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Ameliorative effect of Yeast Extract, IAA and Green-synthesized Nano Zinc Oxide on the Growth of Cu-stressed *Vicia faba* Seedlings

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THE EFFECT of $CuSO_4$ stress on some growth parameters and some metabolites in 14-day-old *Vicia faba* seedlings was studied. Irrigation with 150 mM $CuSO_4$ resulted in remarkable reduction in root length, shoot height, fresh and dry weights of root and shoot, leaf area and photosynthetic pigments (Chl a, Chl b and carotenoids), while it raised Chl a/b ratio. Total soluble carbohydrates and protein contents were significantly depleted under Cu treatment. The ameliorative role of seed priming with different concentrations of yeast extract (25, 50, or 100 %), IAA (0.1, 0.3, 0.5 mM), or the green-synthesized Nano zinc oxide with average size 61 (nm) prepared from leaf extract of *Coriandrum sativum* (10, 50, 100 ppm) for different periods (6 and10h for yeast and IAA, 3 and 6h for Nano ZnO were studied. Results revealed that seed priming with 50% yeast or 0.1 mM IAA for 10h showed a remarkable increase in the measured growth criteria, chla, chl b, soluble carbohydrates and proteins but diminished chl a/b ratio compared with the Cu stress treatment. Seed priming with 100 ppm Nano ZnO for 6 h exhibited the most effective treatment in mitigating the harmful effects of Cu stress on growth criteria, Chla, Chl b, soluble carbohydrates and proteins.

Keywords: Copper, IAA, Metabolites, Nano ZnO, *Vicia faba*,Yeast.

Introduction

Heavy metal contamination is one of the most deleterious stresses in the natural habitats, as they are not biodegradable and persist in the environment indefinitely (Gangwaret et al., 2014 and Kasim et al., 2014). Among heavy metals, copper is a redox-active micronutrient essential for normal growth and development of plants, acts as a structural element in regulatory proteins and participates in photosynthetic electron transport, mitochondrial respiration, oxidative stress responses, cell wall metabolism and hormone signaling (Yruela, 2005 and Emamverdian et al., 2015). At higher concentrations than that required for optimal growth, Cu is proved to be phytotoxic at morphological, physiological, biochemical and molecular levels. It was shown that it inhibits plant growth and interferes with many important cellular processes such as photosynthesis, respiration, electron transport, cell wall metabolism, nitrogen assimilation and senescence(Kasim, 2005; Sánchez-Pardo & Zornoza, 2014 and Adrees et al., 2015).

Hammad & Ali (2014) reported that application of yeast extract significantly alleviates drought stress by increasing the relative water content, photosynthetic pigments, total carbohydrates, total free amino acids, and enzyme activities in wheat plants. It was reported that the application of yeast extract enhances plant tolerance to heavy metal stress due to its contents of hormones, sugars, amino and nucleic acids, vitamins and minerals (Abdo et al., 2012).

Indole-3-acetic acid (IAA) as one of the signal hormones (auxins) was found not only to act as a plant growth regulator but also to play a crucial role in stress resistance, where it can increasethe growth of root and shoot of plants that were stressed by salinity or heavy metals indicating an alleviating capacity of the toxic impacts of stresses (Singh & Prasad, 2015 and Agami, 2016).

The application of nano-materials has been shown to cause both positive and negative effects in plants (Tripathi et al., 2015). Nano-iron oxide enhanced seedling growth and development and facilitated photosynthesis and iron transfer (Liu et al., 2005). Foliar application of ZnO nanoparticles

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at 1.5(mg/ml) on chickpea improved biomass production (Burman et al., 2013). The role of biologically synthesized ZnO nanoparticles using the marine green alga *Ulva lactucainthe* amelioration of Cd and Pb in plantshas been recently studied by Venkatachalam et al.(2016).

The major object of this study is to examine the effectiveness of priming the *Vicia faba* seeds with different concentrations of green-synthesized plant-based ZnO nanoparticles to choose the effective concentration in alleviating the phytotoxicity of Cu stress as compared with IAA as a chemical tool and yeast extract as a natural biostimulant.

Materials and Methods

The green-synthesized nano ZnO was engineered according to the method of Gnanasangeetha & Thambavani (2013). The size and morphology of the nano ZnO particles were examined by TEM(JEOL model JEM 100SXelectron microscope, Tanta University unit). *Coriandrum sativum* leaves were collected from local market, washed with distilled water, then grinded and filtered. The filtrate was utilized as bio-reducing agent for preparation of nanoparticls with zinc acetate dihydrate. The resultant nano powder was used to prepare different concentrations of nano ZnO solutions (10, 50, 100 ppm) in distilled water.

Yeast extract was prepared according to Hanafy et al. (2012), where10 g of dry pure yeast powder was activated with addition of carbon source, then filtered and completed to 1 L which was considered as a concentration of 100% extract and used to prepare 25 % and 50% yeast extract.IAA solution was prepared with concentrations of 0.1, 0.3 and 0.5 mM.

Growth conditions and treatments

Seeds of *Vicia faba* L. (cv. Giza 716) supplied by the Agricultural Research Center, Giza, Egypt, were selected for apparent uniformity of size and shape and then washed in distilled water. They were divided into four groups, the first group was soaked in distilled water for 10h as control, the second was divided into six subgroups, and each was primed by soaking either in 25 %, 50%, or 100% yeast extract for 6 or 10 h. The third group was also divided into six subgroups; each was soaked in 0.1, 0.3, 0.5 mM IAA for 6 or10 h. The fourth group was also divided into six subgroups; each was soaked either in 10, 50, 100 (ppm) of nano ZnO for 3or 6 h. Six of the primed seeds of every treatment were then sown in plastic pots (15 cm diameter and 10 cm depth)containing one kg of clay-sandy soil (2:1 w/w) and three pots were used as replica for each treatment. Seeds were irrigated every other day with tap water and left to grow in the green house for 7 days, before the Cu treatment, under normal environmental conditions (11h light and 13h dark at 28°C ±2 and 16°C ± 2, respectively with 62% relative humidity).The 7-day-old seedlings were irrigated once at 70 % capacity, with 150 mM $CuSO_4$ solution except the control treatment which was irrigated with water. The 14-day-old seedlings were collected for sampling.

Measurement of germination and physiological analysis

Seeds were considered germinated when there was a visible radicle. The germinated seeds were counted on day 7, and then the germination and inhibition percentages were calculated (Cayuela et al., 2007).

Inhibition %= [1-(number of germinated seeds/ total number of sowed seeds)] x 100

Growth criteria including root depth, shoot height were measured and fresh and dry masses of root and shoot, as well as the leaf area were determined. The photosynthetic pigments, chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoids were estimated in the leaves of the 14-day-old seedlings according to Arnon (1949) for chlorophylls and Horvath et al. (1972) for carotenoids. Extraction of total soluble carbohydrates and proteins of shoots was carried out according to the method described by Naguib et al. (1968). The phenol sulfuric acid method was used to estimate total soluble carbohydrates according to Dubois et al. (1956). Total soluble protein content was determined as described by Bradford (1976).

