Alleviation of Copper Stress in Tomato Plants using Proline and Melatonin in an Open Soilless System

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ABSTRACT

This experiment was conducted to study the response of tomato plants (*Solanum lycopersicum* L. cv. 'Thuria') to different levels of copper and to reduce the harmful effects of high levels. The plants were grown during two summer seasons between March and July (2022) and repeated between March and July (2023) on a private soilless farm in New Noubaria City, Beheira Governorate. The plants were grown in sandy and peat moss as substrate, copper was added to the tomato plants through the nutrient solution. The Cu concentrations in the nutrient solutions were: control 48 ppb (the Cu concentration in the standard irrigation solution), 96 ppb, 144 ppb, and 192 ppb. On the other hand, protective treatments tested as foliar sprays are proline (Pro1) 40 mmol, proline (Pro2) 80 mmol, melatonin (MT1) 50 µmol, melatonin (MT2) 100 µmol, and the control distilled water. The results obtained from the two seasons indicated that increasing the Cu concentration led to a significant decrease in the fresh weight of roots and aerial parts, fresh and dry weight of the tomato plant, number of fruits per plant, average fruit weight, and plant productivity in both seasons. The highest mean values for the previous characteristics were achieved by applying Cu at 48 ppb (control treatment). However, the lowest mean values were obtained with the application of Cu at 192 ppb. Regarding the elemental content of tomato plants, the results showed that leaf nitrogen, phosphorus, and potassium contents decreased. In contrast, Cu contents in roots, stems, leaves, and fruits increased with increasing Cu concentrations in the nutrient solution. The chlorophyll content in leaves increased from 48 ppb to 96 ppb copper, decreasing sharply with increasing Cu concentration up to 192 ppb. Meanwhile, the protein and proline contents of the leaves decreased as the Cu concentration increased. The roots and leaf's contents of hydrogen peroxide, MDA, catalase enzyme activity, and superoxide dismutase increased with increasing Cu concentration. However, using proline (Pro1) 40 mmol, proline (Pro2) 80 mmol, melatonin (MT1) 50 µmol, and melatonin (MT2) 100 µmol, led to the plant's leaves having more nitrogen, phosphorus, potassium, and chlorophyll, which was reflected in improved plant growth characteristics compared with the control under different copper levels. It also improved the antioxidant content in the roots and leaves, which helped increase the number of fruits per plant, average fruit weight, and plant productivity. The outcome of this research suggested the possibility of using 100 µmol melatonin (MT2) and proline(Pro2) 80 mmol to reduce the harmful effects of excess copper in tomato plants.

Keywords: Tomato, Copper, Nutrient solution, Proline, Melatonin, Vegetative growth, Antioxidant, Yield.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.), a member of the family Solanaceae, is one of the most important crops that are grown on a large scale, as the total cultivated area worldwide in 2022 reached 4.92 million hectares and gave a productivity of 186 million tons, according to the FAOSTAT (2024). During the growing season, tomato plants are exposed to many fungal and bacterial diseases, whether soil or aerial (Panno *et al*., 2021). On the other hand, bactericides, fungicides, and fertilizers that contain Cu are widely used in open fields and greenhouses (Sonmez *et al*., 2006 and Adrees *et al.*, 2015).

Copper is essential for plant growth and development (Yruela, 2005). It is a micronutrient that binds to the plastocyanin protein and works in photosynthesis and metabolism. It also is involved in carbon metabolism and protects against oxidative stress (Yruela, 2013; Dalcorso *et al*., 2014 and Chen *et al*., 2022). Many enzymes need Cu to work properly. These include cytochrome c oxidase, laccase, amino oxidase, polyphenol oxidase, and phycocyanin (Nazir *et al*., 2019). Lafuente *et al*. (2023) found that the Cu deficiency in tomato leaves delayed lycopene content and fruit colour changes but increased acidity, antioxidant capacity, and vitamin C content during the transition from green to light red in Moneymaker tomato cultivar. In addition, Cu is vital in plants' photosystem II (PSII)-mediated electron transfer. This process is responsible for the decomposition of water molecules in photosynthetic cells. Copper ions can regulate plant hormones, such as auxin, abscisic acid, and melatonin, in plant root cells, affecting root growth (Hong *et al*., 2015; Batool *et al*., 2015; Cui *et al*., 2019 and Marques *et al*., 2019). Moreover, high levels of copper disrupt the structure of chloroplast and thylakoid membranes, leading to oxidative stress in plant cells. This change lowers the number of photosynthetic pigments and electron carriers, which stops the transfer of electrons during photosynthesis (Vassilev *et al*.,

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2003; González-Mendoza *et al*., 2013; Tan, 2014 and Hossain *et al*., 2020). However, excess copper adversely affects various growth parameters of plants cultivated in a solution-based system (Zheng *et al*., 2004). In addition, high levels of Cu reduce root length, root dry weight, total dry weight, root-to-shoot ratio, leaf area, and specific leaf area, as well as damage root cell membranes (Wainwright & Woolhouse, 1977 and Nazir *et al*., 2019), and induce structural changes to mitigate reactive oxygen species (ROS) production. Plants possess antioxidant mechanisms that include non-enzymatic molecular antioxidants such as ascorbic acid (ASC) as well as enzymatic antioxidants such as catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), superoxide dismutase (SOD), and glutathione reductase (GR), which prevent oxidative damage caused by excess copper (Kumar *et al*., 2008; Ivanova *et al*., 2010 and Azooz *et al*., 2012).

Melatonin, an indole tryptamine melatonin (MT), an N-acetyl-5-methoxytryptamine, is abundant in both animals and plants and plays a role in growth metabolism, seed germination, root structure, and flowering regulation, as well as mitigating the harmful effects of heavy metal toxicity and other environmental stresses on plants (Posmyk *et al.*, 2009; Shi *et al*., 2016; Sharif *et al*., 2018; Ibrahim *et al*., 2020; Nawaz *et al*., 2020 and Brengi *et al*., 2022). In addition, it acts as an internal scavenger of free radicals, which improves stress tolerance (Paredes *et al*., 2009). Melatonin is a bio-stimulant with a significant antioxidant effect, and MT ranked first among plant hormones in terms of antioxidant potential (Korkmaz *et al*., 2017). According to Nawaz *et al*. (2020), melatonin controls cellular activities by reducing the chemical activity of harmful substances and directly removing free radicals. Evidence suggests that MT can bind and neutralize the harmful effects of metals such as Cd, Cu, Al, Pb, and Fe in plant tissues. Moreover, melatonin binds to Cu^{2+} and Cu^{1+} to prevent free radical damage to plant cells (Tan, 2015). Melatonin alleviated the effects of Cu toxicity on plants that were treated with high levels of Cu; it led to an increase in flowering time and survival rate of cauliflower (Zhang *et al*., 2017), red cabbage (Posmyk *et al*., 2009) and cucumber (Cao *et al*., 2019). According to Oloumi *et al*. (2024), melatonin improves the ability of basil seedlings to tolerate Cu stress by controlling mineral nutrition, growth, and antioxidant responses. Zhao *et al*. (2017) found that applying melatonin from an external source significantly raised the levels of proline and antioxidants in the leaves of kiwifruit seedlings (*Actinidia deliciosa*), which, when exposed to Cu stress, improved the cells' ability to regulate osmotic balance and enhanced their ability to counteract the harmful effects of copper, thus reducing its toxicity.

