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Effect of copper and zinc toxicity on physiological and biochemical parameters in Vigna mungo (L.) Hepper

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ABSTRACT

The present study aimed at investigating the effects of copper and zinc in *Vigna mungo* (L.) growth and metabolism. Physiological parameters decrease consequently with increasing copper and zinc concentration in nutrient medium. Copper at 0.2mM concentration led to 32.12% and 47.82% reduction in fresh weight and dry weight respectively whereas, zinc at 1.50mM concentration resulted in 23.83% and 70.56% decrease in fresh weight and dry weight of seedlings with respect to control. The combined effect of copper and zinc on seedling growth was antagonistic at low concentration but showed additive effect at high concentrations. The results demonstrated an increase in DNA content/mg fr. Wt. of seedlings up to 124.45% at 0.2mM Cu + 1.50mM Zn and RNA content was adversely affected in all treatment conditions. Moreover, seedlings exposed to copper and zinc metal ions have shown significant variations in phosphate hydrolysis and mobilization from source to sink and specific activity of acid phosphatase.

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KEYWORDS

Heavy metal stress, Seedling biomass, Nucleic acids, Phosphate metabolism.

INTRODUCTION

Anthropogenic activities associated with agricultural practices, industrial processing, mining activities, application of waste water for irrigation and solid waste management are the major contributors to heavy metal pollution of agricultural lands. The heavy metals have received special attention at the global level because of their adverse effects on plants growth and metabolism. Accumulation of metals and their toxic effects through food chain can lead to serious ecological and health problem (Malik 2004). Toxicity threshold of plants have been shown to be highly variable. A number of plants have exceptionally high ability to tolerate heavy metals whereas some are highly sensitive (Akinola and Ekiyoyo 2006). Although metals are required as structural and catalytic components of enzymatic proteins involved in various physiological processes but they can still be toxic to a plant if present at supraoptimal concentrations (Sharma et al. 2010). When heavy metals are accumulated in excess in plant tissues, these may cause alteration in various physiological processes such as transpiration, photosynthesis and photosynthetic electron transport, biosynthesis of chlorophyll as well as cell membrane integriety (Molina et al. 2008; Jayakumar and Jaleel 2009; Hussain et al. 2010). Heavy metal may chemically or physically interact with the natural compounds which change their form of existence in the environment. Heavy metal may be bound or sorbed by particular natural substances which may increase or decrease their mobility rate and the migration ability in the natural environment. The toxicity of heavy metals may arise as a result of the generation of reactive oxygen species that may cause wide-ranging damage to proteins, nucleic acids and lipids, eventually leading to cell death (Mittler 2002). In particular, copper and zinc in excess has been reported to have a negative effect on mineral nutrition and enzyme activities related to metabolism of plants (Kopittke and menzies 2006; Peng et al. 2009; Jain et al. 2010). But, the combined effect of copper and zinc may be quite different due to their interaction. To understand the fundamentals of plant growth under heavy metal stress is very important to meet the demands for food, fuel and fibre. Thus, the aim of the present study was to determine the effect of copper and zinc on growth, nucleic acids and phosphate metabolism in *Vigna mungo* (L.).

MATERIAL AND METHOD

Seeds of *Vigna mungo* (L.) Hepper Cv. T-9 (Black Gram) was obtained from National Seed Corporation Unit, I.A.R.I., New Delhi.

Growth Conditions - Healthy seeds of uniform size were sorted and sterilized with 0.1% $HgCl_2$ solution for 5 min. and washed with distilled water. Then, seeds were germinated in petriplates containing Whatman filter paper No. 1, moistened with Arnon and Hoagland media (Control). Copper metal was added to the nutrient solution at concentration 0.05, 0.1 and 0.2 mM as $CuSO_4.5H_2O$. Zinc metal was added as 0.25, 0.50, 1.00 and 1.50 mM of $ZnSO_4.7H_2O$. And, a mixture of both salts was added to study the interaction of both the metals. Various treatment conditions are mentioned below-

(1) Control **(2)** 0.05 mM $CuSO_4$ **(3)** 0.1 mM $CuSO_4$ **(4)** 0.2 mM $CuSO_4$ **(5)** 0.25 mM $ZnSO_4$ **(6)** 0.50 mM $ZnSO_4$ **(7)** 1.00 mM $ZnSO_4$ **(8)** 1.50 mM $ZnSO_4$ **(9)** 0.05 mM $CuSO_4$+ 0.25 mM$ZnSO_4$ **(10)** 0.05 mM $CuSO_4$+ 0.50 mM$ZnSO_4$ **(11)** 0.05 mM $CuSO_4$ + 1.00 mM$ZnSO_4$ **(12)** 0.05 mM $CuSO_4$ +1.50 mM$ZnSO_4$ **(13)** 0.1 mM$CuSO_4$ + 0.25mM $ZnSO_4$ **(14)** 0.1 mM$CuSO_4$ + 0.50mM $ZnSO_4$ **(15)** 0.1 mM$CuSO_4$ + 1.00mM $ZnSO_4$ **(16)** 0.1 mM$CuSO_4$ + 1.50mM

$ZnSO_4$ **(17)** 0.2 $mMCuSO_4$ + 0.25mM $ZnSO_4$ **(18)** 0.2 $mMCuSO_4$ + 0.50 mM $ZnSO_4$ **(19)** 0.2 $mMCuSO_4$ + 1.00 mM $ZnSO_4$ **(20)** 0.2 $mMCuSO_4$ + 1.50 mM $ZnSO_4$

Sterile conditions were maintained by adding 20µg/ml of streptomycin sulphate in the medium to suppress microbial growth. All experiments were carried out for 7 days at 28±2^{0}C in dark. At regular interval of time required number of seeds were withdrawn and used for analysis of various growth parameters. On the indicated days, seedlings were taken and washed thoroughly with Distilled water and then used.

