Effect of copper on seed germination, root elongation and shoot elongation of seedlings of commercially cultivated tea varieties

Sima Mandal, Aniruddha Saha¹ and Dipanwita Saha* Plant Biotechnology Laboratory, Department of Biotechnology, University of North Bengal, Siliguri-734013, India. ¹Molecular Plant Pathology and Fungal Biotechnology Laboratory, Department of Botany, University of North Bengal, Siliguri-734013, India.

Abstract

Tea (*Camellia sinensis* L. (O.) Kuntze) is an economically important plantation crop of India but is prone to attack by several fungal pathogens. Copper based fungicides are being used for decades to control fungal disease in tea which may lead to accumulation of copper in the soil. The aim of the present work was to investigate toxic effect of Cu^{2+} on seed germination, growth and morphological changes in tea seedlings. Different concentration of copper sulphate was applied on three different commercially cultivated tea varieties (TS-462, TS-520 and TS-463). The effect of different concentrations of copper (0.5 - 8 mM) on seed germination, root elongation and shoot elongation of the tea plant were evaluated. Percent germination was found to decrease progressively with increasing concentrations of Cu^{2+} . Maximum reduction of seed germination and reduction in length of root and shoot were observed when the germinated seedlings were allowed to grow at higher concentrations of copper solutions. Decrease in the dry mass of both root and shoot were also recorded. The results showed that excess copper have negative effect on germination of tea seeds and subsequent growth of the seedlings.

Key words: Tea seedlings, copper, germination, growth.

Introduction

Tea (Camellia sinensis (L.) O. Kuntze) is the most important and popular non-alcoholic beverage. Seeds or vegetative clones are the main sources of tea plant propagation. Successful production of crops is ordinarily associated with healthy shoot and root growth. Tea plants are prone to attack by several fungal pathogens which cause diseases such as blister blight, brown blight, grey blight, black rot, pink disease and thread blight leading to major economic losses (Tripathi 2006). To control the various diseases, copper-fungicides are used excessively in tea gardens of north-east India including Assam and sub-Himalayan West Bengal (Barua 1988, Singh 2005). The fungicides that are used most commonly include basic copper sulphate, Bordeaux mixture, Bicoxy (copper oxychloride 50% WP) and various customized formulations of copper sulphate and copper oxychloride (Worthing 1983, Sanjay et al. 2008). Agricultural practices

Corresponding author: E-mail: dsahanbu@yahoo.com with a long history of copper fungicide application have resulted in high levels of copper in soil that has affected a large portion of agricultural land (Brun *et al.* 1998).

Copper is a constituent of certain enzymes such as cyctochrome oxidase, polyphenol oxidase, tyrosinase, amine oxidase and superoxide dismutase. Thus copper in trace amount is essential for various metabolic processes in the plant but at higher concentrations it causes physiological stress (Dat et al. 2000, Saha et al. 2012). Copper content of whole plants exceed 20 ppm (on dry weight basis) and this value is most often considered to indicate the threshold limits (Singh et al. 2007). High levels of Cu2+ application to soil and leaves have been found to disrupt normal plant growth. The toxic effect of Cu²⁺ has been attributed to the redox nature of this element that induces over-production of reactive oxygen species which in turn interferes with the photosynthetic electron transport and damages the cell ultrastructure (Babu et al. 2001, Yruela 2005 and Quian et al. 2009).

Applications of high levels of Cu^{2+} usually inhibit root growth and shoot production (Wisniewski and Dickinson, 2003, Peralta *et al.* 2000, Lombardi & Sebastiani, 2005). Sonmez *et al.* (2006) reported an increasing reduction in total yield, fruit number, dry root weight and plant height with increasing levels of Cu^{2+} application to soil and leaves. Increased copper concentrations have been reported to reduce percent germination, root and shoot elongation in maize (Hunter 1981), tomato (Mazhoudi *et al.* 1997), citrus (Alva *et al.* 2000), wheat (Singh *et al.* 2007), rice (Chen *et al.*2000, Lidon & Henriques, 1992) etc.

An initial survey of several tea gardens of North Bengal has revealed that copper-fungicides are extensively used in the tea gardens of the Dooars and Terai region and also in the Hilly region of West Bengal. This extensive use of copper over a long time has necessitated research on effect of copper on tea plants. The aim of the present work was to investigate toxic effect of Cu²⁺ on seed germination, growth and morphology of tea seedlings.