Proline improves the growth of stressed plants and other physiological characteristics. With heavy metals, proline acts as a messenger molecule, antioxidant defense molecule, and metal chelator (Hayat *et al*., 2012). The accumulation of proline in *Vigna radiata* plants improved their ability to tolerate Cu stress by restricting the generation of H_2O_2 (Fariduddin *et al*., 2014). In addition, the increase in the proline accumulation caused by H_2O_2 treatment of Cu-stressed plants may be attributed to the activation of genes involved in the proline biosynthesis pathway (Li *et al*., 2013). Nazir *et al*. (2019) showed an increase in proline content with H_2O_2 under conditions of excessive Cu stress. Also, in stress-free plants, proline improves water uptake. It positively affects photosynthesis through its protective effect on the photosynthesis machinery, resulting in increased PSII efficiency and enhanced chlorophyll content, cumulatively protecting photosynthetic ability under stress from copper. Applying proline topically to wheat plants improves their nutrient uptake and decreases the stress caused by Cu (Noreen *et al*., 2018).

As tomatoes are one of the most common vegetable crops infected by many fungal, bacterial, and nematode diseases that require Cu in its various forms, whether metallic, chelated, or complex copper, it may exceed the safe limit for the plant. Therefore, this study aimed to study the effect of different levels of Cu on tomatoes and the possibility of alleviating the harmful effects of excess Cu using melatonin or proline in an open soilless system.

MATERIALS AND METHODS

The current experiment was conducted during the two summer seasons between 15th March and 15th July (2022) and repeated between 15th March and 15th July (2023) on a private farm, in New Noubaria City, Beheira Governorate, Egypt (30°40′00″N 30°04′00″E). This study aimed to investigate the effect of melatonin or proline on elevating the negative impact of Cu stress on tomato vegetative growth, yield, and chemical composition.

Plant cultivation and growth:

Tomato cultivar "Thuria" seeds were collected from Kanza Group. Seeds were sown in 209-cell trays filled with vermiculite and peat moss (1:1 vol) in a controlled growth chamber. After the development of the second-true-leaf, equal size tomato seedlings, 35 days old, were transplanted into white plastic containers (40 *40*40 cm) with 16 kg of soil substitute (peat moss 20:80 sand by volume) and fertigated with the appropriate nutrient solution for each stage, according to Van der Lugt *et al*. (2020) (Table 1). Subsequently, the plants were grown under greenhouse conditions at 28/20 ± 1 °C (day/night), a midpoint humidity was around 70% to 80%, and 12-14/12-10 h (day/night) photoperiods. Each experiment included 20 treatments consisting of four levels of copper: Control (standard nutrient solution fertigation copper at level of 48 ppb), 96 ppb, 144 ppb, and 192 ppb in the form of Copper EDTA 15%; and five protective treatments: proline (Pro1) 40 mM, proline (Pro2) 80 mM, melatonin (MT1) 50 µM, melatonin (MT2) 100 µM in addition to the untreated control (distilled water). The treatments were applied as a foliar spray a week after transplanting and repeated three times at 15-day intervals. Transplants were sprayed to run off. Supplementary requirements and agronomic practices for growing tomato plants are based on the recommendations of the Ministry of Agriculture for cultivating tomato plants grown in soil alternatives.

Experimental layout

The experimental layout was a randomized complete block design (RCBD) with four replicates in a split-plot system. Copper levels were arranged in the main plots, and protective treatments were randomly placed in the sub-plots. Six pots were planted for each treatment in each replicate.

Measurement of data:

- **Vegetative growth**:

Sixty days after transplanting, three plant samples were harvested, and the following data were recorded: plant height (cm), fresh weight of aerial parts (gm), root fresh weight (gm), plant fresh weight (gm) and plant dry weight (gm) were measured after drying the vegetative parts and roots at 70 °C to a constant weight.

- **Leaf and fruits chemical constituents:**

The total leaves chlorophyll content was measured using a chlorophyll meter (SPAD-502-metre, Konica Minolta, Japan). Leaves N content was determined, as Baker and Thompson (1992) outlined. Leaves P and K contents were determined as described by Cottenie *et al*. (1982). Protein content was calculated by multiplying the nitrogen concentration by 6.25. The total Cu content in plants (roots, stems, and leaves) and fruits was determined according to Wieteska *et al.* (1996).

Enzyme and hydrogen peroxide extract from tomato leaves and roots:

Enzymes were extracted from tomato leaves and roots and homogenized in phosphate buffer (pH 7.0) in a cold pestle and mortar. The mixture was then centrifuged at 9000g for 20 minutes in a cooled centrifuge. The resultant supernatant was examined for the presence of malondialdehyde (MDA), catalase (CAT), and superoxide dismutase. Catalase (CAT) activity in tomato leaves was measured according to Aebi (1984) method. Superoxide dismutase (SOD) was measured using the modified-nitro blue tetrazolium (NBT) method. Aluminium foil was used to shield the samples from the fluorescent light that began the procedure (Beauchamp and Fridovich, 1971). Landi (2017) method for determining the content of malondialdehyde (MDA) included reacting thiobarbituric acid (TBA) with tricarboxylic acid and then measuring the absorbance at 532 and 450 nm. leaf's hydrogen peroxide (H_2O_2) concentration was determined according the technique outlined by Velikova *et al.* (2000). Additionally, Bates *et al.* (1973) method, which required homogenizing leaves in salicylic acid, was used to quantify the proline concentrations.

Table 1. The composition of nutrients in the standard solution intended for irrigation was as follows:

Soluble ions \ Tomato growth stages		Start	Fruit Set	End season
$N\text{-}NH_4$	ppm	9.5	17	6.5
K	ppm	351	401.25	364
Mg	ppm	64.25	54.88	58
Ca	ppm	226.5	205.5	220
$N\text{-}NO_3$	ppm	207	207	207
P	ppm	47	47	31
S	ppm	141	141	141
Cl	ppm	35	35	35
Fe	ppb	1965	1680	1680
Zn	ppb	327	327	327
Mn	ppb	550	550	550
Mo	ppb	48	48	48
B	ppb	383	324	324
Cu	ppb	48	48	48

The nitrogen source was NO_3- derived from $Ca(NO_3)_2$ and KNO_3, and the iron source was Fe-DTPA 6% chelate, manganese EDTA 13, zinc EDTA 15%, and copper EDTA 15%. The nutrient solution pH was 5.3 and the EC was 2.6 m/cm (Van der Lugt *et al.*, 2020).

