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Toxicological and significant execute of biocompatible CuO nanoparticles and their influences on biochemical and molecular markers of wheat genotypes under saline conditions

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Abstract:
***Background**: **Due to its broad applications in many fields, such as medicine, medicinal drugs, catalysis, energy and materials, nanotechnology technique has gained intense interest in recent years. Such nanoparticles (NPs), small to wide surface area (1-100 nm) and called the magic bullets have many possible functions. Sustainable agriculture is required these days. The development of biocompatible nanoparticles, which can withstand various stressors such as salinity, has appeared as promising agents for plant growth but there are controversial issues about their safety**.*
***Materials and Methods**: In field experiments were held at Ras Sudr station (an area of salinity stress) for two successive seasons and two wheat genotypes have been cultivated Misr 1 (tolerant) and Gimmeza 11 (sensitive). Engineered biocompatible copper oxide nanoparticles (CuO NPs) prepared by chemical precipitation method with a size ranged approximately 24.3-40.7 nm by Debye-Scherer equation. Concentrations used were (0, 0.25, 0.50, 1.00 ppm) and achieved better viability for wheat genotypes by increasing growth characteristics, total pigments, and biological markers such as: superoxide protection system first line defense system superoxide dismutase also, activating glutathione reduced and enhancing of some polypeptides accumulation which may be related to saline stress tolerance and CuO NPs application. On the other hand, CuO NPs had negative effect on the toxic product released from peroxidation to plant cell membranes malondialdehyde (MDA).*
***Results**: The most effective dose was CuO NPs (1.0ppm) which gave highest total pigments in both genotypes, lesser MDA amount by Misr1 and highest growth parameters. A unique SOD isozymic band was noticed by Gimmeza11 when treated with (CuO NPs 1.00ppm). In addition to that, ISSR assay profile indicated changes in the number of bands in all samples examined. The highest polymorphic bands observed in samples treated with higher levels of CuO NPs (1.00 ppm) compared with non - treated plants. In addition to the appearance and disappearance of bands in ISSR profiles, a decrease in the stability values of the genetic template was registered as concentration increased. Moreover, we used the model of Wistar albino rats to estimate the effectiveness of CuO NPs as a foliar spray on two genotypes of wheat to clarify their safety. We explored the median lethal dose (LD_{50}) and repeated doses for 28 days.*
***Conclusion:** It can be noted that CuO NPs have no negative effects on the levels of liver or kidney functions and histopathological cases, all of which remained within the standard values.*
Key Word**:* **Wheat;** ***salinity stress; CuO nanotechnology; chlorophyll; superoxide dismutase; reduced glutathione; malondialdehyde; DNA ISSR; Wistar Albino rats

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I. Introduction

Nanotechnology is rapid growth and has drawn attention to the environmental effects of NPs, voicing concern about environmental challenges and adverse health impacts. Nanoparticles are classified as particles with dimensions ranging from 1 to 100 nm in diameter and have received great attention due to their characteristics and beneficial agricultural applications, crop improvement and several stimulators of environmental stress tolerance **(Ambily et al. 2017)**.

Metal and metal oxide NPs have distinct physiochemical properties and vary in many properties, such as surface, optical, thermal, and electrical characteristics, from their native bulk compounds. Nanoparticles of metal and metal oxide are formed during their manufacture by addition of reducing or oxidizing / precipitating

agents **(Sanchez-Dominguez et al. 2009)**. The reactivity of biomolecules with NPs is responsible for several determinants, including height, core composition, shape, surface characteristics, purity as well as stability and production method of NPs **(Wang et al. 2016)**.

Nanoparticles may interfere with plant metabolism by disrupting different oxidative operations in plants resulted in oxidative burst. Clearly that, several NPs when present in excess concentration, resulted in reactive oxygen species (ROS) output **(Cvjetko et al. 2017).** Once ROS were accumulated as a result of NPs interactions, they interfere with almost all cellular compartments and may produce protein modifications, lipid peroxidation, and DNA threats **(Van Breusegem and Dat, 2006).** Concerning toxic effects of NPs, several reports have elucidated an increase in lipid peroxidation and DNA damage in (nanoparticle-plants) interaction Therefore, it can be reported that the toxic effect of NPs in the plant is mainly mediated through ROS. (**Belava et al. 2017).**

In our study, copper is a serious element which is significant for growth and development of plants, it incorporated in many enzymes and proteins and contributes to several substantial physiological processes that act as a catalyst for oxidation-reduction reactions in chloroplast, cytoplasm and mitochondria or as electron transporter in plant respiration processes **(Fargašová, 2004 and Yruela, 2009)**.

A positive effect was noticed when wheat seeds were treated with CuO NPs (0.1 and 0.01 gm/l) on the early stages of growth. Only a residual impact of CuO NPs was detected at concentrations of 0.10 gm/l and 1.00 gm/l on the antioxidant enzymes peroxidase and catalase activities **(Zakharova et al. 2019)**.

Earthy plants face enormous difficulties when growing in the saline environment due to increasing osmotic pressure in the soil so that roots lose their function in the efficient absorption of water. Furthermore, high concentrations of Na^+ and Cl^-ions due to salinity stress induced toxicity to plant cells so that these ions should be removed **(Sanders, 2020)**. Salinity is a significant abiotic stressor as it affects approximately 45 million hectares of irrigated soil and decreases the quantity and quality of crop production. Over salinity conditions, plants have two response phases: a rapid phase (water deficit) and a slow phase (salt accumulation and toxicity) **(Machado and Serratheiro, 2017).**

For both phases, stomatal conductivity, transpiration, and abundance of CO_2 decrease, and the photosynthetic system is altered. As a result of this scenario, oxidative stress is caused by higher output and presence of ROS and/or reactive nitrogen species (RNS) **(Turkan, 2017).** Both ROS and RNS serve as markers under stress conditions as they cause a non-enzymatic and enzymatic response that helps the plant resisting stress. Some non-enzymatic components are glutathione, phenolic compounds, flavonoids, alkaloids, tocopherols and carotenoids **(Gill and Tuteja, 2010)**. Among the main enzymatic compounds in the removal of ROS are superoxide dismutase, catalase, peroxidase**.** The response of plants to abiotic stress is complex and involves changes in their morphology, physiology and metabolism **(De Cássia Alves et al. 2018)**.

DNA-based markers have gained popularity in the evaluation of genetic linkages between species and genotypes in recent years. Of these markers, ISSR (Inter Simple Sequence Repeat) markers **(Zietkiewicz et al. 1994)** are very commonly used in genotypic studies. The advantages of SSR (Simple Sequence Repeat) markers are these markers, which are polymorphic **(Bornet and Branchard, 2001)** and ubiquitous in the genome while avoiding the main obstacle to the SSR marker, that is the need to know the flanking sequences. Hence, in species where comprehensive knowledge on DNA sequences is not yet available, ISSR markers are suitable for use **(Meloni et al. 2006)**, and scored as dominant markers and inherited in Mendelian fashion **(Ratnaparkhe et al. 1998)**. ISSRs are DNA-based markers declare detection of polymorphisms in inter - microsatellite loci (**Karaca and Izbirak, 2008).**

Through this, the aim of this study is to decrease the hazards of salinity stress effect on two wheat genotypes (Misr1 and Gimmeza11) by using some engineered biocompatible metal oxide NPs like CuO. Moreover, genetic stability and genotoxic effects through DNA ISSR technique including genetic diversity parameters, number of fragments, unique profiles and polymorphic levels per assay unit was also detected. Moreover, testing CuO NPs toxicity, safety and effectiveness on model Wistar albino rats via direct injection with NPs and feeding on resultant wheat grains. So, to clarify their safety we explored the median lethal dose (LD50) and repeated doses for 28 days.

II. Material And Methods

Field experiment layout:

A field experiments was carried out at the Agricultural Experimental Station of Desert Research Center (DRC) located in Ras Sudr, South of Sinai, Egypt to study the influence of NPs on two wheat genotypes under salinity conditions.The grains of two wheat genotypes Misr1 (tolerant) and Gimmeza11 (sensitive) were obtained from the Field Crop Institute, Agriculture Research Center, Giza, Egypt.

