

EFFECT OF COPPER NANOPARTICLES AND MAGNETIZED SALTY WATER IN ENZYMATIC AND NON-ENZYMATIC ANTIOXIDANTS OF TOMATO (*LYCOPERSICON ESCULENTUM* L.)

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Abstract

This study was conducted in the department of Biology, College of Science, University of Babylon, Iraq, to evaluate the effect of copper nanopartical and magnetized salty water in superoxide dismutase enzyme activity, catalase enzyme activity, glutathione content, proline content and malondialdehyde content of tomato (Lycopersicon esculentum L.). The overall number of pots at 144 (48 treatments and 3 replicates per treatment). The results of study showed that Cu nanoparticles at concentration of (0.5ppm and 9ppm) and the magnetic intensity (2000 Gauss) caused decreasing in SOD activity of tomato leaves significantly. The rest of the treatments increased SOD activity significantly comparing with the control treatment. The treatment of the drainage water (E.C.4 mmohs/cm) with Cu NPs. of 5ppm caused the highest value of SOD (666.7 unit), the Cu NPs of the concentration (9ppm) caused decreasing in CAT activity of tomato leaves significantly comparing with control also with treatment of magnetic river water (E.C. 2.83mmohs/cm) at 2000 Gauss. The concentrations of almost treatments increased the CAT activity significantly comparing with control, the treatment of drainage water (7 mmohs/cm) with the concentration (5ppm) for Cu NPs. caused the highest value of CAT (158.6 unit). that Cu NPs. at concentration of 5 ppm with magnetized distilled water caused decreasing in GSH content of tomato leaves significantly. The highest value at the drainage water (E.C.4 mmohs/cm) caused enhancement in GSH content (0.304 µmol) comparing with control and treatment of drainage water at (7 mmohs/cm) alone, the Cu NPs at concentration of 5ppm caused decreasing of proline content of tomato leaves significantly. All concentrations of all treatments increased the content of proline significantly comparing with control. The treatment of drainage water (E.C. 4mmohs/cm) with magnetic water of 2000 Gauss and the concentration (0.5ppm) caused the highest value of proline content (8.87 μ mol/g), the Cu NPs at concentration of 5ppm and the magnetic intensity 3000 Gauss with the drainage water (7 mmohs/cm) caused decreasing of MDA content of tomato leaves significantly. The treatment of river water (E.C.2.83mmohs/cm) with magnetic water of 3000 Gauss and the concentration (5ppm) caused the highest value of MDA content (14.91mmoler).

Key words: Salinity, Cu NPs, Magnetic water, Lycopersicon esculntum L.

Introduction

A saline soil have a high concentration of soluble salts, high enough to affect plant growth .Salt stress is one of the major abiotic threats to plant life and significantly reduces crop yield in affected areas. Excessive salt above what plants need limits plant growth and productivity and can lead to plant death. About 20% of all irrigated land is affected by soil salinity, decreasing crop yields (Kader and Lindberg, 2010). Salinity posses two major threats to plant growth: osmotic stress and ionic stress (Flowers and Colmer, 2008). Several studies

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have been conducted to investigate the salinity effect in plant growth and productivity (Shah, 2007). Nanoparticles are minute's finites with lengths ranging from 1-100 nanometers. They have unique physical and chemical properties. They have a large surface area to their size, making them highly motivating on influencing the growth and development of different types of plants, these effects may be either positive or negative (Ma *et al.*, 2010).