Materials and Methods

Plant Materials and Chemicals: Tea seeds of three different biclonal seed stocks (viz. TS-520, TS-463 and TS-462) were procured from Gayaganga Tea Estate, Siliguri, India. Sixteen different concentrations (0.5–8.0 mM) with 0.5 mM increments of copper solutions were prepared freshly and used for application. Control sets were treated with distilled water.

Application of copper and germination of tea seeds: To determine the percent germination in the treated and untreated seeds of TS-520, TS-463 and TS-462, metal application procedure as described by Munzuroglu and Geckil (2002) was followed with some modifications. Seed surfaces were sterilized with 0.01% HgCl₂ washed twice with sterile distilled water and sowed in earthen pots (6 cm diameter) containing moist sterilized sand. The pots were placed in dark in growth chamber at 25°C. Following rupturing of seeds which occurred after 20 d, the seeds were placed in sand and wetted with 4 ml of Cu²⁺ solution of each concentration. The pots were kept at 25°C in

the growth chamber and the germination was noted every 24h. Seeds were considered to be germinated at one mm of radical emergence and percent germination was calculated. The length of the shoots as well as roots of each variety was recorded at 15, 18, 21, 24 and 27d after treatment and deformities, if any were noted. The dry weight of treated and untreated shoots and roots of the tested varieties were measured after 27d of treatment. This time duration was followed based on observations obtained during preliminary studies which showed that this time was necessary to test appropriate inhibitory effects. A set of plants (exposed to 6.5 mM Cu²⁺ concentration) were allowed to grow until 60 days for observing the progressive deformations in the roots over a longer period.

Results and Discussion

Figure 1 summarizes the effect of different concentrations of copper on seed germination of three tested tea seed varieties of north east India. In general there was a progressive reduction in seed germination as metal concentration increased in all the tested varieties. Minimum seed germination was recorded when 8 mM copper solution was applied. Germination percentage was found to be lowest in TS-463 (17%) and highest in TS-462 plants (25%). Peralta *et al.* (2000) investigated the individual effects of several doses of heavy metals on the growth of live alfalfa plants using solid media. They used 0, 5, 10, 20 and 40 ppm doses for experiment and according to them 20 ppm concentration of Cu²⁺ significantly affected

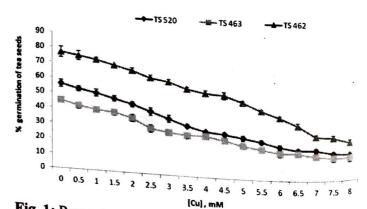


Fig. 1: Percent germination of seeds of TS-520, TS-463 and TS-462 at different concentrations of copper solution.

the seed germination and plant growth. Claire *et al.* (1991) obtained similar results in a study using copper and other heavy metals on cabbage, lettuce, millet, radish, turnip, wheat and alfalfa plants. Singh *et al.* (2007) observed that the germination (60%), plumule and radicle length, and number of lateral roots of wheat decreased with increase in copper concentration (5, 25, 50 and 100 mgl⁻¹) after 14th and 21st day of treatment.

Both root and shoot elongation was hampered with increase in the concentration of Cu²⁺ for all the three varieties. Maximum reduction in length of shoot (91%) was observed in TS-463 variety followed by TS-520 (86%) and TS-462 (68%) (Tables 1, 2, 3). Root elongation was most affected in TS-462 (96%) followed by TS- 463 (91%) and TS-520 (84%) (Tables 4, 5, 6). Higher concentration of Cu²⁺ (>6.5 mM) showed several damaging effects such as reduced root hair proliferation, reduction in the number of root hairs, blacking of the root tips, stunted growth, deformed root and shoot structure and substantial reduction in the length of the root and shoots in all tested varieties (Fig. 2). Sheldon and Menzies (2004) observed that excess copper in Chloris gayana seeds caused

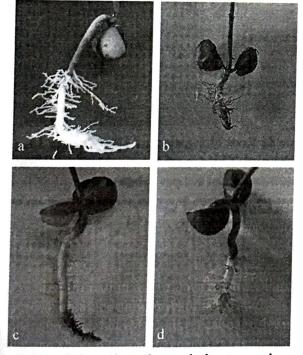


Fig. 2: Root deformations observed when tea seeds were allowed to germinate in high Cu^{2+} concentrations (6.5 mM) for 60 days. a: Control, b: TS-463; c: TS-520; d: TS-462.

damage to the cuticle on the main root, reduction in the number and length of root hairs on the main root and damage to the root meristem. According to Peralta *et al.* (2000), Cu (II) exerts detrimental effects at the dose of 40 ppm and 10 ppm causing a shoot and root elongation reduction of 70.0%, 54% respectively in (*Medicago sativa*). Ali *et al.* (2006) observed that root treated with 50 μ M copper resulted in 52% and 89% growth inhibition after 20 & 40 days of treatment respectively in *Panax ginseng*. Manivasagaperumal *et al.* (2011) reported that 100-200 mg/kg concentration of copper reduces the growth of the shoots and roots of *Vigna radiata*.