The wheat grains sown in 30 November at rate of 70 Kg/fad, the experiments were designed in split plot design with three replicates. The plot was $4x4m^2$; each plot was fertilized with super phosphate at the rate of 200 Kg/fad. Before planting, potassium sulphate at the rate of 100 K_2O Kg/fad and ammonium nitrate at rate of

180 Kg/fad were added. The fertilizers were added in two equal doses after 35 and 65 days from sown. Mechanical and chemical analysis of soil and water are presented in table (1)**.** The results were determined according to **Estefan et al. (2013).**

Table (1): Water and soil chemical analysis

Level	pH	EC ppm	Cations (meq/L)				Anions (meq/L)			
			Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{-}	HCO_3^{-}	Cl^{-}	$SO_4^{=}$
Water analysis										
Well	7.82	4557	10.8	7.15	53.6	0.35	-	5.30	39.1	26.8
Soil analysis										
Soil	7.76	6195	4.6	3.2	88.3	0.67	-	4.95	65.7	26.1

Study Design: Split plot design by Duncan's multiple range
Study Location: Agricultural Experimental Station of Desert Research Center (DRC) located in Ras Sudr, South of Sinai, Egypt.
Study Duration: November 2018 to September 2020.

Treatments

Copper oxide as NPs suspension (CuO NPs) at 0, 0.25, 0.50, 1.0 ppm and size approximately 40 nm ±5 nm was applied to the two wheat genotypes as foliar spray at early morning on plants at a rate of 400 Litres/fad after 45 and 65 days from sowing.

The CuO NPs used in the present work approximately (36 nm) could be collected by the stomatal pathway in a greater proportion. It should be noted that the rate of absorption of NPs varies according to the morphology of the leaf, the density and stomatal index, the hydrophobicity and roughness of the surface, and the physiological stage of plant organ growth.

Plant sampling

One plant samples were taken randomly from each treatment during the experiment as follows: Samples of fresh plants were collected after 75 days after sowing and preserved at -20 deep freezer. Growth traits including (plant height (cm), fresh and dry weights were determined. Fresh samples were tested for chlorophyll, malondialdehyde (MDA), total glutathione (GSH), antioxidant enzymes (Superoxide dismutase), and electrophoretic behavior proteins (SDS PAGE) and ISSR (Inter simple sequence repeat).

Nanoparticles preparation and characterization

The copper oxide NPs with different concentrations and definite nanometric size were prepared. The preparation, designing and characterization of nanometric compounds will be performed in Advanced Materials Central Lab (NAMCL), Agricultural Research Center, Egypt. The application of nanometric treatments and wheat planting was performed in field experiment at; Ras Sudr station (an area of salinity stress), South Sinai with best doses and their suitable sizes.

Preparation of Copper oxide nanoparticles:

The CuO NPs were prepared by chemical precipitation method using copper (II) chloride dihydrate (99.99% Pure, Sigma-Aldrich, USA) as a precursor according to **Luna et al. (2015)**. In brief, a solution of copper (II) chloride dihydrate (0.5M) in ethanol (≥ 99.8% Pure, Sigma−Aldrich, USA) was prepared separately. Then 100 ml of solution of Sodium hydroxide solution (99.99 %, Sigma−Aldrich, USA) (1M) in ethanol was added drop wise to the copper (II) chloride dihydrate solution under continuous stirring at room temperature. The color of the reaction mixture turned to black. The CuO NPs were separated and washed with ethanol and deionized water by centrifugation. The resultant precipitates were dried under vacuum at 50°C and annealed at 400°C for 4hrs.

Characterization of CuO nanoparticles

The High Resolution Transmission Electron Microscope (HR-TEM) operating at an accelerating voltage of 200 kV (Tecnai G2, FEI, Netherlands) imaged the actual morphology of the as-prepared CuO NPs.

To decrease the aggregation of particles, the diluted CuO NP suspension was ultra-sonicated for 5 minutes. Using the micropipette, approximately three drops were deposited on the carbon-coated copper grid from the sonicated suspension and left to dry at room temperature. For morphological images, HR-TEM images of the CuO NPs were recorded.

The Dynamic Light Scattering (DLS) method was used to estimate the average distribution of particle size determined by the zeta sizer (Malvern, ZS Nano, UK) and the zeta potential for charge and stability was also detected.

The chemical structure and phase of the as-prepared CuO NPs were evaluated using the technique of X-ray diffraction (XRD). In the scanning mode (X'pert PRO, PAN analytical, Netherlands) operated by the Cu K radiation tube (= 1.54 A ˚) at 40 kV and 30 mA, the corresponding XRD pattern was registered. The diffraction pattern obtained was interpreted by the standard ICCD library installed in the software of PDF4. At Nanotechnology and Advanced Materials Central Lab (NAMCL), Agricultural Research Center, Giza, Egypt both preparation and characterization procedures were carried out.

Biochemical analysis:

1. Determination of photosynthetic pigments.

Chlorophyll a, b and carotenoids were extracted and estimated according to **A.O.A.C. (1975)** and **Wettstein, (1957).**

Procedure:

The testing procedures occurred at low light to protect the pigments from breakdown. Fresh leaf samples (0.5 g) were homogenized in mortar with 85 % acetone. The homogenate was then filtered and the residue was washed several times with acetone until the residue became decolorized. The extracts were combined and the volume was made up to 35 ml. The optical density was measured colorimetrically **Unico SQ2800 UV/VIS Spectrophotometer** at 662, 644 nm for chlorophyll a and b respectively and 440 nm for carotenoids. The concentration of chlorophyll a, b and carotenoids were calculated by means of Wettstein's formula, and final concentration of pigments was then expressed as mg/100g fresh weight of wheat leaves.

2. Malondialdehyde content.

The most frequently used approach to lipid peroxidation is the thiobarbituric acid (TBA) process, mainly due to its simplicity and convenience. The ability of malondialdehyde (MDA), a powerful electrophilic species that is one of the secondary lipid peroxidation products, to react with TBA is indicated by this approach. The concept of this method is the reaction of MDA to TBA to form a pink MDA-(TBA)2 adduct under acidic conditions and at higher temperatures that can be spectrophotometrically quantified at 532 nm and 600 nm for nonspecific turbidity **(Keles et al. 2012).**

Procedure:

The level of lipid peroxidation in the wheat leaves tissue was quantified by determination of MDA, a breakdown product of lipid peroxidation according to **Health and Packer, (1968)** with some modifications. Approximately 0.5 g from wheat tissue was homogenized in a 2.5 ml of 0.1 % (w/v) trichloroacetic acid with a prechilled mortar and pestle. The homogenate was spun at 10,000 rpm for 15 minutes. 2 ml of freshly prepared 0.5% thiobarbituric acid (98%, Acros organics) in 20% (w/v) TCA in TBA reagent (was added to 0.5 ml aliquot of the supernatant. The mixture was heated at 95^{o}C for 15 minutes and immediately cooled. After cooling the absorbance was read at 532 nm and the value was corrected for non–specific absorption at 600 nm was subtracted. The used device was Unico SQ2800 UV/VIS Spectrophotometer. The concentration of MDA-TBA complex in wheat leaves was converted from ppm (calculated from MDA standard curve) the standard material: 1,1,3,3 tetramethoxy propane (Sigma Aldrich, USA), as a source of malondialdehyde when acid hydrolyzed, and then diluted to cover the range (0.015 to 1.0 ppm). The values were expressed as µmol / g fresh weight.

