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Response of wheat seed germination and seedling growth under copper stress

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Abstract: *The experiment was performed to study the seed germination and seedling growth in wheat under the influence of different concentrations of copper. The germination %, plumule and radicle length, and number of lateral roots decreased with increase in copper concentration (5, 25, 50 and 100 mgl^{-1}). Total chlorophyll contents declined on 14th day from 1.605 of control to 1.581, 1.242, 1.275 and 1.107 mg g^{-1} fresh weight in respective treatments. Similarly, on 21st day the decline in total chlorophyll contents was 1.288, 1.123, 1.077 and 0.985 mg g^{-1} fresh weight in respective treatments against 1.724 of control. Likewise the pheophytin contents also declined showing the same pattern. However, carotenoid contents increased in different treatments, ranging between 0.366 to 0.464 mg g^{-1} fresh weight in comparison to control (0.328) on 14th day, but showed adverse effects on 21st day as the carotenoid contents decreased in different copper treatments. The activity of amylase was found to be gradually reduced 14th day from 29.73 of control to 27.80, 27.33, 21.86 and 20.00 mg g^{-1} and at 21st day from 14.40 of control to 11.46, 11.01, 9.86 and 5.60 mg g^{-1} fresh weight with increase in concentrations of copper. The catalase activity increased 14th day from 97.33 of control to 134.66, 161.33, 216.00 and 232.00 and on 21st day from 140.00 of control to 245.33, 274.66, 278.66 and 300.66 ml H_2O_2 hydrolyzed/g fresh weight in different increased concentrations of copper. Similarly, the peroxidase activity was also increased with increase in copper concentration. Likewise fresh weight and moisture contents decreased with increase in copper concentration. The dry weight was increased with increase in concentration of copper treatment. Total protein contents were initially decreased on 14th day from 80.69 of control to 66.75, 60.41, 56.41 and 48.48 μg/mg and on 21st day 81.37 of control to 67.06, 62.31, 54.92 and 46.47 μg/mg fresh weight in different copper concentrations respectively. Sugar contents were significantly decreased in all the doses of copper on both 14th and 21st days i.e. (5.53 of control to 4.76, 3.69, 3.68 and 2.86 μg/mg in different copper treatments on 14th day and 4.81 of control to 4.49, 3.40, 2.79 and 2.15 μg/mg on 21st day respectively.*

Key words: *Copper, Chlorophyll, Pheophytin, Carotenoid, Amylase, Catalase, Peroxidase*

Introduction

Metal pollution is continously increasing and the root cause is the anthropogenic behavior which interferes with the environmental activities and makes conditions toxic for living organism. Copper is widely prevalent in our environment and was considered as an essential element for all living organisms including plants (Underwood, 1977; Goyer, 1991). Copper occurs in the environment as hydrated ionic species, forming complex compounds with inorganic and organic legends. Subsequent nutritional studies have demonstrated that copper and other metals are essential for optimal growth of plants and animals (Woolhouse, 1983). Living organisms require certain metals for their growth and metabolism and so, they evolved an appropriate uptake mechanism for metals. Some plant species have capacity to grow in the metal contaminated soil and accumulate elevated amount of heavy metals (hyper-accumulation) as an eco-physiological adaptation in metaliferous soil. *Phaseolus vulgaris* has been reported a good accumulator of lead and cadmium (Garay *et al.,* 2000). The mechanisms involved in heavy metal tolerance may range from exclusion, inclusion and accumulation of heavy metals depending on the plant species (Raskin and Ensley, 2000; Munzuroglu and Geckil, 2002; Kaushik *et al*., 2005). Distinct concentrations of metals induce different biochemical responses in plants. In sensitive plants, high concentration of these metals inhibit enzymes involved in photosynthetic reaction (Singh *et al*., 1997; Wang and Zhou, 2005; Smirnoval *et al*., 2006). *Brassica juncea* (Indian mustard), a high biomass producing plant can accumulate lead (Pb), chromium (Cr VI), cadmium (Cd), copper (Cu), nickel (Ni), zinc (Zn), boron (B) and selenium (Se) (Raskin and Ensley, 2000; Palmer *et al.,* 2001). Even trace elements have been shown to have toxic effect on different plant traits such as leaf, stem, root flower *etc.* (Sivakumar *et al.,* 2001). Copper causes injury at cellular level by the formation of free radicals. Cellular injury by this type of mechanism is well documented for copper as well as other metals (Shi *et al*., 1993; Gupta and Kalra, 2006). Copper being one of the common heavy metals in industrial discharge of aeronautic, metal and metallurgy, and refinery industries shows toxic effects on plants and animals. Previously, the copper concentration in soil and water was usually lower than 5mgl^{-1} but it has increased during the last decade reaching occasionally 50 mgl^{-1} because of heavy industrialization. Keeping in view the importance of wheat as major edible crop and the adverse effects of copper, the present experiment was planned to explore the effect of different concentrations of copper on the germination and growth of wheat.