Effect of high concentrations of copper on dry weight of shoots and roots were also tested and the results are summarized in Fig 3 & 4. Dry weight of both shoots and roots were decreased with increasing concentration of Cu^{2+} . Dry weight of roots reduced to 0.042 mg, 0.013 mg and 0.017 mg in tea seedlings treated with 8 mM concentration

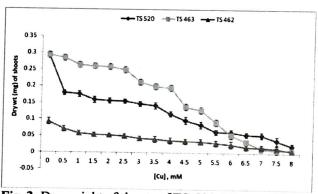


Fig. 3: Dry weight of shoots of TS-520, TS-463 and TS-462 at different concentrations of copper solution after 27 days of treatment

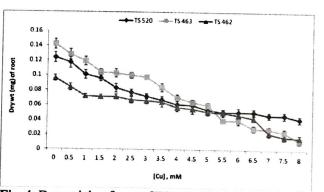


Fig. 4: Dry weight of root of TS-520, TS-463 and TS-462 at different concentrations of copper solution after 27 days of treatment

Table 1: Shoot elongation of TS-520 after 15, 18, 21, 24 and 27 days of treatment with different concentrations of copper solution

Concentration of CuSO ₄ (mM)	Shoot length variety (in cm) was measured at different days after treatment in TS520					
	15d	18d	21d	24d	27d	
0	9.2 ± 0.5	10.4 ± 0.45	11.2 ± 0.3	13.5 ± 0.25	15.2 ± 0.3	
0.5	6.7 ± 0.3	8.4 ± 0.3	9.6 ± 0.3	10.3 ± 0.5	10.6 ± 0.45	
1	6.5 ± 0.4	7.9 ± 0.2	8.9 ± 0.5	10.1 ± 0.65	10.5 ± 0.35	
1.5	6.2 ± 0.4	7.2 ± 0.3	8.9 ± 0.2	10.1 ± 0.45	10.5 ± 0.1	
2	5.7 ± 0.25	7.2 ± 0.15	8.7 ± 0.3	9.2 ± 0.4	9.9 ± 0.5	
2.5	5.2 ± 0.2	6.3 ± 0.5	7.8 ± 0.15	8.8 ± 0.4	9.1 ± 0.15	
3	4.7 ± 0.3	6.3 ± 0.4	7.3 ± 0.4	8.3 ± 0.5	9 ± 0.25	
3.5	4.6 ± 0.3	6.2 ± 0.4	7.1 ± 0.3	8.3 ± 0.55	8.8 ± 0.5	
4	4.6 ± 0.3	5.8 ± 0.4	6.7 ± 0.4	7.2 ± 0.15	8.1 ± 0.2	
4.5	4.6 ± 0.2	5.5 ± 0.2	6.4 ± 0.5	6.9 ± 0.6	7.7 ± 0.3	
5	3.6 ± 0.2	4.9 ± 0.7	5.2 ± 0.2	6.3 ± 0.6	6.5 ± 0.4	
5.5	3.5 ± 0.3	4.2 ± 0.5	5.2 ± 0.2	5.4 ± 0.25	6.2 ± 0.4	
6	2.1 ± 0.1	3.6 ± 0.4	4.8 ± 0.45	5.3 ± 0.2	5.7 ± 0.4	
6.5	2.1 ± 0.45	3.6 ± 0.2	4.2 ± 0.2	4.7 ± 0.1	5.1 ± 0.4	
7	1.5 ± 0.45	3.1 ± 0.3	3.8 ± 0.2	4.2 ± 0.3	4.6 ± 0.35	
7.5	1.5 ± 0.1	2.7 ± 0.3	3.2 ± 0.3	4.2 ± 0.2	4.5 ± 0.3	
8	1.2 ± 0.3	1.4 ± 0.3	1.6 ± 0.2	1.8 ± 0.5	2 ± 0.4	

Table 2: Shoot elongation of TS-463 after 15, 18, 21, 24 and 27 days of treatment with different concentrations of copper solution

