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Toxic effect of Copper on rooting and sprouting characteristics of the mulberry (*Morus alba* L.) cuttings

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Abstract: **Copper is necessary for the growth and development of plants. However, high concentration of Cu exerts extreme toxic effects, resulting in inhibition of growth and development. In the present communication, results on toxic effects of copper on rooting and sprouting regeneration and growth characteristics of the mulberry cuttings, *Morus alba* L. are reported under hydroponic culture experiments. In total, 8 concentrations of copper chloride solutions, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, 1.0 and 1.5 mg/l were considered, apart from control, distilled water alone. Distilled water/$CuCl_2$ solutions in the experimental beakers were replaced every alternative day. Regeneration characteristics of rooting and sprouting like rooting %, sprouting %, time (in days) taken for initiation of rooting and sprouting were considered. Growth attributes root of mulberry cuttings, such as root length, number of roots and weight of roots were taken. Also, growth attributes of mulberry cuttings sprouting like shoot length, number of leaves produced, weight of leaves and leaf area were recorded. Using the recorded data, Tolerance Index (TI) and Phytotoxicity variables were evaluated. Macroscopic data were subjected to regression analysis and ANOVA to indicate the statistical authenticity.**

Rooting in mulberry cuttings was initiated on day 16 of experimentation under control conditions while the same was delayed under experimental conditions. Rooting was 82% for control cuttings while it increased for initial low concentrations of Cu up to 0.05 mg/l and thereafter, it drastically reduced. Shooting in mulberry was initiated on day 8. No change in shooting initiation time was observed for most of Cu concentrations. All root growth attributes (root length, number of roots and root weight) recorded high values for initial two Cu concentrations (0.025 and 0.050 mg/l) over control mulberry cuttings and there after the attributes decreased. In the case of shoot growth attributes, all the four parameters (shoot length, number of leaves, leaf weight and leaf area) did not show any statistical variations up to 0.25 mg/l Cu concentration compared to control. The last two Cu concentrations (1.0 and 1.5 mg/l) induced neither rooting nor shooting in mulberry cuttings. Tolerance Index (TI) was more for mulberry cutting shooting characters while it was low for mulberry cutting rooting characters. On the contrary, phytotoxicity was less for mulberry cutting sprouting compared to mulberry cutting rooting. From the data, it is clear that the effective concentration for Cu ranged between 0.025 to 0.050 mg/l.

Keywords: **mulberry, *Morus alba*, rooting, sprouting copper, toxicity, effective concentration.**

1. INTRODUCTION

Sericulture is a unique field of agriculture for which silkworms are reared for economic important natural fibre, the silk. The mulberry silkworm, *Bombyx mori* is a monophagous insect, feeding exclusively on mulberry leaves. Low leaf yield and any variation in the quality of leaves would naturally affect the growth and productivity of silkworm. Production of leaves by quantity and quality depends on the quality of the soil in which the plants are raised. Although alfisol is naturally preferred for quantity and quality improvement in mulberry leaves, the present day soils are more modified due to various agronomical production and protection measures as well as industrial pollution which directly implicate on mulberry leaf production, both in terms of quantity and quality.

Among micronutrients and heavy metals, Cooper is an important element and micronutrient for plants. The metal activates certain enzymes (catalase, hydrogenase, cytochrome oxidase), it stimulates the formation of chlorophyll, gets involved in the metabolism of carbohydrate and oxidative processes. Copper also stimulates the nitrogen fixation and seed germination (Yruela, 2005, 2009).

Copper is used as an antifungal agent in many pesticides (Almeida *et al*., 1998). Long-term application of fungicides in mulberry fields allows accumulation of copper in soil. Once copper enters the soil, it attaches itself to organic materials in the upper layers of soil and it does not migrate over long distances in soil. This type of metal pollution is a problem caused by the penetration of metal into the structure of the food chains. High Cu concentrations in soil it affects plant tissues and cause deficiencies in other essential nutrients due to antagonistic interaction (Kabata-Pendias and Pendias, 2001). Excess of copper affects the activity of enzymes, impairs the DNA, the protein oxidation and the integrity of membranes which alters the photosynthesis, and it damages plasma membranes and produces functional changes and other metabolic disorders (Lou *et al*., 2004; Azooz *et al*., 2012; Ye *et al*., 2014).

Reports are available on the growth responses of various plants to metal exposure and the level of metal accumulation in different plant parts under controlled conditions. However, such studies pertain to plants grown from seeds (Haghiri, 1973; Beauford *et al*., 1977; Kalita *et al*., 1993; Lidon and Henriques, 1993; Ornes and Sajwan, 1993; Ouzounidou, 1994; Sajwan *et al*., 1996) are scanty. Literature also indicated that only a few reports are available on the impact of heavy metals on regeneration potential of different plant parts, although restoration potential serves as the principal or additional method of propagation in plants (Thangavel and Subburam, 1998). Mulberry (*Morns alba* L.) is a hardy plant and raised from stem cuttings alone. Apart from a report on few growth responses of mulberry plants to heavy metal (Zn and Cd) contaminated environment as a result of emission of heavy metals from smelters (Homma and Shirata, 1977), little information is available on the effect of individual heavy metals such as Copper on the restoration potential of mulberry stem cuttings, their further growth and the quality of the leaves produced.