Estimation of nucleic acids - Nucleic acids were extracted by the method given by Schneider (1957). DNA and RNA were estimated according to Burton (1968) and Schneider (1957) respectively.

Estimation of Inorganic phosphates and Acid phosphatase activity - Fiske and Subbaraw method (1925) was followed for the estimation of inorganic phosphates with slight modifications and Acid phosphatase activity was assayed according to the method of Johnson et al. (1973).

Statistical Analysis- Statistical analysis was done by using Microsoft excel and Graphpad prism 5.0 software. All the experiments were conducted in triplicates. The obtained data were statistically analysed for the mean ±S.D. and difference between the control and treated plants were analyzed by one way ANOVA taking $p \leq 0.05$ as significant level according to Dunnett's multiple comparison test.

RESULT AND DISCUSSION

Effect of copper and zinc metal ions on biomass production in Vigna mungo (L.)

The effect of copper and zinc metal ions on biomass produced has been expressed in terms of fresh weight and dry weight of the seedlings. Though seedling biomass has increased with time but there was a progressive decline in seedling biomass with increasing concentration of copper as well as zinc as shown in **Fig.-1 and 2**. On 7th day, copper reduced the fresh weight of seedlings by 14.50%, 19.17% and 32.12% and dry weight by 25.08%, 30.10% and 47.82% at the concentration of 0.05mM, 0.1mM and 0.2mM respectively. The effect of zinc was severe on seedling growth as it resulted in 10.36%, 20.72%, 22.27% and 23.83% decrease in fresh weight and 48.16%, 55.51%, 62.21% and 70.56% decrease in dry weight at 0.25mM, 0.50mM, 1.0mM and 1.50mM concentration. Minimum reduction in seedling biomass has been noticed at 0.05mM Cu + 0.25mM Zn treatment level. This treatment level has caused only 1.55% reduction in fresh weight and 20.40% reduction in dry weight. The combined treatment of 0.05mM Cu and 0.1mM Cu with 0.25mM Zn and 0.50mM Zn was antagonistic whereas further increase in concentration led to serious consequences including drastic reduction in seedling growth and biomass.

Growth is the best indices for evaluating plant response to any type of stress. Reduction in seedling biomass in response to various heavy metals has been reported by a number of researchers (Maheshwari and Dubey 2008; Al-Qurainy 2009; Luo et al. 2010). John et al. (2009) observed that the reduction in seedling growth during copper and/or zinc stress may be due to low water potential, hampered nutrient uptake and secondary stress such as oxidative stress. Reduced seedling growth under heavy metal treatment could be due to the reduction in meristematic cells present in this region. Barceló et al. (1986) suggested that during seedling growth hydrolysis of food reserves take place which is carried out by hydrolytic enzymes. If the activities of hydrolytic enzymes might have affected then, food do not reach to the embryonic axis leading to the reduction in seedling growth or it could be due to inhibition of cell division/ elongation or extension of the cell cycle.

Figure-1

Effect of copper and zinc metal ions on fresh weight of Vigna mungo (L.) seedlings.

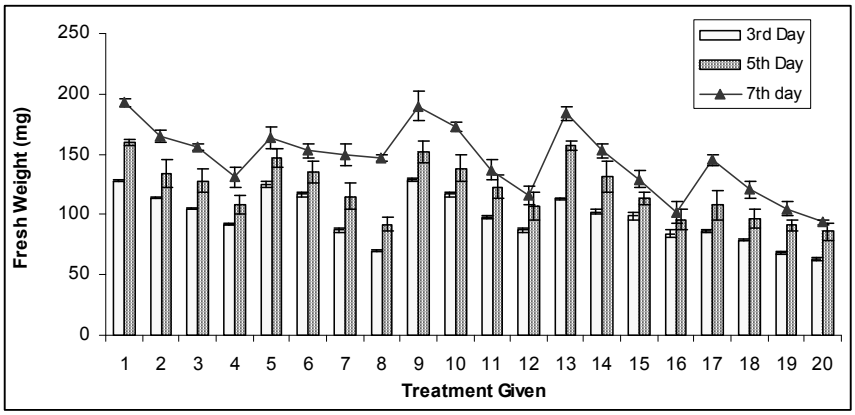
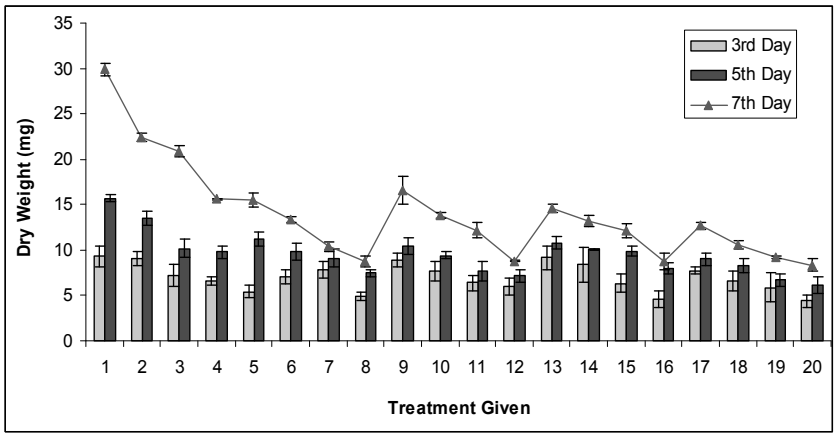


