Evaluation of the Effectiveness of Safflower *(Carthamus tinctorius)* Seeds Amendment with Silicon or Humic Acid During Germination under CuO (NPs) Phytotoxicity

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AMELIORATION with silicon (1mM sodium silicate Na_2SiO_3) or humic acid (50mg L^{-1} HA) was tested in counteracting the phytotoxicity of copper oxide nanoparticles (CuONPs) at 50, 100 and 150mg L^{-1} on Safflower plants (*Carthamus tinctorius*). Si sustained seed growth at (150mg L^{-1} CuO NPs). The application of HA increased copper concentration in seedling by about 5 to 10 fold, relative to the control at 50 and 100mg L^{-1}. Fresh weight of safflower seedlings were reduced significantly at 100mg L^{-1} CuO NPs. Si enhanced fresh and dry matter gain under 100 and 150mg L^{-1} CuO NPs, also HA improved dry matter at 100mg L^{-1} CuO NPs. Chlorophyll a and carotenoid contents reduced by about 60% and 50% at CuO NPs (100mg L^{-1}), respectively as compared to control. Chlorophyll a and carotenoid contents reduced by about 60% and 50% at CuO NPs (100mg L^{-1}), respectively as compared to control. Si or HA sustained Chl.a content unchanged and improved carotenoids contents at 100mg L^{-1} CuO NPs.

Phenolics contents significantly increased under CuO NPs phytotoxicity. External application of HA provoked more phenolics content. Nano-CuO phytotoxicity reduced H_2O_2 scavenging and lipid peroxidation inhibition %. Si or HA enhanced significantly lipid peroxidation inhibition % and H_2O_2 radial scavenging %. Si or HA reflected significant enhancement of SOD and CAT enzymes under elevating concentrations of CuO NPs. Si enhanced significantly POD activity at (50, 100 and 150mg L^{-1}) CuO NPs levels.

Keywords: Antioxidants, *Carthamus tinctorius*, CuO, Humic acid, Nanoparticles, Phenolics, Silicon.

Introduction

Food security is a crucial need which can never be ignored by any society. The parallel extensive increases in environmental damage due to unsuitable anthropogenic activities and human population pressure have the unlucky consequence that global food production may soon become inadequate to feed all of the world's people (Etesami & Jeong, 2018). Contamination of agricultural soils in many parts of the world by heavy metal toxicity such as Cd, Cu, Zn, Ni, Co, Cr, Pb, and As, is a serious problem. Plant response to the toxicity of the heavy metal stress has been studied extensively over the past decade at physiological and molecular levels (Elisa et al., 2007; Sudo et al., 2008; Zhang et al., 2009; Ahmed et al., 2010 and Thounaojam et al., 2012).

Engineered metal oxide nanoparticles (NPs) emissions are increasing, and expected to accumulate in aquatic, terrestrial and atmospheric environments (Tripathi et al., 2017). Timely, there is a new emerging issue that become a serious threat to the ecological system including plants, which is the phytotoxicity of nanoparticles (NPs). Plant nanotoxicology is an emerging and less-explored area of research for the plant stress biologists. However, due to lack of information on nanoparticles (NPs) toxicity, behavior and fate even under laboratory conditions, it is not easy to estimate the severity of nanomaterial impacts on the ecosystem and human health.

Copper-based nanoparticles (NPs) are profusely used due to their optical, thermal, electrical, antibacterial, and catalytic applications (Peralta-Videa et al., 2016). As the ultimate

sink of nanoparticles, soil is supposed to be the most significant exposure venue for the entry of nanoparticles into food webs (Remédios et al., 2012). Therefore, the introduction of nanoparticles in terrestrial ecosystems may change the profile of soil-plant systems (Du et al., 2017). Nano-sized particles of CuO were more phytotoxic than bulk size (Koce, 2017). However, little is known about their physiological and biochemical effects in many agricultural crops (Du et al., 2017 and Ochoa et al., 2017). It is reported that nano-CuO, at different concentrations, alters plant growth and development by increasing the reactive oxygen species (ROS) production and unbalancing homeostasis of essential elements (Singh et al., 2017 a).

Safflower (*Carthamus tinctorius*) is an annual herbaceous plant, belongs to the family *Asteraceae*. It is a high-quality forage crop that can be grown in arid and semiarid regions and it can tolerate hot and drought conditions (Bar-Tal et al., 2008 and Çulha & Çakırlar, 2011). Safflower has long been grown for several purposes, such as a vegetable, an edible and industrial oil, a spice and as birdfeed (Johnston et al., 2002). Historically, it has been mainly cultivated for medicinal purposes and extracting cartamin from its florets which is used for coloring foods and clothes (Knight, 2007). Furthermore, safflower seeds could be used for ruminant's diet production as a possible source of polyunsaturated fatty acids (Kott et al., 2003). Its extracts are composed mainly of phenolic compounds, including flavonoidsand serotonins which have been proved to possess antioxidant effects (Han et al., 2010 and Çulha & Çakırlar, 2011).

Although silicon (Si) is not considered so far as essential nutrient for plants, it is classified by many authors as beneficial or useful element, as it might have an important role in metabolic or physiological and/or structural activity (Liang et al., 2015). According to the newly established definition of essentiality of elements proposed by Epstein & Bloom (2005), the essentiality for Si may be finally recognized in higher plants. Si-fertilizer is a high-quality fertilizer for developing ecologically green agriculture as it is noncorrosive and pollution-free. It has been testified for biomass, yield and quality improvement of a broad range of crops including monocotyledonous, some dicotyledonous, some

vegetables and fruits, which actively take up and accumulate high amounts of Si in their organs (Liang et al., 2015). The content of Si in plants is equivalent to or more than the major nutrients N, P, and K, which are supplied through fertilizers (Meena et al., 2014). Si improved survival of higher plants exposed to different biotic and abiotic stresses (Liang et al., 2015).

Humic substances refer to a class of naturally occurring organic materials commonly found in soils, sediment sand natural waters, which derive from the decomposition of plant and animal residues (MacCarthy, 2001). Humic acids (HA) represent the fraction of humic substances insoluble in water under acidic conditions, which becomes soluble and extractable at higher soil pH. Molecules of HA are characterized by acidic groups such as carboxyl and phenol OH functional groups (Hofrichter & Steinbeuchel, 2001) and when applied to the soil, play an important role in the transport, bioavailability and solubility of HA (Lagier et al., 2000). It has been demonstrated that HA contribute to reducing the physical mobility (diffusion, mass flow) of various metal species (e.g., Cu, Pb, Zn, Ni) in the soil and thus reduce the consequent risk of lateral or vertical contamination of water bodies, as acetic acid extraction of metals which is generally reduced with HA (Halim et al., 2003). A few researches have already found that HA could affect the toxicity of some NPs to algae; where HA adsorbed by NPs weakened the biological toxicity of these NPs (Li et al., 2011 and Lin et al., 2012). However, dissolved nature organic materials (NOMs) were also observed to increase algae toxicity of CuO NPs as the result of higher Cu^{2+} release, lesser degree of aggregation and enhanced internalization of CuO NPs (Wang et al., 2011). Therefore, the influencing mechanism of NOMs to the toxicity of NPs should continue to be explored.

The use of silicon and humic acid in improving the tolerance of plants to nanotoxicity has not been explored extensively so far, and little attention is devoted to it in the current literature. Therefore, the objectives of this research were to determine the impact of silicon and humic acid in alleviating the toxic effect of elevated concentrations of CuO NPs on the physiological, and biochemical traits of safflower plants grown in sand.

Materials and Methods

Source of CuO nanoparticle and suspension preparation

CuO Nanoparticles (CuONP) tested for phytotoxicitywere purchased from Sigma-Aldrich. Sizes, specific surface areas, and purities of compounds were adopted (where available) from the manufacturers. CuO (size <50nm, surface area 29m^2/g). Tested CuO Nano at a concentration of 150mg L^{-1} were sonicated for 30min to ensure dispersion in the solution and diluted further to obtain the remaining concentrations of 50 and 100mg L^{-1}.

Sand matrix preparation and plant growth

Silica sand was sieved for particle sizing and soaked overnight in 1% HCL, then washed thoroughly with distilled water before drying and use. Sand (300g) was weightedper box (8x8cm), lined with polyethylene bags. This solid matrix will provide a more representative indication of the impact of NPs on plants under environmentally relevant conditions. The seeds of Safflower (*Carthamus tinctorius* L.) were surface-sterilized in 10% hydrogen peroxide (H$_2$O$_2$) for 15min and rinsed thoroughly with distilled water and then five seeds were sown at separate locations per box at depths of about 0.5cm. Quartz sand treated with elevated CuO NPs levels (0, 50, 100, and 150mg L^{-1}), individually or in combination with 1mM sodium silicate (Na$_2$SiO$_3$) or 50mg L^{-1} humic acid (HA) at approximately 100% of the field capacity at 11% water content. Only H$_2$O was added for the absolute control. Each box represented as an experimental unit. All experiments were performed in six replications. The seeds were grown at 24°C for 8 days. The root length and shoot height of seedlings were measured over the course of 8 days and compared to the untreated control. After measurement, whole seedlings were washed twice with distilled water, dried gently with filter paper. The seedlings were quickly weighted for fresh weights determination, then oven-dried at 70°C for 48h in order to determine dry weights. The Cu content in seedling tissues from all treatments was measured by Atomic Absorption Spectroscopy (AAS) after HNO$_3$ digestion according to Sawhney & Frink (1991).

Preparation of seedling extract

Fresh seedlings were immediately weighted and ground in a chilled mortar and pestle with 6ml buffer solution containing 50mM TrisHCl (pH 7), 1mM sodium EDTA and 3mM MgCl$_2$. The extract was centrifuged at 4°C for 10min at 5000rpm. The resultant supernatant was used for the enzymatic and non-enzymatic antioxidants determinations, in addition to the antioxidant potential determinations.

