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Effect of increasing levels of soil copper on growth of young rubber *(Hevea brasiliensis)* plants

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Abstract

Although copper is a micro-nutrient which is essential for the growth of plants, its accumulation in soil can have toxic effects on plant growth. Copper fungicides are regularly used in many rubber plantations for controlling fungal diseases. A study was conducted to assess the copper sensitivity of young rubber plants. Three sets of polybag experiments were conducted in the glass house to study the effect of varying levels of copper in soil on growth of young rubber plants. For experiments 1 and 2, the treatments included 0, 10, 20, 30, 40, 50 and 100 mg Cu per kg soil, incorporated as $CuSO_4.5H_2O$ into the soil in the polybag. In the first experiment, treatments of copper were incorporated in the soil used for filling the polybags, before planting budded stumps. In the second experiment, treatment incorporation was carried out in the polybags, at the time of maturity of first whorl of leaves. The third experiment was conducted in soils with inherently low and high copper status, but comparable organic carbon status and pH. Low level of copper (10 mg kg^{-1} soil) had a positive effect on sprouting, growth and dry matter production of young plants when copper was incorporated in soil before planting budded stumps. This effect was not observed beyond the level of 10 mg Cu kg^{-1} soil. Beyond 30 mg Cu kg^{-1} soil, some of the growth parameters, such as height, leaf area and total dry matter were significantly decreased. Copper at a rate of 100 mg kg^{-1} soil adversely affected growth and dry matter production of plants. Copper content of leaf and stem did not increase with increasing levels of soil copper. However, copper content of root showed significant increase with increasing levels of soil copper. Though excess copper was accumulated in roots, it was not translocated to the shoot, indicating locking up in roots as a tolerance mechanism. Similar results were observed in Experiment 2 also where copper was incorporated at the stage of maturity of first whorl. When plants were grown in soils that were inherently low and high in available copper status, no significant difference in growth of plants was observed, indicating that soil copper content of 20 mg kg^{-1} does not adversely influence the growth of young plants of *Hevea*.

Keywords: Copper toxicity, *Hevea*, natural rubber, plant growth, soil

Introduction

Copper is a micro-nutrient which is essential for the growth of plants. As a component of different enzymes, it is involved in several physiological functions and catalyze redox reactions (Marschner, 1986; Hansh and Mendal, 2009). Although copper is essential for plant growth, its accumulation in soil can be toxic to plants (Anderson, 1988; Alva and Chen, 1995). Excess copper inhibits enzyme activity and affects plant biochemistry, including photosynthesis, pigment synthesis and membrane integrity (Fernandes and Henriques, 1991; Upadhyaya and Panda, 2009). Excess copper causes toxic effect in plant cells by over-producing oxy radicals (Sonmez *et al.,* 2006).

Most of the copper in soil is present in organically bound form, which is strongly retained in the uppermost soil layers (Hunter *et al*., 1987). Unlike other heavy metals, copper is not readily bio-accumulated, and therefore its toxicity to man and other animals is relatively low. Compared to animals, plants are more sensitive to Cu toxicity, showing growth retardation at Cu contents slightly higher than normal levels (Fernandes and Henriques, 1991). Copper phytotoxicity was reported in citrus orchards in Florida, orchards in

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Australia, banana plantations in Costa Rica, and is attributed as one of the reasons for the 'replanting sickness' of cocoa in West Africa (Ross, 1994).

Accumulation of copper in different plant parts increases with increase in the soil copper level (Pinochet *et al.,* 1999; Xiong and Wang, 2005). In non-tolerant plants, high levels of soil copper inhibits root elongation and damages root cell membranes (Wainwright and Woolhouse, 1977). Because of the adverse effect of copper on root elongation, the ability of the plant to explore the soil for water and nutrients is reduced (Wong and Bradshaw, 1982). This may be particularly important for young plants than mature trees as they are more sensitive to environmental stresses and grow in the contaminated superficial soil layer (Patterson and Olson, 1983).