Figure-2

Effect of copper and zinc metal ions on dry weight Vigna mungo (L.) seedlings.



Effect of copper and zinc metal ions on nucleic acid content in *Vigna mungo* (L.)

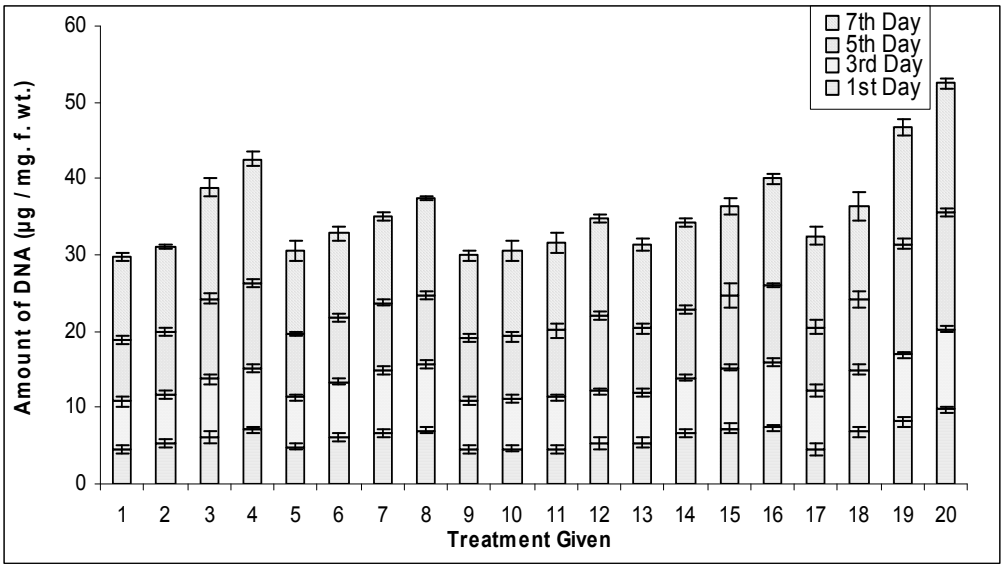
DNA content/ mg. f. wt of the seedlings has found to be increased progressively with increase in concentration of copper and zinc ions. At different levels of metal treatment DNA has shown significant variations in comparison to control as expressed in **Fig.-3**. On 1st day, total amount of DNA / mg. fresh weight got increased up to 124.45% at 0.2mM Cu + 1.50mM Zn, followed by 0.2mM Cu + 1.00mM Zn which have caused 84% rise in DNA content. At 0.05mM, 0.1mM and 0.2mM Cu, DNA content got increased by 19.54%, 39.31% and 62.00% whereas zinc metal ions have raised the DNA content by 9.77%, 40.90%, 52.27% and 59.77% at 0.25mM, 0.50mM, 1.00mM and 1.50mM Zn. This trend of increase in DNA content with increase in copper and zinc ion concentration was followed up to 7th day. Therefore, the DNA content showed a reverse trend with that of seedling growth. On 7th day, maximum increase in DNA was noticed in seedlings grown in 0.2mM Cu + 1.50mM Zn followed by 0.2mM Cu treatment conditions. Whereas, under combined conditions copper and zinc have shown antagonistic effect and the values for DNA content was found intermediate to their individual effect, except at the highest level of combination i.e, 0.2mM Cu

+ 1.50mM Zn which led to 55.24% increase in DNA content.