3. Total glutathione content (GSH).

Being a tripeptide, GSH interacts chemically in plant cells with other free radicals. GSH stabilizes the structure of the membrane by preventing lipid peroxidation reactions produced by acyl peroxides (preserve integrity of the membrane. It participates in the detoxification of xenobiotics as a substrate for glutathione-S-transferase enzyme and a precursor of phytochelatines that function as heavy metals that bind peptides in plants. GSH is the main compound of non-protein thiol that is widely distributed in plants and animals **(Foyer et al. 1994).** GSH was determined by the method of **Moron et al. (1979),** by the reaction with DTNB (5,5'-dithiobis nitro benzoic acid) and produces a yellow colored product that absorbed at 412nm.

Procedure:
A homogenate was prepared from 0.5g of the plant leaves with 2.5ml of 5% TCA. The homogenate was centrifuged at 1000 rpm for 10 minutes for protein precipitation. About 0.1 ml of the supernatant was used for the estimation of GSH.
The supernatant (0.1ml) was made up to 1.0ml with 0.2M sodium phosphate buffer (pH 8.0). Standard GSH (Alfa Aesar, Germany 98 %) in correspondence with concentrations ranging between 100 and 1000 mg/l were also prepared. 2.0 ml of freshly prepared DTNB (Sigma Aldrich, USA) solution was added and the intensity of the yellow color developed was measured by **Unico SQ2800 UV/VIS Spectrophotometer** at 412 nm after 10 minutes. The values were expressed as µmoles GSH/g fresh weight.

5. Electrophoretic behavior of soluble proteins
In fresh wheat leaves, soluble protein was determined following the SDS-PAGE gel electrophoresis protocol which performed in acrylamide glass slab gels following the method of **Laemmli (1970)** and **Studier (1973).**

6. Superoxide dismutase isozymic pattern
Superoxide dismutase (SODs) were extracted from fresh wheat leaves and separated by native polyacrylamide gel electrophoresis (PAGE) followed the methodology of **Weydert and Cullen (2010).**

7. Genomic DNA extraction
Wheat leaves 0.2 g of eight plants treated with CuO NPs were ground into powder in microphage tubes using liquid nitrogen and then DNA was extracted using AxyPrep multisource Genomic DNA Mini-Prep Kit (Axygen Bioscience, USA, cat). In order to assess intra-genotypic variations, DNA samples of each plant were individually analyzed and bulked to test inter-genotype variations.

ISSR Analysis
DNAs of eight plants as isolated above were PCR-amplified with five ISSR primers **(Table 2)**. Primers were reported earlier by **Syamkumar and Sasikumar (2007).** Reactions were carried out in total volume of 25 mL comprising 50 ng template DNA, 2.5µL 10x amplification buffer, 0.2 µM dNTPs, 1 unit Taq, 2.0 mMMgCl2, 0.4 µM primer and water, purified by reverse osmosis. PCR was performed in Eppendorf Master Cycler Personal using the following parameters: initial denaturation at 94 ^{O}C for 5min, then 40 cycles of denaturation at 94 ^{O}C for 30 s, annealing at primer-specific temperatures for 45 s, elongation at 72 ^{O}C for 2 min. A final elongation reaction was performed at 72 ^{O}C for 5min. A 100 bp DNA ladder (Roche) was used as size marker. The amplified products were resolved on 2% agarose gels buffered with 1x TAE, pH 8.0 and containing ethidium bromide at 0.05 mg/ml concentration.

The ISSR banding patterns were visualized and photographed by the UVI save gel documentation system and molecular sizes of well-resolved bands estimated by the accompanying software. Lanes representing the citrus samples were scored for the presence (1) or absence (0) of homologous bands.
The matrix of binary characters (0, 1) was prepared in the NT edit module of the software package **(Rohlf, 2005)**. **Felsenstein, (2004)** used to prepare a consensus phenetic tree based on the UPGMA (Un-weighed Pair Group Method with Arithmetic Mean) algorithm **(Sneath and Sokal, 1973).**
A 100 bp standard DNA ladder was used as the molecular standard to confirm the appropriate markers for analysis.

Primer name	Sequence	Primer name	Sequence
ISSR1	(CAA) 5	ISSR2	(CAG) 5
ISSR3	$(CT)_8$ AC	ISSR4	(GACA) 4
ISSR5	$(GA)_6$ GG		

Table (2): ISSR primers names and their sequences

Statistical data analysis
The discrete and reproducible ISSR molecular data bands were rated as either present (1) or missing (0). For the measurement of Jaccard's genetic similarity coefficient between all possible pairs of collections, binary data (matrix) was used. Using the unweighted pair-group arithmetic averages (UPGMA) method of the NTSYSpc programme, version 2.1. The confidence limits for the dendrogram groupings were performed by bootstrapping using the Win Boot programmer **(Yap and Nelson, 1996).**

8. Effect of CuO nanoparticles in albino rats
Wistar male albino rats (*Rattus norvegicus*), (160-170 g, body weight), were obtained from the farm of the Faculty of Veterinary, Zagazig University, Zagazig, Egypt and stayed in cages (8 animals/cage) to acclimatize

(25 ± 5°C; 60% ± 5 relative humidity and a normal light/dark cycle) for 15 days. The rats were fed of balanced standard diet containing 23 % protein and tap water during the acclimation and experimental time **(Sitohy et al. 2013)**.

Safety evaluation of copper nanoparticles in Wistar albino rats

One dose (LD_{50} estimation)

The acute oral toxicity assay was evaluated in compliance with OECD test guideline 423 **(OECD, 2001)**. Forty Wistar male albino rats were divided into three groups (n=8), group one (control) received distilled water, group two received CuO NPs (1000 mg/kg body weight), group three received CuO NPs (2000 mg/kg body weight). Suspension of CuO NPs was prepared in distilled water and volume was maintained to 2 ml. For registration of any signs of toxicity or death rate, all animals were kept under control for 24 hours and it took 14 days to monitor body weight and animal behavior.

Repeated doses (28 days)

The CuO NPs safety was examined as combined in the diets at three levels (0.25, 0.5, and 1µg g^{-1} diet) compared to the control (0 µg g−1). Fifty−six Wistar male rats were divided into seven groups (n=8).Every rat received, on average, a daily diet of 100 g/kg rat weight. So, the seven groups received a daily meal of a standard balanced diet containing 0, 0.25, 0.5, and 1.0 mg/kg body weight for 28 days **(Osman et al. 2020)**.

Group 1: Rats were fed on the basal diet (healthy control) without any treatments.

Group 2, 3 and 4: Rats were fed on the basal diet was containing 0.25, 0.5, and 1.0 mg CuO NPs /kg body weight as integrated into the powdered standard animal diet. At the end of the experiment after 28 days, all the rats were sacrificed then, blood and organ samples were collected and the serum was isolated from the whole blood samples as described in **(Abdel-Hamid et al. 2020)**.The histopathological examination was estimated in all treatments compared to control. The biochemical parameters (uric acid, urea, creatinine, ALT, AST, ALP, total proteins, albumin and total bilirubin) was estimated using commercial diagnostic kits provided by the Bio Diagnostic Company, Giza, Egypt **(Osman et al. 2019).**

Globulin and albumin globulin ratio (A/G ratio) were calculated mathematically as follow:

$$Globulin = total\ protein - albumin$$

$$A/G\ ratio = albumin/globulin$$

The body weight gain was calculated as follow:

$$Body\ weight\ gain\ (g) = intial\ weight\ (g) - final\ weight\ (g)$$

III. Result and discussion

Characterization of CuO nanoparticles

Figure (1) shows characterization physicochemically of the as-prepared CuO NPs to evaluate its properties using different techniques. The nanoparticle morphology and size determination was determined by HR-TEM electrograph shows semi-spherical shape, smooth surface and size range of about 24.3 – 40.7 nm by Debye-Scherer equation, Fig.1A. Fig. 1B, 1C represents the particle size (DLS) and zeta potential were used to measure hydrodynamic diameter and surface charge in the nanometer range. The size and zeta potential of CuO NPs were 36 nm and 28 mV, respectively. An X-Ray powder diffraction pattern of CuO NPs is shown in Figure 1D. The XRD pattern of the synthesized CuO NPs is shown in fig. (1). The peaks at 2θ = 32.48°, 35.54°, 38.64°, 48.85°, 61.52°, 65.66, 66.34 and 68.02° were assigned to (110), (-111), (111), (-202), (-113), (022), (-311) and (220) of CuO NPs, indicating that the crystalline structure of synthesized CuO NPs presented a monoclinic phase structure of the wurtzite (JCPDS 04-005-4712) and crystalline.