Statistical analysis

The results were statistically analyzed using one way Analysis of Variance (ANOVA) to determine the degree of significance for the obtained results by COSTAT statistical program.Significance level $P \leq 0.05$ was used.

Results

Characterization of green-synthesized nano ZnO

The morphology of the final product of green-synthesized nano ZnO revealed that it is a pale white powder and its characterization using Transmission Electron Microscope (TEM)showed that they are rod shaped nanoparticles with average size of 61 nm(Fig. 1).

Germination and inhibition percentages

Results in Table 1 showed that priming of *Vicia faba* seeds with different concentrations of yeast extract had no significant effect on both germination and inhibition percentages, where their percentages were close to that of the control (soaked inwater). Similarly, priming with different nano ZnO concentrations showed the same trend. However, among the IAA treatments, priming with its highest concentration (0.5 mM) for 10h severely affected the germination and the inhibition percentages comparable to those of the control.

Growth parameters

Irrigation with 150mM $CuSO_4$ caused a drastically detrimental effect on all growth parameters. It: (i) decreased root and shoot lengths by 16 % and 50 %, respectively (Fig. 2). (ii) diminished root and shoot fresh weights by 60 % and 40 %, respectively(Fig. 3),(iii) reduced root and shoot dry weights by 39 % and 38 %, respectively (Fig. 4) and (iv) reduced the leaf area by 50 % (Fig. 5); all effects are relative to the control.

The concentration and the period of priming with yeast extract affected the degree of alleviation of the harmful effect of Cu stress. Priming with 50 % yeast extract for 6h significantly increased fresh weight (Fig.3) and dry weight (Fig.4) of both root and shoot as well as leaf area (Fig.5), compared with their stressed counterparts, but the values were still lower than those of the control. By increasing the period of priming to 10h,the 50 % yeast extract was the most successful concentration in diminishing Cu toxic impacts than all other yeast treatments irrespective of the priming period. This concentration significantly increased root and shoot lengths (Fig.2) by 39 % and 71 %, respectively compared with the treatment of Cu stress, while their fresh weights increased by 126 % and 71 %, respectively than Cu treatment (Fig.3). Similarly, under the same treatment, the root and shoot dry weights (Fig.4) significantly increased than those in the Cu treatment by 47 % and 89 %, respectively, while leaf area (Fig.5) increased by 108 % than the stress treatment and its value became approximately comparable to those of the control.

Seed priming with 0.1 mM IAA for 10h was the most effective treatment in mitigating the toxic effects of Cu stress than the other two concentrations. Seed priming with this concentration for 10h: (i) increased root and shoot lengthsby 19 % and 51 %, respectively (Fig.2), and (ii)increased the fresh weights of roots and shoots of *Vicia faba* seedlings (Fig.3) as well as leaf area (Fig. 5) by 89 %, 31% and 55 %, respectively; relative to their unprimed Cu stressed counterparts. Moreover, dry weights of root and shoot of IAA-primed seedlings (Fig.4) increased by 47% and 56 %, respectively compared with stress treatment.

Seed priming with green-synthesized nano ZnO for 3h had nosignificant effect on the Cu-induced decline of all growth criteria. Priming for 6 h was more effective especially with increasing the concentration, where the data in Fig.2 manifested that priming with 100 ppm of nano ZnO for 6h significantly increased the lengths of both root and shoot by 36 % and 105 %, respectively, compared to Cu treatment and reached the values of the control. This treatment also increased both fresh and dry weights of shoot by 94 % (Fig. 3 and 4), while increased leaf area by 122%, compared to those of stressed samples (Fig.5). Moreover, under the same priming treatment, the fresh and dry weights of roots (Fig. 3 and 4) highly increased than the corresponding samples in the Cu stress treatment by 142 % and 71 %, respectively relative to the control.

Photosynthetic pigments

Exposure of *Vicia faba* seedlings to 150 mM $CuSO_4$ resulted in a general reduction of photosynthetic pigments. Compared with the control, the stress application reduced the contents of Chl a, and Chl b by 50 % (Fig. 6) and carotenoids by 52 % (Fig. 8). On the contrary, the Chl a/b ratio was highly increased (up to 40 %) by Cu stress relative to the control as shown in Fig.8. However, the total pigment contents were significantly decreased with stress treatment by 52 % comparedwith the control (Fig. 7).

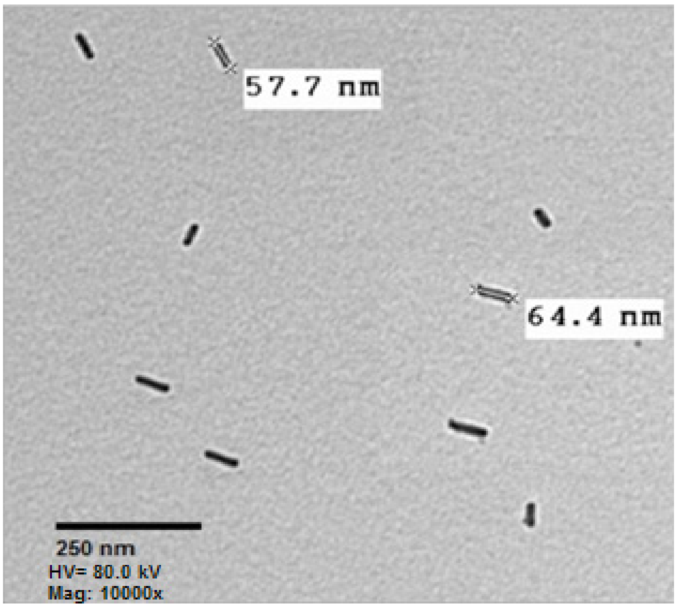

Fig. 1. Green synthesized Nano ZnO using leaf extract of *Coriandrum sativum* with average size of 61 nm.

TABLE 1. Effect of seed priming with yeast extract (Y1=25%, Y2=50%, Y3=100%); IAA (I1= 0.1, I2= 0.3, I3= 0.5 mM) or Nano ZnO (N1=10, N2= 50, N3= 100 ppm)on germination and inhibition percentages of 14-day-old *Vicia faba* seedlings. Values are means of three replicates ± SD. Different letters indicate statistically significant differences (*P*≤0.05).