The result has suggested that the total number of cell per unit weight of embryonic axis was more under the copper and zinc metal stress in comparison to control. But, these cells must be non-dividing which resulted in inhibition of embryonic axis elongation. Many researchers have reported the toxic effect of copper and zinc ions on cell cycle and cell division. Bishnoi et al. (1993) and Zeid (2001) has observed similar results in pea and bean seedlings respectively. Many reports have revealed that copper caused the inhibition of root elongation by metal interference with cell division, inducement of chromosomal aberrations and abnormal mitosis (Jiang et al. 2001). Moreover, zinc metal toxicity has been reported to cause some chromosomal abnormalities like sticky chromosomes that has been attributed to the effect of Zn^{2+} on the physico-chemical properties of DNA, protein or both and the formation of complexes with phosphate groups (Valle and Ulmer 1972). El-Ghamery (2003) has noticed that zinc ions induced an imbalance in the frequency of mitotic phases in root meristem of *Triticum aestivum* (L.) and *Nigella sativa* (L.). The frequency of prophase and/or metaphase stage got increased at the expense of other mitotic stages in both the plants. According to Liu et al. (1996) the accumulation of dividing cells at these stages indicates the influence of zinc ions on sequence of mitotic division. It might have caused reduction in the number of cell entering mitotic division by blocking the process at the end of prophase or may be due to interaction with the spindles which can cause an arrest of the cell division at metaphase.

Figure- 3

Effect of copper and zinc metal ions on DNA content in Vigna mungo (L.) seedlings.

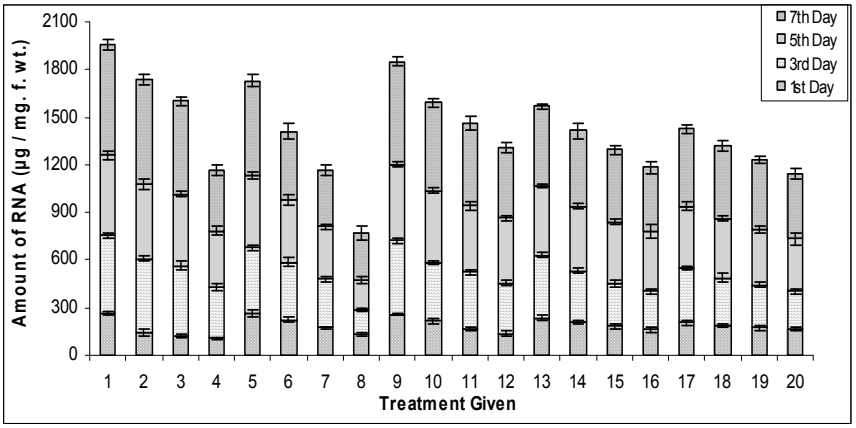


Copper and zinc metal ions treatment has adversely affected the RNA content of *Vigna mungo* (L.). Different levels of copper and zinc stress had significant effect on RNA content, individually as well as in combination. RNA content of the embryonic axis was depleted by about 57.21% in 1.50mM Zn treated seedlings and 0.2mM Cu succeeded it and led to reduction of RNA content by 45.48%. This trend of decrease in RNA content with increase in copper and/or zinc concentration was continuous from 1st to 7th day as shown in **Fig.-**

4. Maximum reduction in RNA content has occurred in case of 1.50mM Zn and minimum reduction has been noticed at the lowest level of combination i.e, 0.05mM Cu + 0.25mM Zn, as per results it was only 6.39% on 7th day. RNA content is negatively affected by the uptake of these heavy metals and resulted in decreased RNA content on each experimental Day. The present results are corroborated with the findings of Zeid and Ghate 2007; Hamid et al. 2010. Element such as copper, nickel, cadmium and lead(Pb) have been reported to decrease RNA synthesis and to activate ribonucleases (RNase) activity leading to further decrease in RNA content (Schmidt 1996).

Figure- 4

Effect of copper and zinc metal ions on RNA content in Vigna mungo (L.) seedlings.



Effect of copper and zinc metal ions on phosphate metabolism in Vigna mungo (L.).

The cotyledons have shown increased level of inorganic phosphates with time (1-7 days) but it got decreased with increase in copper and zinc ions concentration. This decrease in phosphate content was consistent during the entire period of experimental study. On 1st day, the phosphate content of cotyledon attached to seedlings grown in control condition was 0.892mg/g.fr.wt. and it increased up to 1.537mg/ g.fr.wt. in 7 days. But, in case of seedlings grown in copper and zinc metal stress the rate of increase in phosphate level was very low in comparison to control as shown in **Table- 1**. In cotyledons, copper at 0.05mM, 0.1mM and 0.2mM concentration have caused 3.25%, 21.18% and 28.13% decline in phosphate content in comparison to control after 1 day of germination. There was 13.22%, 28.36%, 38.78% and 42.15% decrease in phosphate content at 0.25mM, 0.50mM, 1.00mM and 1.50mM zinc metal ion concentration. At low level the combined effect of both the metal was found antagonistic but at high concentration it was proved severe. At 0.05mM Cu + 0.25mM Zn, 0.1mM Cu + 0.25mM Zn and 0.2mM Cu + 0.25mM Zn the reduction in phosphate level was 9.08%, 13.45% and 15.35% respectively as compared to control.