-Yield components:

Total plant yield was recorded as the weight of all marketable fruits throughout the harvest period. The average fruit weight was calculated by dividing the total weight by the number of fruits.

Statistical analysis and experimental design:

The experimental data were analyzed as a split-plot system in randomized complete block design. The CoStat software programme (Version 6.4, Co. Hort, USA, 1998–2008) was used for all data obtained for statistical analysis. Means were compared using the revised least significant difference (RLSD) test at $p < 0.05$.

RESULTS AND DISSECTION

Vegatitive growth:

The data in Table (2) demonstrate that the levels of copper in the nutrient solution affected plant height, number of branches, number of leaves, weight of vegetative parts, weight of roots, fresh plant weight, and dry weight.

Table 2. Effect of different levels of copper as fertigation, foliar application of proline or melatonin, and their interaction on plant height, fresh weight of aerial parts, root fresh, plant fresh weight and plant dry weight of tomato plants during the 2022 and 2023 seasons.

Treatments		Plant height (cm)		Fresh weight of aerial parts (g)		Root fresh weight (g)		Plant fresh weight (g)		Plant dry weight (g)	
		2022	2023	2022	2023	2022	2023	2022	2023	2022	2023
Cu levels	48 ppb	65.77	64.01	1579.70	1537.12	76.35	77.36	1656.05	1614.48	238.39	231.95
	96 ppb	63.69	63.52	1440.13	1436.97	66.87	68.30	1507.00	1505.27	193.52	192.99
	144 ppb	52.07	51.53	1035.10	1024.46	55.45	55.79	1090.55	1080.25	162.12	160.44
	192 ppb	45.98	45.91	821.58	820.59	46.26	46.05	867.83	866.64	132.74	132.58
RLSD A		1.159	1.292	26.465	21.921	0.694	0.936	26.757	22.012	5.488	5.174
Pro and Mt	C	54.80	54.15	1189.61	1176.17	58.66	59.87	1248.27	1236.04	175.29	172.83
	Pro1	57.44	56.81	1204.65	1192.54	61.19	61.01	1265.84	1253.56	179.24	177.39
	Pro2	56.62	56.25	1223.90	1214.41	61.62	61.88	1285.52	1276.29	183.11	181.80
	MT1	56.20	55.25	1222.48	1199.44	61.76	63.23	1284.24	1262.67	182.17	178.81
	MT2	59.30	58.74	1255.00	1241.34	62.93	63.39	1317.93	1304.73	188.65	186.61
RLSD B		1.348	1.507	29.250	34.82	0.689	1.121	29.331	34.629	6.248	6.405
Cu at 48 ppb	C	64.18	61.55	1581.00	1516.58	75.15	77.69	1656.15	1594.26	241.42	231.55
	Pro1	66.43	66.38	1572.75	1571.63	76.15	73.55	1648.90	1645.18	235.48	235.30
	Pro2	64.98	63.70	1560.75	1530.33	75.95	76.21	1636.70	1606.54	236.94	232.30
	MT1	64.43	61.68	1553.75	1486.90	76.15	78.66	1629.90	1565.56	231.76	221.80
	MT2	68.83	66.75	1630.25	1580.18	78.35	80.67	1708.60	1660.85	246.34	238.78
Cu at 96 ppb	C	63.83	65.78	1478.90	1525.25	63.70	64.31	1542.60	1589.56	188.36	194.23
	Pro1	63.18	62.68	1401.33	1389.90	67.13	68.80	1468.45	1458.70	191.58	190.00
	Pro2	63.88	62.90	1439.60	1418.03	68.15	69.51	1507.75	1487.53	193.75	190.88
	MT1	63.55	62.58	1437.83	1415.80	67.55	69.42	1505.38	1485.22	195.63	192.58
	MT2	64.00	63.68	1443.00	1435.85	67.83	69.49	1510.83	1505.34	198.27	197.28
Cu at 144 ppb	C	51.05	49.85	937.43	915.15	52.83	53.54	990.25	968.69	146.02	142.45
	Pro1	52.08	51.35	1025.30	1010.78	55.48	55.90	1080.78	1066.68	159.72	157.48
	Pro2	51.88	51.68	1050.38	1046.48	55.58	55.52	1105.95	1102.00	165.52	164.88
	MT1	51.10	50.65	1071.90	1062.05	56.00	56.38	1127.90	1118.43	167.88	166.38
	MT2	54.23	54.10	1090.50	1087.83	57.38	57.63	1147.88	1145.46	171.46	171.00
Cu at 192 ppb	C	40.15	39.43	761.10	747.70	42.98	43.97	804.08	791.67	125.36	123.10
	Pro1	48.10	46.83	819.23	797.88	46.00	45.80	865.23	843.67	130.18	126.78
	Pro2	45.75	46.73	844.88	862.80	46.80	46.29	891.68	909.09	136.24	139.15
	MT1	45.73	46.10	826.43	833.03	47.35	48.45	873.78	881.48	133.41	134.50
	MT2	50.15	50.45	856.25	861.53	48.15	45.76	904.40	907.28	138.54	139.38
RLSD A×B		2.696	3.013	58.50	69.635	1.379	2.243	58.663	69.258	12.497	12.81

Proline (Pro1) 40 mM, proline (Pro2) 80 mM, melatonin (MT1) 50 µM, melatonin (MT2) 100 µM and control distilled water (C).

These characteristics decreased significantly as the level of copper increased; the lowest mean values were associated with the highest level of copper (192 ppb). The results agree with those obtained from Sonmez *et al*. (2006). Plant height and dry root weight decreased with increasing Cu application to the soil, whereas these characteristics fell below the highest level compared to the control. Consistent with the results obtained by Gong *et al*. (2021), stress at high levels significantly reduced spinach biomass and total fresh and dry weight. The findings were in line with those of Batool *et al*. (2015); Cui *et al*. (2019) and Marques *et al*. (2019), who contributed to our understanding of copper toxicity, which can restrict and damage plant root growth. High concentrations of copper in soil can lead to decreased nutrient and water uptake, resulting in reduced root formation, root size, and root hairs. The results agree with those of previous studies by Azmat and Riaz (2012). Increased copper concentration causes root length inhibition in mung beans, and it has been found (Işeri *et al*., 2011) in tomatoes and cucumber. Copper was more toxic to cucumber roots than tomatoes at the same copper concentration. By controlling hormones in plant root cells, which in turn change the growth of roots, Park & Back (2012) and Tasar (2022) found that copper ions can change the root meristem cell division rate and root development. It also contributes to this understanding. Overall, copper toxicity has significant effects on plant health and growth.

The application of proline or melatonin led to a significant improvement in growth characteristics, namely plant height, weight of aerial parts, weight of fresh roots, weight of fresh plant, and weight of dry plant. The results agreed with Brengi and Nasef (2023), who found that foliar spraying with proline improved the growth of under stress conditions parsley plants. The highest values were obtained under melatonin (MT2) 100 µM treatment, followed by proline (Pro2) 80 µM (Table 2). Adding melatonin, lowering H_2O_2 and MDA levels, and raising the activity of PAL and antioxidant enzymes helped basil seedlings under copper stress grow better (Oloumi *et al*., 2024).