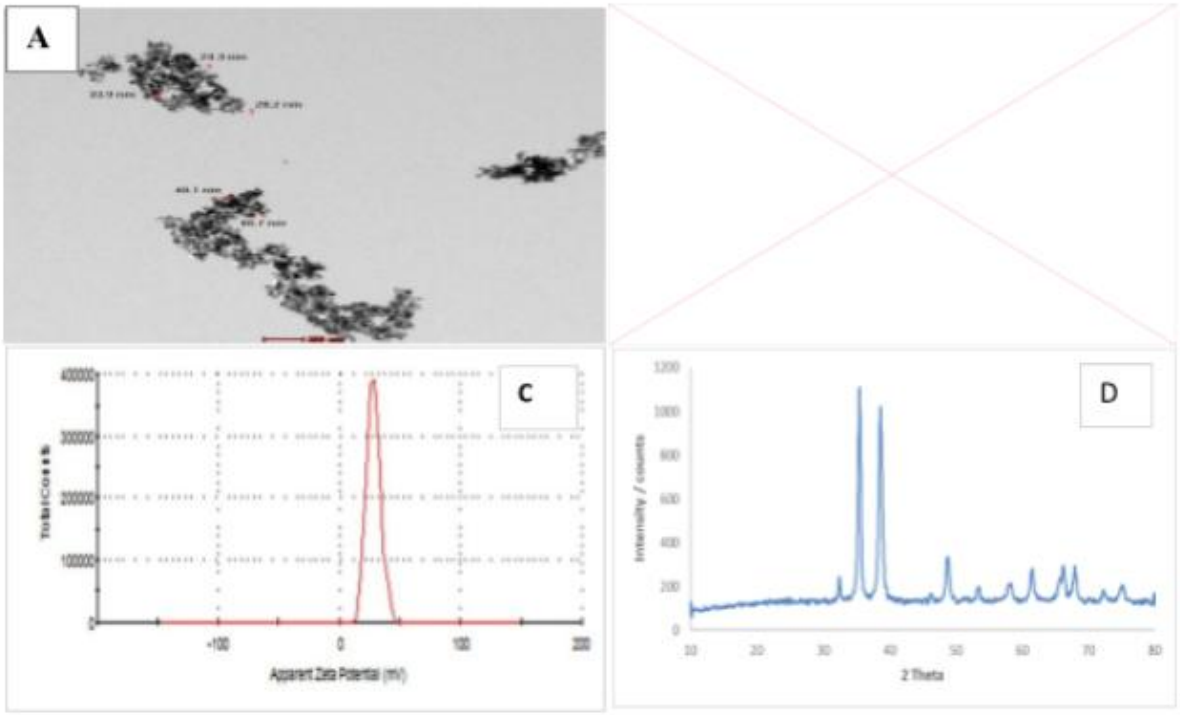


Figure (1): Characterization of CuO-NPs. **(A):** HRTEM image showing nearly spherical shape of prepared CuO NPs with average size 33.4 nm. **(B):** Particle size distribution of prepared CuO NPs showing the average size of 36 nm. **(C):** zeta potential of prepared CuO NPs showing surface charge, zeta potential,+28 mV. **(D):** XRD pattern analysis indicating the formation of CuO NPs.

Biochemical parameters:

1. Growth traits

Table (3) reported the enhancing behavior of CuO NPs treatment on growth parameters, the highest values of plant height, fresh weight and dry weight were noticed by application of CuO NPs 1.0 ppm when compared to the control. Regarding to genotypes, Gimmeza11 surpassed Misr1 in plant height, fresh weight and dry weight. Interaction between genotypes and treatments showed that, maximum value of plant height, fresh weight and dry weight was achieved by Gimmeza11 when treated with CuO NPs (1.0 ppm).

The same was noticed by **Nguyen et al. (2020)** where Cu NPs priming positively regulated drought stress responses in maize by enhancing drought tolerance indicated by their higher leaf water content and plant biomass under drought as compared with water treated plants. However, a slight decrease in tomato plant height, fresh weight and dry weight under saline conditions and applied 250 mg/l Cu NPs when compared to control. But individual Cu NPs size 50 nm, 250 mg/l application without saline stress increased plant height, fresh and dry weight **(Pérez-Labrada et al. 2019).**

Clearly from data the NPs application had positive role in enhancing growth parameters so that, the enhancing effects of Cu NPs to growth may be due to NPs induced increased activity of chloroplast rubisco, antioxidant enzyme system **Nekrasova et al. (2011)** and nitrate reductase. Depending on the form of NPs, concentration, scale, shape, morphology and stage of application, as well as the biological material used the responses in the growth and development of the plants discovered with the application of NPs can vary **(Pérez-de-Luque, 2017).**

It is speculated that, increasing in chlorophyll, antioxidant enzymes, minerals content may be related to an improved growth and development besides NPs which can overwhelm the hazards of salinity. Plants are subjected to numerous stresses during their development that harm their productivity.

Nanoparticles treatment	Growth parameters								
	Plant height (cm)			Fresh weight (g)			Dry weight (g)		
	Misr 1	Gim*	Mean	Misr 1	Gim*	Mean	Misr 1	Gim*	Mean
	CuO nanoparticles (ppm)								
Control	55.67 e	60.67 d	**58.17 C**	28.04 d	28.02 d	**28.03 D**	8.480 e	8.260 e	**8.370 D**
0.25	56.33 e	65.33 c	**60.83 B**	31.08 c	30.33 c	**30.71 C**	10.54 d	10.75 cd	**10.65 C**
0.50	67.67 c	78.33 b	**73.00 A**	30.28 c	50.57 b	**40.42 B**	11.56 c	16.21 b	**13.88 B**
1.0	66.67 c	81.00 a	**73.83 A**	31.29 c	65.13 a	**48.21 A**	10.51 d	20.18 a	**15.66 A**
Mean	**61.58 B**	**71.33 A**		**30.17 B**	**43.51 A**		**10.27 B**	**14.01 A**	

-Values followed by the same letter in columns are not different at p < 0.05 by Duncan's multiple range test.
Gim* : Gimmeza11

2. Chlorophyll content

Applied nanoparticles CuO NPs had positive behavior on photosynthetic pigments in wheat shoots (Cha, Chb, carotenoids and total pigments) as introduced in table (4). Regarding to CuO NPs, the highest mean value of Cha was detected at (0.5ppm), the dose CuO NPs (1.0ppm) gave the maximum value of Chb and total pigments, however, the same treatment had slight negative effect on carotenoid content when compared to the control. Misr1 surpassed Gimmeza11 in Chb but there was no significance in remnant pigments between the both genotypes. The above results were identical to **Hernández-Hernández et al. (2018)** who cited an increase in the content of photosynthetic parameters after application of Cu NPs (100ppm) to tomato plants under salinity conditions. But others **Pérez-Labrada et al. 2019** found a different behavior to Cu NPs on tomato plants under salinity stress. The positive effect of CuO NPs may be due to Cu is recognized as an important component of the control of plant growth and development, including chlorophyll formation and seed production **(Viera et al. 2019)**.