Further, increase in metal ion concentration led to decrease in phosphate level up to greater extent. In case of combined metal treatment as a result of high concentration of copper and zinc there was 21.41%, 26.90 % and 43.60% reduction was noticed at 0.05mM Cu + 1.50mM Zn, 0.1mM Cu + 1.50mM Zn and 0.2mM Cu + 1.50mM Zn. This trend was continuous till seven days and drastic change in phosphate level was reported during fifth to seventh day of germination. In

seven day old seedlings, copper at the concentration of 0.05mM, 0.1mM and 0.2mM concentration led to 26.87%, 37.28% and 46.19% reduction in phosphate level and there was 17.17%, 19.38%, 24.07% and 28.56% decrease at varying concentrations of zinc metal ions. Combined treatment of copper and zinc at the concentration less than 0.1mM Cu + 1.50mM Zn was relatively less harmful than individual effect of copper and zinc metal. However, further increase in concentration has caused deleterious effects on phosphate metabolism. Maximum reduction was noticed at 0.2mM Cu (46.19%) followed by 0.2mM Cu + 1.50mM Zn (44.17%).

The inorganic phosphate content got increased as a function of time as well as metal concentration in embryonic axis grown under copper and zinc ion stress. Inorganic phosphate content was found to increase with increase in copper and zinc concentration. In one day old seedlings, copper and zinc below 0.1mM and 1.00mM concentration led to increase in phosphate level. Further increase in metal ion concentrations was found to decrease phosphate content in embryonic axis. In copper and zinc treated seedlings the phosphate level raises below 0.1mM Cu + 0.50mM Zn concentration whereas high concentration of copper and zinc were observed to decrease inorganic phosphate level in embryonic axis. But this trend was not constant during all the days of experiment because there was increase in phosphate content under all treatment conditions except in case of 0.2mM Cu and 0.2mM Cu + 1.50mM Zn, till five days after germination. In seven day old seedlings whether grown under individual metal treatment or combined treatment, there was increase in phosphate level. In case of 0.05 mM Cu it got raised up to 78%. But, the percentage of increase in phosphate content was relatively less at high concentrations of copper and zinc. The obtained data was subjected to Dunnett's multiple comparison test to find out the significant differences in phosphate level in seedlings grown in control and treated conditions.

Mishra and Dubey (2008) reported the decline in phosphate content in rice seedlings exposed to As (III) is correlated with decreased activity of phosphatases under heavy metal stressed conditions which proved detrimental for proper growth of seedlings and establishment of plants. An increased activity of acid phosphatase enzyme facilitates the release of phosphate reserve from stored food for the developing embryo. But, in the present study we have observed that the phosphate content in cotyledons was decreasing continuously with increase in copper and zinc metal ion. This decrease in phosphate content seems to be correlated with acid phosphatase activity because, decrease in acid phosphatase activity will decrease the rate of release of inorganic phosphate from reserves as it is present in the form of phytate in *Vigna mungo*. These results are in agreement with Tabaldi et al. 2007, who have observed that a number of metal ions are known to serve as inhibitors for acid phosphatase activity.

However, increased phosphate content in embryonic axis under copper and zinc ion stress might be due to increase in activity of acid phosphatase enzyme to overcome phosphate starvation resulted from metal stress. Mohamad et al. (2009) noticed that the activity of acid phosphatase in plant tissues increase under phosphorous deficient conditions to improve plant capacity to obtain phosphorous from organic source.

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Table-1

Effect of copper and zinc on inorganic phosphate (mg/ g.f.wt.$^{-1}$) in Vigna mungo (L.).