The effect of the interaction between copper levels in the nutrient solution and foliar spraying with proline and melatonin on tomato vegetative growth was significant in both seasons (Table 2). The combined treatment of copper at 48 ppb and foliar spraying with melatonin (MT2) at 100 µM resulted in the highest mean values of the above-mentioned growth parameters. However, the lowest values were found in the control treatment (spraying with distilled water) combined with copper at 192 ppb.

Mineral nutrients:

Data in Table (3) indicates that as the copper level in the nutrient solution increased, the leaves' nitrogen, phosphorus, and potassium decreased. Meanwhile, the results, Table (4), showed that copper content was significantly affected as a result of increasing copper concentration in the nutrient solution. Adding copper at any level increases copper content in roots to a much greater extent than in leaves, stems, or fruits.

According to Baldi *et al*. (2018), high copper concentrations in soil can delay root growth and the absorption of potassium and phosphorus. Hippler *et al*. (2018) found that copper increases the levels of nitrate reductase, low-affinity nitrate transporters, and transcription factors, which makes plants take in and store less nitrogen. According to Gong *et al*. (2021), spinach leaves and roots significantly increase copper concentrations under high copper stress.

The data in Table (3) indicate that foliar spraying with proline and melatonin increases nitrogen, phosphorus, and potassium leaf content. Spraying melatonin (MT2) showed the highest mean values of leaf's N, P, and K content than those of the other four treatments. On the other hand, the data in Table 4 indicate that the copper content in roots, leaves, stems, and fruits was significantly decreased when tomato plants received proline or melatonin compared with the control. Moreover, the lowest mean values were obtained by applying melatonin (MT2) at the rate of 100 µM treatment. Melatonin was found to regulate mineral distribution in basil seedlings under Cu stress conditions (Oloumi *et al*., 2024). It is known that melatonin stimulates the activation of excess Cu^{2+} capture, which is then transported and stored in the apoplast (Cao *et al*., 2019).

Concerning the interaction effect between copper levels and the foliar sprays with proline and melatonin, the results showed that leaf's N, P, and K contents exhibited the highest mean values when treated with copper level at 48 ppb and melatonin at (MT2) 100 µM. Conversely, the control treatment with copper at 192 ppb had the lowest values. In contrast, the copper content in the roots and stems was higher than that in the leaves and fruits when the control treatment was applied at a lower copper level. However, the lowest values were observed when melatonin (MT2) 100 µM treatment was applied under copper at 48 ppb.

Proline, protein, and chlorophyll content:

The data in Table (5) generally indicate that protein and chlorophyll contents decreased when copper content in the nutrient solution increased. However, proline content increased due to increasing copper in the nutrient solution. These results align with previous studies on stonehead cabbage, cucumber, and sunflower, which showed a significant reduction in total chlorophyll (Chl a+b) and carotenoid content when exposed to copper stress (Kabata-Pendias & Pendias, 2001;Vinit-Dunand *et al*., 2002; Zengin & Kirbag, 2007; Ali *et al*., 2015; Hossain *et al*. 2020 and Gong *et al*., 2021). Multiple studies have shown that copper affects both photosystems, as documented by Mishra and Dubey (2005). The most vulnerable site to copper toxicity is PS II, as documented by Panou-Filotheou *et al*. (2001); Yruela (2009); Xu *et al*. (2013) and Cambrollé *et al*. (2015).

The reduction in protein content in leaves could result from plants reducing their ability to absorb nitrogen, which is caused by an imbalance in nitrogen assimilation and transport enzymes due to increased copper (Hippler *et al*., 2018). Moreover, Shahid *et al*. (2014) and Gong *et al*. (2021) showed that higher copper levels led to higher proline levels. During the two seasons of the study, foliar spraying with Proline and Melatonin improved the protein, chlorophyll, and proline content in tomato leaves compared with the control treatment. The treatment with melatonin (MT2) at 100 µM resulted in the highest mean values of protein and chlorophyll in the leaves, whereas treatment with proline (Pro2) at 80 mM gave the highest mean values of proline content in the leaves.

Table 3. Effect of different levels of copper as fertigation, foliar application of proline or melatonin, and their interaction on nitrogen, phosphorus and potassium content in tomato leaves during the 2022 and 2023 seasons.

Treatments		Nitrogen%		Phosphorus %		Potassium %	
		2022	2023	2022	2023	2022	2023
Cu levels	48 ppb	3.41	3.38	0.64	0.62	3.81	3.83
	96 ppb	3.34	3.32	0.60	0.59	3.58	3.61
	144 ppb	3.06	3.01	0.56	0.54	3.34	3.39
	192 ppb	2.84	2.84	0.49	0.48	3.02	3.00
RLSD A		0.0246	0.0482	0.00558	0.014	0.0283	0.0338
Pro and Mt	C	3.10	3.10	0.55	0.53	3.33	3.33
	Pro1	3.15	3.11	0.57	0.56	3.40	3.40
	Pro2	3.15	3.15	0.58	0.56	3.47	3.51
	MT1	3.19	3.16	0.58	0.56	3.46	3.48
	MT2	3.22	3.18	0.58	0.57	3.53	3.56
RLSD B		0.0168	0.0598	0.0108	0.017	0.0297	0.0258
Cu at 48 ppb	C	3.38	3.42	0.63	0.62	3.77	3.79
	Pro1	3.41	3.38	0.63	0.63	3.77	3.80
	Pro2	3.37	3.31	0.64	0.62	3.86	3.86
	MT1	3.45	3.38	0.65	0.61	3.79	3.81
	MT2	3.43	3.42	0.65	0.63	3.85	3.87
Cu at 96 ppb	C	3.26	3.28	0.59	0.57	3.42	3.51
	Pro1	3.35	3.33	0.61	0.60	3.53	3.57
	Pro2	3.31	3.32	0.60	0.58	3.62	3.60
	MT1	3.37	3.29	0.59	0.59	3.66	3.63
	MT2	3.41	3.40	0.60	0.59	3.69	3.73
Cu at 144 ppb	C	2.97	2.90	0.54	0.49	3.23	3.29
	Pro1	3.04	3.00	0.56	0.55	3.33	3.36
	Pro2	3.08	3.03	0.56	0.55	3.38	3.45
	MT1	3.08	3.04	0.56	0.55	3.39	3.39
	MT2	3.12	3.06	0.57	0.55	3.39	3.48
Cu at 192 ppb	C	2.78	2.80	0.46	0.43	2.91	2.73
	Pro1	2.82	2.74	0.48	0.47	2.99	2.85
	Pro2	2.85	2.94	0.51	0.50	3.03	3.14
	MT1	2.86	2.92	0.51	0.51	2.99	3.11
	MT2	2.91	2.82	0.51	0.51	3.17	3.16
RLSD A×B		0.033	0.1197	0.0217	0.034	0.059	0.052

Proline (Pro1) 40 mM, proline (Pro2) 80 mM, melatonin (MT1) 50 µM, melatonin (MT2) 100 µM and control distilled water (C).