Copper is one of the essential nutrients for plant growth in low concentrations because it needs very small quantities, copper is involved in many vital processes to form protein and is the main component in the synthesis of many plant enzymes that activate oxygen reduction reactions such as cytochrome oxides, ascorbic acid oxides and lactase (Xiong et al., 2006). Copper is widely distributed in plant tissues and is essential micronutrient for growth and involved in many physiological processes (Sommer, 1931 and Chibber et al., 2013). It is widely used in agricultural industries, cosmetics, coatings, environmental remediation, fungicides, food industry, chemical industry, textile industries, medical industry, paints, plastics, wastewater treatment and electronics (Rafique, et al., 2017). Copper as an element converts toxic above a threshold level, which depends on the type of crop plants (An, 2006). The magnetic field is defined as the magnetic force that arises in the area surrounding the magnetic body or in other words can be described as the area surrounded the magnet and shows the effect (in a given material), the magnetization of matter under the influence of an external magnetic field is due to the alignment of atoms or molecules of matter when the material exposed to the magnetic field becomes dipole of its atoms and its molecules are aligned towards the field used (AL-Qaisi, 2004). Magnetic treatment of water has been reported to change some of the physical and chemical properties of water, mainly hydrogen bonding, polarity, surface tension, conductivity, pH and solubility of salts, these changes in water properties may be capable of affecting the growth of plants (Grewal and Maheshwari, 2011). Lin and Votvat, (1990) reported an increase in water productivity in both crop and livestock production with magnetically treated water. Maheshwari and Grewal, (2009) showed that the magnetic treatment of irrigation water resulted in statistically significant increases in the yield and water productivity for celery and snow pea plants in some instances. Antioxidants are an inhibitor of the process of oxidation, antioxidant constituents of the plant material act as radical scavengers and helps in converting the radicals to less reactive species, a variety of free radical scavenging antioxidants is found in dietary sources like fruits, vegetables and tea (Hall, 2001). Superoxide dismutase (SOD) enzyme is present in the cytoplasm, mitochondria and chloroplast, that free radicals are often associated with photochemical reactions the cells in which photosynthesis takes place are prone to oxidative stress because they contain rows of lightsensitive molecules that produce and consume oxygen (Stofieth, 2012). The activity of the SOD enzyme in the roots of two cowpea varieties, one of which is sensitive to salinity and the other is resistant to salinity is different, as it is not affected in the first category, while increasing in the second (Maia, et al., 2010). It was also found that different types of tomato (Lycopersicon pannellii L.

and Lycopersicon esculentum L.) showed different directions in their response to saline stress, as the treatment of plants with salt concentrations (70 and 140) m mol of different periods, this cause an increase in the activity of the enzyme for the first hit at the concentration of 140 mM on the sixth day and for the second hit at the concentration of 70 mM for the same period (Koca, et al., 2006). Catalase is mainly localized in the peroxisomes, in higher plants, it is present in all differentiated peroxisomes including the peroxisomes of leaves, cotyledons, roots, and glyoxysomes and unspecialized peroxisomes (Su, et al., 2012). Sivanadanam, et al., (2012) also found that high salinity concentrations caused an increase in the activity of the enzyme when treating Rhizophora apiculata and Acanthus ilicifolius. The effectiveness of the enzyme CAT decreases when the plant produces large quantities of free radicals and therefore the enzyme is unable to break them (Esfandiari, et al., 2007). Caval-cantia, et al., (2007) reported that the treatment of 200 mM Phaseolus vulgaris with six days caused a rapid and permanent decrease in the activity of the enzyme one day after saline treatment. glutathione is a short peptide consisting of three amino acids glutamic acid, cysteine and Glycine (Y-Glu-Cys-Gly) (Gangwar, et al., 2014). Glutathione is an important source of nonprotein sulfur in both animal and plant cells, and it has a primary function in the defense of cells, it defends the plant cell as it acts as an antioxidant (Rahier, et al., 2008). This triple peptide is part of a cycle Ascorbate glutathione that helps protect or reduce the risk from free oxygen radicals (ROS), this mechanism is summarized by oxidizing the sulfur group as a compound GSSG (Glutathione-disulfate-G) the danger of free radicals lies in changing the level of peptides in tissues and plant fluids subject to environmental stress conditions such as saline stress and stress of heavy metals such as uranium and cadmium (Chen and Viljoen, 2010 and Wang, et al., 2001). In many species, compatible osmoprotectant, such as proline and soluble sugars, is produced to protect the cells against the adverse effects from salt stress. High accumulation of proline is associated with tolerance to stress (Hokmabadi, et al., 2005). Consequently, the ability to accumulate proline has often been suggested as a valuable criterion for the selection of salt tolerant genotypes (Ashraf and Harris, 2004). The relationship between cell membrane stability and salinity tolerance is sufficient to differentiate between crop types (Sairam and Srivastava, 2002), for example, lipid peroxidation in an important physiological process that determines and chooses a stress in tolerant plant (Sanchez-Rodriguez, et al., 2010).

Tomato (*Lycopersicon esculentum* L.) belongs to the solanaceae and genus solanum is grown in temperate and warm regions (Rick, *et al.*, 1990). Tomato, is a widespread crop that is widely adapted to the different conditions of agriculture and is used in food industries as well as for fresh consumption (Delaplace, *et al.*, 2009). The aim of this study is to treat the salinity by the use of magnetized water as well as the copper nanoparticale and the interaction between them, when apply in tomato.