Concentration		In cotyledons (mg/ g.f.wt.$^{-1}$)				In embryonic axis(mg/ g.f.wt.$^{-1}$)			
$CuSO_4.5H_2O$ (mM)	$ZnSO_4.7H_2O$ (mM)	1st	3rd d	5th d	7th d	1st d	3rd d	5th d	7th d
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
0.0	0.0	0.892 ± 0.010	0.924 ±0.020	1.033 ±0.020	1.537 ±0.005	0.619 ±0.020	0.729 ±0.020	0.774 ±0.020	0.917 ±0.020
0.05	0.0	0.863 ±0.020	0.879 ±0.015	1.026±0.011	1.124±0.020[a]	0.676 ±0.032	0.761 ±0.004	1.234 ±0.020 [a]	1.639 ±0.009 [a]
0.1	0.0	0.703 ±0.023[a]	0.841 ±0.017 [a]	0.816±0.018[a]	0.964±0.020[a]	0.615 ±0.030	0.732 ±0.016	0.921 ±0.020 [a]	1.164 ±0.005 [a]
0.2	0.0	0.641 ±0.011[a]	0.790 ±0.024 [a]	0.740±0.010[a]	0.827±0.013[a]	0.471±0.014[a]	0.629 ±0.014[a]	0.724 ±0.030	0.998 ±0.025 [a]
0.0	0.25	0.774 ±0.022[a]	0.875±0.011	0.904±0.025[a]	1.273±0.026[a]	0.807±0.026[a]	0.919 ±0.023 [a]	1.130 ±0.030 [a]	1.164 ±0.006 [a]
0.0	0.50	0.639 ±0.005[a]	0.857±0.032[b]	0.896±0.027[a]	1.239±0.011[a]	0.784±0.018[a]	0.859 ±0.019 [a]	1.052 ±0.023 [a]	1.135 ±0.024 [a]
0.0	1.00	0.546 ±0.024[a]	0.799±0.020 [a]	0.882±0.037[a]	1.167±0.027[a]	0.622 ±0.021	0.808 ±0.016 [a]	0.939 ±0.031 [a]	1.120 ±0.036 [a]
0.0	1.50	0.516 ±0.018[a]	0.700±0.022[a]	0.853±0.030[a]	1.098±0.023[a]	0.529±0.036[a]	0.754 ±0.010	0.930 ±0.026 [a]	0.965±0.013
0.05	0.25	0.811 ±0.023[a]	0.858±0.014[b]	0.924±0.021[a]	1.288±0.018[a]	0.803±0.022[a]	1.029 ±0.012 [a]	1.109 ±0.013 [a]	1.277 ±0.011 [a]
0.05	0.50	0.789 ±0.022[a]	0.817±0.012[a]	0.904±0.025[a]	1.211±0.028[a]	0.760±0.012[a]	0.921 ±0.024 [a]	1.043 ±0.027 [a]	1.173 ±0.019 [a]
0.05	1.00	0.729 ±0.016[a]	0.788±0.011[a]	0.858±0.016[a]	1.157±0.008[a]	0.746±0.022[a]	0.912 ±0.027 [a]	1.000 ±0.027 [a]	1.048 ±0.018 [a]
0.05	1.50	0.701 ±0.017[a]	0.720±0.027[a]	0.784±0.021[a]	1.015±0.023[a]	0.728±0.026[a]	0.833 ±0.021 [a]	0.884 ±0.028 [a]	0.981 ±0.014 [b]
0.1	0.25	0.772 ±0.021[a]	0.825±0.010[a]	0.885±0.020[a]	1.134±0.024[a]	0.753±0.019[a]	0.906 ±0.022 [a]	0.995 ±0.032 [a]	1.173 ±0.025 [a]
0.1	0.50	0.710 ±0.016[a]	0.794±0.022[a]	0.858±0.031[a]	1.056±0.015[a]	0.698±0.023[c]	0.857 ±0.024 [a]	0.916 ±0.022 [a]	1.064 ±0.029 [a]
0.1	1.00	0.694 ±0.025[a]	0.734±0.024[a]	0.743±0.037[a]	1.039±0.026[a]	0.552±0.023[b]	0.811 ±0.016 [a]	0.880 ±0.038 [a]	1.040 ±0.021 [a]
0.1	1.50	0.652 ±0.025[a]	0.678±0.024[a]	0.714±0.024[a]	0.961±0.010[a]	0.534±0.016[a]	0.783 ±0.013 [c]	0.805 ±0.023	1.007 ±0.016 [a]
0.2	0.25	0.755 ±0.025[a]	0.803 ±0.019[a]	0.871±0.032[a]	0.931±0.007[a]	0.583 ±0.015	0.883 ±0.016 [a]	0.897 ±0.014 [a]	1.065 ±0.022 [a]
0.2	0.50	0.704 ±0.022[a]	0.764 ±0.025[a]	0.827±0.025[a]	0.904±0.018[a]	0.564 ±0.028	0.768 ±0.014 [b]	0.862 ±0.015 [b]	0.935 ±0.012
0.2	1.00	0.626 ±0.015[a]	0.668 ±0.024[a]	0.712±0.016[a]	0.890±0.023[a]	0.481±0.014[a]	0.745 ±0.035	0.777 ±0.027	0.923 ±0.015
0.2	1.50	0.503 ±0.027[a]	0.630 ±0.024[a]	0.701±0.020[a]	0.858±0.013[a]	0.414±0.027[a]	0.717 ±0.020	0.761 ±0.026	0.870 ±0.018

1) Above values are averages of three replicates ± SD
2) **a**, **b** and **c** indicates the values that differ significantly from the control at p≤ 0.05, p≤ 0.01 and p≤ 0.001 level respectively.

Acid phosphatase activity- A significant inhibition in acid phosphatase activity was noticed in cotyledons of *Vigna mungo* seedlings at different concentrations of copper and zinc individually as well as in combination as shown in **Table- 2**. On 1st day the specific activity of acid phosphatase in cotyledons of control seedlings was 1.045 µg p-nitrophenol liberated/min./mg of protein. It increased with the passage of time and reached up to 5.672 µg p-nitrophenol liberated/min./mg of protein. During early stage (1-5 days) the rate of increase in enzymatic activity in cotyledons was less but a rapid increase was noted during (5-7 days). Copper at various concentrations i.e, 0.05mM, 0.1mM and 0.2mM resulted in decline in acid phosphatase activity in cotyledons of seedlings by 39.13%, 47.84% and 58.66% as compared to control. Whereas, 23.63%, 31.38%, 44.88% and 51.00% decrease was observed in 0.25mM, 0.50mM, 1.00mM and 1.50mM zinc concentration. The effect of copper and zinc combined treatment seemed to be antagonistic at low concentration but become very harmful at high concentration. At 0.05mM Cu + 0.25mM Zn, 0.05mM Cu + 0.50mM Zn and 0.1mM Cu + 0.25mM Zn there was 2.39%, 16.17% and 17.51% decrease in acid phosphatase activity with respect to control. Whereas, at high concentration the specific activity of acid phosphatase decreased by 86.41% at 0.2mM Cu + 1.50mM Zn. At 0.05mM Cu + 1.50mM Zn decline in enzymatic activity was 57.12% and 61.91%.