Agronomists focused on about 25 elements for fixing allowable concentration of arable soils (Kloke, 1980, 1982). However, researches are restricted to limited number of metals like Fe, Zn, Cu, Mn, Co, Mo, Hg, Cr, Cd, Pb, As, Ni, Se and Li in view of their role as micronutrients or as toxicants. For the present study, only Copper was selected based on its occurrence in the agricultural soils. Copper is an essential element which acts in either stimulatory or inhibitory mode depending on its biological availability (Engel and Sunda, 1981). Based on their levels of accumulation and effects on plants and animals in the food chain, Cu is grouped under passage poison elements. It is toxic both to plants and animals depending on levels of availability in the food chain components and accumulation in plants and animals. Copper, being an essential nutrient, most plants tend to maintain in relatively constant concentration because of internal regulatory mechanism (Timperley *et al*., 1970). However, high external concentration of Cu is reported to be toxic to plants (Fernandes and Henriques, 1991). Copper is reported to cause a general chlorosis and stunted growth (Foy *et al*., 1978). Thus, it is felt useful to have a detailed study of copper in rooting and sprouting characteristics of mulberry, *Morus alba* in laboratory condition, employing hydroponic culture system.

2. MATERIALS AND METHODS

Mulberry, *Morus alba* L. (V1 variety) was selected as experimental plant in the present study to assess the effect of Copper on their regeneration characteristics through hydroponic culture experiments. Six months old uniform mulberry stem cuttings of 10 cm length and 8-10 mm width with 3-4 active buds were selected. Cuttings were prepared from stem branches of healthy mulberry plants grown under natural conditions in the mulberry field of Sri Krishnadevaraya University, Anantapur, Andhra Pradesh, India, using sharp secateurs. Care was taken to keep both the ends of the cuttings intact, without splits or peeling-off of bark. Prior to the experiment, mulberry cuttings were thoroughly washed with tap and distilled water. The cuttings were subsequently dipped in 0.005% Indole Butyric Acid (IBA) for 2 hours as root promoting hormone.

Analytical grade chloride salt of copper from s.d. fine chem. Ltd, Mumbai, India was utilized for preparation of test solutions. As a stock solution, 1000 mg of Copper chloride ($CuCl_2$) was dissolved in 1 litre of distilled water and kept for further use in preparing solutions of different concentrations. Three mulberry cuttings were kept in each beaker of 500 ml capacity containing distilled water (200 ml control) or a known concentration of $CuCl_2$ (200 ml) to a height of 45 mm. In total, 8 concentrations of copper chloride solutions, 0.025, 0.050, 0.100, 0.200, 0.400, 0.800, 1.000 and 1.500 mg/l (diluting stock solutions appropriately in distilled water), apart from control (distilled water alone) were employed.

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Experimental set-up was kept under laboratory conditions, with a photoperiod of 16 h/day. For the light period, a white light with an intensity of 600 E/m^2/S was used. Room temperature maintained was 26 ± 2°C with RH (relative humidity) of 70 ± 5%. Distilled water/$CuCl_2$ solution in experimental beakers was replaced every alternative day.

From the experimental set-up, count on cuttings rooted and sprouted was noted initially, from which the percentage of rooting and sprouting was calculated. The time in days (from initiation of experimentation to the initiation of rooting and sprouting) for the above two characteristics of regeneration of cuttings was also noted. The other parameters of regeneration characteristics of mulberry cutting rooting, such as root length, number of roots and weight of roots were taken on 40th day of initiation of experiment. For regeneration characteristics of mulberry cutting sprouting, observations on number of leaves produced, weight of leaves and area of leaves were recorded on 40th day of initiation of study. Mulberry leaf area was measured using the leaf area meter (Model, LI-COR- 300).

Using the recorded data, indices values of tolerance of roots and leaf to $CuCl_2$ were calculated, employing formula of Baker *et al.* (1994), IT (%) = [(Mean length in treatment/Mean root length in control)/Mean length in treatment] x 100. Also, phytotoxicity of shoots and roots was calculated by using formula of Chou and Lin, (1976), Phytotoxicity of shoot (%) = [(length of shoot in control - length of shoot in treated)/length of shoot in control] x 100 and phytotoxicity of root (%) = [(length of root in control - length of root in treated)/length of root in control] x 100.