Nanoparticles treatment	Photosynthetic pigments											
	Chlorophyll a (mg/g)			Chlorophyll b (mg/g)			Carotenoids			Total pigments		
	Misr 1	Gim*	Mean	Misr 1	Gim*	Mean	Misr 1	Gim*	Mean	Misr 1	Gim*	Mean
	CuO nanoparticles (ppm)											
Control	0.955 b	0.800 c	**0.877 B**	0.380 b	0.263 d	**0.321 A**	0.387 a 0.368 ab	0.400 a 0.271 c	**0.394 A**	1.721 ab	1.463 cd	**1.592 A**
0.25	0.977 b	0.810 c	**0.894 AB**	0.374 b	0.341 b-d	**0.358 A**	0.348 ab	0.314 bc	**0.320 B**	1.719 ab	1.423 d	**1.571 A**
0.50	1.132 a	0.818 c	**0.975 A**	0.356 bc	0.356 bc	**0.356 A**	0.338 ab	0.349 ab	**0.331 B**	1.836 a	1.488 cd	**1.662 A**
1.0	0.877 bc	1.003 b	**0.940 AB**	0.488 a	0.273 cd	**0.381 A**			**0.343 B**	1.703 ab	1.624 bc	**1.664 A**
Mean	**0.985 A**	**0.858 A**		**0.399 A**	**0.308 B**		**0.360 A**	**0.334 A**		**1.745 A**	**1.499 A**	

Gim* : Gimmeza11
-Values followed by the same letter in columns are not different at p < 0.05 by Duncan's multiple range test.

Table (4): Effect of CuO nanoparticles treatment on photosynthetic pigments of two wheat genotypes under saline conditions

3. Lipid peroxidation product

It could be noticed that, CuO NPs had negative effects on malondialdehyde content as noticed in table (5) and figure (2), the lowest mean value of MDA was detected in CuO 0.25 ppm.Misr1 had lesser amount of malondialdehyde than Gimmeza11. The lowest amount of lipid peroxidation product was obtained by Misr1 at 1.00 CuO NPs in the interaction.

The results were compatible with **Da Costa and Sharma, (2016)** who used CuO NPs on rice, they found a slight decrease of MDA accumulation by CuO NPs (50 mg/l) when compared to the control plants. On the other hand, **Dimkpa et al. (2012)** reported an increase in MDA toxic product by using (CuO NPs < 50 nm) on wheat grown in solid matrix, sand.

Along with that, the positive role of Cu NPs on decreasing lipid peroxidation product may be due to Cu ions enter the plant cells and used as co-factor for some antioxidant enzymes like SOD which can dismute ROS and decrease their content, preventing them from the penetration to cell membrane and affecting membrane integrity. According to **Gao et al. (2008)** by using Cu metal with *Jatropha curcas* L. seedlings caused a significant increase in POD activity in roots under Cu stress so that, **Passardi et al. (2005)** suggested an increased POD activity in *Jatropha curcas* might be sufficient to protect proteins, chlorophyll and lipids of some parts of plants against ROS attack.

Nanoparticles	Malondialdehyde content (nmol/g fr.wt.)			Reduced Glutathione content (µmol/g fr.wt.)		
	Misr1	Gim*	Mean	Misr1	Gim*	Mean
			CuO NPs (ppm)			
Control	10.64 e	18.10c	**14.37 B**	11.15 g	9.460 h	**10.30 D**
0.25	11.51 de	10.29 e	**10.90 C**	45.36 a	38.09 b	**41.73 A**
0.50	12.22 d	25.96 a	**19.09 A**	15.68 f	36.83 c	**26.26 B**
1.0	7.34 f	22.08 b	**14.71 B**	23.49 d	18.55 e	**21.02 C**
Mean	**10.43B**	**19.11A**		**23.92 B**	**25.73 A**	

Gim*: Gimmeza11

-Values followed by the same letter in columns are not different at $p < 0.05$ by Duncan's multiple range test.

Table (5): Effect of CuO nanoparticles treatment on malondialdehyde and reduced glutathione content of two wheat genotypes under saline conditions

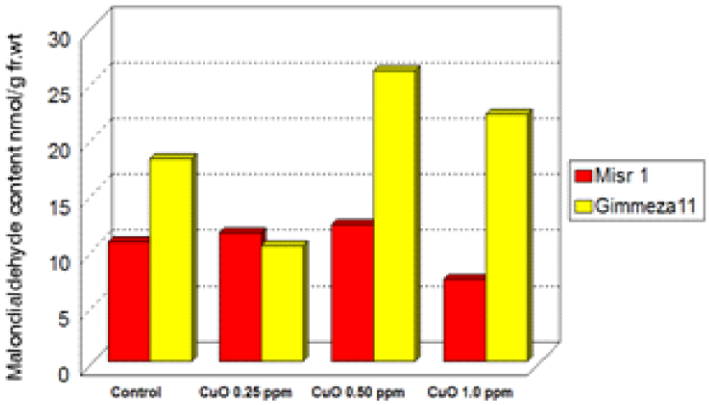


Figure (2): Effect of CuO nanoparticles application on malondialdehyde content of two wheat genotypes under salinity conditions

4. Reduced glutathione

In table (5) and figure (3) the highest mean value of GSH was detected by the lowest concentration of CuO NPs (0.25ppm). Gimmeza11 surpassed Misr1 in GSH content. The interaction showed that, Misr1 gave the mean value of GSH (0.25ppm) of CuO NPs.

These findings go in the same direction with **Hassan et al. (2018)** on heat stressed wheat when treated with ZnO and Fe_3O_4 NPs and **Pérez-Labrada et al. 2019** where the GSH content in the leaves of tomato plants treated with Cu NPs + NaCl was increased by 13% (compared to the Control. This indicates that GSH synthesis may be a mechanism by which plants tolerate salinity stress because due to its high reduction potential, this non-enzymatic antioxidant has great flexibility, by which it removes H_2O_2, $^1O_2$, $OH^{\bullet}$, and $O_2^{\bullet -}$ **(Kaushik and Roychoudhury, 2014).**

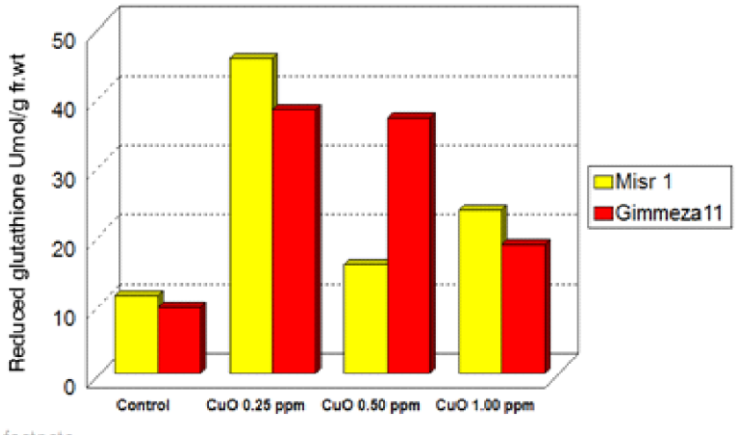


Figure (3): Effect of CuO nanoparticles application on reduced glutathione content of two wheat genotypes under salinity conditions

5. Superoxide dismutase

Copper oxide NPs had positive effects on SOD isozymic pattern and intensity. The native PAGE exhibited 3 bands. There was a marked increase in all treated genotypes in band no1. A unique band was noticed at Gimmeza11 when treated with CuO NPs (1.00 ppm) and this may be related to increase the plant tolerance to salinity stress.

The highest value of band intensity which related to increased enzyme activity was recognized CuO NPs (1.00 ppm) by Misr1 and in Gimmeza11 by 0.50 ppm.

Data in table (6) cleared the effect of CuO NPs on superoxide dismutase banding pattern of two wheat genotypes subjected to salinity hazards. SOD patterns revealed the presence of about 3 bands and this is dependent on cultivar, stress type and treatment with nanoparticles.

Concerning CuO NPs, a marked increase was noticed in 1st banding pattern intensity and highest value recorded at CuO NPs (0.50 ppm) in Gimmeza11. The second band was unique to Gimmeza11 when received 1.00 ppm CuO NPs. The highest value of band No.3 intensity was due to treatment of Gimmeza11 with CuO NPs 0.50 ppm.