In rubber plantations, copper is introduced into the soil mainly through the application of copper based fungicides. Use of commercial organic fertilizers such as city compost and sewage sludge also adds copper to the soil. Soil acidification enhances the mobility and bioavailability of copper in soil (Ulrich, 1994). A collaborative study conducted by Kerala Agricultural University (KAU) and Rubber Research Institute of India (RRII) during 1998-2000 on the impact of continuous application of copper fungicides in the rubber growing tracts of Kerala and Tamil Nadu showed that rubber plantations sprayed with copper fungicides accumulate copper in the soil compared to unsprayed plantations and virgin lands. However, extremely high levels of Cu was not observed in soils of the sprayed plantations, and Cu phytotoxicity was not reported in any of the plantations studied (KAU-RRII, 2000).

Study on heavy metals in central region of traditional rubber growing tract showed that available copper content in rubber growing soils ranged from 3.8 to 5.3 mg kg^{-1} in small holdings, and 25.4 to 34.2 mg kg^{-1} in estate soils, while it was 2.3 mg kg^{-1} in a forest within the study area (Prasannakumari *et al*., 2014). Abraham (2015) reported higher available copper status in rubber based systems (28.5 to 44.0 mg kg^{-1}) compared to virgin forest (1.9 mg kg^{-1}) in a tropical region of Kerala. However, data of the large scale survey, undertaken by RRII, in collaboration with National Bureau of Soil Survey and Land Use Planning (NBSS&LUP), Bangalore, on soil fertility evaluation and mapping of soils in the traditional rubber growing regions of Kerala and Tamil Nadu showed that available copper content of 80 per cent of the rubber growing soils in the traditional area was less than10 mg kg^{-1} soil, and values above 20 mg kg^{-1} were observed in less than one per cent area. A study was conducted to assess the copper sensitivity of young rubber plants as there are no previous reports on the effect of soil copper on growth of rubber plants.

Materials and methods

Three sets of polybag experiments were conducted with green budded stumps of clone RRII 105 in the glass house. For the first two experiments, the treatments were 0, 10, 20, 30, 40, 50 and 100 mg kg^{-1} copper added as copper sulphate ($CuSO_4.5H_2O$). There were 30 polybag plants under each treatment, and each polybag contained 10 kg soil. In the first experiment, copper treatments were incorporated in soil before filling the polybags. In the second experiment, treatments of copper were imposed in polybags at the stage of maturity of first whorl of leaves.

In the third experiment, instead of imposing treatments of copper, budded stumps were grown in soils which were inherently low and comparatively high in copper status but comparable in organic carbon status and pH. There were 30 polybag plants under each set, and each polybag contained 10 kg soil. Soil with low available copper status (2.2 mg kg^{-1} soil) was collected from RRII farm and soil with higher available copper content (20.1 mg kg^{-1}) was collected from Mundakkayam Estate of Harrison Malayalam Ltd. at Mundakkayam. The soil used for filling polybags in the first two experiments was acidic in reaction (pH- 4.53), medium in organic carbon (0.77%), available phosphorus (23.6 mg kg^{-1}) and potassium (153 mg kg^{-1}), low in available calcium (294 mg kg^{-1}) and high in available magnesium (82 mg kg^{-1}). Available copper content of the soil (0.1 N HCl extracted) was 2.2 mg kg^{-1}. Recommended quantity of N:P:K:Mg mixture (10:10:4:1.5) was applied at the time of maturity of first and second whorls, and the plants were irrigated to maintain soil at field capacity. Percentage of sprouting in each treatment was recorded. Observations on stem diameter, height, number of leaves per whorl and leaf area were recorded. Leaf area was recorded with a LI-COR portable leaf area meter model LI-3000C. Dry matter accumulation and contents of copper and other nutrients in different plant parts were recorded by destructive sampling, after six months.

Data of experiments 1 and 2 were analyzed using Duncan's multiple range test, while that of experiment 3 was analyzed by T-test.