Treatments	Germination %			Inhibition %		
	Priming period (h)			Priming period (h)		
	3	6	10	3	6	10
control			94±10 a			5.6±9.6 b
Y1		89±20 a	89±10 a		11.1±19 b	11.1±9.6 b
Y2		94±10 a	100±0 a		5.6±9.6 b	0.0±0.0 b
Y3		89±10 a	94±10 a		11.1±9.6 b	5.6±9.6 b
I1		94±10 a	100±0 a		5.6±9.6 b	0.0±0.0 b
I2		100±0 a	89±10 a		0.0±0.0 b	11.1±9.6 b
I3		82±6 a	59±6 b		22.2±9.6 b	44±9.6 a
N1	94±10 a	94±10 a		5.6±9.6 b	5.6±9.6 b	
N2	89±20 a	94±10 a		5.6±9.6 b	5.6±9.6 b	
N3	94±10 a	94±10 a		5.6±9.6 b	5.6±9.6 b	

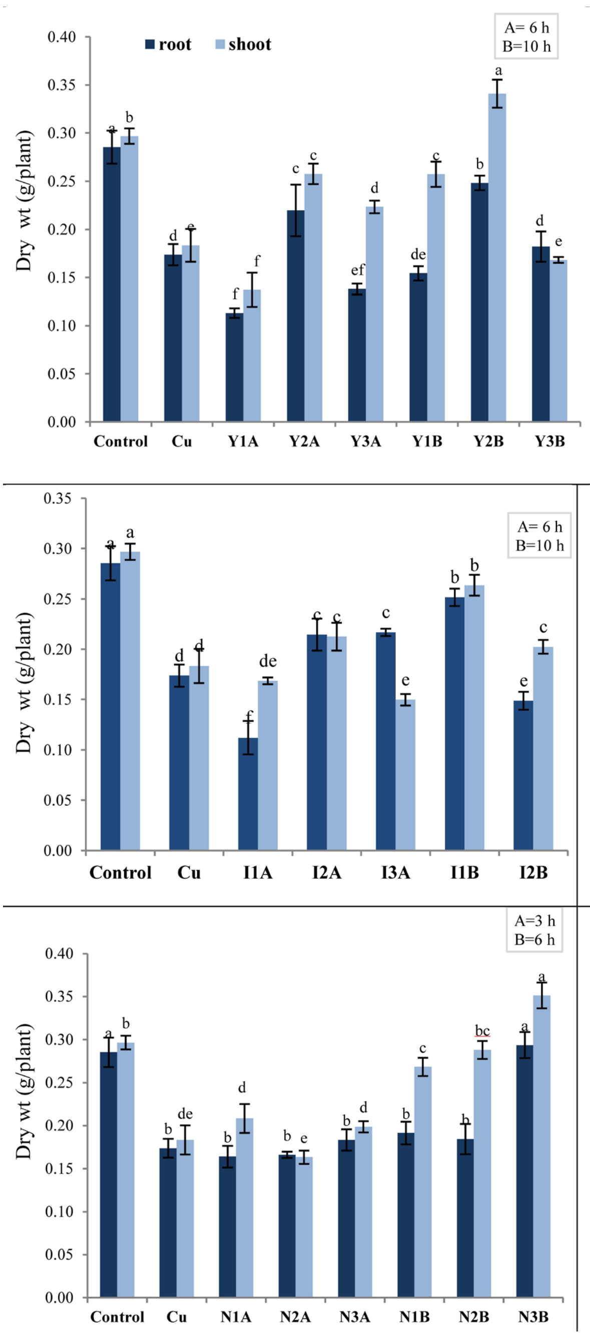

Fig. 2. Effect of seed priming with yeast extract (Y1=25%, Y2=50%, Y3=100%), IAA (I1= 0.1, I2= 0.3, I3= 0.5 mM), or Nano ZnO (N1=10, N2= 50, N3= 100 ppm) on root and shoot lengths of Cu-stressed 14-day-old *Vicia faba* seedlings. Values are means of three replicates ± SD. Different letters indicate statistically significant differences (*P*≤0.05).

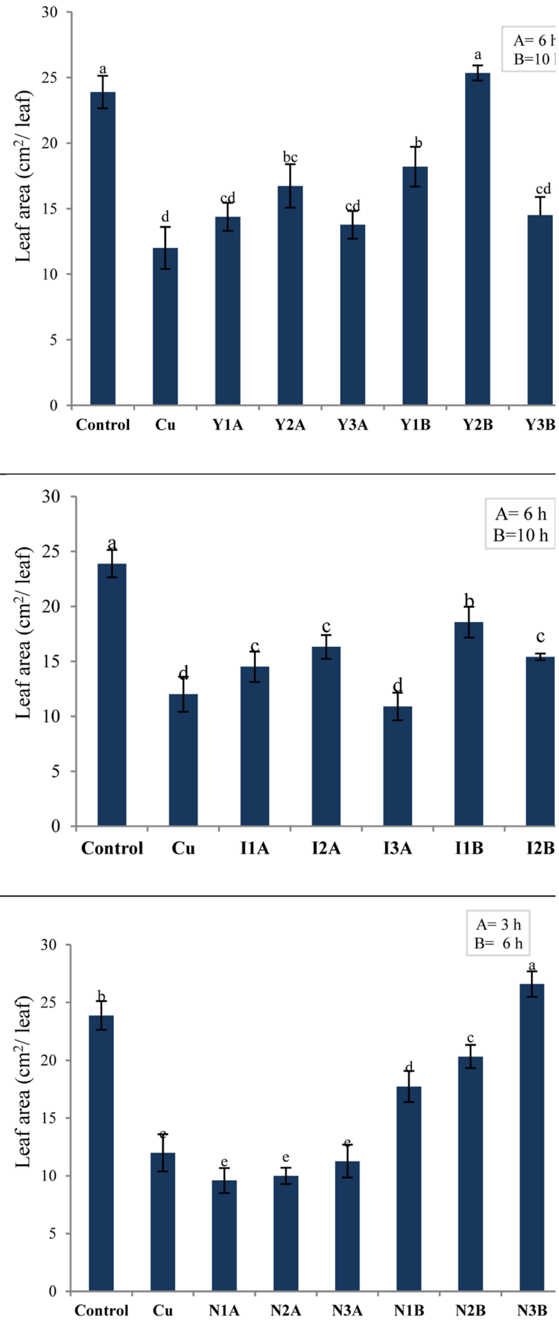

Fig. 3. Effect of seed priming with yeast extract (Y1=25%, Y2=50%, Y3=100%), IAA (I1= 0.1, I2= 0.3, I3= 0.5 mM), or Nano ZnO (N1=10, N2= 50, N3= 100 ppm) on fresh weight of Cu-stressed 14-dayold *Vicia faba* seedlings. Values are means of three replicates ± SD.Different letters indicate statistically significant differences (*P*≤0.05).

