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Effect of Copper Sulphate on Seed Germination, Plant Growth and Peroxidase Activity of Mung Bean (*Vigna radiata*)

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Abstract: The aim of this study was to find the effect of copper as a micronutrient for enhanced plant growth, protein content and antioxidant enzyme activity of mungbean (*Vigna radiata*) under the influence of different concentrations of copper. The effect of various copper sulphate solution has insignificant effect on the percent germination of mungbean while plumule and radicle length decreased with increase in copper concentration (50, 200, 500 and 1000 µM copper sulphate solution). Total protein content in root was significantly higher (127.7%) than shoot (83.7%) as compared to control. Peroxidase activity, in case of shoot, increases with increase in copper sulphate concentration i.e., (1.423 of control to 1.713, 2.29, 2.52, 2.88 and 3.02 OD/min/mg protein in different copper sulphate solutions treatments after 72 h) while for the root copper sulphate solution has negative effect on the peroxidase activity i.e., (11.41 of control to 11.19, 10.85, 10.04, 9.73 and 9.40 OD/min/mg protein in different copper sulphate solutions treatments after 72 h). The present experiment revealed that copper is an essential micronutrient which promoted the seedlings growth at less than 50 µM concentration. Higher concentration significantly decreases shoot and root length. The protein concentration in both shoot and root of the seedling was recorded to be enhanced with increase in the concentration of copper sulphate as compared to control. POD activity in root decreased at higher levels of copper concentration while POD activity in shoot increased with increasing concentration.

Key words: Mungbean (*Vigna radiata*), copper sulphate, germination, protein, peroxidase activity

INTRODUCTION

Copper is widely prevalent in our environment and was considered as an essential element for all living organisms including plants (Singh *et al.*, 2007). It plays a key role in many metabolic mechanisms but it can be toxic when the copper (Cu) content in tissues is slightly higher than its optimal levels. Some plant species have capacity to grow in the metal contaminated soil and accumulate elevated amount of heavy metals (hyper-accumulation) as an ecophysiological adaptation in metaliferous soil (Singh *et al.*, 2008). *Phaseolus vulgaris* has been reported a good accumulator of lead and cadmium (Garay *et al.*, 2000). Root growth is particularly sensitive to the Cu and Zn toxicity (Hajiboland *et al.*, 2006). Shen *et al.* (1998) has studied the effect of copper (Cu) and zinc (Zn) toxicity on growth of mung bean (*Phaseolus aures* Roxb. cv VC-3762) in a solution culture. The mechanisms involved in heavy metal tolerance may range from exclusion, inclusion

and accumulation of heavy metals depending on the plant species (Munzuroglu and Geckil, 2002; Kaushik *et al.*, 2005; El-Tayeb *et al.*, 2006).

Distinct concentrations of metals induce different biochemical such as enzyme inhibition involved in photosynthetic reaction (Wang and Zhou, 2005; Smirnov *et al.*, 2006). Generally, Cu can induce many alterations of the plant cells. Owing to its redox properties, Cu can catalyze the formation of some harmful free radicals, such as reactive oxygen species and peroxide compounds which cause an oxidative burst (Gupta and Kalra, 2006; Hameed *et al.*, 2003). This toxic effect coming from the cellular oxidative state may be allayed by several antioxidative systems such as peroxidase (POD), catalase and superoxide dismutase (SOD) (Patra and Panda, 1998; Agarwal *et al.*, 2006; Joseph and Jini, 2010).

Peroxidases may remove excess H₂O₂ caused by metal stress. They are involved in several physiological and

biochemical processes, such as cell growth and expansion (Fang and Kao, 2000), auxin catabolism (Passardi *et al.*, 2004), lignification (Brownleader *et al.*, 2000).

Keeping in view this experiment was designed to study the effect of copper toxicity on the seedling growth of mung bean (*V. radiata*).

MATERIALS AND METHODS

Plant material and metal treatment: The present research work has been completed in the microbiology laboratory at Department of Soil Science and Agricultural Chemistry, BHU, Varanasi in 2007-08. *Vigna radiata* (variety 30 and 35) seeds were used for the Petri dish experiments. Seeds were surface sterilized with 0.1% HgCl₂ and 70% ethyl alcohol for the prevention of surface fungal/bacterial contamination. The 50, 100, 200, 500 and 1000 µM copper sulphate solutions were prepared in pure distilled water in laboratory by using copper sulphate (CuSO₄.5H₂O) and pure distilled water was used as control for the study in triplicate. Hundred selected healthy seeds of same size were soaked in copper sulphate solutions of different concentration along with distilled water as control for 3 h. The seeds were then transferred to Petri dish with cotton bed containing respective solution of copper sulphate concentration and distilled water. The Petri dishes were incubated at room temperature for 24 h in dark condition. It was transferred to light condition for 48 h and then again for another 24 h (total 72 h). Then percent germination was calculated on the basis of seed germination.