Results and discussion

Experiment 1: Treatments imposed in soil used for filling polybags

Sprouting percentage was highest (88%) for plants treated with 10 mg Cu kg^{-1} soil, and lowest for treatment of 100 mg Cu kg^{-1} soil (Table 1), indicating adverse effect of excess soil copper on sprouting. Stem diameter and height of plants recorded three and five months after planting budded stumps in polybags is shown in Table 2. Stem diameter of plants was significantly higher for the low level of copper, *viz.,* 10 mg Cu kg^{-1} soil, during both recordings. Significant decrease in stem diameter was observed at the highest level of copper, *ie.,* 100 mg Cu kg^{-1} soil. Height of plants was also highest for the treatment of 10 mg Cu kg^{-1} soil and significantly lower for Cu @ 100 mg kg^{-1} soil, compared to lower levels, during both recordings. After five months, significant decrease in plant height compared to control plants was observed beyond the level of 30 mg Cu kg^{-1} soil. Number of leaves per whorl showed significant reduction at the highest level of Cu @ 100 mg kg^{-1} soil (Table 3). Leaf area was significantly higher at the level of 10 mg Cu kg^{-1} soil, but showed significant reduction beyond the level of 30 mg Cu kg^{-1} soil. These observations are in agreement with the earlier reports that application of copper has a stimulatory effect on growth at lower concentrations and inhibitory effect at higher concentrations (Lidon and Henriques, 1993; Manivasagapperumal *et al.*, 2011). Alva and Chen (1995) reported significant reduction in stem diameter and canopy volume in three and five year old "Hamlin" orange trees grown in soils with excess copper. Reduction in leaf area due to high levels of copper was observed by Mocquot *et al.* (1996) and Zeng *et al.* (2004). The inhibitory action of excess copper on plant growth may be due to reduction in cell division, and toxic effect on photosynthesis, respiration and protein synthesis (Kupper *et al.,* 1996; Sonmez *et al.,* 2006).

Table 1. Sprouting of RRII 105 in Experiment 1 (copper applied in soil before planting budded stumps)

Levels of applied Cu (mg kg^{-1} soil)	Sprouting (%)
0	83
10	88
20	79
30	77
40	81
50	76
100	43

Table 2. Stem diameter and height of plants, three and five months after planting (copper applied in soil before planting budded stumps)

Levels of applied Cu (mg kg^{-1} soil)	Diameter (mm)	Height (cm)	Diameter (mm)	Height (cm)
	3 months after planting		5 months after planting	
0	3.6 ^{bcd}	27.8 ^{ab}	4.8 ^{b}	30.1 ^{ab}
10	4.5 ^{a}	29.5 ^{a}	5.4 ^{a}	31.4 ^{a}
20	3.9 ^{b}	24.8 ^{bcd}	4.8 ^{b}	26.4 ^{ab}
30	3.8 ^{bc}	23.3 ^{bcd}	4.6 ^{b}	25.8 ^{bc}
40	3.3 ^{cd}	20.5 ^{cd}	4.5 ^{b}	21.6 ^{c}
50	3.3 ^{d}	20.4 ^{cd}	4.3 ^{b}	21.2 ^{c}
100	3.0 ^{e}	15.6 ^{d}	3.8 ^{c}	16.8 ^{d}

Mean followed by a common letter are not significantly different at P<0.05

Table 3. Effect of copper treatments on number of leaves per whorl and leaf area (copper applied in soil before planting budded stumps)

Levels of applied Cu (mg kg^{-1} soil)	No. of leaves per whorl	Leaf area (cm^2)
0	5.9 ^{a}	43.9 ^{b}
10	6.2 ^{a}	49.5 ^{a}
20	5.8 ^{a}	42.2 ^{bc}
30	5.6 ^{a}	43.5 ^{b}
40	5.8 ^{a}	39.4 ^{cd}
50	5.6 ^{a}	37.8 ^{d}
100	3.8 ^{b}	19.7 ^{e}

Mean followed by a common letter are not significantly different at P<0.05

Table 4. Dry matter accumulation in different plant parts (copper applied in soil before planting budded stumps)