Materials and Methods

Wheat (*Triticum aestivum* L., Var. - PBW 343) seeds were used for the petri dish experiments. Seeds were surface sterilized with 0.1% $HgCl_2$ for the prevention of surface fungal/bacterial

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contamination (Young, 1926). The 5, 25, 50 and 100 mg l^{-1} copper solutions were prepared in pure distilled water in laboratory by using copper sulphate ($CuSO_4.5H_2O$) and pure distilled water was used as control for the study in triplicate. After quantification of copper, as per percent availability in $CuSO_4.5H_2O$ the 5, 25, 50 and 100 mg l^{-1} doses of this compound were taken. Twenty seeds were placed on filter paper in each petri dish and 10 ml solution was used as prepared above. The fresh solutions were applied every alternate day for the prevention of contaminants and also for the maintenance of concentration (Nath *et al*., 2005). The nutrient solution was provided once in a week, replacing the copper treatment for 24 hr. The petri dishes were monitored daily for fungal and other type of infections.

The growth parameters like germination, plumule and radicle length and number of lateral roots were observed on 14th and 21st day after seedling emergence. The seedling's fresh weight was taken with the help of digital balance (Shimadzu AY-220) and dry weight was measured by placing seedlings at 80 ± 1^0C in a hot air oven for 24 hr until constant weight was observed. Average of three replicates in data are presented in Tables.

Pigment estimation was done by using the method of Arnon (1949) as amended by Lichtenthalce (1987). Amylase, catalase and peroxidase activity were measured by the methods of Katsuni and Fekuhara (1969), Euller and Josephson (1927) and Luck (1963) respectively. The total protein was estimated by the method of Lowry *et al*. (1951), while total sugar was determined by the method of Dubais *et al*. (1956). The data observed in the experiment, were statistically analyzed for the calculation of standard error (S.E.). Student 't' test was administered for testing the hypothesis with the help of computer software sigma stat 2.0 programmes. The data shown are the averages of three replicates ± S.E and statistically significant at $p< 0.05$ level.

Results and Discussion

The results obtained in petri dish culture experiment are shown in Table 1 to 5 and Fig. 1. The results after 14th and 21st day of aqueous exposure of copper to wheat (*Triticum aestivum* L.), showed considerable reduction in seed germination, plumule elongation, radicle elongation and number of lateral roots. An over all inhibition was observed in seed germination and seedling growth as compared to control with increasing copper concentration. Number of lateral roots increased in 5 mg $l^{-1}$and 25 mg l^{-1} of copper treatment and then decreased in 50 and 100 mgl^{-1} of copper treatment. The fresh weight, dry weight and moisture contents (biomass production in seedling) slightly decreased with increasing copper concentration on 14th day. But on 21st day dry weight was decreased in 5 mgl^{-1} copper concentration, but it was equal to control in 25 mg l^{-1}. Increase was observed in 50 mg l^{-1} and 100 mg l^{-1} of copper concentration (Fig. 1 and Table 1).