The growth parameters like germination after 24 h, plumule and radicle length after 48 and 72 h were observed. The experiment was conducted in triplicates.

Protein content analysis: Folin-Lowry method (Lowry *et al.*, 1951) was used to determine the total protein content of root and shoot.

Peroxidase (POD) activity: The activity of peroxidases was determined using guaiacol as a substrate. The POD activity was measured according to Kochhar *et al.* (1979).

Statistical analysis: Experiment was conducted with three replications and six treatments. Statistical analysis was conducted using one-way analysis of variance (ANOVA) using SPSS 12.0 software. Comparisons of means were performed by the Fisher's Protected LSD test at $p \leq 0.05$.

RESULTS

Percent germination: Seed germination was recorded 97% in variety-30 and 69% in variety-35 at 24 h incubation at room temperature. Protein contents in seed of variety 30

and 35 were estimated 79.24 and 85.54 µg/10 µL protein solution, respectively. Protein contents in root of variety 30 and 35 were recorded 34.62 and 32.45 µg/10 µL protein solution, respectively after 24 h growth (Table 1). Based on the quality of seed germination, variety 30 was selected for further experiments. *Vigna radiata* (variety 30) was showed maximum germination 97 to 99% when seeds treated with different concentration 50, 100, 200, 500 and 1000 µM CuSO₄ solution after 24 h incubation at room temperature. There was no significant difference in the percent germination at different concentration of copper sulphate after 24 h (Table 2).

Length of shoot: Result pertaining to the shoot length revealed that higher concentrations of CuSO₄ had significant effect on reduction of shoot length over control. At concentration 50 and 100 there were no significant difference in shoot length after 48 h. Reduction in shoot length was observed 48.5% at 500 µM of CuSO₄ solution over control at 48 h incubation of room temperature while 68.7% reduction in shoot length was recorded at 72h incubation. Reduction in shoot length was recorded 16.9 and 8.5% at 48 h and 72 h, respectively at 50 µM CuSO₄ (Table 2).

Protein content: A marked increase in protein concentration was observed in shoot and root of the seedling at 72 h compared to control. This increase was halted at a concentration of 1000 µM of CuSO₄ solution which was found to be lower as compared to that of 500 µM. In case of shoot and root, at 500 µM concentration, the protein content showed 83.7 and 127.7%, respectively increase as compared to control while 1000 µM showed lesser protein content as compared to 500 µM concentration. The protein content accumulation was more in case of root as compared to shoot. In case of both shoot and root, protein content at 50 µM had no significant difference as compared to control (Table 2).

Activities of antioxidant enzymes: In case of shoot POD, no significant change in the activity of POD was observed in seedlings shoot and root raised in the presence of 50 µM copper as compared with that of control (Table 3). However, the shoots of seedlings raised in the presence of high levels of copper showed a significant enhancement (up to 112.23%) in the activity of POD compared with controls. The plants with 1000 µM copper

Table 1: Germination profile and protein content in *Vigna radiata* seed variety P-30 and P-40

Seed variety	Germination (%) after 24 h	Protein (µg/10 µL)	
		Seed	Root
P-30	97	79.24	34.62
P-35	69	85.54	32.45

Table 2: Effect of copper on shoot length and protein content in *Vigna radiata*

Treatments	Germination (%) after 24 h	Shoot length (cm)		Protein ($\mu\text{g}/10 \mu\text{L}$) after 72 h	
		After 48 h	After 72 h	Shoot	Root
Control	98 ^a	1.204 ^d	3.66 ^d	21.5 ^a	18.0 ^a
50 μM CuSO ₄	97 ^a	1.004 ^c	3.35 ^c	23.0 ^{ab}	18.4 ^a
100 μM CuSO ₄	98 ^a	0.916 ^{bc}	2.12 ^b	24.5 ^b	22.5 ^b
200 μM CuSO ₄	99 ^a	0.772 ^b	1.948 ^b	35.0 ^c	35.5 ^c
500 μM CuSO ₄	99 ^a	0.620 ^{ab}	1.144 ^a	39.5 ^c	41.0 ^d
1000 μM CuSO ₄	99 ^a	0.956 ^a	0.956 ^a	38.0 ^d	37.5 ^c