Photosynthetic pigments

The photosynthetic pigments (chlorophyll a, b and carotenoids) were extracted from fresh plumule samples by suspending them in 5ml of 95% C$_2$H$_5$OH at 60°C, until colorless. Then the total volume completed to 10ml with 95% C$_2$H$_5$OH and absorbance readings were determined at 663, 644 and 452nm spectrophotometerically. Chlorophylls and carotenoids concentrations were calculated as cited by Lichtenthaler (1987) as mg g^{-1} FW.

Soluble proteins

Soluble protein contents were determined in the plant extract by Folin reagent according to Lowry et al. (1951). A calibration curve was constructed using bovine serum albumin (BSA) and the data were expressed as mg BSA g^{-1} fresh matter.

Enzymatic antioxidants
Super oxide dismutase (SOD)

SOD activity was assayed according to of Beauchamp & Fedovich (1976). The amount of enzyme causing the reduction of NBT by 50% was expressed as SOD Unit. The expression of specific activity was in terms of units per mg of protein.

Catalase (CAT)

CAT activity wasassayedaccording to Aebi (1984). The decrease in H$_2$O$_2$ absorbance at A$_{240}$ nm was used to calculate the activity.

Guaiacol peroxidase (GPX)

GPX activity was determined following the method of Tatiana et al. (1999). The increase in absorbance at A$_{470}$ nm due to the formation of tetraguaiacol was measured.

Non enzymatic antioxidants
Total phenolics

Total phenolic contents were determined according to Singleton & Rossi (1965). Folin-Ciocalteau reagent method was used. The measurements were carried out at A$_{765}$nm. Gallic acid equivalents were used to express the data

as µg g^{-1} FW using Molar Coefficient of 120µg^{-1} cm^{-1} ml^{-1}.

Antioxidants

Activity of total antioxidant

The contents of total antioxidant were measured according to Prieto et al. (1999). The absorbance was measured at A$_{695}$ nm.

Hydrogen peroxide (H$_2$O$_2$) scavenging

H$_2$O$_2$ radical scavenging assay carried out according to Long et al. (1999). Sodium pyruvate was used as the reference compound. The absorbance of the ferric-xylenol orange complex was measured at A$_{560}$ nm.

Inhibition of lipid peroxide formation

The % inhibition of lipid peroxidation was carried out according to Janero (1990). The absorbance of the upper organic layer was measured at A$_{532}$ nm. The inhibition in percent (I) was calculated by the formula:

I= (Abs control – Abs sample)/Abs control × 100

Statistical analysis

The experiments were factorial in complete Randomized (CR) design. Each treatment was replicated in six boxes. One-way ANOVA was performed on the data from two independent experiments with three replicates. The expressions of data as (mean±SE). Analysis was performed using the SPSS statistical 11.0 package. Comparing of means for significant differences Duncan's multiple range tests at (P<0.05) were used. All the assessed attributes analyzed with Principle Component Analysis (PCA) variance regression ordination, using the Sørensen coefficient as the distance measure, to check the magnitude of change in attributes along the Si or HA and CuO gradientsby the PAST softwareversion 2.11 for Windows (Hammer et al., 2001).

Results

Plant growth parameters

Data indicated that, Cu reached to its highest values (152.2µg g^{-1} DW) in the plants grown in 100mg. L^{-1} CuONP + HA, followed by 88.7µg g^{-1} DW in the plants grown in 50mg. L^{-1} CuO amended with HA (Table 1). While Cu concentration showed lower values in plants

grown at 50 and 100mg L^{-1} CuO NPs individually or amended with Si. Generally, the safflower seedling growth was completely inhibited under 150mg L^{-1}CuONP. While, external application of silicon sustained the seedling growth under this concentration 150mg L^{-1}CuONP.

Results revealed that plant fresh and dry weights exhibited different trends under CuO NPs amended with Si or HA. Fresh matter reduced significantly under 100mg L^{-1} CuO NPs. While lower concentration of CuO NPs (50mg L^{-1}) induced the fresh matter values with about 65%, as compared to control. The external application of HA reduced significantly the fresh weight values at 0, 50 and 100mg. L^{-1}CuO NPs. On the other hand, amendment with Si enhanced significantly the fresh matter of safflower seedlings at 0, 50 and 100mg L^{-1} levels of CuO NPs.

According to the data shown in Table 1, there is no significant change in dry weight at 50mg L^{-1} CuO NPs with or without Si or HA, as compared to 0.0 CuO NPs (absolute control). There was a significant increase in dry weight at 0.0 CuO NPs with Si, as compared to control. Another significant increase was observed in dry weight recorded at 100mg L^{-1} CuO NPs with Si, as compared to the corresponding concentration (100mg L^{-1} CuO NPs) without (Si or HA) and as compared to absolute control. External application of Si to the treatment of 150mg L^{-1} CuO NPs resulted in a significant increase in dry weight, as compared to control.

The response of root length was shown in Table 1. Lower CuO NPs concentration (50mg L^{-1}) reduced significantly shoot height by about 26%, while no significant change was observed in root length. At 100mg L^{-1} shoot height reduced by about 35% and root length reduced by about 45%. External application of Si showed non-significant increase in root length, while HA amendment showed significant enhancement in root length, as compared to the corresponding values at 100mg L^{-1} CuO NPs without external amendment with Si or HA. On the other hand, when Si was applied at 150 mg L^{-1} CuO NPs, an obvious reduction in root length was recorded. Shoot height showed significant reduction at 50 and 100mg L^{-1} CuO NPs with or without (Si or HA), as compared to control. The same trend was observed at 150mg L^{-1} CuO NPs with Si.

TABLE 1. Growth attributes and copper concentration of Safflower (*Carthamustinctorius* L.) seedlings as influenced by CuO Nano (mg. L⁻¹) phytotoxicity, silicon (1 mM Na₂SiO₃) orhumic acid (50 mg L⁻1).

Treatments	Fresh weight (g plant⁻¹)	Dry weight (g plant⁻¹)	Shoot height (cm)	Root length (cm)	Cu (µg g⁻¹ DW)
Control	0.169±0.003[f]	0.026±0.001[a]	3.4±0.07[d]	3.5±0.14[de]	15.02±1.98[c]
0+Si	0.247±0.003[h]	0.041±0.002[c]	3.6±0.05[d]	4.0±0.08[de]	6.87±1.11[b]
0+HA	0.119±0.006[b]	0.029±0.002[a]	2.5±0.06[c]	3.9±0.08[de]	4.60±1.32[a]
50 Nano-CuO	0.279±0.003[i]	0.025±0.003[a]	2.5±0.06[c]	3.5±0.20[de]	29.57±1.98[g]
50 Nano-CuO +Si	0.193±0.002[g]	0.029±0.002[a]	2.5±0.04[c]	3.5±0.09[de]	30.57±1.71[h]
50 Nano-CuO +HA	0.130±0.005[c]	0.027±0.001[a]	2.0±0.10[ab]	4.5±0.09[e]	88.70±3.01[i]
100 Nano-CuO	0.158±0.001[e]	0.030±0.001[a]	2.2±0.15[bc]	1.9±0.21[b]	25.68±1.63[e]
100 Nano-CuO +Si	0.282±0.005[j]	0.039±0.003[bc]	2.5±0.09[c]	2.3±0.09[bc]	26.64±3.11[f]
100 Nano-CuO +HA	0.148±0.002[d]	0.031±0.002[ab]	2.5±0.11[c]	3.2±0.20[cd]	152.22±2.51[j]
150 Nano-CuO +Si	0.079±0.004[a]	0.039±0.002[bc]	1.6±0.09[a]	0.5±0.10[a]	22.24±1.20[d]

Different litters are significantly different at P<0.05 (mean±SE; n=3).

Photosynthetic pigments

Chlorophyll a content exhibited no significant change at 50mg L⁻¹ CuO NPs without or with external application of Si or HA (Fig. 1). The higher CuO NPs level (100mg L⁻¹) showed significant reduction in Chl. a content. Amendment with Si or HA sustained non-significant change in Chl. a content at 100mg L⁻¹ CuO NPs, as compared to control. Another significant reduction was recorded at 150mg L⁻¹ CuO NPs with Si application.
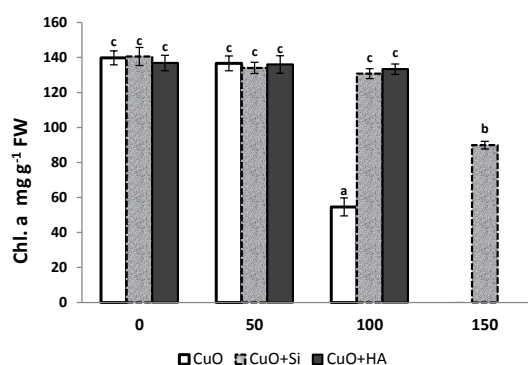

Fig. 1. Effects of nano-CuO (mg L⁻¹) stress on chlorophyll a (Chl. a) content in safflower *(Carthamus tinctorius* L.) seedlings as influenced by silicon (1mM Na₂SiO₃) or humic acid (50mg L⁻¹) [Data are expressed as the mean values ± standard errors (n= 3). Values in columns with different letters are significantly different at 5% level according to Duncan's Multiple Range Test].

Chlorophyll b content exhibited fluctuated trend as shown in Fig. 2, external application of HA at 0mg L⁻¹ CuO NPs level enhanced its content significantly. An enhancement in Chl. b content was also recorded at 50mg L⁻¹ CuO NPs with and without (Si or HA) and the highest value was recorded in case of 50mg L⁻¹ CuO NPs plus Si. With increasing CuO NPs concentration to 100mg L⁻¹ significant increase in Chl.b content occurred. Another enhancement was recorded in Chl. b content with external application of HA at 100mg L⁻¹. While significant reduction was determined at 100mg L⁻¹ CuO NPs plus Si. The highest concentration of CuO NPs, (150mg L⁻¹) plus Si, exhibited significant raise, as compared to control.

