.

Elena B (2014). Elemente de bioinginerie agricolă. Editura Sitech, Craiova, pp 12.

Gebaly S, Ahmed F, Namich A (2013). effect of spraying some organic, amino acids and potassium citrate on alleviation of drought stress in cotton plant. Journal of Plant Production 4(9):1369-1381. *https://doi.org/10.21608/jpp.2013.74149*

Haghighi M, Saadat S, Abbey L (2020). Effect of exogenous amino acids application on growth and nutritional value of cabbage under drought stress. Scientia Horticulturae 272:109561. *https://doi.org/10.1016/j.scienta.2020.109561*

Horoszkiewicz J, Jajor E, Danielewicz J, Korbas M, Schimmelpfennig L, Mikos-Szymańska M, Klimczyk M, Bocianowski, J (2023). The assessment of an effect of natural origin products on the initial growth and development of maize under drought stress and the occurrence of selected pathogens. Agriculture 13(4):815. *https://doi.org/10.3390/agriculture13040815*

Hussain M, Farooq S, Hasan W, Ul-Allah S, Tanveer M, Farooq M, Nawaz A (2018). Drought stress in sunflower: Physiological effects and its management through breeding and agronomic alternatives. Agricultural Water Management 201(September 2017):152-166. *https://doi.org/10.1016/j.agwat.2018.01.028*

Ikechukwu OA (2014). Crude Extracts of *Senna alata* (L.) Roxb. mimics plant growth hormones in promotion of vegetative and reproductive growth in *Celosia argentea* L. American Journal of Plant Sciences 5:1918-1925. *https://doi.org/10.4236/ajps.2014.513205*

Im Kim J, Baek D, Park HC, Chun HJ, Oh DH, Lee MK, Chung WS (2013). Overexpression of *Arabidopsis* YUCCA6 in potato results in high-auxin developmental phenotypes and enhanced resistance to water deficit. Molecular Plant 6(2):337-349.

Kapoor D, Bhardwaj S, Landi M, Sharma A, Ramakrishnan M, Sharma A (2020). The impact of drought in plant metabolism: How to exploit tolerance mechanisms to increase crop production. Applied Sciences 10(16):5692. *https://doi.org/10.3390/app10165692*

Khazaei Z, Estaji A (2020). Effect of foliar application of ascorbic acid on sweet pepper (*Capsicum annuum*) plants under drought stress. Acta Physiologiae Plantarum 42:1-12. *https://doi.org/10.1007/s11738-020-03106-z*

Khazaei Z, Esmaielpour B, Estaji A (2020). Ameliorative effects of ascorbic acid on tolerance to drought stress on pepper (*Capsicum annuum* L) plants. Physiology and Molecular Biology of Plants 26:1649-1662. *https://doi.org/10.1007/s12298-020-00846-7*

Kong CH, Xuan TD, Khanh TD, Tran HD, Trung NT (2019). Allelochemicals and signaling chemicals in plants. Molecules 24(15):2737. *https://doi.org/10.3390/molecules24152737*

Lalarukh I, Al-Dhumri SA, Al-Ani L KT, Hussain R, Al Mutairi KA, Mansoora N, … Galal TM (2022). A combined use of rhizobacteria and moringa leaf extract mitigates the adverse effects of drought stress in wheat (*Triticum aestivum* L.). Frontiers in Microbiology 13:813415. *https://doi.org/10.3389/fmicb.2022.813415*

Lichtentahler (1989). The Science of Photobiology. In: Smith KC (Ed). The Science of Photobiology. Boston, MA: Springer US. *https://doi.org/10.1007/978-1-4615-8061-4*

Lowry OH, Rosebrough, NJ, Farr AL, Randall RJ (1951). Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 193:265-275. *https://doi.org/10.1016/S0021-9258(19)52451-6*

Maharshi A, Rashid MM, Teli B, Yadav SK, Singh DP, Sarma BK (2021). Salt stress alters pathogenic behaviour of *Fusarium oxysporum* f. sp. *ciceris* and contributes to severity in chickpea wilt incidence. Physiological and Molecular Plant Pathology 113:101602. *https://doi.org/10.1016/j.pmpp.2021.101602*

Mangena P (2022). Evolving role of synthetic cytokinin 6-benzyl adenine for drought stress tolerance in soybean (*Glycine max* L. Merr.). Frontiers in Sustainable Food Systems 6:992581. *https://doi.org/10.3389/fsufs.2022.992581*

Marklund S, Marklund G (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European Journal of Biochemistry 47(3):469-474. *https://doi.org/10.1111/j.1432-1033.1974.tb03714.x*

Matta A, Diamoned C (1963). Symptoms of Fusarium wilt in relation to quantity of fungus and enzyme activity in tomato stems. Phytopathology 53(5):574-587.