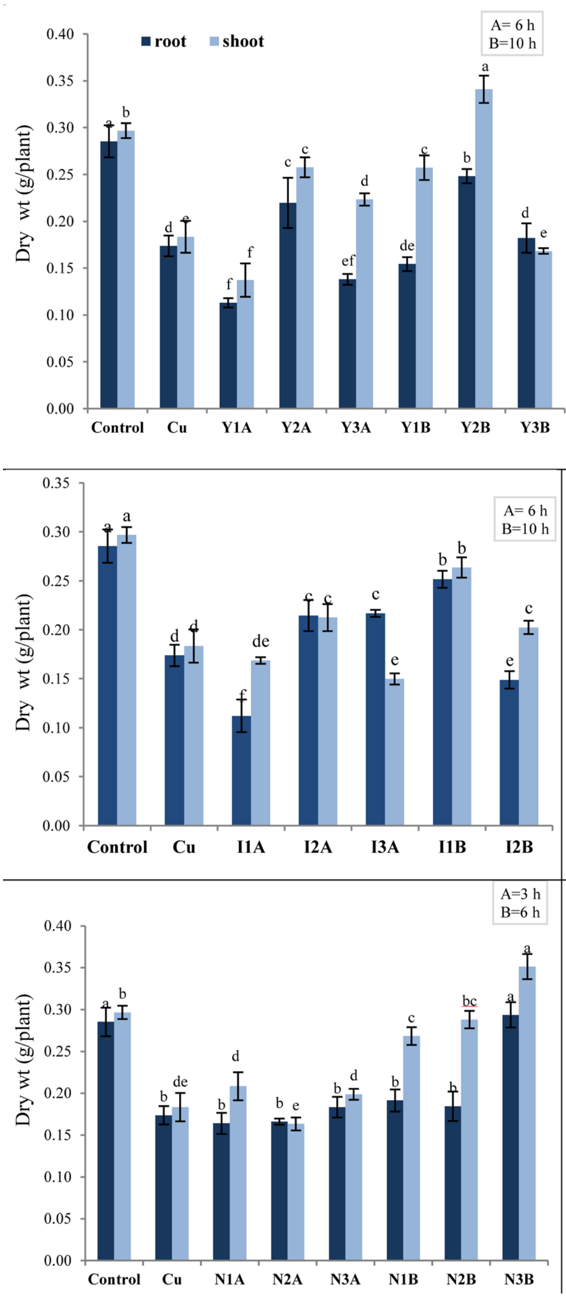

Fig. 4. Effect of seed priming with yeast extract (Y1=25%, Y2=50%, Y3=100%), IAA (I1= 0.1, I2= 0.3, I3= 0.5 mM), or Nano ZnO (N1=10, N2= 50, N3= 100 ppm) on dry weight of Cu-stressed 14-day-old *Vicia faba* seedlings. Values are means of three replicates ± SD. Different letters indicate statistically significant differences ($P \leq 0.05$).

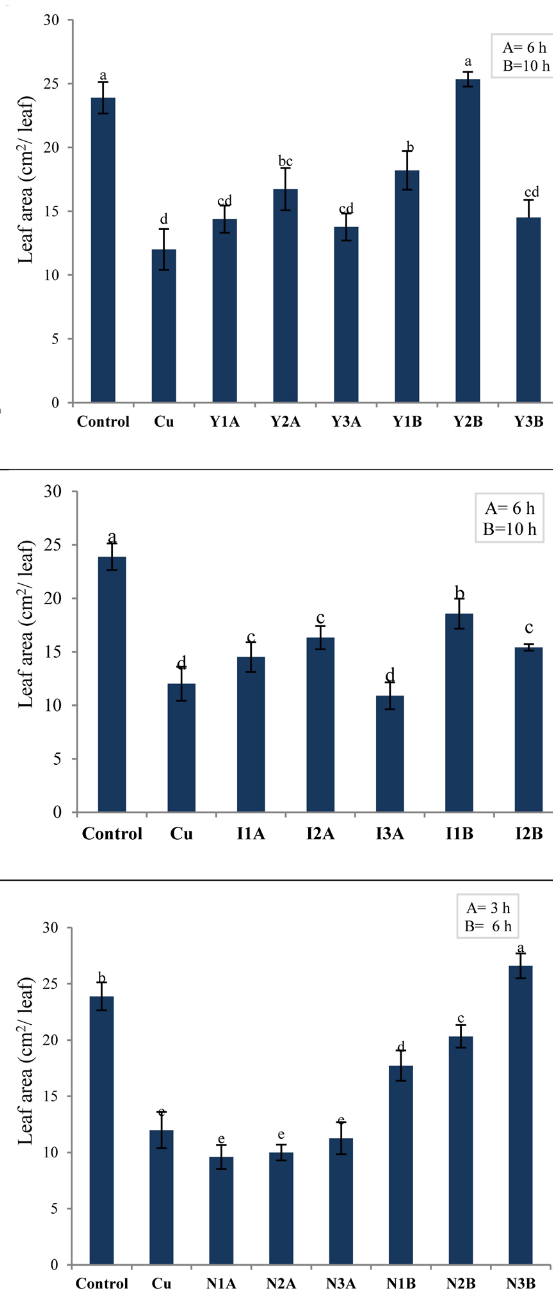

Fig. 5. Effect of seed priming with yeast extract (Y1=25%, Y2=50%, Y3=100%), IAA (I1= 0.1, I2= 0.3, I3= 0.5 mM), or Nano ZnO (N1=10, N2= 50, N3= 100 ppm) on leaf area of Cu-stressed 14-day-old *Vicia faba* seedlings. Values are means of three replicates ± SD. Different letters indicate statistically significant differences ($P \leq 0.05$).