Table 4. Effect of different levels of copper as fertigation, foliar application of proline or melatonin, and their interaction on copper content in roots, stems, leaves and fruits of tomato plants during the 2022 and 2023 seasons.

Treatments		Cu in roots (ppm)		Cu in stems (ppm)		Cu in leaves (ppm)		Cu in friut (ppm)	
		2022	2023	2022	2023	2022	2023	2022	2023
Cu levels	48 ppb	182.61	184.84	7.13	7.39	25.23	25.94	2.07	2.27
	96 ppb	424.15	433.05	11.95	11.73	30.31	30.34	3.56	3.52
	144 ppb	931.95	937.87	21.05	21.23	44.96	45.71	5.46	5.76
	192 ppb	1395.33	1390.53	30.28	30.75	54.78	54.41	7.12	7.38
RLSD A		12.26	9.781	0.608	0.376	1.216	0.544	0.121	0.098
Pro and Mt	C	770.32	784.35	18.33	19.03	41.69	40.97	4.91	5.12
	Pro1	747.01	748.24	18.05	18.58	39.04	39.73	4.68	4.77
	Pro2	739.39	737.61	16.94	16.87	37.38	37.36	4.48	4.58
	MT1	712.47	726.01	17.54	17.41	39.22	39.69	4.42	4.51
	MT2	698.34	686.65	17.15	16.99	36.76	37.74	4.27	4.67
RLSD B		12.486	9.867	0.691	0.548	1.166	0.803	0.149	0.109
Cu at 48 ppb	C	174.60	180.28	6.65	7.08	24.68	24.98	2.13	2.45
	Pro1	189.28	182.78	7.07	7.27	25.18	25.40	2.10	2.18
	Pro2	186.08	186.70	7.01	7.20	25.68	26.38	2.00	2.10
	MT1	182.18	188.18	7.47	7.82	25.73	27.03	2.09	2.17
	MT2	180.95	186.28	7.46	7.58	24.90	25.93	2.03	2.45
Cu at 96 ppb	C	452.55	456.68	13.63	12.86	33.60	32.15	3.70	3.61
	Pro1	430.35	441.08	12.60	12.40	33.10	33.53	3.58	3.54
	Pro2	419.73	428.10	11.45	11.63	28.23	28.50	3.51	3.52
	MT1	409.93	421.28	11.08	10.93	28.85	29.73	3.55	3.54
	MT2	408.18	418.13	10.98	10.85	27.78	27.80	3.48	3.39
Cu at 144 ppb	C	961.58	974.58	21.28	22.05	46.90	47.93	5.73	6.33
	Pro1	938.45	945.73	21.41	21.85	45.02	45.63	5.53	5.75
	Pro2	934.85	933.98	20.10	20.00	42.05	42.73	5.38	5.55
	MT1	919.60	925.83	21.16	21.28	46.44	47.13	5.45	5.68
	MT2	905.25	909.23	21.30	20.98	44.39	45.15	5.23	5.48
Cu at 192 ppb	C	1492.58	1525.88	31.75	34.13	61.58	58.83	8.10	8.11
	Pro1	1429.95	1423.40	31.13	32.80	52.89	54.38	7.54	7.61
	Pro2	1416.93	1401.65	29.19	28.68	53.55	51.85	7.02	7.15
	MT1	1338.18	1368.75	30.48	29.60	55.89	54.88	6.58	6.64
	MT2	1299.00	1232.95	28.88	28.55	49.98	52.10	6.35	7.38
RLSD A×B		24.97	19.733	1.383	1.097	2.33	1.606	0.298	0.218

Proline (Pro1) 40mM, proline (Pro2) 80mM, melatonin (MT1) 50 µM, melatonin(MT2) 100 µM and control distilled water (c).

Applying proline resulted in an improvement in proline and protein accumulation as well as a decrease in reactive oxygen species formation in wheat subjected to copper stress, as shown by Noreen *et al.* (2018) Concerning the interaction effects between copper levels and the foliar sprays with proline and melatonin on tomato leaves protein, chlorophyll, and proline contents. The results indicated that the highest mean values of characters, as mentioned earlier, were achieved when tomato plants were treated with 48 ppb Cu and sprayed with melatonin (MT2) at 100 µM.

Antioxidant activities:

Tables (6 and 7) show the rise in the activity of antioxidant enzymes, such as CAT and SOD, as well as the levels of MDA and H_2O_2 as a result of increasing copper concentrations in the nutrient solution. Similar patterns were noticed between leaves and roots.

Peak values were observed at 192 ppb Cu. Results of Işeri *et al*. (2011); Chen *et al*. (2000) and Younis *et al*. (2018) focused on the levels of copper in the roots of tomatoes, cucumbers, and common beans. Their results indicated that high copper levels elevated CAT and APX enzyme activities, but low levels boosted SOD and POD contents in rice seedlings. However, high copper concentrations led to a decrease in enzyme activity. Furthermore, there was an increase in SOD and POD activities.

Table 5. Effect of different levels of copper as fertigation, foliar application of proline or melatonin, and their interaction on protein, proline, and chlorophyll-content in tomato leaves during the 2022 and 2023 seasons.

Treatments		Protein%		Proline		Chlorophyll content (SPAD unit)	
		2022	2023	2022	2023	2022	2023
Cu levels	48 ppb	21.30	21.13	100.18	101.3	46.55	45.40
	96 ppb	20.86	20.78	134.79	135.30	47.45	46.45
	144 ppb	19.10	18.79	205.97	208.20	44.00	43.00
	192 ppb	17.77	17.77	270.48	271.10	40.60	39.60
RLSD A		0.154	0.300	2.546	3.309	0.477	0.565
Pro and Mt	C	19.36	19.37	175.38	177.13	42.38	41.69
	Pro1	19.71	19.45	186.43	189.56	44.50	43.44
	Pro2	19.69	19.68	191.64	191.56	45.38	44.31
	MT1	19.94	19.73	171.96	172.56	45.13	43.94
	MT2	20.09	19.85	163.85	164.06	45.88	44.69
RLSD B		0.1052	0.374	2.360	3.340	0.708	0.753
Cu at 48 ppb	C	21.14	21.34	93.08	93.75	44.50	44.00
	Pro1	21.33	21.14	107.58	107.75	45.75	44.75
	Pro2	21.05	20.67	112.83	115.00	47.00	46.00
	MT1	21.58	21.13	93.30	94.50	47.50	46.00
	MT2	21.42	21.34	94.13	95.50	48.00	46.25
Cu at 96 ppb	C	20.38	20.52	133.08	129.25	45.75	45.50
	Pro1	20.91	20.78	139.80	141.25	47.25	46.00
	Pro2	20.67	20.75	144.03	146.50	47.50	46.25
	MT1	21.05	20.58	131.13	133.00	48.25	47.00
	MT2	21.31	21.27	125.90	126.50	48.50	47.50
Cu at 144 ppb	C	18.53	18.14	211.30	216.50	41.50	40.50
	Pro1	19.00	18.75	222.10	225.25	44.00	43.00
	Pro2	19.25	18.94	230.63	231.50	45.00	44.00
	MT1	19.23	18.97	187.93	189.50	44.25	43.25
	MT2	19.47	19.14	177.90	178.25	45.25	44.25
Cu at 192 ppb	C	17.39	17.48	264.08	269.00	37.75	36.75
	Pro1	17.61	17.11	276.23	273.25	41.00	40.00
	Pro2	17.78	18.36	279.10	284.00	42.00	41.00
	MT1	17.89	18.23	275.50	273.25	40.50	39.50
	MT2	18.17	17.64	257.48	256.00	41.75	40.75
RLSD A× B		0.210	0.748	4.721	6.681	1.417	1.506