Noteworthy decline in pigments with increased copper exposure was noted in total chlorophyll, chlorophyll a/b ratio (Table 2) and pheophytin contents (total, 'a' and 'b' pheophytin). However carotenoid contents increased on 14th day but decreased on 21st day in all copper treatment (Table 3). The amylase activity (total amylase α and β amylase) was inhibited in all the copper concentrations (Table 4). Marked increase in catalase and

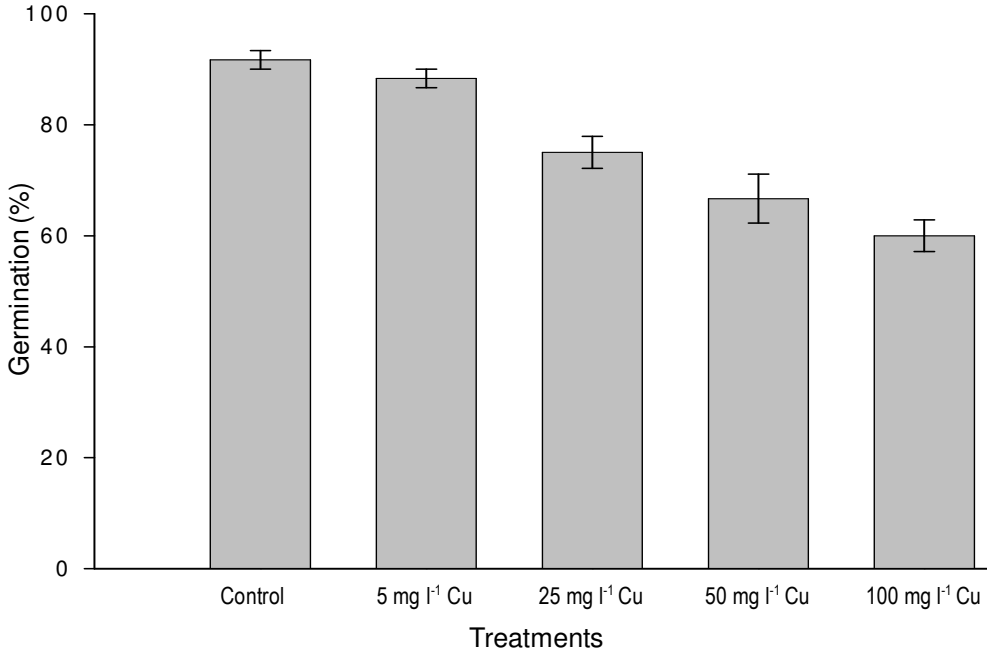

Fig. 1: Effect of copper on seed germination in wheat (*Triticum aestivum*)

Table - 1: Effect of copper on seedling growth in wheat (*Triticum aestivum*)

Treatments	14th Day						21st Day					
	Plumule length (cm)	Radicle length (cm)	Lateral roots (No.)	Fresh weight (g)	Dry weight (g)	Moist-ure (%)	Plumule length (cm)	Radicle length (cm)	Lateral roots (No.)	Fresh weight (g)	Dry weight (g)	Moist-ure (%)
Control	10.40 ± 0.15	9.30 ± 0.25	6.50 ± 0.17	0.1501 ± 0.002	0.0170 ± 0.001	88.67 ± 0.46	17.06 ± 0.51	11.63 ± 1.02	7.33 ± 0.43	0.1685 ± 0.002	0.0194 ± 0.001	88.48 ± 0.74
5 mg l^{-1} copper	8.96* ± 0.17	2.42* ± 0.23	7.33* ± 0.24	0.1230* ± 0.004	0.0161 ± 0.001	86.91 ± 0.64	14.30* ± 0.50	3.09 ± 0.10	7.83 ± 0.43	0.1328* ± 0.003	0.0180 ± 0.001	85.53 ± 0.96
25 mg l^{-1} copper	6.66* ± 0.29	1.26* ± 0.03	7.66* ± 0.06	0.1206* ± 0.001	0.0157 ± 0.003	86.98* ± 0.22	10.33* ± 0.94	1.53 ± 0.13	7.86 ± 0.57	0.1265* ± 0.006	0.0194 ± 0.001	84.66* ± 0.48
50 mg l^{-1} copper	5.73* ± 0.24	1.03* ± 0.06	5.56* ± 0.12	0.1128* ± 0.002	0.0147 ± 0.001	86.96* ± 0.28	8.73* ± 0.24	1.28 ± 0.11	5.66 ± 0.12	0.1230* ± 0.008	0.0198 ± 0.002	84.10* ± 1.20
100 mg l^{-1} copper	4.86* ± 0.16	0.64* ± 0.01	5.13* ± 0.17	0.1106* ± 0.004	0.0138 ± 0.001	87.52* ± 0.57	7.20* ± 0.28	0.81* ± 0.01	5.33 ± 0.24	0.1216* ± 0.002	0.0204 ± 0.001	83.23* ± 1.30