Concentration of CuSO4 (mM)	Shoot length (in cm) was measured at different days after treatment in TS-463					
	15d	18d	21d	24d	27d	
0	5.2 ± 0.5	6.5 ± 0.3	7.5 ± 0.3	9.8 ± 0.3	11.5 ± 0.7	
0.5	5.1 ± 0.3	6.3 ± 0.1	7.1 ± 0.2	9.5 ± 0.45	11.5 ± 0.55	
1	4.4 ± 0.3	6 ± 0.4	6.8 ± 0.1	9.2 ± 0.45	11.1 ± 0.6	
1.5	4.3 ± 0.5	5.6 ± 0.4	6.4 ± 0.2	9.2 ± 0.2	10.5 ± 0.5	
2	4.2 ± 0.2	5.3 ± 0.2	6.3 ± 0.1	9.2 ± 0.5	10.2 ± 0.5	
2.5	4.1 ± 0.4	5.2 ± 0.2	6.3 ± 0.2	7.7 ± 0.5	10.1 ± 0.2	
3	3.7 ± 0.4	5.1 ± 0.25	6.1 ± 0.2	7.7 ± 0.4	9.4 ± 0.5	
3.5	3.3 ± 0.2	4.2 ± 0.1	5.9 ± 0.1	7.5 ± 0.4	8.5 ± 0.5	
4	3.1 ± 0.3	3.9 ± 0.4	5.9 ± 0.1	7.1 ± 0.4	8.3 ± 0.2	
4.5	3.1 ± 0.2	3.7 ± 0.2	4.9 ± 0.2	6.9 ± 0.3	7.3 ± 0.2	
5	2.1 ± 0.2	3.2 ± 0.1	4.3 ± 0.1	6 ± 0.3	6.3 ± 0.25	
5.5	1.8 ± 0.2	2.9 ± 0.15	4.2 ± 0.1	5.8 ± 0.3	6.2 ± 0.1	
6	1.3 ± 0.2	2.8 ± 0.1	4 ± 0.2	4.8 ± 0.3	5.1 ± 0.1	
6.5	1.2 ± 0.15	1.5 ± 0.2	1.9 ± 0.1	2.3 ± 0.2	2.7 ± 0.1	
7	1 ± 0.1	1.2 ± 0.2	1.8 ± 0.1	2.1 ± 0.1	2.5 ± 0.1	
7.5	0.4 ± 0.1	1.1 ± 0.15	1.3 ± 0.1	1.9 ± 0.15	2.1 ± 0.1	
8	0.3 ± 0.2	0.6 ± 0.45	0.7 ± 0.1	0.9 ± 0.25	1 ± 0.3	

Table 3: Shoot elongation of TS-462 after 15, 18, 21, 24 and 27 days of treatment with different concentrations of copper solution

Concentration of CuSO ₄ (mM)	Shoot length (in cm) was measured at different days after treatment in TS-462					
	15 d	18 d	21d	24d	27d	
0	4.4 ± 0.5	4.9 ± 0.15	5.5 ± 0.3	6.1 ± 0.1	7±0.2	
0.5	4.1 ± 0.4	4.8 ± 0.2	5.1 ± 0.5	5.9 ± 0.4	6.6 ± 0.2	
1	3.9 ± 0.2	4.2 ± 0.5	4.2 ± 0.3	5.3 ± 0.3	6.2 ± 0.3	
1.5	2.6 ± 0.1	3.8 ± 0.4	3.9 ± 0.2	4.2 ± 0.2	4.9 ± 0.1	
2	2.5 ± 0.2	3.1 ± 0.2	3.8 ± 0.2	4 ± 0.3	4.5 ± 0.1	
2.5	2.2 ± 0.4	3.1 ± 0.3	3.7 ± 0.1	4 ± 0.1		
3	2.1 ± 0.1	3 ± 0.3	3.6 ± 0.4	3.9 ± 0.2	4.5 ± 0.4	
3.5	1.8 ± 0.3	2.9 ± 0.6	3.1 ± 0.1	3.7 ± 0.1	4.3 ± 0.1	
4	1.4 ± 0.3	2.5 ± 0.3	3.1 ± 0.1	3.7 ± 0.1	4.2 ± 0.1	
4.5	1.3 ± 0.1	2.4 ± 0.3	3.1 ± 0.15	3.7 ± 0.1	4.2 ± 0.1	
5	1.3 ± 0.2	2.3 ± 0.4	3 ± 0.5	3.1 ± 0.3	4.1 ± 0.15	
5.5	1.2 ± 0.1	1.9 ± 0.4	2.5 ± 0.3	2.9 ± 0.3	3.9 ± 0.1	
6	1.1 ± 0.1	1.6 ± 0.2	2.1 ± 0.1	2.9 ± 0.3 2.4 ± 0.2	3.2 ± 0.3	
6.5	1.1 ± 0.1	1.6 ± 0.15	1.9 ± 0.1		2.8 ± 0.4	
7	1 ± 0.1	1.5 ± 0.1	1.9 ± 0.1	2.3 ± 0.4	2.8 ± 0.2	
7.5	0.9 ± 0.1	1.5 ± 0.3	1.9 ± 0.2 1.9 ± 0.25	2.1 ± 0.15	2.5 ± 0.1	
8	0.7 ± 0.1	1.3 ± 0.5	1.9 ± 0.25 1.8 ± 0.3	2 ± 0.2	2.3 ± 0.2	
		1.5 - 0.5	1.0 ± 0.3	2 ± 0.2	2.2 ± 0.1	