The combined effect of copper and zinc was more harmful on acid phosphatase activity

rather than their individual effect at the same concentration. Though there was progressive increase in enzymatic activity in all treatment conditions but the rate of enzyme activity with time was found to be affected. At 0.05mM, 0.1mM and 0.2mM, there was 10.57%, 27.29% and 65.72% decrease in enzymatic activity and zinc at various studied concentration i.e, 0.25mM, 0.50mM, 1.00mM and 1.50mM has caused 45.55%, 61.51%, 71.70% and 80.18% decrease in acid phosphatase activity. The combined effect of copper and zinc at 0.05mM Cu + 0.25mM Zn, 0.1mM Cu + 0.25mM Zn and 0.2mM Cu + 0.25mM Zn concentration level have been reported to cause 51.69%, 62.16% and 66.16% reduction in enzymatic activity as compared to control. Moreover, the acid phosphatase activity was continuously decreased with corresponding increase in copper and zinc concentration. Maximum reduction (84.92%) was noticed at 0.2mM Cu + 1.50mM Zn. The Dunnett'smultiple comparison test was applied to find out the significant differences in acid phaospatase activity between control and treated seedlings.

These results are in agreement with Skrebsky et al. (2008) who reported decrease in acid phosphatase activity in *Pfaffia glomerata* due to cadmium metal stress and according to Kuriakosa and Prasad (2008) the decrease in acid phosphatase activity have occurred due to loss of one or more isozymes. Other factors contributed to decrease in enzyme activity could be modulation of enzyme activity by divalent cations through delayed enzyme solubilization and activation (Prisco et al. 1998) or inhibition of enzyme activity by heavy metals due to interference with the PO_4^{3-} binding sites (Tabaldi et al. 2007).

The present study reveals that in embryonic axis of copper and zinc treated seedlings there was significant increase in acid phosphatase activity with time and increase in metal concentration. In seven day old seedlings it was found that in 0.05mM, 0.1mM and 0.2mM Cu treated seedlings there was 144.63%, 115.28% and 36.64% increase in acid phosphatase activity. Zinc metal ions at 0.25mM, 0.50mM, 1.00mM and 1.50mM concentration resulted in 57.06%, 53.18%, 43.23% and 24.91% increase in enzymatic activity. As a result of combined treatment of copper and zinc, there was 140.32%, 105.05% and 12.53% increase in acid phosphatase activity at 0.05mM Cu + 0.25mM Zn, 0.1mM Cu +0.25mM Zn and 0.2mM Cu + 0.25mM Zn. Copper and zinc meta ions at high concentration has resulted in copper and zinc concentration has resulted in low rate of increase in acid phosphatase activity.

These results are in agreement with Mohamed et al. 2009; and Bhardwaj et al. 2009. It has been long recognized that acid phosphatase activity in plants increased when plants become phosphorus (Pi) deficient. The increase in acid phosphatase activity is correlated with low level of inorganic phosphates in cell (Mohamed et al. 2009), and intracellular and extracellular acid phosphatase are integral component of plant response to phosphorus deficiency (Duff et al. 1993). Yadav and Tarafdar (2001) reported that phosphatases are adaptive enzymes and their activity increases with the difficulty of hydrolysis of organic phosphorus presented in the plants. The increase in acid phosphatase activity with the age of plants may be attributed to the development of the root system with age and increase in total root surface area. But phosphorus deficiency can significantly alter the composition of root exudates in a way that in some plant species it is related to the increased ability for the mobilization of sparingly soluble phosphorus sources (Jones 1998). Moreover, these results are in agreement with Neumann and Romheld (2000) who reported increase root exudation is a P-deficiency response particularly in dicotyledonous plant species.

Table-2
Effect of copper and zinc on specific activity of acid phosphatase (µg p-nitrophenol liberated/min./mg of protein) in *Vigna mungo* (L.)