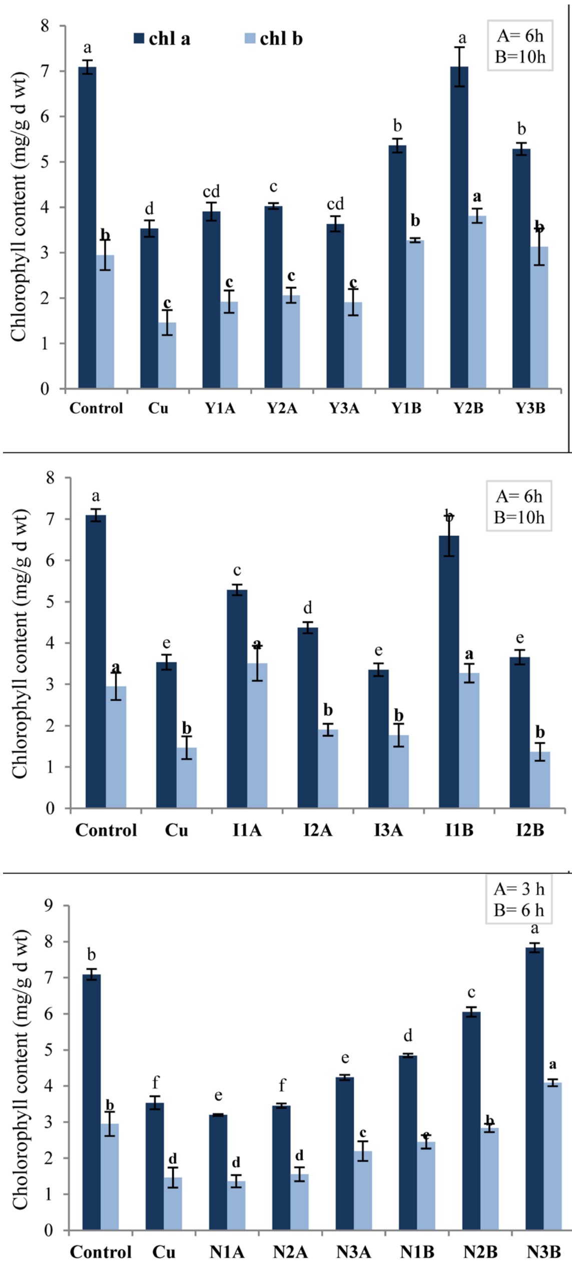

Fig . 6. Effect of seed priming with yeast extract (Y1=25%, Y2=50%, Y3=100%), IAA (I1= 0.1, I2= 0.3, I3= 0.5 mM), or Nano ZnO (N1=10, N2= 50, N3= 100 ppm) on Chla and Chl b of leaf of Cu-stressed 14-day-old *Vicia faba* seedlings. Values are means of three replicates ± SD. Different letters indicate statistically significant differences (*P*≤0.05).

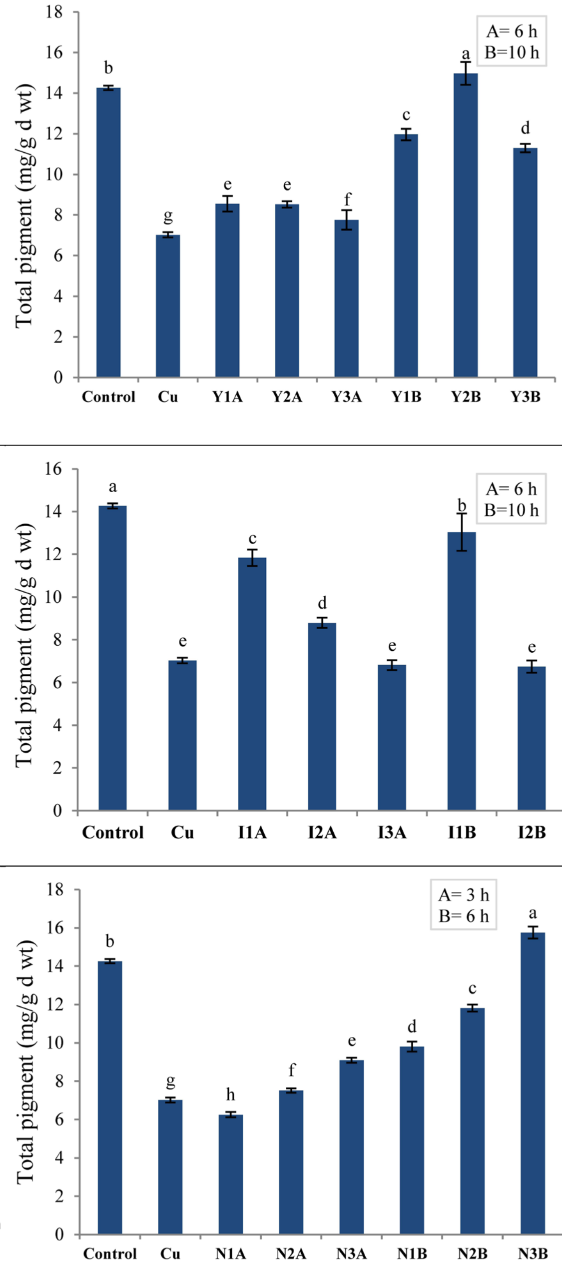

Fig. 7. Effect of seed priming with yeast extract (Y1=25%, Y2=50%, Y3=100%), IAA (I1= 0.1, I2= 0.3, I3= 0.5 mM), or Nano ZnO (N1=10, N2= 50, N3= 100 ppm) on total pigments content of leaf of Cu-stressed 14-day-old *Vicia faba* seedlings. Values are means of three replicates ± SD. Different letters indicate statistically significant differences (*P*≤0.05).