Levels of applied Cu ($mg\ kg^{-1}$ soil)	Dry matter accumulation ($g\ plant^{-1}$)			
	Leaf	Stem	Root	Total
0	2.1 ^{b}	6.43 ^{bc}	7.5 ^{ab}	16.0 ^{b}
10	3.9 ^{a}	10.2 ^{a}	8.8 ^{a}	21.6 ^{a}
20	2.1 ^{b}	7.1 ^{bc}	6.3 ^{bc}	15.7 ^{b}
30	1.9 ^{b}	6.3 ^{bc}	5.1 ^{cd}	12.9 ^{bc}
40	2.1 ^{b}	5.8 ^{bc}	4.5 ^{cd}	12.3b ^{cd}
50	2.0 ^{b}	5.67 ^{bc}	3.9 ^{de}	11.3 ^{cd}
100	1.1 ^{c}	4.0 ^{c}	2.1 ^{e}	7.2 ^{e}

Mean followed by a common letter are not significantly different at P<0.05

Treatment of 10 mg Cu kg^{-1} soil showed positive effect on dry matter accumulation of leaf, stem and root, while Cu @ 100 mg kg^{-1} soil resulted in significant decrease in leaf and root biomass (Table 4). Root biomass was highest for treatment of 10 mg Cu kg^{-1} soil, which was on par with control, and showed a significant reduction for 20 mg Cu kg per soil, indicating that root growth is more sensitive to higher copper levels. Reduction in root elongation and root biomass due to excess soil copper was reported in several crop species, like *Lolium perenne* L. (Wong and Bradshaw, 1982) and *Brassica chinensis* (Wong *et al.*, 1986; Meharg, 1983). Study conducted in *Pinus pinea* L. and *Pinus pinaster* Ait. seedlings on the influence of copper on root growth and morphology in culture solutions showed that copper was much more toxic to root growth than cadmium, and exposure to 1 µM copper greatly decreased root elongation (Arduini *et al.*, 1994). Copper binds strongly to the root surface, decreasing transport potential which is essential for water and ion uptake (Kennedy and Gonsalves, 1987).

Table 6. Nutrient status of stem (copper applied in soil before planting budded stumps)

Levels of applied Cu ($mg\ kg^{-1}$ soil)	Nutrient status (%)				
	N	P	K	Ca	Mg
0	1.79	0.13	0.46	0.85 ^{a}	0.25 ^{a}
10	1.72	0.11	0.55	0.62 ^{ab}	0.16 ^{b}
20	1.66	0.08	0.37	0.50 ^{b}	0.13 ^{b}
30	1.68	0.08	0.25	0.43 ^{b}	0.12 ^{b}
40	1.56	0.08	0.27	0.46 ^{b}	0.12 ^{b}
50	1.56	0.08	0.22	0.49 ^{b}	0.12 ^{b}
100	1.53	0.08	0.39	0.48 ^{b}	0.09 ^{b}

Mean followed by a common letter are not significantly different at P<0.05

Nutrient status of leaf, stem and root are shown in Tables 5, 6 and 7, respectively. Leaf nitrogen status was significantly lower for treatment of 100 mg Cu kg^{-1} soil, while it was comparable for other treatments including control. Compared to the treatment of 10 mg Cu kg^{-1} soil, leaf nitrogen status was significantly lower beyond 40 mg Cu kg^{-1} soil. Nitrogen status of stem and root did not show significant difference among treatments. P and K status of leaf, stem and root did not vary significantly with varying levels of copper.

Significant reduction in calcium content of leaves was observed for plants treated with copper compared to control plants (Table 5). Calcium

Table 5. Nutrient status of leaf (copper applied in soil before planting budded stumps)

Levels of applied Cu ($mg\ kg^{-1}$ soil)	Nutrient status (%)				
	N	P	K	Ca	Mg
0	4.63 ^{bc}	0.13	1.05	0.68 ^{a}	0.24
10	4.97 ^{ab}	0.12	1.27	0.57 ^{b}	0.32
20	4.71 ^{ab}	0.13	1.26	0.53 ^{b}	0.37
30	4.49 ^{bcd}	0.13	1.00	0.51 ^{b}	0.27
40	4.30 ^{cd}	0.13	1.13	0.55 ^{b}	0.31
50	4.53 ^{bcd}	0.14	1.19	0.53 ^{b}	0.25
100	4.20 ^{d}	0.15	1.01	0.51 ^{b}	0.32