Table - 2: Effect of copper on chlorophyll (total, a and b) and a/b ratio in wheat (*Triticum aestivum*)

Treatments	14th Day				21st Day			
	Chlorophyll (mg/gm)			Chl. a/b	Chlorophyll (mg/gm)			Chl. a/b
	a	b	Total	Ratio	a	b	Total	Ratio
Control	0.950 ± 0.013	0.556 ± 0.010	1.605 ± 0.034	1.70	0.959 ± 0.021	0.598 ± 0.013	1.724 ± 0.011	1.60
5 mg l^{-1}copper	0.934 ± 0.004	0.547 ± 0.008	1.581 ± 0.015	1.70	0.700* ± 0.006	0.487* ± 0.003	1.288* ± 0.009	1.43
25 mg l^{-1}copper	0.812* ± 0.002	0.533 ± 0.004	1.416* ± 0.019	1.51	0.574* ± 0.024	0.448* ± 0.013	1.123* ± 0.011	1.28
50 mg l^{-1}copper	0.776* ± 0.030	0.489* ± 0.029	1.366* ± 0.060	1.58	0.557* ± 0.003	0.386* ± 0.005	1.077* ± 0.028	1.44
100 mg l^{-1}copper	0.647* ± 0.022	0.450* ± 0.017	1.242* ± 0.032	1.36	0.507* ± 0.001	0368* ± 0.004	0.985* ± 0.003	1.37

Table - 3: Effect of copper on pheophytin (total, a and b) and total carotenoid in wheat (*Triticum aestivum*)

Treatments	14th Day				21st Day			
	Pheophytin mg g^{-1} (F. wt.)			Total carotenoid (mg/gm F.wt.)	Pheophytin mg g^{-1} (F. wt.)			Total carotenoid (mg/gm F.wt.)
	a	b	Total		a	b	Total	
Control	0.728 ± 0.001	0.608 ± 0.004	1.336 ± 0.004	0.328 ±0.005	0.832 ± 0.008	0.644 ± 0.001	1.476 ± 0.007	0.250 ±0.002
5 mg l^{-1}copper	0.689* ± 0.002	0.586* ± 0.001	1.275* ± 0.001	0.366 ±0.002	0.788* ± 0.003	0.582* ± 0.004	1.370* ± 0.001	0.244 ±0.012
25 mg l^{-1}copper	0.673* ± 0.006	0.542* ± 0.001	1.215* ± 0.005	0.407* ±0.006	0.749* ± 0.003	0.526* ± 0.002	1.275* ± 0.005	0.207* ±0.004
50 mg l^{-1}copper	0.659* ± 0.002	0.523* ± 0.003	1.182* ± 0.005	0.425* ±0.001	0.713* ± 0.007	0.490* ± 0.001	1.203* ± 0.007	0.187* ±0.002
100 mg l^{-1}copper	0.605* ± 0.008	0.502* ± 0.004	1.107* ± 0.010	0.464* ±0.003	0.669* ± 0.028	0.472* ± 0.010	1.141* ± 0.039	0.158* ±0.003

The averages of three replicates ± S.E and (*) statistically significant at $p < 0.05$ level

Table - 4: Effect of copper on α, β and total amylase activity in wheat (*Triticum aestivum*)