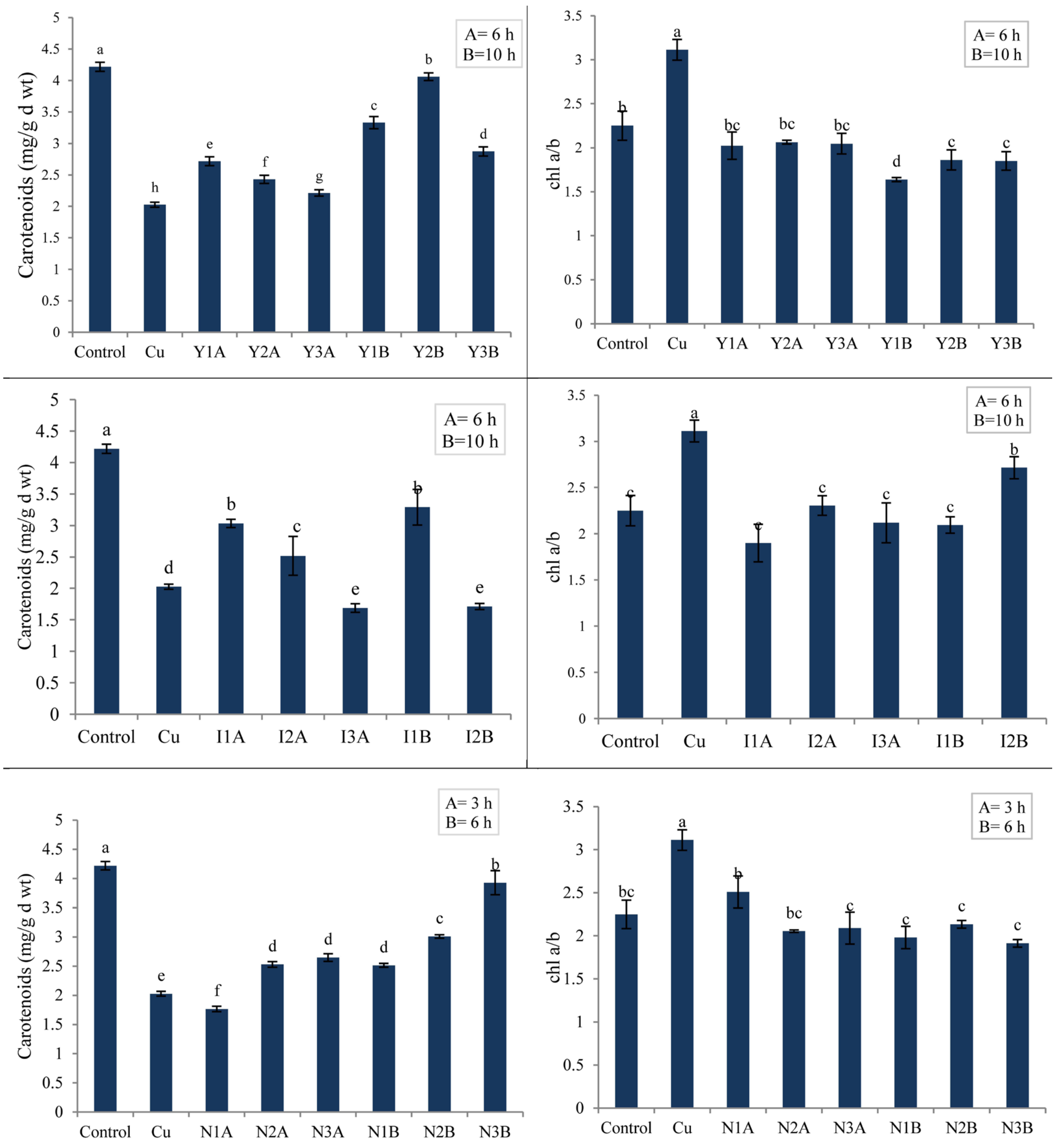

Fig 8. Effect of seed priming with yeast extract (Y1=25%, Y2=50%, Y3=100%), IAA (I1= 0.1, I2= 0.3, I3= 0.5 mM), or Nano ZnO (N1=10, N2= 50, N3= 100 ppm) on carotenoids content and chl a/b ratio of leaf of Cu-stressed14-day-old *Vicia faba* seedlings. Values are means of three replicates ± SD. Different letters indicate statistically significant differences (P≤0.05).

Furthermore, the results indicated that seed priming with any of the three concentrations of yeast extract for 6h had no effect on Chla or Chlb content compared with Cu treatment (Fig. 6). By increasing priming period to 10h, all the yeast extract concentrations significantly increased Chl a, Chl b, carotenoids and the total pigment contents, while they decreased Chl a/b ratio relative to the Cu treatment. However, the most pronounced effect was recorded in case of priming with 50% yeast extract for 10h, where it increased Chla and b (Fig. 6) and total pigments (Fig. 7) contents by 101%, 159 %, and 119%, respectively than stress treatment and their values nearly reached to those of the control. In addition, carotenoids were also increased by100% while Chl a/b ratiowas decreased by 42 %, relative to Cu treatment (Fig. 8).

The best concentration of IAA in alleviating the effect of Cu stress by increasing the photosynthetic pigment content was 0.1 mM for 6 or 10 h. Priming for 10h was more favorable than for 6h Chl a, chl b (Fig. 6), carotenoids (Fig. 8) and total pigments (Fig. 7) significantly increased by 87 %, 122 %, 65% and 85 %, respectively on priming with the lowest concentration of IAA (0.1 mM) for 10 h; while Chl a/b ratio was significantly reduced than Cu stress treatment to reach the control value (Fig. 8).

Regarding nano ZnO treatments, results showed that the increase of concentration and priming hours induced a gradual increase in photosynthetic pigments compared with the Cu stress treatment reaching the highest value on priming with 100ppm nano ZnO for 6h,where it significantly increased Chl a, Chl b (Fig. 6), carotenoids (Fig.8) and total pigments (Fig.7) by 123%, 173%, 95% and 126%, respectively relative to Cu treatment. However Chl a/b ratio was significantly reduced than that of Cu treatment and became near the control value (Fig.8).

Carbohydrates and proteins

Cu stress resulted in a severe decrease in each of the total soluble carbohydrates and proteins of shoots by 70% and 65%, respectively compared with the control (Fig. 9).

Seed priming with all yeast concentrations reversed the inhibitory effect of Cu stress and caused a significant increase in their carbohydrate and protein contents relative to the treatment of Cu stress. Comparison between the tested yeast concentrations revealed that priming with 50% yeast for 10h showed the highest ameliorative effect on the carbohydrate and protein contents (Fig. 9) with the percentage of increase 171% and 125%, respectively compared with Cu stress treatment but their values did not match those of the control.

Data represented in Fig. 9 showed that priming with the different concentrationsof IAA increased carbohydrate and protein contents of *Vicia faba* seedlings than those detected in Cu stress treatment. However, seed priming with 0.1 mMIAA for 10h caused the highest increase in carbohydrates and proteins by 284% and 210%, respectively,compared to Cu stress.

Priming of *Vicia faba* seeds with nano ZnO caused a significant increase in the carbohydrates and proteins compared with Cu treatment. The most effective concentration was 100 ppm nano ZnO when used for 6h which increased carbohydrate content of seedling shoot by 277% and the protein contentby 195%,compared with the stress treatment (Fig.9).

Discussion

Heavy metal stress by Cu not only affects the growth criteria of *Vicia faba*, but it also had an adverse effect on its metabolic components especially carbohydrates and proteins (Yruela, 2005 and Kasim, 2007). The data presented here showed that application of $CuSO_4$ resulted in a remarkable reduction in the lengths, fresh weight and dry weight of root and shoot, as well as leaf area of *Vicia faba* seedlings. These reductions might be attributed to an inhibition in cell division and cell elongation by Cu stress which is finally translated into impaired shoot and root growth (Agami, 2016).

The results indicated that Cu treatment reduced Chla and b, as well as carotenoids, compared with the control. Cu is known to inhibit some enzymes associated with the biosynthesis