Mean followed by a common letter are not significantly different at P<0.05

Table 7. Nutrient status of root (copper applied in soil before planting budded stumps)

Levels of applied Cu ($mg\ kg^{-1}$ soil)	Nutrient status (%)				
	N	P	K	Ca	Mg
0	1.83	0.06	0.36	0.29 ^{a}	0.07
10	2.07	0.06	0.34	0.28 ^{a}	0.06
20	1.87	0.07	0.35	0.18 ^{b}	0.05
30	1.78	0.07	0.33	0.18 ^{b}	0.05
40	1.93	0.09	0.33	0.19 ^{b}	0.06
50	1.86	0.06	0.32	0.15 ^{b}	0.06
100	1.72	0.06	0.32	0.18 ^{b}	0.05

Mean followed by a common letter are not significantly different at P<0.05

content of stem and root was comparable for control and treatment of Cu @ 10 mg kg^{-1} soil, while it was significantly lower for higher levels of copper (Tables 6 and 7). This negative interaction between copper and calcium may also have implications in disease management, as calcium has been reported to reduce disease incidence in several plants (Huber *et al.*, 2012; Campanella *et al*., 2002). Decline in calcium content of leaf and stem observed in this study is in agreement with the earlier reports (Lidon and Henriques, 1993; Gussarsson, 1994; Mateos-Naranjo, 2008). Ouzounidou *et al.* (1995) observed a sharp decline in calcium content in the roots and shoots of *Alyssum montanum* when copper was applied in higher concentration. Manivasagapperumal *et al.* (2011) observed reduction in calcium content of green gram shoot due to high levels of soil copper. All these observations are in agreement with the earlier reports that copper ions tend to displace Ca^{++} ions from exchange sites and are strongly bound in root free space (Mateos-Naranjo, 2008). Copper toxicity induced deficiency of magnesium was also reported by Ouzounidou *et al.* (1995) and Lequeux *et al.* (2010). In this study, this was not observed in leaf and root, but significant decline in magnesium content of the stem was observed in copper treated plants compared to control plants.

Copper content in leaf and stem (Fig. 1) ranged from 22.3 to 23.9 mg kg^{-1} dry weight and 21.4 to 27.7 mg kg^{-1} dry weight respectively, and did not vary significantly with treatments. Critical toxicity level of copper in most plant species range between 20 to 30 mg kg^{-1} dry matter and critical copper deficiency level in vegetative plant parts is 3 to 5 mg kg^{-1} dry weight (Marschner, 1986). Copper content in leaf and stem in all the treatments, including control, were within the critical toxicity limit, but did not increase with increasing levels of soil copper, indicating that excess copper in the soil was not transferred to the shoot.

Copper content of root varied from 18.5 mg kg^{-1} dry weight for control to 96.2 mg kg^{-1} dry weight for the treatment of Cu @100 mg kg^{-1} soil, and increased significantly with increasing levels of soil copper (Fig. 1). Similar observation was reported by Alva and Chen (1995) in "Hamlin" orange trees where copper application up to 120 kg ha^{-1} did not show any change in copper content of the foliage, while the copper concentration of the feeder roots varied from 40 to 450 mg kg^{-1} depending on soil copper levels. Accumulation of copper in the roots is one of the mechanisms of tolerance of excess copper in the root environment, and its translocation to the shoots is an indication of breakdown of the tolerance (Graham, 1981, Alva and Chen, 1995). Assarah *et al*. (2008) reported 17 times increase in concentration of copper and zinc in the root tissue compared to shoot tissue in three species of *Eucalptus* and concluded that *Eucaliptus* is able to grow in areas where some metals are accumulated in soils.