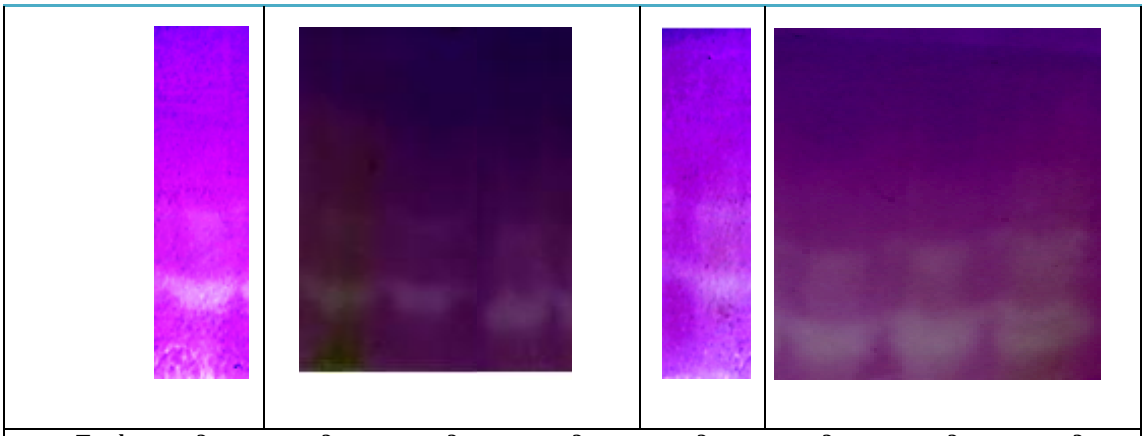
The first line of protection to reduce the damage caused by ROS is the protein of SOD and its isoenzymes (Cu / Zn-SOD, Mn-SOD and Fe-SOD) and it is most effective in the $O^{2-}$to H_2O_2 and O_2 dismutation reaction. **(Kaushik and Roychoudhury, 2014).**

The increase in the antioxidant activity induced by the application of Cu NPs found in salinity stressed tomato by **Pérez-Labrada et al. (2019)** who reaffirms the conclusions by **Huang et al. 2019** who proposed that NPs stimulate the concentration and activity of antioxidants. This bio-stimulation may be due to the interaction of NPs with intracellular structures as a result of their high reactivity and ease of penetrating cell barriers **Shobha et al. (2014)** by endocytosis, forming pores or transport proteins.

The foliar treatment of NPs induces more quickly and efficiently the output of ROS and antioxidant compounds as they are absorbed by the porous spaces and move through the cell wall; once within, the NPs can bind to the outer side of the cell membrane through electrostatic interactions (with the positive part of the membrane) that benefit the NP reaction (negatively charged).

Finally, the obfuscation of the NPs in the cytosol, plastid, vacuole, or nucleus is likely to be carried out by the plasma membrane 's potential or, to a lesser degree, by **Da costa and Sharma, 2016, Marslin et al. 2017**, endocytosis. Oxidative stress is caused by the entry of NPs and, as a result, transcriptional reprogramming of the secondary metabolism takes place; that is, enzymatic and non-enzymatic antioxidant mechanisms are activated **Marslin et al. 2017** and to improve the resistance of the plant to salinity stress. It should be noted that some of the NPs applied to tomato plant leaves can react electrostatically with the leaf surface metabolites formed by trichomes **Schilmiller et al. (2008).**

Band intensity								
Band intensity	**Control**	**Misr 1 CuO NPs**			**Control**	**Gimmeza11 CuO NPs**		
		0.25	**0.50**	**1.00**		**0.25**	**0.50**	**1.00**
1	1.1	1.6	1.6	1.3	1.0	1.1	1.8	1.4
2	0	0	0	0	0	0	0	1.2
3	2.2	3.0	2.9	3.5	1.7	3.1	4.0	1.0



Total bands	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**3**

Table (6): Effect of CuO nanoparticles treatment on superoxide dismutase isozyme pattern of two wheat genotypes under saline conditions

6. Protein banding pattern:

The effect of CuO NPS on protein banding pattern of two wheat genotypes (Misr1 and Gimmeza11) was well introduced in table (7) and figure (4) under saline conditions. The polypeptides were ranged from (147-19 kDa). The bands of molecular weights (45, 34, 28, 25, 22, 19 kDa) were completely present at control and in both genotypes except molecular weight 22 kDa was disappeared from control of Gimmeza11 only.

The first band 147 kDa was appeared at control of both genotypes and all CuO NPs doses of Gimmeza11 and completely eliminated from all CuO NPs doses. The second band 124 kDa was noticed only in Misr1 CuO NPs 1.0 ppm and Gimmeza11 CuO NPs 0.25 ppm. The 3rd and 6th bands with molecular weights (113, 63 kDa) was present only on Misr1 and Gimmeza11 control and completely absent from all treatments. The 4th band 102 kDa was synthesized by using CuO NPs 1.0 ppm in Misr1 and Gimmeza11 (0.5, 1.0 ppm). Band number 5 with molecular weight 88 kDa was present at control of both genotypes when treated with CuO NPs (0.5, 1.0 ppm).

A slight increase in band intensity was noticed in the polypeptide 22 kDa in Misr1 when the genotypes treated with 0.25 ppm. The same effect was noticed in Misr1 19 kDa polypeptide (1.00ppm) and Gimmeza11 (1.00 ppm).

This was identical with our earlier work, where there was a complete destruction to most of polypeptides of Gimmeza7 genotype under heat stress conditions when treated with CuO NPs. Authors supposed that action may be due to synthesis of new and unique bands at antioxidant isozymes as a defense mechanism against stress. They also reported a detectable negative effect of some NPs on the biosynthesis of essential and non-essential amino acids in the grains of the same cultivar **(Mahdi, 2017)**.

Concerning the negative effects of copper, **Zengin and Kirbag, (2007)** reported that, Cu negatively affected total protein content in sunflower plants. Protein content in organisms, as an important indicator of reversible and irreversible changes in metabolism, is known to respond to a wide variety of stressors such as heavy metals **(Singh and Tewari, 2003)**. It is showed that, excessive Cu may reduce the protein amount of many plant species.

Band number	MW (KDa)	CuO nanoparticles treatments							
		Misr 1	**Gimmeza 11**	**Misr 1**			**Gimmeza 11**		
		Control	**Control**	**0.25ppm**	**0.5ppm**	**1.0 ppm**	**0.25 ppm**	**0.5 ppm**	**1.0 ppm**
1	147	2.2	1.8	0	0	0	1.7	2.2	2.3
2	124	0	0	0	0	2.5	2.0	0	0
3	113	2.6	1.8	0	0	0	0	0	0
4	102	0	0	0	0	1.9	0	2.3	2.8
5	88	3.1	2.2	0	0	0	0	1.9	1.9
6	63	3.3	3.0	0	0	0	0	0	0
7	45	3.5	3.2	1.9	1.5	1.8	2.6	2.9	3.0

8	34	2.8	2.1	2.0	1.5	1.4	1.8	2.5	2.6
9	28	3.2	2.0	2.2	2.1	2.4	1.4	2.0	2.0
10	25	2.6	1.9	1.3	1.6	1.5	1.5	1.5	1.2
11	22	1.7	0	1.8	1.7	1.6	1.6	1.8	1.2
12	19	1.8	2.0	2.0	2.0	2.3	1.9	1.6	2.1
Total bands		**10**	**9**	**6**	**6**	**8**	**8**	**9**	**9**

Where;

- **0 = no bands.**
- **1.2 = refers to the lowest band intensity.**
- **3.5 = refers to the highest band intensity.**

Table (7): Effect of CuO nanoparticles treatment on SDS-PAGE profile (soluble protein) of two wheat genotypes under saline conditions