Table 4: Root elongation of TS-520 after 15, 18,21, 24 and 27 days of treatment with differentconcentrations of copper solution

Concentration of	Root length (in cm) was measured at different days after treatment in TS-520					
CuSO4 (mM)	15 d	18 d	21d	24d	27d	
0	4.5 ± 0.3	6.8 ± 0.3	7.5 ± 0.6	-7.7 ± 0.1	7.8 ± 0.1	
0.5	4.3 ± 0.2	5.2 ± 0.3	5.9 ± 0.3	6.2 ± 0.2	6.7 ± 0.1	
1	4.1 ± 0.2	5.1 ± 0.1	5.3 ± 0.3	5.7 ± 0.2	6.2 ± 0.2	
1.5	4.1 ± 0.2	4.5 ± 0.2	4.8 ± 0.2	5.5 ± 0.6	5.7 ± 0.1	
2	3.2 ± 0.1	4.2 ± 0.4	4.5 ± 0.45	5 ± 0.3	5.3 ± 0.1	
2.5	3.2 ± 0.15	3.9 ± 0.35	4.3 ± 0.55	4.7 ± 0.2	5.1 ± 0.1	
3	3.2 ± 0.2	3.5 ± 0.3	3.9 ± 0.5	4.1 ± 0.1	4.3 ± 0.2	
3.5	2.9 ± 0.3	3.1 ± 0.1	3.5 ± 0.3	3.6 ± 0.1	3.8 ± 0.1	
4	2.7 ± 0.2	3.1 ± 0.4	3.3 ± 0.4	3.4 ± 0.3	3.8 ± 0.1	
4.5	2.7 ± 0.4	2.9 ± 0.4	3 ± 0.3	3.2 ± 0.1	3.5 ± 0.1	
5	2.2 ± 0.1	2.8 ± 0.4	3 ± 0.5	3.1 ± 0.25	3.3 ± 0.1	
5.5	2.1 ± 0.3	2.3 ± 0.2	2.6 ± 0.2	2.7 ± 0.1	2.9 ± 0.2	
6	2.1 ± 0.06	2.3 ± 0.15	2.4 ± 0.1	2.6 ± 0.1	2.8 ± 0.1	
6.5	1.5 ± 0.3	1.9 ± 0.3	2 ± 0.1	2.2 ± 0.3	2.5 ± 0.2	
7	1.4 ± 0.3	1.7 ± 0.2	2 ± 0.25	2.1 ± 0.3	2.4 ± 0.1	
7.5	1 ± 0.06	1.6 ± 0.35	1.8 ± 0.15	1.9 ± 0.2	2.1 ± 0.1	
8	0.6 ± 0.3	0.7 ± 0.2	0.9 ± 0.1	1 ± 0.1	1.2 ± 0.1	

Table 5: Root elongation of TS-463 after 15, 18,21, 24 and 27 days of treatment with differentconcentrations of copper solution