The content of carotenoids increased significantly with the external amendment with Si or HA at 0mg L⁻¹ CuO NPs (Fig. 3). Furthermore, at (50mg L⁻¹) of CuO NPs, an enhancement was recorded in the carotenoids content except for HA application. Toxicity of CuO NPs was observed at 100mg L⁻¹, where the reduction in carotenoids content was about 50%, as compared to control. Amendment with Si or HA enhanced the content of carotenoids under this concentration significantly, as compared to control. Higher level of CuO NPs (150mg L⁻¹) amended with Si showed significant reduction in carotenoids content, as compared to control.
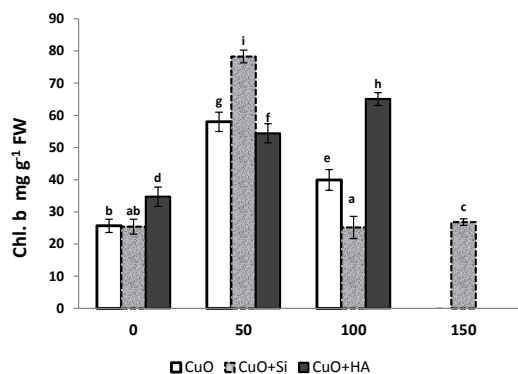
Fig. 2. Effects of nano-CuO (mg L^{-1}) stress on chlorophyll b (Chl. b) content in safflower *(Carthamus tinctorius* L.) seedlings as influenced by silicon (1 mM Na$_2$SiO$_3$) or humic acid (50 mg L^{-1}). Data are expressed as the mean values ± standard errors (n = 3). Values in columns with different letters are significantly different at 5% level according to Duncan's Multiple Range Test.
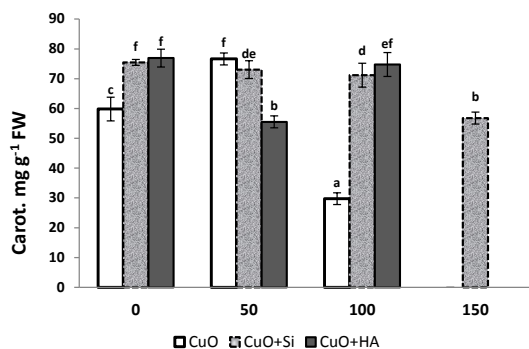

Fig. 3. Effects of nano-CuO (mg L^{-1}) stress on carotenoids content in safflower *(Carthamus tinctorius* L.) seedlings as influenced by silicon (1mM Na$_2$SiO$_3$) or humic acid (50mg L^{-1}) [Data are expressed as the mean values ± standard errors (n= 3). Values in columns with different letters are significantly different at 5% level according to Duncan's Multiple Range Test].

Total phenolics

Data of total phenolics was shown in Fig. 4. Application of external HA at (0mg L^{-1}) CuO NPs markedly enhanced the content of phenolics. Other significant increase in phenolics content was recorded at 50 and 100mg L^{-1} CuO NPs relative to absolute control. Furthermore, external application of HA follows the same trend and induced significant increase in phenolics content at 50 and 100mg L^{-1} CuO NPs. On the other hand, Si amendment showed somewhat different trends toward these two concentrations of CuO NPs and non-significant change in phenolics content recorded as compared to control. The significant enhancement recorded for Si application was only at 150mg L^{-1} CuO NPs, as compared to control.
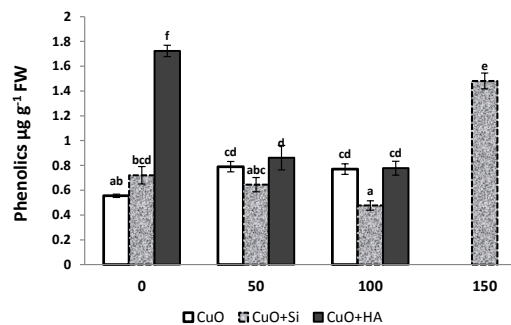
Fig. 4. Effects of nano-CuO (mg L^{-1}) stress on accumulations of phenolics in safflower *(Carthamus tinctorius* L.) seedlings as influenced by silicon (1mM Na$_2$SiO$_3$) or humic acid (50mg L^{-1}) [Data are expressed as the mean values ± standard errors (n= 3). Values in columns with different letters are significantly different at 5% level according to Duncan's Multiple Range Test].

Total antioxidant

The external application of Si or HA without CuO NPs toxicity enhanced total antioxidant content, as compared to control as shown in Fig. 5. CuO NPs at 50mg L^{-1} increased significantly the total antioxidants content and another significant increase was detected with the external application of HA or Si at 50mg L^{-1}, as compared to control. At higher level of CuO NPs (100mg L^{-1}), another increase in total antioxidants was recorded. Amendment with HA exhibited similar trend, while Si application caused no significant change in the total antioxidants contents, as compared to the control at 100mg L^{-1} CuO NPs. While pronounce significant raise was detected at 150mg L^{-1} CuONP amended with Si.
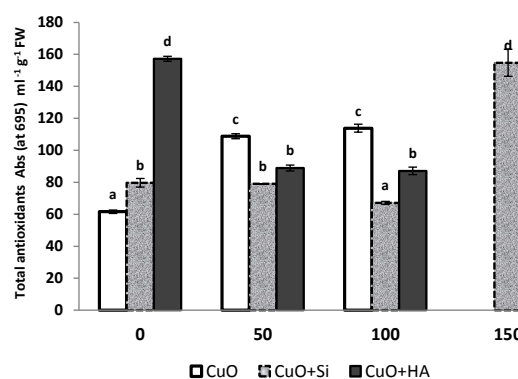

Fig. 5. Effects of nano-CuO (mg L^{-1}) stress on total antioxidants in safflower *(Carthamus tinctorius* L.) seedlings as influenced by silicon (1mM Na$_2$SiO$_3$) or humic acid (50mg L^{-1}) [Data are expressed as the mean values ± standard errors (n= 3). Values in columns with different letters are significantly different at 5% level according to Duncan's Multiple Range Test].

Lipid peroxidation

The percent of lipid peroxidation inhibition was found to be retarded significantly at 50 and 100mg L^{-1} CuO NPs. On the other hands, external application of Si or HA improved significantly the percent of lipid peroxidation inhibition at 50 and 100mg L^{-1} CuO NPs, as compared to relative treatments without Si or HA. While at 150mg L^{-1} CuO NPs, significant decrease was observed with the application of Si, as compared to control (Fig. 6).
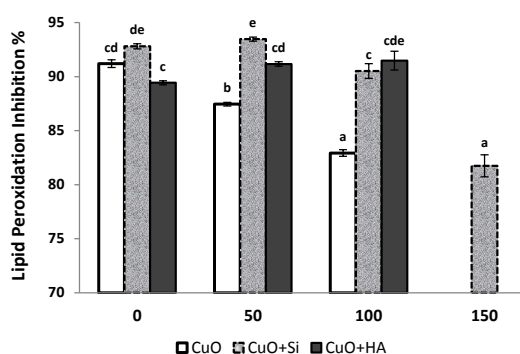

Fig. 6. Effects of nano-CuO (mg L^{-1}) stress on lipid peroxidation inhibition % in safflower *(Carthamus tinctorius* L.) seedlings as influenced by silicon (1mM Na$_2$SiO$_3$) or humic acid (50mg L^{-1}) [Data are expressed as the mean values ± standard errors (n= 3). Values in columns with different letters are significantly different at 5% level according to Duncan's Multiple Range Test].

Significant reduction in the percent of hydrogen peroxide radical scavenging recorded at 50mg L^{-1} CuO NPs and at the same concentration plus HA (Fig. 7), while Si application enhanced significantly H$_2$O$_2$ radical scavenging % at 50mg L^{-1} CuO NPs as compared to control. All treatments with or without Si or HA at 100mg L^{-1} CuO NPs showed general reduction in the percentof H$_2$O$_2$ radical scavenging, as compared to control. Meanwhile, as comparing H$_2$O$_2$ radical scavenging % under the application of Si or HA and relative treatment without their application under 100mg L^{-1} CuO NPs, significant enhancement was recorded. Higher toxicity level of CuO NPs (150mg L^{-1}) plus Si resulted in a significant reduction in H$_2$O$_2$ radial scavenging %, as compared to control.

Enzymes activities

Data of SOD enzyme activity was shown in Fig. 8. It seems to be unaffected at the first level of CuO NPs (50mg L^{-1}), even when Si

was externally applied. While, HA amendment induced the activity of SOD significantly under 50mg L^{-1}. Furthermore, significant induction in the SOD activity was resulted with increasing the toxicity of CuO NPs to100mg L^{-1} as compared to control. In addition, external application of Si or HA at 100mg L^{-1}CuO NPs and application of Si at 150mg L^{-1} CuO NPs showed even higher activity of SOD, as compared to corresponding copper concentration without their application and to absolute control as well.
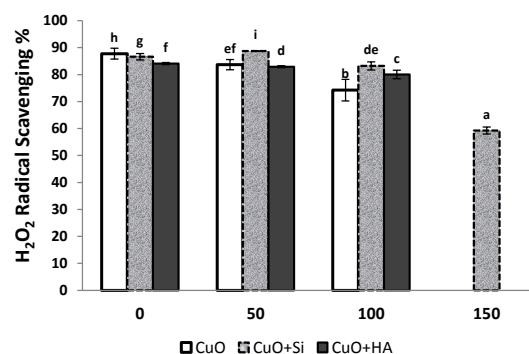

Fig. 7. Effects of nano-CuO (mg L^{-1}) stress on H$_2$O$_2$ radical scavenging % in safflower *(Carthamus tinctorius* L.) seedlings as influenced by silicon (1mM Na$_2$SiO$_3$) or humic acid (50mg L^{-1}) [Data are expressed as the mean values ± standard errors (n= 3). Values in columns with different letters are significantly different at 5% level according to Duncan's Multiple Range Test].