Treatments	14th Day			21st Day		
	Amylase			Amylase		
	α	β	Total	α	β	Total
Control	24.80 ± 0.69	4.93 ± 0.35	29.73 ± 0.35	8.80 ± 0.24	5.60 ± 0.06	14.40 ± 0.23
5 mg l^{-1}copper	22.74 ± 0.78	5.06 ± 0.48	27.80* ± 0.30	6.80* ± 0.17	4.66 ± 0.30	11.46* ± 0.13
25 mg l^{-1}copper	22.53 ± 1.06	4.80 ± 0.40	27.33* ± 0.66	6.75* ± 0.01	4.26* ± 0.13	11.01* ± 0.13
50 mg l^{-1}copper	17.60* ± 1.05	4.76 ± 0.26	21.86* ± 0.87	6.60* ± 0.11	3.26* ± 0.01	9.86* ± 0.35
100 mg l^{-1}copper	15.54* ± 0.26	4.46* ± 0.13	20.00* ± 0.40	3.74* ± 0.35	1.86* ± 0.26	5.60* ± 0.40

Where – amylase activity in starch hydrolyzed mg g^{-1} fresh weight of tissue, Three replicates ± S.E and (*) statistically significant at $p< 0.05$ level

Table - 5: Effect of copper on catalase activity, peroxidase activity, protein and sugar content in wheat (*Triticum aestivum*)

Treatments	14th Day				21st Day			
	Catalase	Peroxidase	Protein	Sugar	Catalase	Peroxidase	Protein	Sugar
Control	97.33 ± 5.81	42.78 ± 1.31	80.69 ± 1.47	5.53 ± 0.14	140.00 ± 7.42	44.32 ± 1.72	81.37 ± 1.15	4.81 ± 0.04
5 mg l^{-1}copper	134.66* ± 7.42	44.74 ± 0.15	66.75* ±4.24	4.76* ± 0.14	245.33 ± 2.66	49.86* ± 0.46	67.06* ± 2.18	4.49* ± 0.09
25 mg l^{-1}copper	161.33* ± 9.61	45.77 ± 0.65	60.41* ± 1.28	3.69* ± 0.63	274.66* ± 11.85	52.44* ± 1.33	62.31* ± 2.90	3.40* ± 0.57
50 mg l^{-1}copper	216.00* ± 6.11	50.69* ± 0.23	56.41* ± 1.09	3.68* ± 0.04	278.66* ± 17.33	55.10* ± 2.34	54.92* ± 4.85	2.79* ± 0.13
100 mg l^{-1}copper	232.00* ± 6.92	59.72* ± 1.04	48.48* ± 0.79	2.86* ± 0.10	300.66* ± 1.76	61.52* ± 2.11	46.47* ± 5.03	2.15* ± 0.06

Where – catalase activity in ml H_2O_2 hydrolysed/gm fresh weight, peroxidase activity in Δ O.D. gm^{-1} fresh weight, and protein and sugar content in μg mg^{-1} fresh weight tissue. The average value of three replicates ± S.E and (*) statistically significant at $p< 0.05$ level

peroxidase activity was found in seedlings on exposure to 5 mg l^{-1} copper and above that producing, noticeably higher amount of catalase and peroxidase than control. The plants with 100 mg l^{-1} copper treatment showed the notably higher catalase and peroxidase activity levels than all other treatments (Table 5). In leaf tissue significant decline was evident in protein and sugar content in each treatment. Protein and sugar were appreciably reduced at the higher concentration of copper exposure (Table 5).