Proline (Pro1) 40 mM, proline (Pro2) 80 mM, melatonin (MT1) 50 µM, melatonin (MT2) 100 µM and control distilled water (C).

Catalase activity CAT was reduced, in contrast. White lupine with elevated copper levels showed increased enzymatic activities in shoots and maize roots, chickpeas, and rice, according to various studies (Tanyolac *et al*., 2007; Kumar *et al*., 2008; Azooz *et al*., 2012; Sánchez-Pardo *et al*., 2012; Thounaojam *et al*., 2012; Tripathi *et al*., 2013; Younis *et al*., 2018 and Saleem *et al*., 2020). High copper concentrations raised the levels of superoxide dismutase (SOD) and peroxidase (POD) activities in flax plants. Studies by Shahid *et al*. (2014) and Gong *et al*. (2021) demonstrated that increased copper levels resulted in elevated activation of antioxidant enzymes. These enzymes are crucial in eliminating plants' reactive oxygen species (ROS). A concentration of 700 mg/kg copper in the soil markedly enhanced plant defense. Spinach plants grown in soil containing 700 mg/kg of copper show improved survival and increased copper tolerance because of proline (Claussen, 2005; Gong *et al*., 2019 and 2021).

Tables (6 and 7) show the effects of proline and melatonin on the activity of antioxidant enzymes (CAT, SOD, and MDA) and H_2O_2 levels in roots and leaves. The results in leaves exhibited a similar pattern to those in roots, with proline and melatonin decreasing CAT and SOD activity and being higher and lower, respectively.

Values were elevated with the melatonin (MT2) 100 µM treatment, whereas MDA and H_2O_2 levels in the leaves were increased with the proline (Pro2) 80 mM treatment compared to the control. Increasing the amount of proline in *Vigna radiata* plants reduces the generation of H_2O_2, enhancing their tolerance to copper stress (Fariduddin *et al*., 2014). Melatonin addition improved growth parameters in basil seedlings exposed to copper by reducing the levels of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) and increasing the activity of PAL and antioxidant enzymes (Oloumi *et al*., 2024). The interaction effect between Cu levels and foliar spraying with proline and melatonin on the activity of antioxidant enzymes CAT and SOD, as well as the levels of MDA and H_2O_2, showed that the highest values were observed when the control treatment was applied at 192 ppb Cu with control foliar spraying.

Table 6. Effect of different levels of copper as fertigation, foliar application of proline or melatonin, and their interaction on activity of catalase, superoxide dismutase enzymes, malondialdehyde and the hydrogen peroxide content in tomato roots during the 2022 and 2023 seasons.

Treatments		CAT		SOD		MDA		H_2O_2	
		2022	2023	2022	2023	2022	2023	2022	2023
Cu levels	48 ppb	25.99	26.42	127.08	129.14	16.94	17.20	98.66	99.94
	96 ppb	30.63	31.30	150.56	153.84	20.20	20.64	108.54	113.57
	144 ppb	36.25	36.49	387.97	390.60	25.50	25.67	121.89	124.70
	192 ppb	48.96	48.78	438.15	436.64	41.31	41.18	143.78	143.92
RLSD A		0.538	0.571	1.748	4.47	0.265	0.473	1.195	2.09
Pro and Mt	C	37.46	38.31	292.75	298.88	27.69	28.29	124.08	131.77
	Pro1	35.53	35.54	276.86	277.18	26.46	26.46	118.89	118.16
	Pro2	34.54	34.57	268.21	267.79	24.82	24.82	114.26	114.47
	MT1	35.49	36.25	272.69	277.72	26.11	26.66	118.87	121.45
	MT2	34.26	34.08	269.19	266.20	24.84	24.63	114.99	116.82
RLSD B		0.557	0.684	2.069	5.178	0.352	0.559	1.419	2.087
Cu at 48 ppb	C	25.33	26.65	124.00	130.10	16.80	17.59	101.45	104.76
	Pro1	25.85	24.95	127.05	122.63	16.93	16.33	100.20	96.71
	Pro2	27.00	27.14	129.05	129.70	17.28	17.36	96.20	96.68
	MT1	26.03	26.86	126.65	130.77	16.33	16.86	98.83	102.02
	MT2	25.75	26.52	128.63	132.50	17.35	17.87	96.63	99.55
Cu at 96 ppb	C	31.50	31.81	160.35	161.99	22.30	22.53	112.63	127.00
	Pro1	31.25	32.03	154.48	158.34	20.51	21.02	109.05	111.78
	Pro2	30.10	30.70	151.18	154.20	18.92	19.30	105.00	107.10
	MT1	31.18	32.11	138.93	143.10	20.13	20.74	109.15	112.43
	MT2	29.13	29.86	147.88	151.57	19.13	19.61	106.88	109.55
Cu at 144 ppb	C	41.30	41.93	427.20	433.65	28.18	28.60	129.90	137.36
	Pro1	36.65	36.93	388.13	391.04	25.53	25.72	124.20	121.39
	Pro2	33.20	33.20	365.83	365.82	24.58	24.57	117.33	117.33
	MT1	35.88	36.06	385.75	387.69	25.88	26.01	123.58	124.20
	MT2	34.20	34.37	372.95	374.80	23.33	23.44	114.43	123.25
Cu at 192 ppb	C	51.70	52.85	459.45	469.80	43.48	44.44	152.33	157.97
	Pro1	48.38	48.25	437.78	436.71	42.88	42.77	142.10	142.77
	Pro2	47.85	47.25	426.78	421.44	38.53	38.04	138.53	136.77
	MT1	48.88	49.97	439.45	449.32	42.10	43.05	143.93	147.16
	MT2	47.98	45.58	427.30	405.92	39.55	37.58	142.03	134.93
RLSD A×B		1.114	1.369	4.138	10.357	0.705	1.118	2.837	4.175

Proline (Pro1) 40 mM, proline (Pro2) 80 mM, melatonin (MT1) 50 µM, melatonin (MT2) 100 µM and control distilled water (C).