Fig. 8. Effects of nano-CuO (mg L^{-1}) stress on SOD activity in safflower *(Carthamus tinctorius* L.) seedlings as influenced by silicon (1mM Na$_2$SiO$_3$) or humic acid (50mg L^{-1}) [Data are expressed as the mean values ± standard errors (n= 3). Values in columns with different letters are significantly different at 5% level according to Duncan's Multiple Range Test].
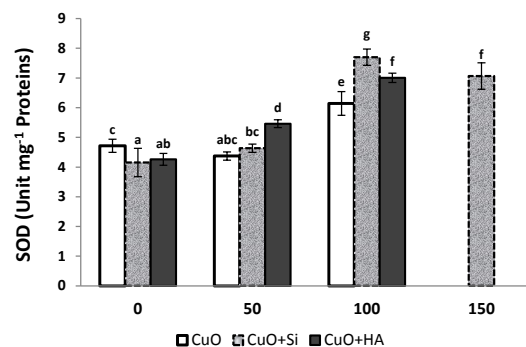
Copper toxicity at 50 and 100mg L^{-1} induced significantly the activity of POD enzyme

(Fig. 9). The same trend was observed with applying Si at 50, 100 and 150mg L^{-1} of CuO NPs concentrations. While external application of HA showed significant decrease or non-significant change at 50 and 100mg L^{-1} CuO NPs levels, respectively.
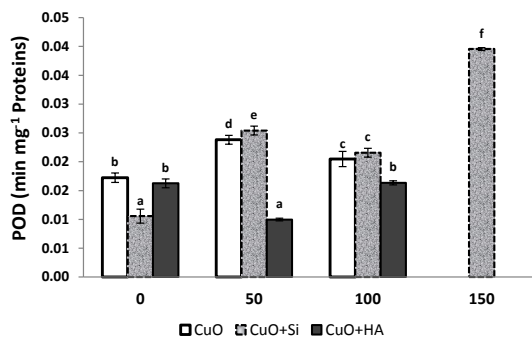

Fig. 9. Effects of nano-CuO (mg L^{-1}) stress on POD activity in safflower (*Carthamus tinctorius* L.) seedlings as influenced by silicon (1mM Na$_2$SiO$_3$) or humic acid (50mg L^{-1}) [Data are expressed as the mean values ± standard errors (n= 3). Values in columns with different letters are significantly different at 5% level according to Duncan's Multiple Range Test].

The activity of CAT enzyme wassignificantly retarded at 50 and 100mg L^{-1} CuO NPs, as compared to control (Fig. 10). Amending safflower seedlings with Si resulted in significant increase of CAT activity at 100mg L^{-1} CuO NPs, while at 50mg L^{-1} CuO NPs significant reduction was recorded. On the other hand, external application of HA exhibits the highest increment in CAT activity at 50 and 100mg L^{-1} CuO NPs, as compared to control (Fig. 10).

As shown in Fig. 11, the two concentrations of CuO NPs (50 and 100mg L^{-1}) resulted in significant enhancement in the content of soluble proteins, as compared to control. The trend of soluble proteins content fluctuated under Si amendment. First, there was no significant changeat 50mg L^{-1} CuO NPs, followed by significant decrease at 100mg L^{-1} CuO NPs and finally, significant increase recorded at 150mg L^{-1} CuO NPs, as compared to absolute control. Concerning the data of soluble proteins as a result of HA amendment, the greatest significant increase recorded at 0mg L^{-1} CuO NPs, followed by 50 and 100mg L^{-1} CuO NPs, respectively, as compared to control.

Subjection of the original data of all measured parameters to the principle component analysis

(PCA) was interpreted in Fig. 12, which revealed the previously mentioned correlations on its first two axes. PCA axis 1 captures about 43.48% of the cumulative percentage followed by the second axis (17.2%). The distances between the attributes on axis 1 illustrate the degree of similarity; the closer the distance, the greater the resemblance and *vice versa*. Thus PCA biplot indicated contrariness between Copper (upper side) and the growth indicators, enzymatic and non-enzymatic antioxidants contents (lower side of Fig. 12).
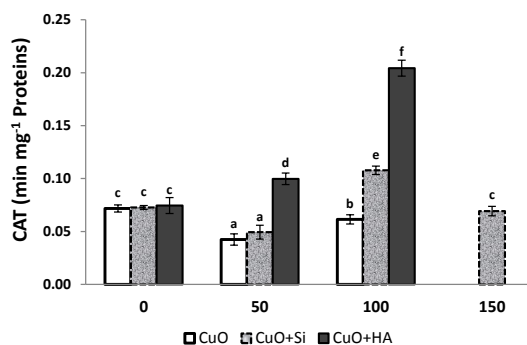

Fig. 10. Effects of nano-CuO (mg L^{-1}) stress on CAT activity in safflower *(Carthamus tinctorius* L.) seedlings as influenced by silicon (1mM Na$_2$SiO$_3$) or humic acid (50mg L^{-1}) [Data are expressed as the mean values ± standard errors (n= 3). Values in columns with different letters are significantly different at 5% level according to Duncan's Multiple Range Test].

Fig. 11. Effects of nano-CuO (mg. L^{-1}) stress on soluble proteins in safflower *(Carthamus tinctorius* L.) seedlings as influenced by silicon (1mM Na$_2$SiO$_3$) or humic acid (50mg L^{-1}) [Data are expressed as the mean values ± standard errors (n= 3). Values in columns with different letters are significantly different at 5% level according to Duncan's Multiple Range Test.]
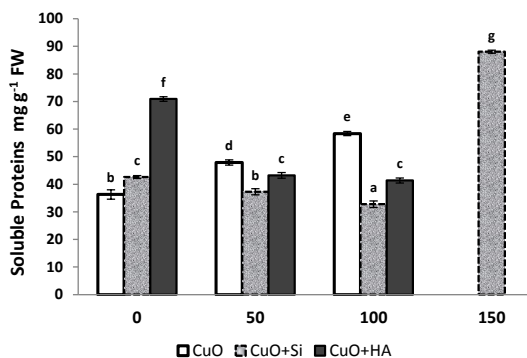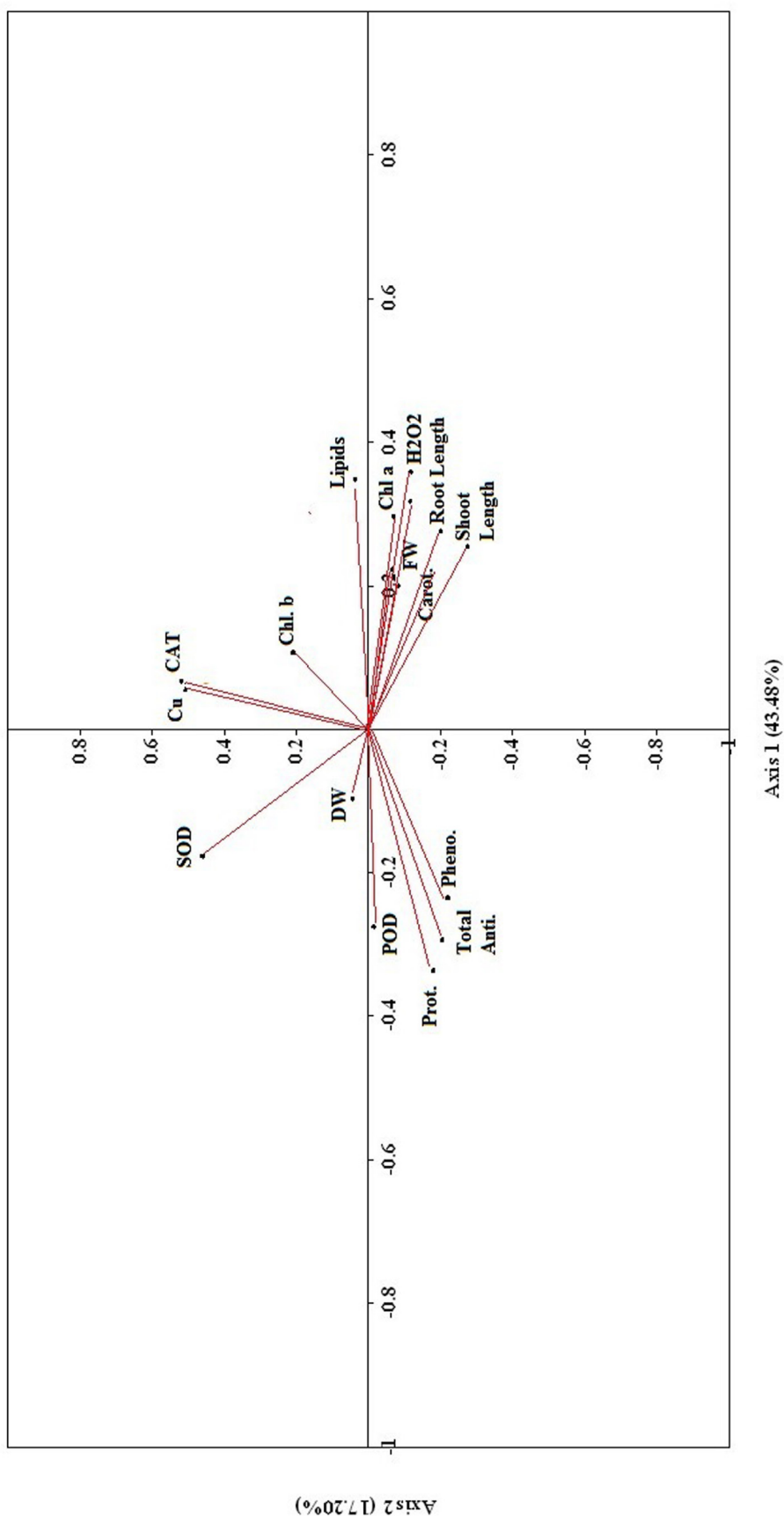
Fig. 12. Loading plot of different studied attributes correlations to the first two principle component analysis (PCA) axes [Abbreviations: Pheno.= Total Phenolics, Total-anti= Total Antioxidants, Prot.= Proteins, POD= Peroxidase, SOD= Superoxide dismutase, CAT= Catalase, Chl. a= Chlorophyll a, Chl. b= Chlorophyll b, Carot.= Carotenoids, Lipids= Lipid peroxidation inhibition, H_2O_2 %= Hydrogen peroxide scavenging %, DW= Dry weight, FW= Fresh weight].