Concentration of CuSO ₄ (mM)	Root length (in cm) was measured at different days after treatment in TS-463						
,	15 d	18 d	21d	24d	27d		
0	3.8 ± 0.1	4.5 ± 0.2	6.3 ± 0.3	7.1 ± 0.2	8±0.15		
0.5	2.9 ± 0.5	3.1 ± 0.3	3.8 ± 0.3	4.7 ± 0.2	5.1 ± 0.1		
1	2.5 ± 0.1	3.1 ± 0.1	3.6 ± 0.3	4.2 ± 0.15	4.9 ± 0.3		
1.5	2.1 ± 0.3	2.9 ± 0.5	3.4 ± 0.3	4.1 ± 0.1	4.5 ± 0.3		
2	2.1 ± 0.2	2.8 ± 0.6	3.2 ± 0.6	3.7 ± 0.1	4.4 ± 0.15		
2.5	1.8 ± 0.4	2.1 ± 0.1	2.9 ± 0.2	3.3 ± 0.3	4.1 ± 0.15		
3	1.7 ± 0.1	2.1 ± 0.5	2.8 ± 0.3	3.1 ± 0.15	3.8 ± 0.1		
3.5	1.3 ± 0.2	1.9 ± 0.1	2.7 ± 0.1	3 ± 0.2	3.2 ± 0.1		
4	1.3 ± 0.25	1.8 ± 0.2	2 ± 0.15	2.5 ± 0.2	2.9 ± 0.15		
4.5	1.3 ± 0.2	1.7 ± 0.1	2 ± 0.75	2.4 ± 0.1	2.9 ± 0.15		
5	1 ± 0.2	1.6 ± 0.2	1.8 ± 0.1	2.1 ± 0.1	2.8 ± 0.1		
5.5	1 ± 0.4	1.4 ± 0.1	1.7 ± 0.2	2.1 ± 0.2	2.5 ± 0.1		
6	0.9 ± 0.2	1.1 ± 0.1	1.6 ± 0.2	2 ± 0.1	2.2 ± 0.1		
6.5	0.9 ± 0.3	1 ± 0.2	1.3 ± 0.2	1.9 ± 0.2	2.1 ± 0.1		
7	0.7 ± 0.1	0.9 ± 0.45	1.1 ± 0.15	1.3 ± 0.1	1.6 ± 0.1		
7.5	0.6 ± 0.1	0.8 ± 0.3	0.8 ± 0.1	0.9 ± 0.2	1 ± 0.1		
8	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.9 ± 0.2 0.4 ± 0.15	0.7 ± 0.1		

Table 6: Root elongation of TS-462 after 15, 18, 21, 24 and 27 days of treatment with different concentrations of copper solution

Concentration of CuSO ₄ (mM)	Root length (in cm) was measured at different days after treatment in TS-462					
	15 d	18 d	21d	24d	27d	
0	3.8 ± 0.1	4.7 ± 0.2	5.2 ± 0.1		6.5 ± 0.1	
0.5	3.5 ± 0.1	3.8 ± 0.1	3.2 ± 0.1 4.1 ± 0.1	5.8 ± 0.3	4.4 ± 0.15	
1	3.5 ± 0.1	3.8 ± 0.1	4.1 ± 0.1 4 ± 0.2	4.2 ± 0.15	4.4 ± 0.15 4.3 ± 0.15	
1.5	3.1 ± 0.2	3.1 ± 0.1		4.1 ± 0.2		
2	2.8 ± 0.1		3.5 ± 0.1	3.8 ± 0.25	4 ± 0.2	
2.5	2.8 ± 0.1	3 ± 0.2	3 ± 0.1	3.2 ± 0.2	3.5 ± 0.1	
3		2.9 ± 0.1	2.9 ± 0.1	3.1 ± 0.2	3.5 ± 0.2	
3.5	2.6 ± 0.15	2.8 ± 0.1	2.8 ± 0.1	3.1 ± 0.1	3.3 ± 0.1	
4	2.5 ± 0.15	2.7 ± 0.1	2.8 ± 0.2	3 ± 0.1	3.3 ± 0.15	
4.5	2.5 ± 0.1	2.7 ± 0.1	2.8 ± 0.4	3 ± 0.2	3.2 ± 0.1	
5	2.4 ± 0.2	2.6 ± 0.2	2.8 ± 0.3	3 ± 0.15	3.2 ± 0.15	
5.5	1.8 ± 0.1	2.1 ± 0.2	2.7 ± 0.1	2.8 ± 0.3	3.2 ± 0.2	
6	1.5 ± 0.1	2 ± 0.1	2.5 ± 0.2	2.5 ± 0.2	3.1 ± 0.1	
	1 ± 0.1	1.8 ± 0.1	2 ± 0.15	2.3 ± 0.2 2.1 ± 0.1	2.2 ± 0.1	
6.5	0.9 ± 0.1	1 ± 0.15	1.1 ± 0.1		1.5 ± 0.15	
7	0.8 ± 0.2	1 ± 0.1		1.4 ± 0.1	1.5 ± 0.1	
7.5	0.4 ± 0.1		1.1 ± 0.1	1.3 ± 0.2	1.5 ± 0.1	
8	0.2 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.2	
	1 0.2 2 0.1	0.2 ± 0.1	0.2 ± 0.05	0.2 ± 0.1	0.2 ± 0.1	