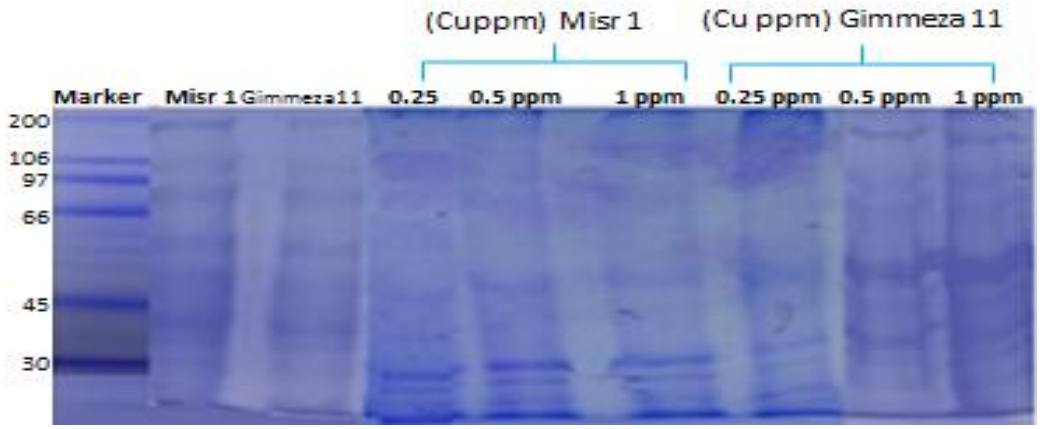


Figure (4): Effect of CuO nanoparticles treatment on SDS-PAGE profile (soluble protein) of two wheat genotypes under salinity stress.

7. Genomic DNA

Five of the ISSR primers amplified reproducible and polymorphic bands. The results are summarized in (Table 8 and 9).The five primers amplified 61 bands of distinct molecular sizes. The number of bands per primer ranged from 8 bands in primer ISSR4 to 17 bands in primer ISSR5 with an average of 12 bands per primer. A representative ISSR banding pattern amplified by the primers is presented in Figure **(5)** the 61 loci were detected of which 49 (80%) were polymorphic and Number of
unique bands 34 markers (Table 8 and 9).

Amplification of a large number of bands by the five primers (Table 2) and 80% polymorphism of these bands suggests that microsatellite sequences, based on which the primers were built, are ubiquitous in the genomes of the wheat genotypes of the current study and also that the sequences are highly variable among the CuO NPs of wheat genotypes. The primers ISSR1, 2, 3 and 4 (Table 2) were based on (TC)n, (GA)n and (CAC)n repeats. Primers based on these were reported earlier **(Sankar and Moore, 2001)** to exhibit only around 69% polymorphism, the variation may be attributed to the difference in the 30 anchors in both studies. **Syamkumar and Sasikumar (2007)** reported amplification of 100% polymorphic bands by the primer ISSR5 in the present study, in genetic diversity analysis. This result suggests the genome-wide presence of the microsatellite motif GC (GTG)n in the plants belonging to two discrete families.

All living organisms are subjected to damage by chemical and physical agents in the environment (e.g. UV and ionizing radiations, chemical mutagens and toxins.) and by free radicals which endogenously generated in metabolism. DNA is also damaged because of errors during its replication. The DNA lesions produced by these damaging agents could be altered base, missing base, mismatch base, deletion or insertion, linked pyrimidines, strand breaks, intra- and inter-strand cross-links. DNA is also impaired during its replication due to some noticed errors such as: alteration in bases, missing, or mismatching, deletion or insertion and may cause DNA lesions formed by these damaging agents. These DNA injuries could be cytotoxic or genotoxic to the cell. To prevent the harmful effects of DNA damage and maintain the genome integrity, all organisms have developed various strategies to reverse, excise, or tolerate the persistence of DNA damage products by generating a network of DNA repair mechanisms. A variety of different DNA repair systems have been introduced including direct reversal, base excision repair, nucleotide excision repair, double-strand break repair

pathway, and mismatching repair pathway. This may be an illustration to the continuing of studied wheat plants to its life cycle under the treatment of CuO NPs and positive effects of such treatment **(Tuteja et al. 2001)**.

Primers	Number of monomorphic bands	Number of unique bands	Number of polymorphic without Unique bands	Number of polymorphic with Unique bands	Total bands amplified	Polymorphism (%)
ISSR1	**1**	**10**	**2**	**12**	**13**	**92.3**
ISSR2	**1**	**9**	**3**	**12**	**13**	**92.3**
ISSR3	**7**	**1**	**2**	**3**	**10**	**30**
ISSR4	**3**	**0**	**5**	**5**	**8**	**62.5**
ISSR5	**0**	**14**	**3**	**17**	**17**	**100**

Table (8): ISSR analyses primer data and the percentage of polymorphic bands in two wheat genotypes treated with CuO NPs under salinity conditions

Primer		Misr1 control	Gimmeza11 control	0.25 ppm Misr1	0.50 ppm Misr1	1.00 ppm Misr1	0.25 ppm Gimmeza11	0.50 ppm Gimmeza11	1.00 ppm Gimmeza11
ISSR 1	(+)	2	3	5	3	3	6	3	4
	(-)	11	10	8	10	10	7	10	9
ISSR 2	(+)	5	4	4	5	4	6	4	5
	(-)	7	9	9	8	9	7	9	8
ISSR 3	(+)	7	9	9	9	9	9	9	9
	(-)	3	1	1	1	1	1	1	1
ISSR 4	(+)	7	7	8	8	4	8	8	8
	(-)	1	1	0	0	4	0	0	0
ISSR 5	(+)	4	5	8	3	3	3	4	7
	(-)	13	12	9	14	14	14	13	10
Total (+)		18	28	34	28	23	32	28	33
(-)		35	33	27	33	38	29	33	28

Table (9) Number of new appeared (+), bands disappeared (-), as related to control in Wheat plants treated with different concentrations of CuO NPs using five ISSR primers.

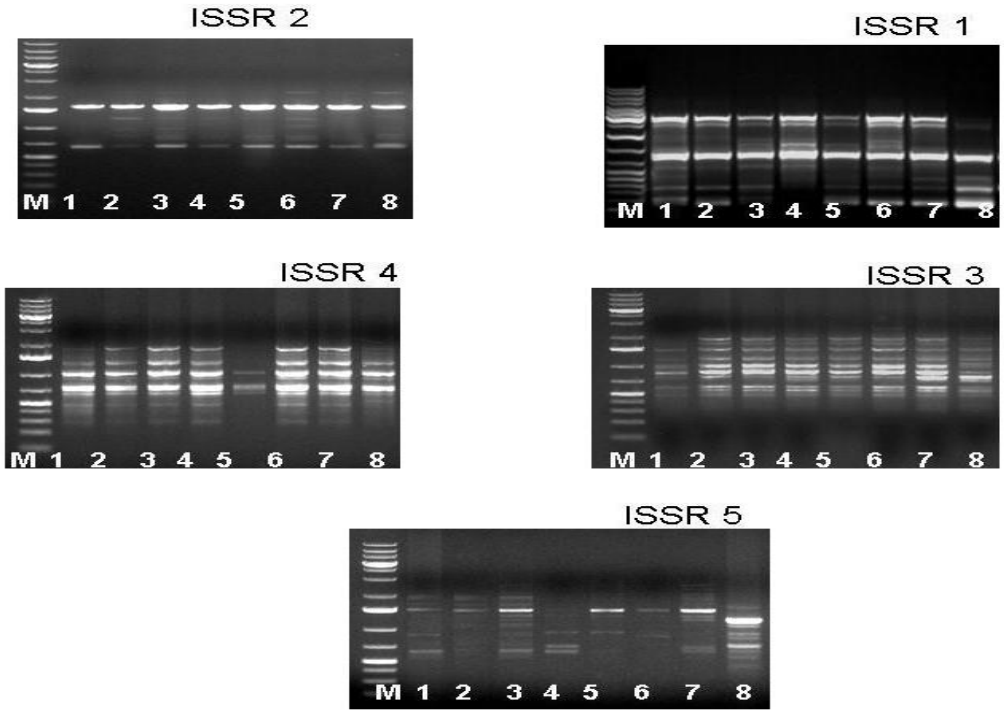


Figure (5): ISSR electrophoresis patterns. Lane M, DNA marker; lanes 1-2 were control Misr1, Gimmeza11 and lanes 3-8 were CuO NPs treated

Safety analysis of copper nanoparticle in Wistar albino rats

One dose

Acute toxicity means the ability of a drug to cause harmful effects within a short period of dosage or exposure, which is usually the first step in toxicological investigations of unknown products. LD_{50}, is also used as a general indicator of the acute toxicity of unknown substances **(Lu et al. 2014)**. In the acute oral toxicity analysis, no irregular clinical signs and mortality were found, and all rats acquired weight during the 14 days after gavage introduced CuO NPs orally. There was no substantial difference in body weight and feed intake in all groups compared to control. In the LD_{50} study no rats died at various doses (0, 1000, and 2000 mg / kg body weight) after the oral conduction of CuO NPs. Thus, while conducting orally through gavage, the CuO NPs were looked as non toxic.