Discussion

The accumulationfrom NPs of metals in soils severely retard agricultural production and the phytotoxicity of these metals NPs affects adversely crop growth (Asli & Neumann, 2009 and Atha et al., 2012). Furthermore, this contamination not only impact plant growth, but could pose a route for contamination of the food chainas plants primary producers and a key point-of-entry of NPs for other living organisms (Dimkpa et al., 2012 and De la Rosa et al., 2017). In the present study, 150mg L^{-1} was the phytotoxic concentrations of CuO NPs to safflower plant which completely inhibited seedling growth, while they can withstand till 100mg L^{-1}. External amendment with 1mM sodium silicate (Na$_2$SiO$_3$) improved seedling grow that the phytotoxic concentration (150mg L^{-1} CuO NPs). On the other hand, the application of 50mg L^{-1} humic acid (HA) failed to sustain seed germination and seedling establishment under 150mg L^{-1} CuO NPs.

CuO NPs concentrations (50 and 100mg L^{-1}) affected differently on fresh and dry weights. Fresh weight showed significant change under CuO NPs treatments. The first level of CuO NPs (50mg L^{-1}) enhanced significantly the fresh matter gain, this could be attributed to the fact that lower concentration of Cu is essential for plant development. Being an essential micronutrient for plants, Cu at low concentration participates in photosynthetic electron transport, mitochondrial respiration, cell-wall metabolism, hormone signaling, protein trafficking and iron mobilization and significantly improves plant growth and development (Raven et al., 1999; Yruela, 2005, 2009 and Anjum et al., 2015 a). However, the higher level (100mg L^{-1}) reduced fresh weight significantly which could be attributed to lower rates of water uptake due to phytotoxicity of NPs as reported by Tassi et al. (2017). In addition, it was reported that nano-CuO mediated plant growth restriction and DNA damage in radish (*Raphanus sativus*) and ryegrass (*Lolium perenne* and *Lolium rigidum*) (Atha et al., 2012). On the other hand, dry matter unchanged under the different concentrations of CuO NPs. Amending safflower seedlings with silicon enhanced significantly fresh matter at 50 and 100mg L^{-1} CuO NPs and improved dry weight significantly at 100 and 150mg L^{-1} CuO NPs. Adrees et al. (2015) reported that Si may decrease metal toxicity by a dilution effect that increase the growth of plants and boosting the xylem sap under metal stress. On the other hand, the stimulatory effect of HA observed as the increase in dry weight at 100mg L^{-1} CuO NPs. The bio-stimulation of humic substances to plant growth was previously documented (Nardi et al., 2009; Rose et al., 2014 and Canellas & Olivares, 2014).

By 8 days of safflower seedlings development, root growth was more impacted by the phytotoxicity of CuO NPs than shoot growth. NPs has previously been reported to reduce root length (Adams et al., 2017). Shi et al. (2014) and Stampoulis et al. (2009) reported that Cu nanoparticles reduced root length, compared to the control. Nair & Chung (2014) recorded drastic roots-growth retardation under 400-500mg L^{-1} CuO NPs in soybean. In previous experiment on the nano-CuO on *Hordeum vulgare*, a restriction in root and shoot growth with decreased photosynthetic performance index were recorded (Shaw et al., 2014).

External application of HA enhanced root growth at 100mg L^{-1}. Such improvement could not be observed in shoot growth. While, Si amendment did not show any significant improvement to shoot and root growth under CuO NPs phytotoxicity. As described by Bandieraa et al. (2009), application of HA resulted in marked modification of root morphology, such as weight, length, diameter, specific root length. Humic stimuli root growth promotion (acid growth theory) according to Rayle & Clealand (1992). The acid growth theory revealed the possible impact of humic substances (HS) on plant signaling via Ca^{2+} waves. This model is directly linked to nutrient uptake, lateral root emergence and root hairs. Such process can alter the redox potential and pH at the root surface, through promotion of secondary transport and over expression of ion transporters (Ramos et al., 2015).

The highest Cu contents among all treatments were found in the safflower seedlings amended with HA. Exposure of safflower to 50 and 100mg L^{-1} CuO NPs plus HA was found to be by at least 5-fold and 10-fold higher than control, respectively. This high content of Cu could be connected to high root length induced under HA application. This suggests a higher number of cells available to accumulate Cu. Root tissues also is considered the most direct contact with Cu upon sand treatment application. Hence, buildup of Cu

is more likely to occur within the root system and this is may be due to the high binding ability of Cu to cell wall compounds (Apodaca et al., 2017). In addition, formation of soluble HS complexes with micronutrients, i.e., metal–humic complexes, is often reported as a strategy to prevent metals from leaching and become more bioavailable to plants (Halim et al., 2003 amd García-Mina et al., 2004). However, 20–30µg Cu g^{-1} leaf dry weight was considered as acritical toxicity level of Cu for most crop species (Yruela, 2005, 2009 and Anjum et al., 2015 b). External application of Si sustained Cu^{+2} at the lowest levels even at elevating concentrations of external CuO NPs. This low content of Cu could be connected to safflower seedling growth induction under Si application at 150mg L^{-1}CuO NPs.

Adams et al. (2017) reported the negative effect of NPs in lowering content of photosynthetic pigments and reducing photosynthesis. Furthermore, it has been widely reported that CuO NPs can induce chlorosis (Dimkpa et al., 2012; Trujillo-Reyes et al., 2014 and Nair & Chung, 2015). The present data showed insignificant change in Chl. a content combined with significant raise in Chl. b and carotenoids at 50mg L^{-1}CuO NPs. while higher concentration of CuO NPs (100mg L^{-1}), reduced Chl. a content by about 60%, as compared to control. Another significant reduction under this concentration was detected in carotenoids content (by about 50%) which was combined with significant raise in chl.b content. In this connection, Gadallah & El-Enany (1999) reported lowered total chlorophyll content in lupin (*Lupinus* spp.) plants exposed to Cu up to 50mg L^{-1}

The reduction in chlorophyll content, in the present results, could be attributed to dampened protochlorophyllide reductase activity, that contributes to chlorophyll synthesis and enhanced activity of chlorophyllase, a chlorophyll degrading enzyme under stress conditions, caused by Cu toxicity (Eser & Aydemir, 2016 and Apodaca et al., 2017). Cu^{2+} could hinder chloroplast reactions by restricting electron transport, consequently alter an essential factor of the energy-transfer mechanism (Uribe & Stark, 1982). External application of Si or HA sustained Chl. a content unchanged under 100mg L^{-1} as compared to relative treatment without Si or HA. The application of Si obviously enhanced Chl. b and carotenoids under 50mg L^{-1}, increasing CuO NPs concentration to

100mg L^{-1} enhanced significantly carotenoids content and reduced Chl. b content. The effect of Cu toxicity at 150mg L^{-1} was found to induce significantly Chl. b content while it reduced significantly Chl. a and carotenoids contents. On the other hands, HA increased significantly Chl. b at 50 and 100mg L^{-1} CuO NPs, while the carotenoid content was significantly reduced at 50mg L^{-1} then, unexpectedly it increased at 100mg L^{-1} CuO NPs. In this regard, Calabrese & Blain (2011) reported the biological phenomenon of hormesis (the resulting U-shaped dose-response curves), where opposite effects are seen in low doses of a toxin (inhibition) compared to high doses (stimulation). This biphasic response is not well understood, but has received considerable interest within toxicological studies (Apodaca et al., 2017) which could be used to explain these results. Nano-CuO can mediate significant elevations in ROS generation and its subsequent consequences of the modulation of antioxidant defense system components and cellular redox homeostasis in plants (Shaw et al., 2014). Plant antioxidant defense system comprises of enzymes (such as superoxide dismutase, SOD; catalase, CAT; guaiacol peroxidase POD) as well as non-enzymes (such as carotenoids, phenolics) (Anjum et al., 2015 a).

Carotenoids are proved to be potent quenchers of ROS, particularly singlet oxygen ($^1O_2$) by intercepting the triplet-chlorophyll (Young, 1991). They also act as light harvesting pigments. In the present investigation, 50mg L^{-1} CuO NPs treated seedlings at 8 days showed significant increase in carotenoids content. Whereas, 100mg L^{-1} nano-CuO NPs stressed seedlings exhibited significant decreases (50%) in plumule carotenoids contents. In contrast, 100mg L^{-1} nano-CuO with external amendment by HA or Si maintained high carotenoids level (Table 1), this could be involved in protecting chlorophylls and photosynthetic apparatus by quenching ROS, and hence, improving safflower seedling growth. Decreased carotenoids levels might render the nano-CuO exposed seedlings more vulnerable to stress (Shaw & Hossain, 2013).

Data of this study revealed significant increase in total phenolics with increasing CuO NPs concentration (Fig. 4). Amendment with HA provoked more phenolics content, as compared to Si application under 0, 50 and 100mg L^{-1} CuO NPs. While Si amendment at 150mg L^{-1} CuO

NPs enhanced greatly total phenolic content. Kim et al. (2007) reported that phenolic compounds in safflower may play a role as protective phytochemical antioxidants against reactive oxygen-mediated pathological diseases. Phenolic compounds (secondary metabolites) play multiple roles in plants, including scavenging of ROS induced under different stresses, they promote roles in plant protection against damaging effects (Solecka, 1997 and Sonar et al., 2011).

Statistical evaluation by principle component analysis (PCA) showed that total antioxidants content exhibited trend close to that of the total phenolics. This indicates that phenolic compounds might be a major contributor to the antioxidant capacities under Cu toxicity. A high correlation between the total phenolic content and antioxidant activity has been previously reported (Wang et al., 2009).