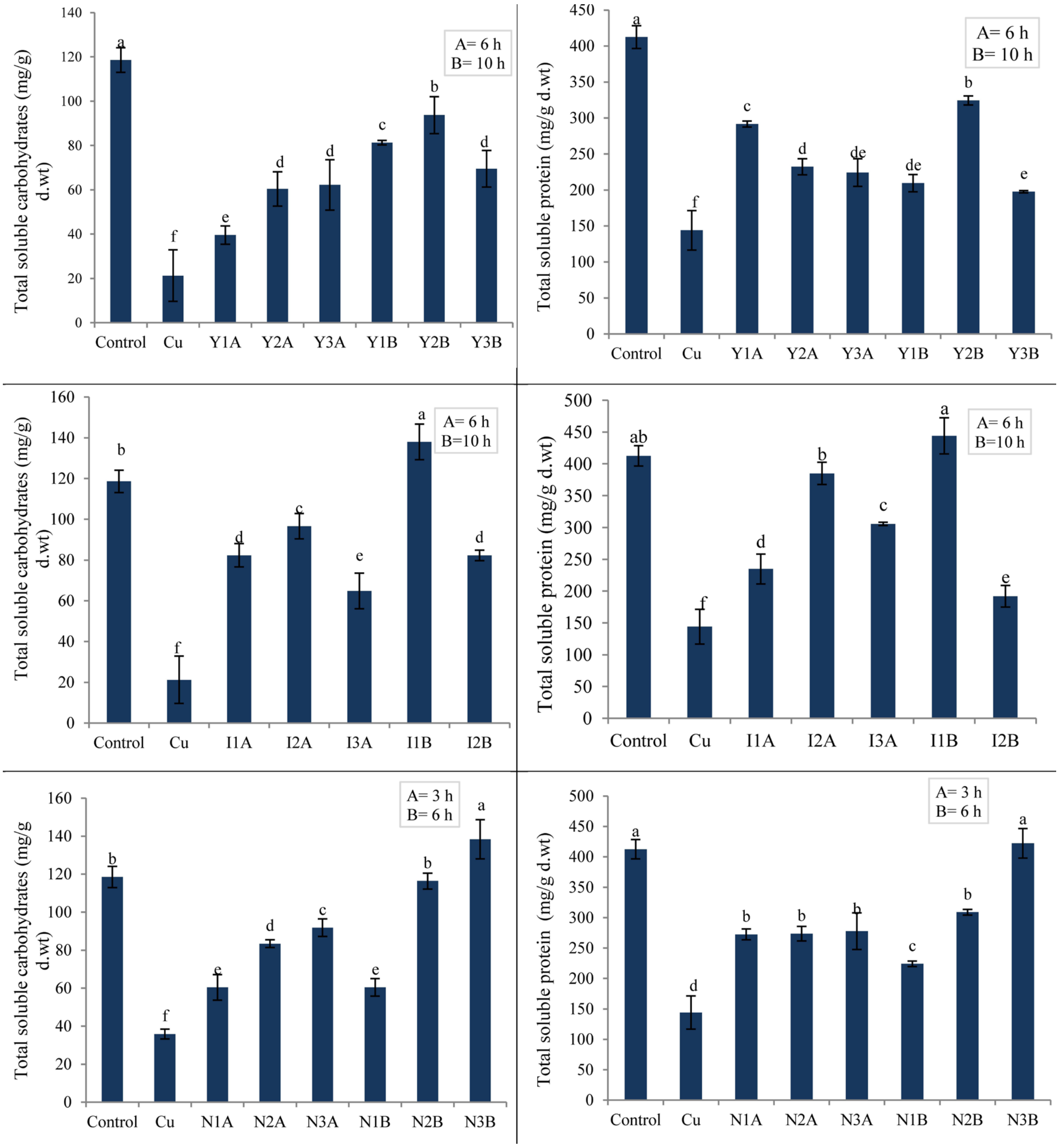

Fig 9. Effect of seed priming with yeast extract (Y1=25%, Y2=50%, Y3=100%), IAA (I1= 0.1, I2= 0.3, I3= 0.5 mM), or Nano ZnO (N1=10, N2= 50, N3= 100 ppm) on total soluble carbohydrates and proteins of shoots of Cu-stressed 14-dayold *Vicia faba* seedlings. Values are means of three replicates ± SD. Different letters indicate statistically significant differences (P≤0.05).

of the photosynthetic pigments (Yruela, 2009 and Chen et al., 2015). The recorded reduction of chlorophyll might be attributed to the Cu-induced Fe deficiency (Pätsikkä et al., 2002) or the substitution of the central Mg ion of chlorophyll by Cu (Küpper & Kroneck, 2005).The resultant increase of the Chl a/b ratio under Cu stress reflected higher reduction in Chl b, the main chlorophyll of photosystem II, which could be attributed to the disruption of the oxygen-evolving complex fluidity and composition (Yruela, 2005 and Kasim et al., 2014). Moreover, Cu toxicity causes destruction in acceptor and donor sides of photosystem II which is more sensitive to Cu than photosystem I (Yruela, 2009).

The presence of heavy metals alters carbohydrate accumulation and distribution in plants (Nazar et al., 2012).The present results indicated that Cu treatment decreased the total soluble carbohydrates in shoots of *Vicia faba* seedlings which is consistent with the findings of Azmat & Riaz (2012). The Cu-induced inhibition in carbohydrate content could be attributed to a reduction in CO_2 fixation that showed a direct relation with the Cu inhibited biosynthesis of chlorophyll (Azmat & Khan, 2011). It may also bedue to the stimulation in the activity of dark respiration causing great depletion in carbohydrate content under Cu toxicity as stated by Al-Hakimi & Hamada (2011). Cu application caused a significant decrease in soluble protein level which is in accordance with the results of Qi et al. (2006) and Yadav & Mohanpuria (2009). This reduction might be attributable to either reduced protein synthesis (Guzel & Terzi, 2013), enhanced protein hydrolysis (Kasim, 2005),or inhibition of nitrate reductase in the cytoplasm and nitrite reductase in the plastids (Lin et al., 2008 and Agami, 2016).

Priming of *Vicia faba* seeds with 50 % yeast extract for 10h has the most ameliorating effect of Cu stress, where it resulted in the increase of growth parameters, Chl a, Chl b and carotenoids. These results were in agreement with those of El-Gamal & Hammad (2003) and Abdo et al. (2012) using yeast extract to overcome Cd stress in tomato and soybean, respectively. This resultant positive effect of yeast on growth might be due to its high content of phytohormones (especially cytokinins), vitamins, enzymes, amino acids and minerals (Mahmoud, 2001 and Sakr et al., 2012) indicating stimulatory effects on cell division, cell elongation, protein and nucleic acid synthesis (Neseim et al., 2014). The increase in photosynthetic pigment could be attributed to the cytokininsin yeast which can delay the aging of leaves by reducing the degradation of chlorophyll and enhancing the protein and RNA synthesis (Agamy et al., 2013). In addition, such promotional effects of yeast extracts might be due to increasing the synthesis of α-amino levulinic acid, the precursor of chlorophyll biosynthesis (Abdo et al., 2012).

The resultant enhancement in total soluble carbohydrates and total soluble protein by priming with yeast treatments agreed with the results of El-Gamal & Hammad (2003) on tomato and Abdo et al. (2012) on soybean. However, this increase in total soluble carbohydrates is considered as a direct result for high rates of photosynthesis with great efficiency due to the increase in leaf area, which was manifested with high content of photosynthetic pigments (Agamy et al., 2013 and Hammad & Ali, 2014). The increase in total soluble protein may be due to direct stimulation of protein synthesis (Stino et al., 2009) and providing plants with essential minerals required for protein formation (Hayat, 2007 and Agamy et al., 2013). This enhancing effect of yeast might be due to its hormonal content that enhances the accumulation of soluble proteins (Khalil & Ismael, 2010 and Neseim et al., 2014).