Different letters in each column indicate significant difference between treatments at $p < 0.05$

Table 3: Effect of copper on peroxidase activity in *Vigna radiata*

Treatments	Change in OD/min/mg protein	
	POD of root	POD of shoot
Control	11.41 ^d	1.423 ^a
50 μM CuSO ₄	11.19 ^{cd}	1.713 ^a
100 μM CuSO ₄	10.85 ^c	2.29 ^b
200 μM CuSO ₄	10.04 ^b	2.52 ^b
500 μM CuSO ₄	9.73 ^a	2.88 ^b
1000 μM CuSO ₄	9.40 ^a	3.02 ^c

Different letters in each column indicate significant difference between treatments at $p < 0.05$

sulphate treatment showed the notably higher peroxidase activity levels than all other treatments (Table 3). The roots of seedlings rose in the presence of high levels of copper solution showed a significant negative reduction in POD compared with control. There were no significant difference in the POD activities at 500 and 1000 μM concentration in case of seedlings root and shoot. Also POD, in case of shoot, had significant difference between treatments at concentration of 100 and 200 μM copper solutions (Table 3).

DISCUSSION

Present study was focused on the seed germination and plant growth at initial stage. The result of seed germination of mung bean was found to be more than 95% and it was non-significantly influenced by different concentration of copper sulphate solution. Growth of the root system was significantly decreased with increasing concentration of copper solution. Present result has been supported by Shen *et al.* (1998) that Cu and Zn toxicity might cause multiple direct and indirect influences on practically all processes of physiological metabolism in plants. The primary toxicity mechanism of heavy metals were shown as altering the catalytic function of enzymes, damaging cellular membranes, inhibiting root growth. Addition of Ca alleviated the Cu and Zn suppression of growth of mung bean seedlings.

This decrease in root growth was due to reduction in cell division. Similar finding has been reported by Souguir *et al.* (2008) who investigated the potential genotoxicity of Cu²⁺ in *Vicia faba* and *Pisum sativum* seedlings under hydroponic culture conditions. Cu²⁺ caused a dose-dependent increase in micronuclei

frequencies in both plant models. Cytological analysis of root tips cells showed clastogenic and aneugenic effects of this heavy metal on *V. faba* root meristems.

Being an essential micronutrient, copper promoted the growth of *V. radiata* seedlings when present at lower concentrations but, if present at high level, copper retarded growth by interfering with normal cellular metabolic events, as was reported by us and other researchers (Alia *et al.*, 1995). Harmens *et al.* (1993) has reported that first visible damage due to zinc was on root growth due to reduction in cell division. Bouazizi *et al.* (2008) and Pourakbar *et al.* (2007) had reported that overall accumulation of copper was significantly higher in the root tissues than in the young leaves. According to Yurekli and Porgali (2006), the higher accumulation of copper in roots results from a tolerance mechanism developed by the plant in order to reduce the effect of heavy metal stress.

The protein concentration in both shoot and root of the seedling was recorded to be enhanced with increase in the concentration of copper sulphate as compared to control. While peroxidase activity in shoot increased and in root it decreased with increasing concentration of copper sulphate solution. This might be due the defense mechanism resulting in higher concentration of various enzymes involved in different protein synthesis pathway. But due to accumulation of copper ion in roots might cause decrease in the peroxidase activity. Excess Cu²⁺ is known to mediate free radical formation in intact roots (De Vos *et al.*, 1993). They suggest that free radical-induced lipid peroxidation is part of the overall expression of Fe²⁺, Cu²⁺ or Zn²⁺ toxicity. The induction of POD by Fe²⁺, Cu²⁺ or Zn²⁺, suggesting that the induction of POD activity is due to de novo POD biosynthesis. The change in lipid peroxidation, antioxidative enzyme activity, H₂O₂ level and cell wall peroxidase activity in Cu-stressed roots of *Phaseolus* seedling and their relation with root growth inhibition were investigated. CuSO₄ was effective in inhibiting root growth. Treatment with CuSO₄ resulted in an increase in lipid peroxidation and modulated antioxidative enzyme activity in root. CuSO₄ also increased H₂O₂ level and cell wall peroxidase in root of *Phaseolus* seedlings. It also appears that the increase in POD activity is a defensive response to most if not all

metals which may cause damage or disturb normal function of the plants. To mitigate and repair the damage initiated by oxygen species, plants have evolved complex antioxidant (both enzymatic and non-enzymatic) systems. The induction of the activities of a particular group of enzymes is considered to play an important role in the cellular defense strategy against oxidative stress, caused by toxic metal concentrations.

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