In the present study, nano-CuO phytotoxicity resulted in deleterious effect on the ability of safflower cells to scavenge H_2O_2 and to inhibit peroxidation of membranes lipids as shown in Fig. 6 and 7. Shaw et al. (2014) reported that nano-CuO extremely disrupt the plant defense system through triggering oxidative burst in terms of elevated levels of H_2O_2 and malondialdehyde (MDA), the indicators of ROS and membrane lipid peroxidation (LPO), respectively. Song et al. (2016) reported that the MDA content of *Lemna minor* increased with the increase of CuO NPs in culture media. An increased rate of lipid peroxidation has also been reported from plants and other biological models under CuO NPs stress (Wang et al., 2012 and Melegari et al., 2013). Data of the present study revealed negative impact of CuO NPs stress on lipid peroxidation inhibition % and H_2O_2 radical scavenging % at elevated concentration of CuO NPs, this could be proposed according to Singh et al. (2017 a) who reported that plants might produce more free radicals under the NPs stress and the activities of antioxidants were not stimulated to the optimum level, thus the damage caused by free radicals was not counteracted, leading to the production of more MDA content which triggers the lipid peroxidation or damage of membrane in the seedlings. Nevertheless, external application of Si or HA enhanced significantly lipid peroxidation inhibition % and H_2O_2 radical scavenging % as compared to corresponding treatments without Si or HA. Application of HA or Si could protect the cell from ROS generation and could modulate

the antioxidant defense system components and cellular redox homeostasis in plants under nano-CuO phytotoxicity.

Within plant cell, SOD acts as the first line of defense against oxidative stress as its activity directly modulates the amount of O_2^- and H_2O_2, the two important Haber–Weiss reaction substrates (Bowler et al., 1992 and Shaw & Hossain, 2013). In the present investigation, the nano-CuO phytotoxicity mediated increase in SOD activity and external application of Si and HA reflected significant enhancement of SOD enzyme as treated with elevating concentrations of CuO NPs. Previous studies revealed that decreased membrane LPO may accompany with increases in SODs that dismutate O_2^- into H_2O_2 (Nekrasova et al., 2011 and Kim et al., 2012).

The present study showed that nano-CuO phytotoxicity (50 and 100mg L^{-1}) induced significant change in POD activity, which is in agreement with some previous reports on POD activity in *Lemna minor* by Song et al. (2016) and Koce (2017). In rice seedlings, nano-CuO treatment led to an increase in activity of antioxidant enzymes and elevated MDA concentration (Shaw & Hossain, 2013). Amending stressed safflower seedlings with HA at 50mg L^{-1} showed significant reduction in POD activity, while higher level, 100mg L^{-1}, showed no change in POD activity. On the other hands, Si application enhanced significantly POD activity at all applied nano-CuO levels 50, 100 and 150mg L^{-1}.

Catalase (CAT) is the main H_2O_2 decomposer enzyme at peroxisome (Scandalios, 2005). Data herein showed that activity of CAT was retarded significantly under 50 and 100mg L^{-1} nano-CuO phytotoxicity. Similar results were reported in rice, alfalfa roots and green pea (Ye et al., 2014; Hong et al., 2015 and Ochoa, 2017). Amendment with HA enhanced CAT activity at 50 and 100mg L^{-1}, while with Si application at 50mg L^{-1}, the CAT activity was reduced, then enhanced significantly at 100mg L^{-1}, while it did not change with 150mg L^{-1}.

Data revealed an increase in soluble proteins under CuO NPs applied solely or in combination with HA. While, external application of Si retarded soluble proteins content at 50 and 100mg L^{-1}. Adversely, higher nano-CuO (150mg L^{-1}) enhanced greatly soluble proteins content. To

survive under stress, plants accumulate proteins that protect cells from stress effects (Wang et al., 2003). The diverse environmental stresses often promote similar cell signaling pathways (Zhu, 2001) and cellular responses, such as the production of stress proteins and up regulation of antioxidants (Vierling & Kimpel, 1992 and Zhu et al., 1997). Previous proteomic study conducted on the roots of *Eruca sativa* under Ag NPs and AgNO$_3$ stress showed that both nanoparticle and bulk metal can cause alteration in the forms of proteins linked with redox regulation as well as disturbance of cellular homeostasis (Vannini et al. 2013 and Singh et al., 2017 b).

Conclusion

Safflower (*Carthamus tinctorius*) seedlings used to evaluate the potential impact of Si or HA in counteracting phytoxicity of CuO NPs. Seedling growth in *C. tinctorius* were coherent with physiological changes implying that plants exposed to CuO were severely stressed, while plants exposed to Si followed by those exposed to HA, were stressed to a lesser extent. This response could be attributed to the recorded activation in the enzymatic and non-enzymatic antioxidant system provoked due to amendment with Si or HA.

References

Adams, J., Wright, M., Wagner, H., Valiente, J., Britt, D. and Anderson, A. (2017) Cu from dissolution of CuO nanoparticles signals changes in root morphology. *Plant Physiol. Bioch*. **110**, 108-117.

Adrees, M., Ali, S., Rizwan, M., Zia-ur-Rehman, M., Ibrahim, M., Abbas, F., Farid, M., Qayyum, M.F. and Irshad, M.K. (2015) Mechanisms of silicon-mediated alleviation of heavy metal toxicity in plants: a review. *Ecotoxicol. Environ. Saf*. **119**, 186-197.

Aebi, H. (1984) Catalase. In:"*Methods in Enzymology*", L. Paker (Ed.), p. 121. Academic Press, Orlando, Fl., USA.

Ahmed, A., Hasnain, A., Akhtar, S., Hussain, A., Abaid-ullah, Yasin G., Wahid, A. and Mahmood, S. (2010) Antioxidant enzymes as bio-markers for copper tolerance in safflower (*Carthamus tinctorius* L.). *Afr. J. Biotechnol*. **9**(33), 5441-5444.

Anjum, N.A., Adam, V., Kizek, R., Duarte, A.C., Pereira, E., Iqbal, M., Lukatkin, A.S. and Ahmad, I. (2015a) Nano scale copper in the soil–plant system – toxicity and underlying potential mechanisms. *Environ. Res.* **138**, 306-325.

Anjum, N.A., Singh, H.P., Khan, M.I.R., Masood, A. and Per, T.S., et al. (2015 b) Too much is bad—an appraisal of phytotoxicity of elevated plant-beneficial heavy metal ions. *Environ. Sci. Pollut. Res*. **22**, 3361-3382.

Apodaca, S.A., Tan, W., Dominguez, O.E., Hernandez-Viezcas, J.A., Peralta-Videa, J.R. and Gardea-Torresdey, J.L. (2017) Physiological and biochemical effects of nanoparticulate copper, bulk copper, copper chloride, and kinetin in kidney bean (*Phaseolus vulgaris*) plants. *Sci. Total Environ.* **599**, 2085-2094.

Asli, S., and Neumann, P.M. (2009) Colloidal suspensions of clay or titanium dioxide nanoparticles can inhibit leaf growth and transpiration via physical effects on root water transport. *Plant Cell Environ.* **32**, 577-584.

Atha, D.H., Wang, H., Petersen, E.J., Cleveland, D., Holbrook, R.D., Jaruga, P., Dizdaroglu, M., Xing, B. and Nelson, B.C. (2012) Copper oxide nanoparticle mediated DNA damage in terrestrial plant models. *Environ. Sci. Technol.* **46**, 1819-1827.

Bandieraa, M., Moscaa, G. and Vamerali, T. (2009) Humic acids affect root characteristics of fodder radish (*Raphanus sativus* L. var. *oleiformis* Pers.) in metal-polluted wastes. *Desalination*, **246**, 78-91.

Bar-Tal, A., Landau, S., Li-xin, Z., Markovitz, T., Keinan, M., Dvash, L., Brener, S. and Weinberg, Z.G. (2008) Fodder quality of safflower across an irrigation gradient and with varied nitrogen rates. *Agron. J.* **100**, 1499-1505.

Beauchamp, B.C. Fedovich (1976) Superoxide dismutase assay and an assay applicable to acrylamide gel. *Anal. Biochem.* **10**, 276-287.

Bowler, C., Montagu, T.V. and Inze, D. (1992) Superoxide dismutase and stress tolerance. *Annu. Rev. Plant. Biol.* **43**, 83-116.

Calabrese, E.J. and Blain, R.B. (2011) The hormesis database: The occurrence of hormetic dose responses in the toxicological literature. *Regul. Toxicol. Pharmacol.* **61**, 73-81.

Canellas, L.P. and Olivares, F.L. (2014) Physiological responses to humic substances as plant growth promoter. *Chem. Biol. Technol. Agric*. **1**, 1-11.

Çulha, Ş. and Çakırlar, H. (2011) Effect of salt stress induced by NaCl on safflower (*Carthamus tinctorius* L.) cultivars at early seedling stages. *Hacettepe J. Biol. Chem.* **39**, 61-64.

De la Rosa, G., Garcia-Castaneda, C., Vazquez-Nunez, E., Alonso-Castro, A.J., Basurto-Islas, G., Mendoza, A., Cruz-Jimenez, G. and Molina, C. (2017) Physiological and biochemical response of plants to engineered NMs: Implications and future design. *Plant Physiol. Bioch.* **110**, 226-235.

Dimkpa, C.O., McLean, J.E., Latta, D.E., Manangón, E., Britt, D.W., Johnson, W.P., Boyanov, M.I. and Anderson, A.J. (2012) CuO and ZnO nanoparticles: Phytotoxicity, metal speciation, and induction of oxidative stress in sand-grown wheat. *J. Nanopart. Res.* **14**, 1125-1139.

Du, W., Tan, W., Peralta-Videa, J.R., Gardea-Torresdey, J.L., Ji, R., Yin, Y. and Guo, H. (2017) Interaction of metal oxide nanoparticles with higher terrestrial plants: Physiological and biochemical aspects. *Plant Physiol. Biochem.* **110**, 210-225.

Elisa, B., Marsano, F., Cavaletto, M. and Berta, G. (2007) Copper stress in *Cannabis sativa* roots: Morphological and proteomic analysis. *Caryologia.* **60**, 96-101.

Epstein, E. and Bloom, A.J. (2005) "*Mineral Nutrition of Plants: Principles and Perspectives*", 2nd ed. Sinauer, Sunderland, MA.

Eser, A. and Aydemir, T. (2016) The effect of kinetin on wheat seedlings exposed to boron. *Plant Physiol. Biochem.* **108**, 158-164.