IAA has a considerable potential to alleviate the harmful effects of Cu stress. Priming of seed with 0.5 mM IAA for 10h reduced the germination percentage with a significant increase in the inhibition percentage. Generally, auxins are reported to increase plant growth when applied at low concentrations and to inhibit growth at higher concentrations (Singh & Prasad, 2015). The concentration of 0.5 mM was considered as high concentration, because it had the ability to generate reactive oxygen species by exciting oxygen intermediates which in turn inhibit cell expansion and division occurring at the initiation of germination (Fässler et al., 2010; Mähönen et al., 2014 and Agami & Mohamed, 2013).

However, seed priming with 0.1 mM IAA for 10h markedly improved the growth parameters, photosynthetic pigments, total soluble carbohydrates and total soluble proteins in the Cu-stressed seedlings. The enhancement of growth criteria may be due that IAA inhibited Cu transport from roots to shoots as proposed by Agami & Mohamed (2013), or to its direct role as agrowth-promoting phytohormone in cell division and cell enlargement, leading to an increase in the fresh weight of shoots and roots and leaf area (Agami, 2016). The enhancement of photosynthetic pigments might be due to the retardation of pigment degradation and increasing activity of chlorophyll biosynthesis enzymes (Singh & Prasad, 2015). Meanwhile, the reduced Chl a/b ratio with IAA might be correlated to increasing photosystem II efficiency by restoring the functional and structural traits such as quantum yield, size and number of the photosynthetic centre and water splitting complex (Singh & Prasad, 2015). Moreover, the ameliorative effect of IAA on total soluble carbohydrates can be explained by inducing photosynthetic processes through pigments up regulation and increasing CO_2 fixation (Kaya et al., 2010 and Agami 2016). Furthermore, the increase in proteins may also be attributed to the role of IAA in improving the nitrogen metabolism(Gangwar et al., 2011), increasing nitrate reductase biosynthesis (Agami, 2016) and altering the expression of stress responsive genes (Jain & Khurana, 2009 and Singh & Prasad, 2015).

It is supposed that nanoparticles have a decreased size and larger specific surface area that rapidly absorbed and transferred, causing their interaction with cellular biomolecules and activation of various biochemical pathways (Dubchak et al., 2010 and Torabian et al., 2015). Seed priming with 100 ppm Nano ZnO for 6h mediated the alleviation of Cu toxicity by increasing growth criteria, Chl a, Chl b, carotenoids, total soluble carbohydrates and protein. Similar to these findings, Venkatachalam et al. (2016) reported that the plant growth tolerance index and biomass were enhanced by ZnO nanoparticle under Cd and Pb stress. Liu et al. (2015) showed that silicon nanoparticles alleviated the Pb phytotoxicity effects and enhanced rice seedlings growth and biomass. The recorded enhancement by priming with nanoparticles under Cu stress might be due to the enhancement of membrane stability, cell elongation and cell division by Zn, leading to an increase in fresh and dry weights (Cakmak, 2000 and Sedghi et al., 2013). Moreover, in the presence of Zn, the biosynthesis of hormones, especially auxins and gibberellins, increased since each is a constituent of an enzyme which influences the secretion of IAA (Cakmak, 2008). The recorded increase in Chl a, Chl b and carotenoids was in harmony with that found by Tripathi et al. (2015) in pea seedlings under Cr stress by silicon oxide nanoparticles. This enhancement may be due to that Zn plays an important role in chlorophyll synthesis (Corredor et al., 2009), chloroplast structure, photosynthetic electron transfer (Fathi et al., 2017). The recorded increase in total soluble carbohydrates and protein on priming with Nano ZnO was in accordance with the findings of Venkatachalam et al. (2016) using Nano ZnO under Cd or Pb stress in *Leucaena leucocephala* seedlings and with those of Soliman et al. (2015) using nano-iron and nano-zinc in salt stressed *Moringa peregrina* plants. This resultant increase might be due tothe involvement of Zn in the structure of various enzymes and transcriptional factors with important roles in protein and carbohydrate synthesis and regulation of nucleic acids and lipid metabolism (Bonnet et al., 2000 and Thounaojam et al., 2013).

In conclusion, the present results revealed that each of yeast extract, IAA, and green synthesized Nano-ZnO have a potential capacity to alleviate copper toxicity at specific concentrations and a specific priming period. The Cu-induced depletion of plant growth, photosynthetic pigments content as well as total soluble carbohydrate and protein contents were reversed with the effective treatments with Nano-ZnO (100 ppm, for 6h), yeast (50 %, for 10h) and IAA (0.1 mM, for 10h). Thus it seems reasonable to suggest that priming the seeds of *Vicia faba* with Nano zinc oxide is the most favorable, effective and economical method which can be used in the alleviation of the inhibitory effects of Cu stress.

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التأثير التخفيفى لمستخلص الخميرة و حمض إندول الخليك و نانو اكسيد الزنك المخلقة خضريا على نمو بادرات الفول المجهدة بالنحاس

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تمت دراسة تأثير إجهاد كبريتات النحاس على بعض مقاييس النمو وبعض المواد الأيضية فى بادرات الفول عمرها 14 يوم. وقد أدى الرى بمحلول كبريتات النحاس تركيزه 150 مللي مول إلى إنخفاض ملحوظ فى طول الجذر وارتفاع المجموع الخضرى والأوزان الرطبة والجافة لكل من الجذر والمجموع الخضرى ومساحة الورقة وكذلك أصباغ البناء الضوئى (كلورفيل أ وكلورفيل ب والكاروتينويدات)، بينما إزدادت نسبة كلورفيل أ/ب. وفى معاملة النحاس تناقص معنويا المحتوى الكلى للبروتينات والسكريات الذائبة. وقد تم دراسة الدور التحسينى لتحفيز البذور بالنقع فى تركيزات مختلفه من مستخلص الخميرة (25، 50، 100%) , و حمض إندول الخليك (0.1، 0.3، 0.5 مللي مول)، أو نانو أكسيد الزنك المصنع خضريا بمتوسط حجم 61 (نانوميتر) والمحضر من مستخلص أوراق الكزبرة (10 ,50 ,100 جزء من المليون) وذلك لفترات مختلفة (6 ،10 ساعات للخميرة; وحمض إندول الخليك و 3،6 ساعات لنانو اكسيد الزنك). وقد أوضحت النتائج أن تحفيز البذور باستخدام 50 % خميرة أو 0.1 مللي مول حمض إندول الخليك لمدة 10 ساعات أظهر زيادة ملحوظة فى مقاييس النمو المسجلة و كلورفيل أ وكلورفيل ب وكذلك البروتينات والسكريات الذائبة ولكن تضاءلت نسبة كلورفيل أ/ب مقارنة بمعاملة إجهاد النحاس. وقد أبدت معاملة تحفيز البذور بواسطة 100 جزء من المليون نانو أكسيد الزنك لمدة 6 ساعات الفاعلية القصوى فى تخفيف التأثيرات الضارة لإجهاد النحاس على مقاييس النمو وكلورفيل أ وكلورفيل ب وكذلك البروتينات والسكريات الذائبة.