Materials and Methods

Tomato (*Lycopersicon esculentum* L.) seedlings class California with two month-old were planted at 2 December, 2018. These seedlings were transferred to plastic pots containing a mixed soil: batmos with a ratio of 1:1, capacity of 1.5 kg and 144 pots (48 treatments and 3 replicates per treatment). The physical and chemical properties of soil were analyzed table 1 in the laboratories of the Soil Department/College of Agriculture/Al-Qasim Green University.

Sand	720	g /kg
Silt	179	g kgm ⁻¹
Clay	101	g kgm ⁻¹
Ph.	7.45	
Ec	1.32	dSm ⁻¹
Са	4.60	mlmolkg ⁻¹
Mg	2.81	mlmolkg ⁻¹
Na	3.39	mlmolkg ⁻¹
K	0.60	mlmolkg ⁻¹
Cl	6.92	mlmolkg ⁻¹
SO_4	3.21	mlmolkg ⁻¹
CO ₃	Nill	mlmolkg ⁻¹
HCO ₃	2.13	mlmolkg ⁻¹

Table 1: Physical and chemical properties of soil.

The seedlings in the greenhouse were grown at a temperature $(25\pm)$, the seedlings were treated with salt water (0,2.83, 4, 7 mmohs /cm), magnetized water (0, 2000, 3000 Gauss) and Cu nanoparticles in concentrations (0, 0.5, 5, 9 M) and interaction experiments between the three factors were treated with salt water and magnetized water by watering, while the nanomaterial was sprayed.

The experiment was completed in February 1, 2019, and the leaves were taken at the age of four months of plant, the leaves between the third and fifth of the top of plant taken to determine of superoxide dismutase enzyme by method (Marklund and Marklund, 1974) and catalase enzyme by method (Goth, 1991) from fresh samples and glutathione by method (Ellman, 1959), prolineby method (Bates, *et al.*, 1973) and malondialdehyde by method (Buege and Aust, 1978) from the dry weight.

Completely Randomized Design (CRD) was used with a three-factor and three-replication, including salinity, magnetic water and Cu nanoparticles concentrations. The values were statistically analyzed by the statistical system GenS tat Release 12.1. Least significant difference (L.S.D.) was used. On the level of probability of 0.05 to compare the differences between the averages (AL-Rawee and Abd AL-Azize, 2000).

Results

Table 2 refers that Cu nanoparticles at concentration of (0.5ppm and 9ppm) and the magnetic intensity (2000 Gauss) caused decreasing in SOD activity of tomato leaves significantly. The rest of the treatments increased SOD activity significantly comparing with the control treatment. The treatment of the drainage water (E.C.4 mmohs/cm) with Cu NPs. of 5ppm caused the highest value of SOD (666.7unit).

Table 2: Effect of copper nanoparticle and magnetized salty water in the activity of SOD enzyme(unit) of tomato (Lycopersicon esculentum L .).

Salt conce- ntration mmohs/cm	Cu NPs. ppmMag. waterGauss	0	0.5	5	9
d.	0	469.9	347.1	453.5	464.2
W0	2000	454.0	375.8	459.0	461.5
	3000	587.9	507.9	468.8	492.7
River	0	494.0	420.5	515.7	435.5
Water	2000	422.1	454.6	484.5	552.4
2.83	3000	499.5	440.1	505.0	441.0
Drainage	0	435.5	572.2	486.5	357.2
Water	2000	543.9	632.1	602.0	602.2
4	3000	536.6	452.6	557.9	562.8
Drainage	0	579.5	583.1	666.7	582.7
Water	2000	517.7	501.6	481.7	597.9
7	3000	564.2	569.4	600.6	594.7
L.S.D. $_{(0.05)} = 5.78$					

Table 3 refers that Cu NPs of the concentration (9ppm) caused decreasing in CAT activity of tomato leaves significantly comparing with control also with treatment of magnetic riverwater (E.C. 2.83mmohs/cm) at 2000 Gauss. The concentrations of almost treatments increased the CAT activity significantly comparing with control. The treatment of drainage water (7 mmohs/cm) with the concentration (5ppm) for Cu NPs. caused the highest value of CAT(158.6 unit).

Table 4 indicates that Cu NPs. at concentration of 5 ppm with magnetized distilled water caused decreasing in GSH content of tomato leaves significantly. The highest value at the drainage water (E.C.4 mmohs/cm) caused enhancement in GSH content (0.304μ mol) comparing with

Table 3: Effect of copper nanoparticle and magnetized salty water in the activity of CAT enzyme(unit) of tomato (*Lycopersicon esculentum* L.).