Etesami, H. and Jeong, B.R. (2018) Silicon (Si): Review and future prospects on the action mechanisms in alleviating biotic and abiotic stresses in plants. *Ecotox. Environ. Safe*, **147**, 881-896.

Gadallah, M.A.A. and El-Enany, A.E. (1999) Role of kinetin in alleviation of copper and zinc toxicity in *Lupinustermis* plants. *Plant Growth Regul.* **29**, 151-160.

García-Mina, J.M., Antolin, M.C. and Sanchez-Diaz, M. (2004) Metal–humic complexes and plant micronutrient uptake: A study based on different plant species cultivated in diverse soil types. *Plant Soil.* **258**, 57-68.

Halim, M. Conte, P. and Piccolo, A. (2003) Potential availability of heavy metals to phytoextraction from contaminated soils induced by exogenous humic substances. *Chemosphere*, **52**, 265-275.

Hammer, O., Harper, D.A.T. and Ryan, P.D. (2001) PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontologia Electronica*, **4**, 4-9.

Han, S.Y., Li, H.X., Bai, C.C., Wang, L. and Tu, P.F. (2010) Component analysis and free radical-scavenging potential of *Panax notoginseng* and *Carthamus tinctorius* extracts. *Chem. Biodivers.* **7**, 383-391.

Hofrichter, M. and Steinbeuchel, A. (2001) "*Biopolymers*", Vol. 1, Lignin, Humic Substances and Coal, Wiley Europe-VCH, Weinheim, New York (Ed.).

Hong, J., Rico, C., Zhao, L., Adeleye, A.S., Keller, A.A., Peralta-Videa, J.R. and Gardea-Torresdey, J.L. (2015) Toxicity effects of seven cu nanoparticles/ compounds to lettuce (*Lactuca sativa*) and alfalfa (*Medicago sativa*). *Environ. Sci.* **17**, 177-185.

Janero, D.R. (1990) Malondialdehyde and thiobarbituric acid reactivity as diagnostic indices of lipid peoxidation and peroxidative tissue injury. *Free Rad. Biol. Med.* **9**, 513-540.

Johnston, A.M., Tanaka, D.L., Miller, P.R., Brandt, S.A., Nielsen, D.C. and Lafond, G.P., et al. (2002**)** Oilseed crops for semiarid cropping systems in the Northern Great Plains. *Agron. J.* **94**, 231-240.

Kim, E.O., Oh, J.H., Lee, S.K., Lee, J.Y. and Choi, S.W. (2007) Antioxidant properties and quantification of phenolic compounds from safflower (*Carthamus tinctorius* L.) seeds. *Food Sci. Biotechnol.* **16**, 71-77.

Kim, S., Lee, S. and Lee, I. (2012) Alteration of phytotoxicity and oxidant stress potential by metal oxide nano particles in *Cucumis sativus*. *Water Air. Soil Pollut.* **223**, 2799-2806.

Knight, S. (2007) Safflower: potential & world adaptability. I*REC Farmers´ Newsletter,* 176, 34.

Koce, J.D. (2017) Effects of exposure to nano and bulk sized TiO_2 and CuO in *Lemna Minor*. *Plant Physiol. Biochem*. **119**, 43-49.

Kott, R.W., Hatfield, P.G., Bergmanb, J.W., Flynn, C.R., Van Wagoner, H. and Boles, J.A. (2003) Feedlot performance, carcass composition and muscle and fat CLA concentrations of lambs fed diets supplemented with safflower seeds. *Small Ruminant Research,* **49**, 11-17.

Lagier, T., Feuillade, G. and Matejka, G. (2000) Interactions between copper and organic macromolecules: Determination of conditional complexation constants. *Agronomie*, **20**, 537-546.

Li, M., Zhu, L. and Lin, D. (2011) Toxicity of ZnO nanoparticles to Escherichia coli: Mechanism and the influence of medium components. *Environ. Sci. Technol.* **45**, 1977-1983.

Liang, Y., Nikolic, M., Bélanger, R., Gong, H. and Song, A. (2015) "*Silicon in Agriculture: From Theory to Practice*". Springer.

Lichtenthaler, H.K. (1987) Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. In: "*Methods in Enzymology*", Academic Press, Orlando, Fl., USA. Vol. 148, 350p.

Lin, D., Ji, J., Long, Z., Yang, K. and Wu, F. (2012) The influence of dissolved and surface-bound humic acid on the toxicity of TiO_2 nanoparticles to *Chlorella* sp. *Water Res*. **46**, 4477-4487.

Long, L.H., Evans, P.J. and Halliwell, B. (1999) Hydrogen peroxide in human urine: Implications for antioxidant defense and redox regulation. *Biochem. Biophys. Res. Commun*. **262**, 605-609.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) Protein measurement with Folin phenol reagent. *J. Biol. Chem*. **193**, 265-275.

MacCarthy, P. (2001) The principles of humic substances. *Soil Sci*. **166**,738-751.

Meena, V., Dotaniya, M., Coumar, V., Rajendiran, S., Kundu, S. and Rao, A.S. (2014) A case for silicon fertilization to improve crop yields in tropical soils. Proc. *Natl. Acad. Sci., India Sect. B: Biol. Sci.* **84**, 505-518.

Melegari, S.P., Perreault, F., Popovic, R.H.R.C. and Radovan Matias, W.G. (2013) Evaluation of toxicity and oxidative stress induced by copper oxide nanoparticles in the green alga *Chlamydomonas reinhardtii*. *Aquat. Toxicol*. **142**, 431-440.

Nair, P.M.G. and Chung, I.M. (2014) A mechanistic study on the toxic effect of copper oxide nanoparticles in soybean (*Glycine max* L.) root development and lignification of root cells. *Biol. Trace. Elem Res.* **162**, 342-352.

Nair, P.M.G. and Chung, I.M. (2015) The responses of germinating seedlings of green peas to copper oxide nanoparticles. *Biol. Plant,* **59**, 591-595.

Nardi, S., Carletti, P., Pizzeghello, D. and Muscolo, A. (2009) Biological activities of humic substances. In: "*Biophysico-Chemical Processes Involving Natural Nonliving Organic Matter in Environmental Systems*". Part I. Fundamentals and Impact of Mineral-Organic-Biota Interactions on the Formation, Transformation, Turnover, and Storage of Natural NonlivingOrganic Matter (NOM) Senesi, N., Xing, B. and Huang, P.M. (Eds.), pp. 305-339. John Wiley & Sons, Hoboken.

Nekrasova, G., Ushakova, O., Ermakov, A., Uimin, M. and Byzov, I. (2011) Effects of copper (II) ions and copper oxide nano particles on *Elodea densa* Planch. *Russ. J. Ecol.* **42**, 458-463.

Ochoa, L., Illya, A., Medina-Velo, I.A., Barrios, A.C., Bonilla-Bird, N.J., Hernandez-Viezcas, J.A., Jose, R., Peralta-Videa, J.R. and Gardea-Torresdey, J.L. (2017) Modulation of CuO nanoparticles toxicity to green pea (*Pisum sativum* Fabaceae) by the phytohormone indole-3-acetic acid. *Sci. Total Environ.* **598**, 513-524.

Peralta-Videa, J.R., Huang, Y., Parsons, J.G., Zhao, L., Lopez-Moreno, M.L., Hernandez Viezcas, J.A. and Gardea-Torresdey, J.L. (2016) Is the green synthesis of engineered nanomaterials a realistic alternative to chemical synthesis? A review of the factors affecting their mass production and applications. *Nanotechnol. Environ. Eng.* **1**, 4.

Prieto, P., Pineda, M., and Aguilar, M. (1999) Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Anal. Biochem.* **269**, 337-341.

Ramos, A.C., Olivares, F.L., Silva, L.S., Aguiar, N.O. and Canellas, L.P. (2015) Humic matter elicits proton and calcium fluxes and signaling dependent on Ca²⁺ dependent protein kinase (CDPK) at early stages of lateral plant root development. *Chem. Biol. Technol. Agric*. **2**, 4.

Raven, J.A., Evans, M.C. and Korb, R.E. (1999) The role of trace metals in photosynthetic electron transport in O₂-evolving organisms. *Photosynth. Res*. **60**, 111-150.

Rayle, D.L. and Cleland, R.E. (1992) The acid growth theory of auxin-induced cell elongation is alive and well. *Plant Physiol*. **99**, 1271-1274.

Remédios, C., Rosario, F. and Bastos, V. (2012) Environmental nanoparticles interactions with plants: Morphological, physiological and genotoxic aspects. *J. Bot*. **2012**.

Rose, M.T., Patti, A.F., Little, K.R. and Brown, A.L. (2014) A meta-analysis and review of plant-growth response to humic substances: practical implications for agriculture. *Adv. Agron*. **124**, 37-89.

Sawhney, B.L. and Frink, C.R. (1991) Heavy metals and their leachability in incinerator ash. *Water Air Soil Poll*. **57**, 289-296.

Scandalios, J. (2005) Oxidative stress: Molecular perception and transduction of signals triggering antioxidant gene defenses. *Braz. J. Med. Biol. Res*. **38**, 995–1014.

Shaw, A.K. and Hossain, Z. (2013**)** Impact of nano-CuO stress on rice (*Oryza sativa* L.) seedlings. *Chemosphere,* **93**, 906-915.

Shaw, A.K., Ghosh, S., Kalaji, H.M., Bosa, K., Brestic, M., Zivcak, M. and Hossain, Z. (2014) Nano CuO stress induced modulation of antioxidative defense and photosynthetic performance of syrian barley (*Hordeum vulgare* L.). *Environ. Exp. Bot*. **102**, 37-47.

Shi, J., Peng, C., Yang, Y., Yang, J., Zhang, H., Yuan, X., Chen, Y. and Hu, T. (2014) Phytotoxity and accumulation of copper oxide nanoparticles to the Cu-tolerant plant *Elsholtzia splendens*. *Nanotoxicol*. **8**, 179-188.

Singh, A., Singh, N.B., Hussain, I. and Singh, H. (2017 a) Effect of biologically synthesized copper oxide nanoparticles on metabolism and antioxidant activity to the crop plants *Solanum lycopersicum* and *Brassica oleracea* var. botrytis. *J. Biotechnol*. **262**, 11-27.