of Cu2+ in comparison to control which recorded 0.124 mg, 0.142 mg and 0.096 mg root dry weight of TS-520, TS-463 and TS-462 respectively. Dry weight in shoots also showed substantial reduction when exposed to high Cu²⁺ concentration. Zheng et al. (2004) worked on the response of three ornamental crops (Dendranthema grandiflorum L. 'Fina', Rosa hybrid L. 'Lavlinger', Pelargonium hortorun L. 'Evening glow') to different solution levels of Cu2+ (ranging from 0.4-40 μ M). They observed that excessive copper reduced the shoot and root dry weight of all three species. Manivasagaperumal et al. (2011) reported the decline of dry weight of Vigna radiata with increasing copper concentration of 100-200 mg/kg. According to Azooz et al. (2012), copper concentration above 10 mM reduced the dry weight of wheat.

From these observations it can be concluded that excess copper had some effect on germination, growth and dry matter yield of three commonly grown tea varieties. Varietal differences in response towards Cu2+ were observed in the shoot elongation study. However, in the roots, the differences was less prominent possibly due to the fact that Cu²⁺ accumulation occurs much more in the roots than in the shoots leading to a greater damage in the roots which minimizes the differential effect within varieties. Differences among cultivars in response to Cu2+ stress have been found in other plants such as Triticum durum (Ciscato et al. 1997), Holcus lanatus (Hartley-Whitaker et al. 2001) and Kummerowia stipulacea (Xiong et al. 2008). Inhibitory action of excess copper in root and shoot elongation and in their damage observed during the present study may be due to reduction in cell division, toxic effect on respiration and protein synthesis (Manivasagaperumal et al. 2011, Kupper et al. 1996 and Sonmez et al. 2006). Our results indicate that a detail study on the effect of excess copper on tea plants is warranted.

Acknowledgement

S. Mandal wishes to thank the University Grants Commission, India for Rajiv Gandhi National Fellowship (No.F.14-2(SC)/2008(SA-III).

References

- Ali MB, Hahn EJ and Paek KY (2006) Copperinduced changes in the growth, oxidative metabolism, and saponin production in suspension culture roots of *Panax ginseng* in bioreactors. Plant Cell Rep 25: 1122-1132.
- Alva AK, Huang B and Paramastuam S (2000) Soil pH affects copper fractionation and phytotoxicity. Soil Sci Soc Am J 64: 955-962.
- Azooz MM, Abou-Elhamd MF and Al-Fredan MA (2012) Biphasic effect of copper on growth, proline, lipid peroxidation and antioxidant enzyme activities of wheat (*Triticum aestivum* cv. Hasaawi) at early growing stage. Australian journal of crop science 6: 688-694.
- Babu TS, Marder JB, Tripuranthakam S, george dixon D, and Greenberg BM (2001) Synergistic effects of a photooxidized polycyclic aromatic hydrocarbon and copper on photosynthesis and plant growth: evidence that in vivo formation of reactive oxygen species is a mechanism of copper toxicity. Environ Toxicol Chem 20: 1351–1358.
- Barua KC (1988) Some aspects of disease control in tea. Field management in tea. Tea Research Association. Tocklai experimental station, pp. 119-124.
- Brun LA, Maillet J, Richarte J, Herrmann P and Remy JC (1998) Relationships between extractable copper, soil properties and copper uptake by wild plants in vineyard soils. Environ Pollut 102: 151-161.
- Chen LM, Lin CC and Kao CH (2000) Copper toxicity in rice seedling: Changes in antioxidative enzyme activities, H_2O_2 level and cell wall peroxidase activity in roots. Bot Bull Acad Sin 41: 99-103.
- Ciscato M, Valcke R, Loven KV, Clijsters H and Navari-Izzo F (1997) Effects of in vivo copper treatment on the photosynthetic apparatus of two *Triticum durum* cultivars with different stress sensitivity. Physiol Plant 100: 901-908.
- Claire LC, Adriano DC, Sajwan KS, Abel SL, Thoma DP, and Driver JT (1991) Effects of

selected trace metals on germinating seeds of six plant species. Water Air Soil Poll 59: 231-240.