Salt conce-	Cu NPs.				
ntration	ppmMag.	0	0.5	5	9
mmohs/cm	waterGauss				
d.	0	111.8	82.6	124.2	110.5
W0	2000	108.0	89.4	109.2	109.8
	3000	143.1	120.9	111.5	117.2
River	0	117.6	100.2	120.8	93.4
Water	2000	117.6	126.2	115.3	78.1
2.83	3000	118.9	102.0	120.2	103.7
Drainage	0	103.6	82.0	115.8	85.0
Water	2000	129.4	147.7	143.3	143.3
4	3000	127.7	129.1	137.5	129.8
Drainage	0	137.9	132.7	158.6	138.6
Water	2000	123.2	119.3	114.6	141.2
7	3000	134.3	135.5	142.9	141.5
$L.S.D_{(0.05)} = 1.34$					

Table 4: Effect of copper Nanoparticle and magnetized salty water on the content of glutathione (µmol) of tomato (*Lycopersicon esculentum* L.).

Salt conce-	Cu NPs.	0	0.5	5	0	
mmohs/cm	waterGauss		0.5	5	9	
d.	0	0.195	0.231	0.095	0.021	
W0	2000	0.052	0.162	0.053	0.099	
	3000	0.149	0.144	0.022	0.193	
River	0	0.218	0.130	0.046	0.245	
Water	2000	0.166	0.110	0.180	0.256	
2.83	3000	0.182	0.237	0.301	0.232	
Drainage	0	0.304	0.170	0.214	0.030	
Water	2000	0.012	0.115	0.128	0.131	
4	3000	0.159	0.247	0.129	0.039	
Drainage	0	0.065	0.117	0.042	0.194	
Water	2000	0.164	0.129	0.176	0.160	
7	3000	0.073	0.273	0.254	0.263	
$L.S.D_{(0.05)} = 0.004$						

control and treatment of drainage water at 7 mmohs/cm alone.

Table 5 refers that Cu NPs at concentration of 5ppm caused decreasing of proline content of tomato leaves significantly. All concentrations of all treatments increased the content of proline significantly comparing with control. The treatment of drainage water (E.C. 4mmohs/cm) with magnetic water of 2000 Gauss and the concentration (0.5ppm) caused the highest value of proline content (8.87µmol/g).

Table 6 refers that Cu NPs at concentration of 5ppm and the magnetic intensity 3000 Gauss with the drainage

Table 5: Effect of copper Nanoparticle and magnetized salty water on the content of Proline (µmol/g) of tomato (*Lycopersicon esculentum* L.).

Salt conce- ntration mmohs/cm	Cu NPs. ppmMag. waterGauss	0	0.5	5	9
d.	0	6.83	3.95	3.60	4.23
W0	2000	3.88	4.96	5.96	4.09
	3000	4.18	2.78	7.78	2.25
River	0	6.77	2.98	5.24	1.65
Water	2000	2.63	3.54	3.43	4.18
2.83	3000	3.25	2.07	3.69	2.86
Drainage	0	2.52	3.30	2.34	2.28
Water	2000	2.67	8.78	3.15	4.26
4	3000	2.27	3.85	3.28	2.09
Drainage	0	1.75	2.30	1.29	1.93
Water	2000	5.10	2.34	2.13	5.11
7	3000	3.15	3.06	4.81	1.63
$L.S.D{(0.05)} = 0.111$					

 Table 6: Effect of copper Nanoparticle and magnetized salty water on MDA (mM) of tomato (Lycopersicone-sculentum L.).

Salt conce- ntration mmohs/cm	Cu NPs. ppmMag. waterGauss	0	0.5	5	9
d.	0	5.10	3.45	4.01	6.37
W0	2000	9.84	6.00	10.68	5.90
	3000	5.36	8.54	5.59	5.25
River	0	9.26	6.10	6.46	10.18
Water	2000	5.48	8.26	12.70	3.81
2.83	3000	9.41	9.02	14.91	10.54
Drainage	0	5.92	10.25	6.24	4.68
Water	2000	5.93	10.09	9.79	5.54
4	3000	7.17	7.01	5.95	5.08
Drainage	0	6.60	4.98	4.54	7.16
Water	2000	6.68	5.66	6.94	5.11
7	3000	3.51	4.51	2.92	7.81
L.S.D. $_{(0.05)} = 0.161$					

water 7 mmohs/cm caused decreasing of MDA content of tomato leaves significantly. The treatment of river water (E.C. 2.83mmohs/cm) with magnetic water of 3000 Gauss and the concentration (5ppm) caused the highest value of MDA content (14.91mM).