Repeated doses

Table (10) showed changes in the body weight gain and biochemical parameters at various levels in rats exposed to copper NPs (0, 0.25, 0.5 and 1 mg / kg body weight). At the beginning and end of treatment, all rats were weighed (after 28 days), and the body weight gain was measured **(Table 10)**.

Copper oxide NPs did not significantly (p<0.05) affect the body weight gain of the rats at a low dose (0.25 mg/Kg body weight).

Renal function parameters (urea, uric acid and creatinine) were estimated and the data also presented in **Table 10**. Copper oxide NPs treatment at low and medium dose (0.25, and 0.5mg/Kg body weight) significantly decreased (p<0.05) the creatinine levels while the high dose did not significantly (p<0.05) effect on the creatinine level compared to control.

The effect of CuO NPs at different levels (0, 0.25, 0.5 and 1 mg/Kg body weight) on liver enzymes activities (ALT, AST, and ALP), protein profile (total protein, albumin, and globulin) and total bilirubin in treated rats were estimated to determine the liver function and the data also presented in **Table 10**.

Copper oxide NPs treatment at low dose (0.25 mg/Kg body weight) did not significantly (p<0.05) effect on the total protein level while the medium and high doses significantly decreased total protein level compared to the control. Copper NPs treatment at different doses (0.25, 0.5, and 1 mg/Kg body weight) significantly increased (p<0.05) the total bilirubin levels compared to control.

Histopathological examination of the liver is presented in **Figure 6.**Histopathological examination of the tissues indicated that CuO NPs at different levels (0, 0.25, and 0.5mg/kg body weight) every day for 28 days showing normal central vein, normal liver cords and normal portal tracts. The liver of groups treated with copper nanoparticles appeared with normal structures apart of mild dilatation of the central vein and hepatic sinusoids. Regarding to the kidneys' tissue presented in **Figure (7),** indicated that CuO NPs at different levels (0, 0.25, and 0.5 mg/kg body weight) every day for 28 days showing normal renal tubules and normal renal glomeruli.

Parameters	**Nanoparticle levels (mg/Kg body weight)**			
	0	**0.25**	**0.5**	**1.0**
		CuO NPs		
Body weight gain (g)	43.30 a	42 ab	37.83 c	32.16 e
Urea (mg/dl)	43.75 c	44.43 c	46.05 b	49.43 a
Uric acid (mg/dl)	2.35 a	2.16 b	1.88 c	1.34 d
Creatinine (mg/dl)	0.517 a	0.423 c	0.473 b	0.503 a
ALT (U/l)	15.16 e	23.33 d	24.83 d	39.16 b
AST (U/l)	22.66 d	23.00 d	26.66 c	32.33 b
ALP (U/l)	129 f	132 e	135 d	147 b
Total protein (g/dl)	6.20 a	6.02 a	5.56 b	5.16 c
Albumin (g/dl)	3.20 a	2.77 b	2.77 b	2.58 c
Globulin (g/dl)	3.00 b	3.24 a	2.79 bc	2.58 cd
A/G ratio	1.07 a	0.862 b	0.997 a	1.01 a
Total bilirubin (g/ml)	0.282 e	0.320 d	0.433 c	0.608 b

The data represent group averages (mean ± SE, n=8). The different letters in the same row indicate significantly (p<0.05) different values.

Table (10) Body weight gain and biochemical parameters in rats exposed copper nanoparticles at different levels (0, 0.25, 0.5 and 1.0 mg/kg body weight)

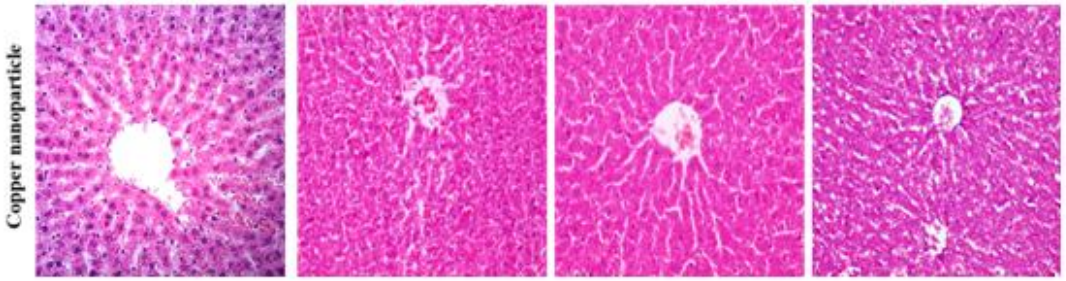


Figure (6): Histopathological examination of the rat liver exposed to CuO NPs at different levels (0, 0.25, 0.5 and 1.0 mg/kg body weight)

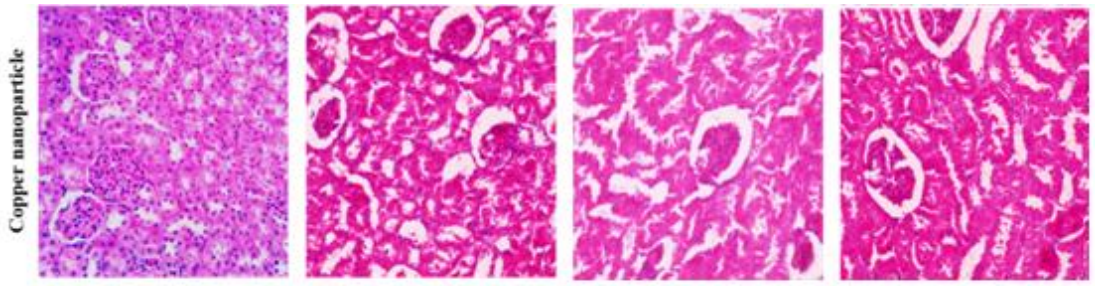


Figure (7): Histopathological examination of the rat kidney exposed to CuO NPs at different levels (0, 0.25, 0.5 and 1.0 mg/kg body weight)

IV. Conclusion

The application of metallic biocompatible nanoparticles such as CuO NPs affected the plant growth traits positively, decreased malondialdehyde content, increased chlorophyll in some extent, increased glutathione content, enhanced accumulation of a unique SOD band especially at 1.0 ppm, and some polypeptides which may be related to the wheat tolerance to salinity stress. Changes were happened in some DNA data obtained from ISSR profiles showed change in band numbers in all tested samples. The highest polymorphic bands recorded are at samples subjected to CuO NPs, as compared with untreated plants. In addition to appearance and disappearance of bands in ISSR profiles, a decrease in genetic template stability values was recorded with increasing the concentrations. These change in DNA may related to the positive indices of biomarkers which may related to salinity stress tolerance. We can't ignore the role of DNA repair system. In our biological study, CuO NPs does not affect the levels of the liver or kidney bio-indicators and histopathological status, which were remained within the typical values and this may be an ending to some controversial approaches towards nanotechnology, so that, in the future CuO NPs could be included as a commercial emulsion instead of hazardous chemicals applied on cultivated fields in wide spectra applications.

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