Concentration		In cotyledons (µg p-nitrophenol liberated/min./mg of protein)				In embryonic axis (µg p-nitrophenol liberated/min./mg of protein)			
$CuSO_4$.$5H_2O$ (mM)	$ZnSO_4$. $7H_2O$ (mM)	1st d	3rd d	5th d	7th d	1st d	3rd d	5th d	7th d
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
0.0	0.0	1.045 ± 0.017	1.557 ±0.008	2.791 ±0.009	5.672 ±0.008	1.447 ±0.008	1.820 ±0.014	2.389 ±0.009	3.717 ±0.008
0.05	0.0	0.636 ± 0.010[a]	1.399±0.006[a]	2.191±0.007[a]	5.072±0.012[a]	2.311 ±0.008[a]	5.825 ±0.002[a]	6.051 ±0.006[a]	9.093 ±0.008[a]
0.1	0.0	0.545 ± 0.013[a]	1.026±0.005[a]	1.442±0.009[a]	4.124±0.015[a]	2.011 ±0.012[a]	5.131 ±0.007[a]	6.015 ±0.007[a]	8.002 ±0.005[a]
0.2	0.0	0.432 ± 0.013[a]	0.747±0.010[a]	1.221±0.006[a]	1.944±0.008[a]	1.083 ±0.009[a]	2.012 ±0.004[a]	3.278 ±0.007[a]	5.079 ±0.004[a]
0.0	0.25	0.798 ± 0.014[a]	1.527 ±0.008	2.357±0.009[a]	3.088±0.007[a]	4.542 ±0.011[a]	5.520 ±0.007[a]	5.633 ±0.007[a]	5.838 ±0.009[a]
0.0	0.50	0.717 ± 0.005[a]	1.055±0.006[a]	1.978±0.066[a]	2.183±0.062[a]	3.615 ±0.004[a]	5.122 ±0.007[a]	5.246 ±0.004[a]	5.694 ±0.003[a]
0.0	1.00	0.576 ± 0.014[a]	1.006±0.004[a]	1.343±0.055[a]	1.605±0.007[a]	3.241 ±0.009[a]	4.194 ±0.008[a]	5.043 ±0.006[a]	5.324 ±0.005[a]
0.0	1.50	0.512 ± 0.014[a]	0.682±0.009[a]	1.200±0.007[a]	1.124±0.006[a]	2.725 ±0.011[a]	3.749 ±0.010[a]	3.729 ±0.008[a]	4.643 ±0.007[a]
0.05	0.25	1.020 ± 0.006	1.481±0.006[a]	1.817±0.007[a]	2.740±0.064[a]	3.016 ±0.006[a]	5.224 ±0.004[a]	6.875 ±0.006[a]	8.933 ±0.006[a]
0.05	0.50	0.876 ± 0.008[a]	1.052±0.010[a]	1.324±0.067[a]	1.662±0.008[a]	2.521 ±0.005[a]	3.466 ±0.005[a]	4.302 ±0.008[a]	4.809 ±0.009[a]
0.05	1.00	0.561 ± 0.006[a]	0.918±0.006[a]	1.008±0.009[a]	1.441±0.007[a]	2.261 ±0.007[a]	3.110 ±0.006[a]	3.345 ±0.008[a]	3.960 ±0.013[a]
0.05	1.50	0.448 ± 0.007[a]	0.750±0.006[a]	0.846±0.009[a]	1.011±0.012[a]	2.183 ±0.012[a]	2.949 ±0.006[a]	3.165 ±0.007[a]	3.747 ±0.006[b]
0.1	0.25	0.862 ± 0.008[a]	1.265±0.009[a]	1.480±0.010[a]	2.146±0.005[a]	2.294 ±0.011[a]	4.206 ±0.009[a]	5.734 ±0.007[a]	7.622 ±0.007[a]
0.1	0.50	0.616 ± 0.007[a]	0.873±0.067[a]	1.213±0.008[a]	1.548±0.006[a]	2.173 ±0.005[a]	3.345 ±0.010[a]	4.111 ±0.007[a]	4.099 ±0.008[a]
0.1	1.00	0.497 ± 0.013[a]	0.690±0.008[a]	1.003±0.006[a]	1.268±0.004[a]	1.911 ±0.006[a]	2.764 ±0.007[a]	3.336 ±0.005[a]	3.397 ±0.006[a]
0.1	1.50	0.398 ± 0.007[a]	0.599±0.007[a]	0.754±0.001[a]	0.922±0.008[a]	1.866 ±0.011[a]	2.233 ±0.004[a]	2.809 ±0.009[a]	2.840 ±0.006[a]
0.2	0.25	0.704 ± 0.012[a]	1.012±0.007[a]	1.436±0.003[a]	1.919±0.003[a]	2.168 ±0.006[a]	2.985 ±0.007[a]	3.332 ±0.007[a]	4.183 ±0.006[a]
0.2	0.50	0.456 ± 0.007[a]	0.786±0.012[a]	1.036±0.012[a]	1.508±0.009[a]	1.965 ±0.007[a]	2.866 ±0.004[a]	2.920 ±0.002[a]	3.219 ±0.008[a]
0.2	1.00	0.396 ± 0.003[a]	0.599±0.005[a]	0.917±0.005[a]	1.019±0.005[a]	1.431 ±0.005	2.764 ±0.011[a]	2.814 ±0.005[a]	2.903 ±0.006[a]
0.2	1.50	0.142 ± 0.012[a]	0.484±0.005[a]	0.657±0.006[a]	0.855±0.006[a]	1.398±0.007[a]	2.515 ±0.004[a]	2.279 ±0.006[a]	2.350 ±0.008[a]

1) Above values are averages of three replicates ± SD
2) **a**, **b** and **c** indicates the values that differ significantly from the control at p≤ 0.05, p≤ 0.01 and p≤ 0.001 level respectively.

CONCLUSION

This study has shown that copper and zinc metal ions have antagonistic effects at low concentration but it turns toxic at high concentrations. The present study suggests that copper and/or zinc ion stress cause alteration in nucleic acid contents and nutritional status which might have resulted in reduction in seedlings growth and development.

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