Discussion

The salinity of water affects the biochemical aspects of enzymatic antioxidants (SOD and CAT) and nonenzymatic antioxidants (GSH, proline and MDA), when irrigated the plant with salty water it may lead to the formation of free radicals by producing ROS and stimulating oxidative stress, and by following tables 2 and 3 an increase in SOD and CAT activity have appeared and this is consistent with the results (Hassanein, et al., 2009). Generation of ROS, which is induced by NPs directly or indirectly, plays a critical role in phytotoxicity mechanism, the production of ROS is based on the physicochemical properties of NPs as well as the test species, various determinants, such as size and shape, solubility and particle dissolution, metal ions released from metal and metal oxide NPs, biotransformation of NPs, light and so forth, may cause the ROS generation and phytotoxicity (Dimkpa, et al., 2012; Rui, et al., 2015 and Zhang, et al., 2015). In the two tables 2 and 3 we observed an increase in the activity of SOD and CAT with increasing salinity and increasing magnetization of water as well as with increasing concentration of Cu NPs and this indicates that they are effective enzymes as antioxidants and also indicates the minimization of the plant for salinity, this is agreement with, (Ma, et al., 2016 and Zhang, et al., 2017), that SOD was related to the increase in tolerance of plants against environmental stress, such as NP toxicity, which means that SOD could act as an indirect selection criterion for researching oxidative stress. While working on tomato and several studies has revealed increased SOD activity in salttolerant genotypes of pea, cotton and tomato, and this induction of SOD activity was suggested as a reason for improved tolerance to salinity in these cases (Hernandez, et al., 2000 and Mittova, etal., 2004). CAT, among all of the antioxidant enzymes, is the enzyme that was first discovered and characterized and plays an indispensable role in ROS detoxification under stress (Garg and Manchanda, 2009). CAT is involved in scavenging of H₂O₂ during salt stress and other abiotic stress conditions (Willekens, et al., 1997). The increase in CAT activity under salt stress may indicate that CAT is a major enzyme detoxifying H₂O₂ in tomato. This increased activity of CAT upon salt stress was often related to the enhanced tolerance to salt stress (Mittova, et al., 2004).

Salt stress is oxidative stress, and these primary effects cause secondary effects ROS, such H_2O_2 as reducing cell amplitude and production, and as a result of these effects, cells die in extreme cases, the concentration GSH also decreased with increased the salinity in table 4 and this is consistent with what was indicated (Tsai, *et al.*, 2004). The treatment of drainage water (E.C. 4mmohs/cm) with magnetic water of 2000 Gauss and the concentration (0.5ppm) caused enhanced the proline content in table 5, in tomato leaves significantly, this indicate as a biochemical marker of salt stress level, that was agree with (Shamshiri and Fattahi, 2014). In the

present study, moderate and high salinityinduced a significant increase in the free proline content in the leaves. Our results are in agreement with those previously reported for *pistacia* species (Chelli-Chaabouni, et al., 2010), P. vera variety (Hokmabadi, et al., 2005 and Karimi and Maleki, 2014), in addition, proline plays a protective role against saltstress in plants. Therefore, the level of MDA, a decomposition product of polyunsaturated fatty acids produced during peroxidation of membrane lipids, is often used as an indicator of oxidative damage (Mittler, 2002). It has been reported that salt treatment increases lipid peroxidation in plant tissues (Hernandez, et al., 1993). Moreover, ROS-mediated membrane damage has been demonstrated to be a major cause of the cellular toxicity by salt stress in rice, tomato and citrus (Dionisio and Tobita, 1998 and Mittova, et al., 2004).

Conclusions

1- The drainage water (7 mmohs/cm) enhanced the SOD and CAT activity and MDA, while it decreased GSH and proline content.

2- The concentration 5 ppm of CuNPs enhanced SOD and CAT activity and inhibition in MDA, GSH and the proline content in the treatments of drainage water (7 mmohs/cm).

The river water (2.83 mmohs/cm) was the suitable for irrigation until with almost treatments of magnetized water and CuNPs, while the drainage water (4 and 7 mmohs/cm) cased increasing in enzymatic and nonenzymatic antioxidants in this research. The treatments with magnetic water especially of 2000 Gauss and CuNPs in different concentrations enhanced the damage mitigation of salinity.

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