Table 7. Effect of different levels of copper as fertigation, foliar application of proline or melatonin, and their interaction on activity of catalase, superoxide dismutase enzymes, malondialdehyde and the hydrogen peroxide content in tomato leaves during the 2022 and 2023 seasons.

Treatments		Catalase (CAT) (unit/g)		Superoxide dismutase (SOD) (µg/g)		MDA		H_2O_2	
		2022	2023	2022	2023	2022	2023	2022	2023
Cu levels	48 ppb	20.94	21.53	15.51	15.95	8.39	8.62	16.53	16.09
	96 ppb	32.16	33.48	24.51	24.56	9.00	9.03	20.77	20.73
	144 ppb	36.25	38.54	51.03	51.60	11.59	11.72	28.41	27.80
	192 ppb	54.27	52.72	57.12	57.29	14.33	14.37	29.91	29.83
RLSD A		1.287	0.869	0.379	0.366	0.331	0.413	0.826	0.939
Pro and Mt	C	39.08	39.72	40.89	41.49	11.39	11.56	26.53	26.20
	Pro1	36.48	37.29	36.84	37.46	11.01	11.18	23.55	22.96
	Pro2	34.34	34.04	35.25	35.19	10.69	10.70	23.26	23.08
	MT1	35.47	37.24	37.13	37.41	10.78	10.91	23.63	23.38
	MT2	34.15	34.54	35.10	35.20	10.27	10.34	22.54	22.43
RLSD B		1.129	1.157	0.481	0.653	0.1802	0.211	0.409	0.537
Cu at 48 ppb	C	19.68	20.51	15.98	16.66	8.20	8.55	16.25	15.59
	Pro1	20.63	20.64	15.38	15.39	8.35	8.36	15.38	15.36
	Pro2	22.90	23.36	15.24	15.54	8.45	8.62	20.90	20.49
	MT1	20.60	21.57	15.65	16.37	8.35	8.73	15.20	14.56
	MT2	20.89	21.60	15.29	15.78	8.60	8.87	14.90	14.47
Cu at 96 ppb	C	36.14	35.89	27.88	27.07	9.36	9.08	22.58	23.26
	Pro1	31.18	33.32	24.18	24.38	9.25	9.32	21.73	21.55
	Pro2	30.68	30.98	23.98	24.34	8.99	9.13	18.93	18.64
	MT1	28.48	34.00	24.48	24.87	8.71	8.85	20.88	20.56
	MT2	34.36	33.20	22.04	22.15	8.71	8.76	19.73	19.62
Cu at 144 ppb	C	35.23	43.35	56.30	57.69	12.55	12.86	31.33	30.58
	Pro1	39.20	40.26	50.43	51.15	11.83	12.00	28.40	27.00
	Pro2	37.38	35.21	48.23	48.41	11.08	11.12	26.50	25.90
	MT1	35.03	37.57	51.88	52.35	11.53	11.63	28.43	28.17
	MT2	34.40	36.30	48.30	48.43	10.95	10.98	27.40	27.33
Cu at 192 ppb	C	65.30	59.16	63.40	64.57	15.45	15.74	35.98	35.36
	Pro1	54.90	54.94	57.38	58.93	14.63	15.03	28.70	27.95
	Pro2	46.43	46.60	53.55	52.45	14.23	13.93	26.73	27.29
	MT1	57.78	55.80	56.50	56.06	14.53	14.41	30.00	30.25
	MT2	46.95	47.08	54.78	54.45	12.83	12.75	28.13	28.29
RLSD A×B		2.257	2.315	0.962	1.306	0.360	0.423	0.817	1.075

Proline (Pro1) 40 mM, proline (Pro2) 80 mM, melatonin (MT1) 50 µM, melatonin (MT2) 100 µM and control distilled water (C).

Yield and its components:

Table (8) shows the impact of copper levels and foliar spray treatments using proline or melatonin on tomato plant productivity and components. The table data indicates that as the copper level in the nutrient solution increased, the number of fruits per plant and the average weight decreased, consequently affecting the plant's overall productivity. The highest number of fruits per plant, average weight, and production per plant were observed when the copper was at 48 ppb, while the lowest values were recorded at 192 ppb, as shown in Tables (2 to 7). Our results indicated that elevating copper in the nutrient solution reduced root, vegetative, growth chlorophyll, nutritional levels, ROS content in roots and leaves, enzyme activity, and H_2O_2 concentration, as previously discussed, which led to lowering plant productivity. The obtained results agreed with those of Sonmez *et al*. (2006) and Hanafy & Ahmed (2017) on tomatoes, and Zheng *et al*. (2010) on cucumbers, where they found that exposure of plants to a high concentration of copper led to a decrease in the number of fruits and ultimately the plant's yield.

Table 8. Effect of different levels of copper as fertigation, foliar application of proline or melatonin, and their interaction on the number of fruit per plant, average weight of fruit, and yield per plant of tomato plants during the 2022 and 2023 seasons.

Treatments		Number of fruit per plant		Avreage Weight of fruit (g)		Yeild per plant (g)	
		2022	2023	2022	2023	2022	2023
Cu levels	48 ppb	49.20	47.35	113.73	111.43	5603.93	5284.49
	96 ppb	30.50	29.55	99.08	98.39	3023.61	2909.17
	144 ppb	17.00	15.20	92.18	91.27	1564.86	1390.60
	192 ppb	14.40	13.35	76.15	74.60	1096.80	999.05
RLSD A		1.075	1.03	1.674	1.115	104.778	128.58
Pro and Mt	C	25.63	24.00	91.62	87.98	2471.67	2260.10
	Pro1	27.31	26.38	97.14	95.59	2824.43	2704.53
	Pro2	27.81	26.56	95.95	95.39	2830.25	2681.25
	MT1	28.88	26.88	95.64	94.14	2954.94	2695.26
	MT2	29.25	28.00	96.06	96.49	3030.20	2887.98
RLSD B		0.760	0.901	1.408	2.018	121.583	119.570
Cu at 48 ppb	C	46.50	44.00	104.98	103.60	4879.13	4555.08
	Pro1	48.00	47.25	115.48	115.20	5543.25	5442.90
	Pro2	48.75	47.25	113.55	111.45	5534.90	5265.78
	MT1	50.50	48.00	115.53	110.95	5837.48	5325.75
	MT2	52.25	50.25	119.10	115.93	6224.90	5832.95
Cu at 96 ppb	C	28.50	27.25	94.83	94.05	2701.53	2562.93
	Pro1	31.75	30.50	100.78	99.45	3199.43	3032.30
	Pro2	30.25	29.25	99.38	99.03	3003.65	2893.08
	MT1	31.25	30.50	100.33	99.50	3136.03	3035.48
	MT2	30.75	30.25	100.10	99.90	3077.43	3022.05
Cu at 144 ppb	C	14.50	13.00	92.03	87.75	1335.18	1141.03
	Pro1	15.50	14.75	94.55	91.93	1465.88	1356.33
	Pro2	17.50	16.00	95.43	94.18	1669.15	1508.40
	MT1	19.00	15.25	91.50	90.23	1738.10	1377.98
	MT2	18.50	17.00	87.38	92.25	1615.98	1569.25
Cu at 192 ppb	C	13.00	11.75	74.65	66.50	970.85	781.38
	Pro1	14.00	13.00	77.78	75.80	1089.18	986.60
	Pro2	14.75	13.75	75.45	76.90	1113.30	1057.75
	MT1	14.75	13.75	75.20	75.88	1108.18	1041.85
	MT2	15.50	14.50	77.68	77.90	1202.50	1127.68
RLSD A×B		2.150	1.80	3.348	4.036	243.165	239.141

Proline (Pro1) 40 mM, proline (Pro2) 80 mM, melatonin (MT1) 50 µM, melatonin (MT2) 100 µM and control distilled water (C).