Mechraoui O, Ladjel S, Nedjimi MS, Belfar ML, Moussaoui Y (2018). Determination of polyphenols content, antioxidant and antibacterial activity of *Nigella sativa* L. seed phenolic extracts. Scientific Study & Research. Chemistry & Chemical Engineering, Biotechnology, Food Industry 19(4):411.

Mehmood H, Abbasi GH, Jamil M, Malik Z, Ali M, Iqbal R (2021). Assessing the potential of exogenous caffeic acid application in boosting wheat (*Triticum aestivum* L.) crop productivity under salt stress. Plos One 16(11):e0259222. *https://doi.org/10.1371/journal.pone.0259222*

Mohammadi A, Habibi D, Rohami M, Mafakheri S (2011). Effect of drought stress on antioxidant enzymes activity of some chickpea cultivars. American-Eurasian Journal of Agriculture and Environmental Sciences 11(6):782-785.

Muhammad N, Hakim UMQ, Quraishi UM, Chaudhary HJ, Munis MFH (2016). Indole-3-acetic acid induces biochemical and physiological changes in wheat under drought stress conditions. Philippine Agriculture Science 99(1):19-24.

Mukherjee SP, Choudhuri MA (1983). Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. Physiologia Plantarum 58(2):166-170. *https://doi.org/10.1111/j.1399-3054.1983.tb04162.x*

Nawaz M, Wang Z (2020). Abscisic acid and glycine betaine mediated tolerance mechanisms under drought stress and recovery in *Axonopus compressus*: A new insight. Scientific Reports 10(1):1-10. *https://doi.org/10.1038/s41598-020-63447-0*

Osakabe Y, Osakabe K, Shinozaki K, Tran LSP (2014). Response of plants to water stress. Frontiers in Plant Science 5:86. *https://doi.org/10.3389/fpls.2014.00086*

Rasheed R, Yasmeen H, Hussain I, Iqbal M, Ashraf M A, Parveen A (2020). Exogenously applied 5-aminolevulinic acid modulates growth, secondary metabolism and oxidative defense in sunflower under water deficit stress. Physiology and Molecular Biology of Plants 26:489-499. *https://doi.org/10.1007/s12298-019-00756-3*

SeleimanM F, Al-Suhaibani N, Ali N, Akmal M, Alotaibi M, Refay Y, Battaglia ML (2021). Drought stress impacts on plants and different approaches to alleviate its adverse effects. Plants 10(2):1-25. *https://doi.org/10.3390/plants10020259*

Shi H, Chen L, Ye T, Liu X, Ding K, Chan Z (2014). Modulation of auxin content in Arabidopsis confers improved drought stress resistance. Plant Physiology and Biochemistry 82:209-217. *https://doi.org/10.1016/j.plaphy.2014.06.008*

Skowron E, Magdalena T (2021). Effect of exogenously-applied abscisic acid, putrescine and hydrogen peroxide on drought tolerance of barley. Biologia 76(2):453-468. *https://doi.org/10.2478/s11756-020-00644-2*

Snedecor GW, Cochran WG (1980). Statistical Methods. 7" ed. lowa State Univ. Press, lowa, USA, pp 325-330. *https://doi.org/10.1038/clpt.1985.194*

Todorova D, Katerova Z, Shopova E, Brankova L, Sergiev I, Jankauskienė J, Jurkonienė S (2022). The physiological responses of wheat and maize seedlings grown under water deficit are modulated by pre-application of auxin-type plant growth regulators. Plants 11(23). *https://doi.org/10.3390/plants11233251*

Umbreit WW, Burris RH, Stauffer JF (1957). Manometric techniques. A manual describing methods applicable to the study of tissue metabolism. Manometric techniques. A manual describing methods applicable to the study of tissue metabolism. Burgess Publishing Co.

Vernon LP, Seely GR (2014). The chlorophylls. Academic press.

Yadav B, Jogawa, A, Gnanasekaran P, Kumari P, Lakra N, Lal SK, Narayan OP (2021). An overview of recent advancement in phytohormones-mediated stress management and drought tolerance in crop plants. Plant Gene 25:100264. *https://doi.org/10.1016/j.plgene.2020.100264*

Zhang Y, Li Y, Hassan MJ, Li Z, Peng Y (2020). Indole-3-acetic acid improves drought tolerance of white clover via activating auxin, abscisic acid and jasmonic acid related genes and inhibiting senescence genes. BMC Plant Biology 20(1):1-12. *https://doi.org/10.1186/s12870-020-02354-y*