Experiment 2: Treatments imposed in polybags during maturity of first whorl of leaves

The stem diameter and height of plants, one month and two months after treatment imposition in polybags at the stage of maturity of first whorl of leaves is shown in Table 8. Diameter of stem

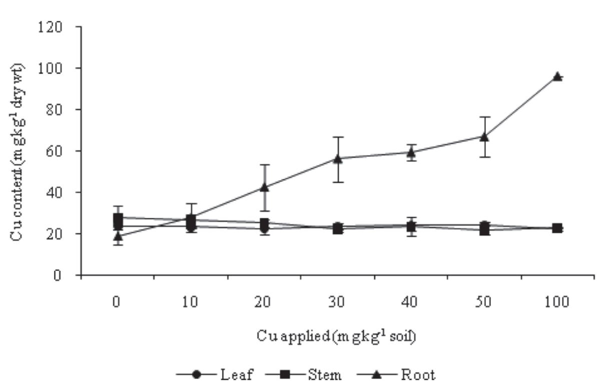

Fig. 1. Effect of copper treatments on copper status of leaf, stem and root (copper applied in soil before planting budded stumps)

Table 8. Stem diameter and height of plants (copper incorporated at maturity of first whorl)

Levels of applied Cu (mg kg^{-1} soil)	Diameter (mm) 1 month after treatment	Height (cm) 1 month after treatment	Diameter (mm) 2 months after treatment	Height (cm) 2 months after treatment
0	5.15 a	26.7 a	5.95 a	29.4 a
10	4.57 b	25.1 ab	5.37 ab	25.9 ab
20	4.36 b	24.8 ab	5.12 abc	25.3 ab
30	4.36 b	25.2 ab	5.15 abc	25.5 ab
40	4.42 b	21.1 bc	4.90 abc	22.4 bc
50	4.36 b	19.7 c	4.90 abc	20.9 c
100	4.50 b	19.1 c	4.62 c	20.4 c

Mean followed by a common letter are not significantly different at P<0.05

Table 9. Nutrient status of soils having low and comparatively higher copper status, used for filling polybags

	OC (%)	Av. P (mg kg^{-1})	Av. K (mg kg^{-1})	Av. Ca (mg kg^{-1})	Av. Mg (mg kg^{-1})	Av. Cu (mg kg^{-1})	Av. Zn (mg kg^{-1})	pH
Low copper soil	2.17	23.5	86.8	71.6	76.6	2.3	1.58	4.45
High copper soil	2.11	19.0	160.9	51.5	16.1	20.1	0.79	4.52

recorded one month after treatment imposition was significantly lower for all copper treatments compared to control. However, stem diameter recorded two months after treatment imposition was comparable for control and copper levels up to 50 mg kg^{-1} soil, and significantly lower at 100 mg Cu kg^{-1} soil. Height of plants recorded one and two months after planting was significantly lower for copper levels above 30 mg kg^{-1}, compared to control.

Experiment 3: Soils having inherently low and comparatively higher copper status

Physico-chemical properties of soils that are inherently low and comparatively higher in soil copper is shown in Table 9. Organic carbon status and pH of these two soils were comparable. Stem diameter and height of plants recorded three and five months after planting is shown in Table 10. No significant difference in stem diameter and height was observed during both the recordings, indicating that soil copper content of 20 mg kg^{-1} did not influence the growth of young plants of *Hevea.*

Table 10. Stem diameter and height of plants grown in soils with low and comparatively higher copper status

	3 months		5 months	
	Diameter (mm)	Height (cm)	Diameter (mm)	Height (cm)
Low copper soil	4.8	28.1	5.1	32.5
High copper soil	4.9	29.2	5.3	33.9
t-stat	NS	NS	NS	NS

Conclusion

From the study, it can be concluded that low level of copper (10 mg kg^{-1} soil) has a positive effect on growth and dry matter production of germinating young plants of *Hevea*, when copper was incorporated in soil before planting budded stumps in polybags. Calcium status of leaf, stem and root declined with increasing levels of soil copper. Growth of plants was poor at the highest copper level of 100 mg kg^{-1} soil. Though excess copper was accumulated in roots, it was not translocated to the shoot, indicating some tolerance mechanism by locking up in roots. When plants were grown in soils that were inherently low and comparatively higher in available copper status, no significant difference in growth of plants was observed. Soil available copper status above 20 mg kg^{-1} was observed in less than one per cent of the soil samples analyzed in the rubber growing soils of South India and hence it may not affect the growth of rubber seedlings.

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