Data presented in Table (8) revealed that the application of proline and melatonin significantly increased the productivity of tomato plants compared to the control treatment. melatonin (MT2) appeared to be the best treatment of all, followed by melatonin (MT1), proline (Pro2), and proline (Pro1), respectively, according to a study by Ibrahim *et al*. (2020), tomato plants treated with melatonin at 20 or 40 ppm produced far more fruit than those not treated. Melatonin protects plants from copper toxicity by making them more antioxidant and getting rid of reactive oxygen species (ROS) when exposed to high levels of copper (Zhang *et al*., 2022). A proline-metal combination formed, which provided this protection, in a study by Sharma *et al*. (1998). Farago and Mullen (1979) also found that proline could form complexes with metals; specifically, they found that proline could do so with Cu in metal-tolerant.

The combined effect between copper levels in the nutrient solution and foliar spraying with proline and melatonin is presented in Table (8). The highest number of fruits per plant, average weight, and yield per plant were obtained from plants treated with melatonin (MT2) at 100 µM and copper fertilization at (48 ppb), while the lowest ones were when adding melatonin (MT2) at 100 μM and copper fertilization (48 ppb).

CONCLUSION

Despite copper's significance for plants, the study demonstrated that its increased existence in a higher concentration which led to disturbances the most vegetative growth, leaf's mineral content and antioxidant enzymes as well as the levels of MDA and H_2O_2 which negatively affected yield potential of tomato plants. Interestingly, application of melatonin or proline was significant for alleviating these harmful effects and correcting disturbances resulting from excess copper in the nutrient solution. Melatonin or proline is a key factor for the use of Cu^{2+} tolerance in tomatoes, and it can be employed as a new tactic to defend against the damage caused by copper stress.

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الملخص العربي

التخفيف من إجهاد النحاس على نباتات الطماطم باستخدام البرولين والميلاتونين تحت النظام المفتوح للزراعة بدون التربة

ساري حسن مصطفي برنجي

اجريت هذه التجربة لدراسة استجابة نباتات الطماطم صنف ثريا لمستويات مختلفة من النحاس وتقليل التأثيرات الضارة للمستويات العالية منه، وقد نفذت تجربتان في الصوبة في مزرعة بدائل تربة تحت النظام المفتوح في بيئة رملية، وبيتموس خلال الفترة من مارس حتي يوليو من عامي ٢٠٢٢، و٢٠٢٣، في مزرعة خاصة في مدينة النوبارية الجديدة– محافظة البحيرة– مصر ولقد أضيف النحاس(Cu) إلى محلول الري المغذي بأربعة مستويات (الكنترول ٤٨ جزء في البليون وهو تركيز النحاس في محلول الري القياسي، ٩٦ جزء في البليون، ١٤٤ جزء في البليون، ١٩٢ جزء في البليون). ولقد تم اختبار خمس من المعاملات من البرولين والميلاتونين رشاً ورقياً لتقليل آثار النحاس الضارة وهي: البرولين (Pro1) ٤٠ ملي مول، البرولين (Pr2) ٨٠ ملي مول، الميلاتونين (MT1) ٥٠ ميكرومول، الميلاتونين (MT2) 100 ميكرومول والكنترول وهو ماء مقطر. وقد أظهر النتائج أن زيادة تركيز النحاس أدت إلى انخفاض معنوي في الوزن الطازج للجذور، والأجزاء الهوائية، والوزن الطازج، والجاف للنبات. كما أدى إلى انخفاض معنوي في عدد الثمار للنبات، ومتوسط وزن الثمرة، وإنتاجية النبات خلال موسمي الدراسة. ولقد تحققت أعلى القيم للصفات السابقة عند مستوى النحاس ٤٨ جزء في البليون وهو تركيز النحاس في محلول الري القياسي، ولكن أقل القيم كانت في تركيز النحاس ١٩٢ جزء في البليون. كما تغيرت قدرة النبات على امتصاص العناصر مع زيادة تركيز النحاس في المحلول المغذي فانخفض محتوى الأوراق من النتروجين والفوسفور والبوتاسيوم، بينما زاد محتوى النحاس في الجذور، والأوراق، والسيقان، والثمار مع زيادته في محلول الري المغذي وتم تراكم كمية أكبر من النحاس في الجذور، والأوراق، والسيقان مقارنة بالثمار وتزداد مع زيادة تركيزات النحاس في محلول الري المغذي. أيضا زاد محتوى الكلوروفيل في الأوراق من تركيز ٤٨ جزء في البليون إلى ٩٦ جزء في البليون نحاس ثم انخفض بشكل حاد. وفي الوقت نفسه، انخفض محتوى البروتين في الأوراق مع زيادة تركيز النحاس في المحلول. علاوة على ذلك ارتفع محتوى البرولين في الأوراق مع زيادة تركيز النحاس في المحلول، وارتفعت مستويات بيروكسيد الهيدروجين، المالونديالدهيد MDA، نشاط إنزيم الكتاليز CAT، وفوق أكسيد ديسموتيز SOD في كل من الجذور والأوراق. باستخدام البرولين (Pro1) ٤٠ ملي مول، البرولين (Pr2) ٨٠ ملي مول، الميلاتونين(MT1) ٥٠ ميكرومول، الميلاتونين(MT2) ١٠٠ميكرومول، أدى ذلك إلى تحسين النمو الخضري للنبات، والمحتوى الكيميائي لأوراقه من النيتروجين، والفوسفور، والبوتاسيوم، والكلوروفيل. مقارنة بالكنترول تحت مستويات النحاس المختلفة وأيضا أدت إلى تحسين محتوى مضادات الأكسدة في الجذور والأوراق، مما ساعد علي زيادة عدد الثمار لكل نبات، ومتوسط وزن الثمرة، وإنتاجية النبات. كانت المعاملة بالميلاتونين عند ١٠٠ ميكرومول والبرولين عند ٨٠ ملي مول من أكثر المعاملات فعالية لتقليل تأثيرات النحاس الزائد.

الكلمات المفتاحية: الطماطم، النحاس،المحلول المغذي، البرولين، الميلاتونين، النمو الخضري، مضادات الاكسدة، المحصول.