Singh, S., Vishwakarma, K., Singh, S., Sharma, S., Dubey, N.K., Singh, V.K., Liu, S., Tripathi, D.K. and Chauhan, D.K. (2017 b) Understanding the plant and nanoparticle interface at transcriptomic and proteomic level: A concentric overview. *Plant Gene*, **11**, 265-272.

Singleton, V.L. and Rossi, J.A. (1965) Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *Ame. J. Enol. Viticult*. **16**, 144-158.

Solecka, D. (1997) Role of phenyl propanoid compounds in plant responses to different stress factor. *Acta Physiol. Plant*. **19**, 257-268.

Sonar, B.A., Nivas, M.D., Gaikwad, D.K. and Chavan, P.D. (2011) Assessment of salinity-induced antioxidative defense system in *Colubrina asiatica* Brong. *J. Stress Physiol. Biochem*. **7**, 193-200.

Song, G., Hou, W., Gao, Y., Wang, Y., Lin, L., Zhang, Z., Niu, Q., Ma, R., Mu, L. and Wang, H. (2016) Effects of CuO nanoparticles on *Lemna minor*. *Bot. Stud*. **57**, 3.

Stampoulis, D., Sinha, S.K. and White, J.C. (2009) Assay-dependent phytotoxicity of nanoparticles to plants. *Environ. Sci. Technol*. **43**, 9473-9479.

Sudo, E., Itouga, M., Yoshida-Hatanaka, K., Ono, Y. and Sakakibara, H. (2008) Gene expression and sensitivity in response to copper stress in rice leaves. *J. Exp. Bot*. **59**, 3465-3474.

Tassi, E., Giorgetti, L., Morelli, E., Peralta-Videa, J.R., Gardea-Torresdey, J.L. and Barbafieri, M. (2017) Physiological and biochemical responses of sunflower (*Helianthus annuus* L.) exposed to nano CeO₂ and excess boron: Modulation of boron phytotoxicity. *Plant Physiol. Bioch*. **110**, 50-58.

Tatiana, Z., Yamashita, K. and Matsumoto, H. (1999) Iron deficiency induced changes in ascorbate content and enzyme activities related to ascorbate metabolism in cucumber roots. *Plant Cell Physiol*. **40**, 273-280.

Thounaojam, T.C., Panda, P., Mazumdar, P., Kumar, D., Sharma, G.D., Sahoo, L. and Panda, S.K.

(2012) Excess copper induced oxidative stress and response of antioxidants in rice. *Plant Physiol. Biochem.* **53**, 33-39.

Tripathi, D.K., Shweta, Singh, S., Singh, S., Pandey, R., Singh, V.P., Sharma, N.C., Prasad, S.M., Dubey, N.K. and Chauhan, D.K. (2017) An overview on manufactured nanoparticles in plants: Uptake, translocation, accumulation and phytotoxicity. *Plant Physiol. Bioch*. **110**, 2-12.

Trujillo-Reyes, J., Majumdar, S., Botez, C.E., Peralta-Videa, J.R. and Gardea-Torresdey, J.L. (2014) Exposure studies of core-shell Fe/Fe(3)O(4) and cu/CuO NPs to lettuce (*Lactuca sativa*) plants: Are they a potential physiological and nutritional hazard?. *J. Hazard. Mater.* **267**, 255-263.

Uribe, E.G. and Stark, B. (1982) Inhibition of photosynthetic energy conversion by cupric ion. *Plant Physiol*. **69**, 1040-1045.

Vannini, C., Domingo, G., Onelli, E., Prinsi, B., Marsoni, M., Espen, L. and Bracale, M. (2013) Morphological and proteomic responses of *Eruca sativa* exposed to silvernanoparticles or silver nitrate. *PLoS One.* **8**, e68752.

Vierling, E. and Kimpel, J.A. (1992) Plant responses to environmental stress. *Curr. Opin Biotechnol*. **3**, 164-170.

Wang, T., Jonsdottir, R. and Ólafsdóttir, G. (2009) Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds. *Food Chem.* **116**, 240-248.

Wang, W., Vinocur, B. and Altman, A. (2003) Plant responses to drought, salinity and extreme temperatures: Toward genetic engineering for stress tolerance. *Planta*, **218**,1-14.

Wang, Z.Y., Li, J., Zhao, J. and Xing, B.S. (2011) Toxicity and internalization of CuO nanoparticles to prokaryotic alga *Microcystis aeruginosa*as affected by dissolved organic matter. *Environ. Sci. Technol*. **45**, 6032-6040.

Wang, Z., Xie, X., Zhao, J., Liu, X., Feng, W., White, J.C. and Xing, B. (2012) Xylem and phloem based transport of CuO nanoparticles in maize (*Zea mays* L.). *Environ. Sci. Technol*. **46**, 4434-4441.

Ye, N., Li, H., Zhu, G., Liu, Y., Liu, R., Xu,W., Jing, Y., Peng, X. and Zhang, J. (2014) Copper suppresses abscisic acid catabolism and catalase activity, and inhibits seed germination of rice. *Plant Cell Physiol*. **55**, 2006-2016.

Young, A.J. (1991) The protective role of caroteniods in higher plants. *Physiol. Plant.* **83**, 702-708.

Yruela, I. (2005) Copper in plants. *Braz. J. Plant Physiol*. **17**,145-156.

Yruela, I. (2009) Copper in plants: acquisition, transport and interactions. *Func. Plant Biol.* **36**, 409-430.

Zhang, H., Lian, C. and Shen, Z. (2009) Proteomic identification of small, copperresponsive proteins in germinating embryos of *Oryza sativa*. *Ann. Bot*. **103**, 923-930.

Zhu, J.K. (2001) Cell signaling under salt, water and cold stresses. *Curr. Opp. Plant Biol*. **4**, 401-406.

Zhu, J.K., Hasegawa, P.M. and Bressan, R.A. (1997) Molecular aspects of osmotic stress in plants. *Critical Rev. Plant Sci*. **16**, 253-277.

تقييم مدي فعالية تحسين نمو نبات القرطم (*Carthamus tinctorius*) بالسيليكون أو حامض الهيوميك أثناء الإنبات تحت سمية اكسيد النحاس النانوى (CuO NPs)

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تعتبر جسيمات أكسيد النحاس النانوية CuO NPs من بين الجسيمات النانوية الأكثر استخدامًا، ومن المتوقع أن تتواجد بتركيزات مرتفعة في جميع البيئات. تهدف الدراسة الحالية إلى تقييم التأثير الذي يمكن ان يلعبه عنصر السيليكون (بأستخدام سيليكات الصوديوم بتركيزا ملل مول) أو حمض الهيوميك HA (بتركيز 50 ملليجرام/لتر) في تحسين الإجهاد الناتج من سمية عنصر النحاس النانوية (بتركيزات 50، 100، و150 مليجرام/لتر) على نمو بادرات نبات القرطم (*Carthamus tinctorius*). أظهرت الدراسة قدرة عنصر السيليكون على تحسين إنبات بذور القرطم تحت تركيز (150 ملليجرام/لتر) من أكسيد النحاس النانوي. ويمكن أن يعزي ذلك إلى قدرة السيليكون على تخفيض معدل دخول النحاس بشكل ملحوظ. من ناحية أخرى، أدت إضافة حمض الهيوميك إلى زيادة محتوي بادرات القرطم من عنصر النحاس بنحو 5 إلى 10 أضعاف تركيزه بالكنترول تحت تركيز (50 و100 ملليجرام/لترأكسيد نحاس). تسبب التركيز (100 ملليجرام /لترأكسيد نحاس) بإنخفاض معنوى في قيم الوزن الطازج. بينما حسن عنصر السيليكون من قيم الوزن الطازج والجاف بصورة معنوية تحت تركيز 100 و150 ملليجرام/لتر اكسيد نحاس نانوي، كما ساهم حمض الهيوميك في تحسين قيم الوزن الجاف تحت تركيز 100 ملليجرام/لتر أكسيد نحاس معنويا. تسببت سمية النحاس النانوى عند تركيز (50 ملليجرام/لتر) في أنخفاض معنوي لمحتوى النبات من الخضوب النباتية وعلى وجه الخصوص (كلوروفيل أ والكاروتينيدات) والتي إنخفضت بمقدار 60% و 50% على التوالى، عند المقارنة مع الكنترول. لوحظ التأثير الأيجابي للسيليكون وحمض الهيوميك في المحافظة على معطيات قيم الكلوروفيل أ بدون انخفاضتحت تركيز(100 ملليجرام/لتر أكسيد نحاس). كما لوحظ ارتفاعا معنويا في قيم الكاروتينيدات عند المعاملة بالسيليكون أو حمض الهيوميك تحت تركيز (100 ملليجرام/لتر اكسيد نحاس). أدت سمية النحاس النانوي لزيادة محتوى بادرات القرطم من الفينولات بصورة معنوية. وقد ساهمت إضافة حمض الهيوميك تحت تركيز (50 و100 ملليجرام/لتر أكسيد نحاس) والسيليكون تحت تركيز (150 ملليجرام/لتر أكسيد نحاس) في تعزيز هذه الزيادة. كما أدت سمية النحاس النانوي إلى إضعاف قدرة الخلية على كبح الشقوق الحرة مثل (فوق أكسيد الهيدروجين) وزيادة معدل الأكسدة للأغشية البلازمية. بينما حافظت معاملة السيليكون أو حمض الهيوميك على مستويات أفضل من كبح الشقوق الحرة (فوق أكسيد الهيدروجين) وتقليل معدلات الأكسدة للأغشية البلازمية تحت ظروف ضغوط الأكسدة. حسنت معاملات السيليكون أو حمض الهيوميك من معدلات نشاط مضادات الأكسدة الإنزيمية متمثلة في انزيمي SOD , CAT . كما ساهمت معاملة السيليكون في تحسين نشاط انزيم POD تحت التركيزات المتزايدة من أكسيد النحاس النانو (50، 100 و150 ملليجرام/لتر).