- Dat J, Vandenabeele S, Vranova E, Van Montagu M, Inze D and Van Breusegem F (2000) Dual action of the active oxygen species during plant stress responses. Cell Mol Life Sci 57: 779-795.
- Hartley-Whitaker J, Ainsworth G and Meharg AA (2001) Copper-and arsenate-induced oxidative stress in *Holcus lanatus* L. clones with differential sensitivity. Plant Cell Environ. 24: 713-722.
- Hunter RB (1981) A reversible phase of copper toxicity in maize roots. J Plant Nutr 3: 375-386.
- Kupper H, Kupper F and Spiller M (1996) Environmental relevance of heavy metal substituted chlorophylls using the example of water plants. J Exp Bot 47: 259-266.
- Lidon FC and Henriques FS (1992) Copper toxicity in rice: diagnostic criteria and effect on tissue Mn and Fe. Soil Sci 154: 130-135.
- Lombardi L and Sebastiani L (2005) Copper toxicity in *Prunus cerasifera*: growth and antioxidant enzymes responses of in vitro grown plants. Plant Sci 168: 797-802.
- Manivasagaperumal R, Vijayarengan P, Balamurugan S and Thiyagarajan G (2011) Effect of copper on growth, dry matter yield and nutrient content of *Vigna radiata* (1.) wilczek. J Phytol 3: 53-62.
- Mazhoudi S, Chaoui A, Ghorbal NH, and El-ferjani E (1997) Response of antioxidant enzymes to excess copper in tomato (*Lycorpersicon esculentum*, Mill.). Plant Sci 127: 129-137.
- Munzuroglu O and Geckil H (2002) Effect of metals on seed germination, root elongation, and coleoptile and hypocotile growth in *Triticum aestivum* and *Cucumis sativus*. Arch Environ Contam Tixicol 43: 203-213.
- Peralta JR, Gardea-Torresdey JL, Tiemann KJ, Gomez E, Arteaga S, Rascon E, and Parsons JG (2000) Study of the effects of heavy metals

on seed germination and plant growth on alfalfa plant (*Medicago sativa*) grown in solid media. Proceedings of the 2000 Conference on Hazardous Waste Research, pp. 135-140.

- Qian H, Li J, Sun L, Chen W, Sheng GD, Liu W and Fu Z (2009) Combined effect of copper and cadmium on *Chlorella vulgaris* growth and photosynthesis-related gene transcription. Aquat Toxicol 94: 56-61.
- Saha D, Mandal S and Saha A (2012) Copper induced oxidative stress in tea (*Camellia* sinensis) leaves. J Environ Biol 33: 861-866.
- Sanjay R, Ponmurugan P and Baby UI (2008) Evaluation of fungicides and biocontrol agents against grey blight disease of tea in the field. Crop Prot 27: 689-694.
- Sheldon AR and Menzies NW (2004) The effect of copper toxicity on the growth and root morphology of Rhodes grass (*Chloris gayana* Knuth.) in solution culture. Super Soil, 3rd Australian New zealand soils conference, www.regional.org.au/au/asssi.
- Singh D, Nath K and Sharma YK (2007) Response of wheat seed germination and seedling growth under copper stress. J Environ Biol 28: 409-414.
- Singh, ID (2005) In the planters guide to tea culture and manufacture, NB Modern Agencies, Siliguri, India, pp. 132-138.
- Sonmez S, Kaplan M, Sonmez NK, Kaya H and Uz I (2006) High level of copper application to soil and leaves reduce the growth and yield of tomato plants. Sci Agric (Piracicaba, Braz.) 63: 213-218.
- Tripathi DP (2006) Introductory Mycology. 1st edition Kalyani Publishers, pp. 546-547.
- Wisniewski L and Dickinson NM (2003) Toxicity of copper to *Quercus robur* (English Oak) seedlings from a copper rich soil. Environ Exp Bot 50: 99-107.
- Worthing CR (1983) The pesticides manual: A world compendium.Croydon, England: The British Crop Protection Council, http://isbndb.com/d

publisher/british_crop_protection_counci.html

- Xiong ZT, Wang T, Liu K, Zhang ZZ, Gan JH, Huang Y and Li MJ (2008) Differential invertase activity and root growth between Cutolerant and non-tolerant populations in *Kummerowia stipulacea* under Cu stress and nutrient deficiency. Environ Exp Bot 62: 17-27.
- Yruela I (2005) Copper in plants. Braz J Plant Physiol 17: 145-156.
- Zheng Y, Wang L and Dixon MA (2004) Response to copper toxicity for three ornamental crops in solution culture. Hort Science 39: 1116-1120.