The various treatments of copper were found to be toxic at sub optimal (5 mg l^{-1}) to supra optimal (25-100 mg l^{-1}) concentrations. A significant reducing effect was found in wheat (*T. aestivum,* L. Var PBW 343) germination percent, plumule length, radicle length, number of lateral roots, fresh weight, dry weight and moisture with increased copper concentration. A slight increase was observed in germination percentage in 5 mg l^{-1} of copper concentration. At the supra optimal copper level (*i.e.*,100 mg l^{-1} Cu) only 60% seed germination was observed (Table 1). Copper is a constituent of certain enzymes such as cyctochrome oxidase, polyphenol oxidase, tyrosinase, amine oxidase and superoxide dismutase. Copper content of whole plants exceed 20 ppm (on dry weight basis) and this value is most often considered to indicate the threshold limits. Allaway (1968) reported normal plants to contain copper higher than 5 to 20 μg g^{-1} dry weight. Saravanan *et al*., 2001 observed that supra optimal concentration of copper drastically inhibited seed germination, growth, biomass and yield of soybean crop. Copper was found accumulated in the roots, with restricted transport to foliage in English Oak *Quercus robur* seedlings. Prolonged exposure of copper caused gross perturbations of root morphology, and finally reduced root and shoot growth (Wisniewski and Dickinson, 2003). Copper was relatively more harmful than nickel to both seed germination and seedling growth of *Raphanus sativus* L. var. Pusa chetki (Gupta *et al*., 2001). The similar effect of Cu containing textile, dye and printing industry effluent on germination and growth performance of two Rabi crops namely, wheat and chickpea was studied by Kaushik *et al*. (2005) and Kumawat *et*

al. (2001). Dragun and Baker (1982) concluded that copper concentrations in the above ground parts of maize are not suitable indicator of copper toxicity. The sensitivity of wheat to the toxicity of the copper and cadmium pollutants was in the order of root elongation > shoot elongation > germination rate (Wang and Zhou, 2005 and Smirnoval *et al*., 2006). The poor germination rate and seedling growth in present study seems to be due to the poor break down of starch by low amylase activity as amylase activity in seeds under the influence of different concentrations of copper solution was found to be decreased in comparison to control. Our results regarding the inhibition of seed germination and seedling growth in copper treatments can be correlated to the decreased amylase activity as worked out by Thevenot *et al*. (1992). Amylase and its role during seed germination through hydrolysis of reserve starch and release of energy have been extensively worked out by Thevenot and his co-workers in 1992. Studies performed by others (Dunn, 1974; Chang, 1982) on the role of amylase correlation with the increased seed germination is due to more hydrolysis of starch and release of energy in different test plants. Copper application to agricultural land in quantities greatly in excess of that required by crops is very common in the case of sewage sludge application (McBride, 1995). It is well known that catalase and peroxidase play an important role in preventing oxidative stress by catalyzing the reduction of hydrogen peroxide (Weckx and Clijsters, 1996). Devi and Prasad (1998) found that catalase and peroxidase activities were increased on copper treatment, suggesting that excess copper may increase the production of hydrogen peroxide (H_2O_2). H_2O_2 is a necessary substrate for the cell wall stiffening process catalyzed by cell wall peroxidase (Schopfer, 1996), which is considered to be one of the mechanisms resulting in growth inhibition.Catalase is an enzyme involved in antioxidant defense that eliminates hydrogen peroxide.

The supra optimal copper concentration in leaf tissue significantly declined the total of protein and sugars contents in each treatment in all over 14th and 21st days of treatment. Protein and sugar were significantly reduced in this cereal wheat (*T. aestivum)* as reported by Tandon and Gupta (2002), at increased doses of heavy metals. Metal induced inhibition of protein synthesis was also earlier reported by Samantary (2000). The decrease in protein content in *Albizia lebbak* has been interpreted either due to reduced *de novo* synthesis of proteins or increased decomposition of proteins into amino acids (Tripathi and Tripathi, 1999).

Presently, increased industrialization in developing countries like India has resulted into enormous deterioration of air, water and soil. Liquid effluents are frequently released in water bodies contains a number of pollutants including metals. The agricultural crops irrigated with such water are largely affected by metal (Cu) toxicity. Wheat is an important edible crop. The copper at lower concentration < 5 mgl^{-1} works as nutrient in plants but when its concentration increases in water and soil it causes copper toxicity. The various parameters like germination, amylase, pigment contents, protein and sugar contents were decreased at high concentration. While antioxidants like catalase and peroxidase were increased. Ultimately the crop production is decreased and affects food production and quality of wheat grains.

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