3. RESULTS

I. Regeneration attributes of rooting and sprouting: Mulberry cuttings generally initiate sprouting from day 8 and rooting from day 16 of plantation. Data on rooting and sprouting attributes, rooting and sprouting (%) and time of initiation of rooting and sprouting are furnished in Table 1. In general, the rooting and sprouting was high for control (distilled water alone) and treatments up 0.05 mg of $CuCl_2$/l of distilled water. Both rooting (%) and sprouting (%) gradually reduced from 0.05 mg/l level and reached zero level at 1.0 mg/l and after. Initiation of rooting and sprouting (days) also recorded similar trends, with less number of days taken for rooting and sprouting for control and increased treatment with 0.05 mg of copper chloride/l. From 0.05 mg of copper chloride/l level, treated cuttings showed an inverse pattern and touched plateau level for copper concentrations of 0.1 mg/l and above, indicating no rooting and sprouting in experimental mulberry cuttings and thus 0% rooting and sprouting as well (Table 1.).

Table 1: Data on rooting (%), sprouting (%), time (days taken for initiation of rooting and sprouting in mulberry cuttings under control (distilled water) and experimental conditions (mg of $CuCl_2$/l) on $CuCl_2$. Values are mean 5 replications (± SD).

Treatment (mg of $CuCl_2$/l)	Rooting (%)		Sprouting (%)		Root initiation (day)		Shoot ignition (day)	
	Average	± SD	Average	± SD	Average	± SD	Average	± SD
Control	81.800	10.780	90.200	5.450	16.600	0.894	8.400	0.548
0.025	94.800	2.950	86.800	5.404	14.200	1.483	8.400	0.548
0.050	91.400	4.561	88.600	6.877	21.200	0.837	8.000	0.707
0.100	86.600	9.503	78.600	8.385	22.400	0.894	7.800	0.447
0.200	68.400	6.656	74.600	5.030	26.200	2.490	7.600	0.894
0.400	69.600	7.197	62.000	6.782	27.600	0.548	8.200	0.447
0.800	34.200	7.950	43.200	8.228	25.800	2.280	8.000	0.707
1.000	0	0	0	0	0	0	0	0
1.500	0	0	0	0	0	0	0	0
Regression	-0.924458233		-0.955247639		-0.685969755		-0.848348132	

a. Rooting percentage: Data on rooting (%) of mulberry cuttings (control and experimental) are plotted in Figure 1. While mulberry cuttings, under control conditions, rooted up to 80% only, those with copper concentration from 0.025 to 0.100 mg of $CuCl_2$/l rooted more than cuttings under control condition (Fig. 1), thus indicating that Cu is essential for better rooting than control at low concentrations. Mulberry cuttings of experimental conditions over 0.100 mg of $CuCl_2$/l recorded decreased rooting percentage compared to cuttings under control condition. On the extreme side, mulberry cuttings under experimental conditions of 1.00 mg of $CuCl_2$/ l and above did not root at all.

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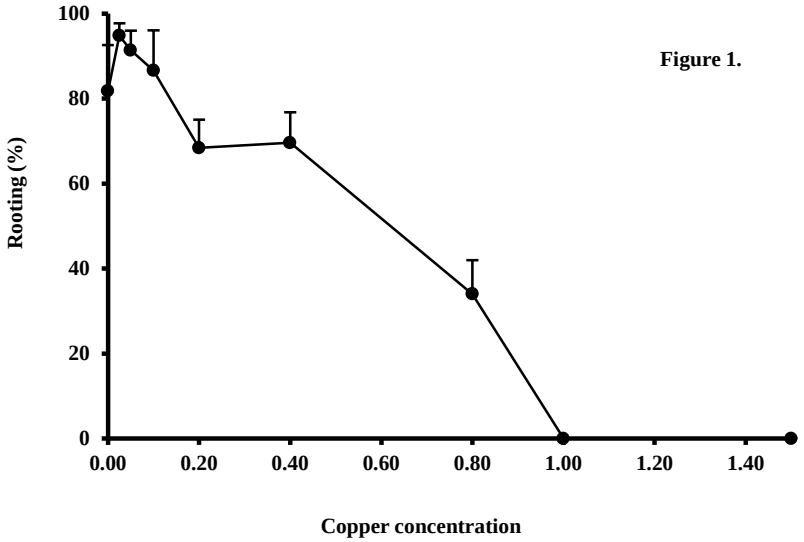

Figure 1: Rooting percentage of mulberry cuttings under control and experimental concentrations of copper (mg of $CuCl_2$/l). Values are mean of 5 replications (± SD).

b. Sprouting percentage: Data on sprouting percentage of mulberry cuttings are presented in Figure 2. Sprouting patterns in mulberry cuttings under control condition was around 90%. The initial experimental copper concentrations (0.025 and 0.050 mg/l) recorded sprouting percentage very close to mulberry cuttings under control conditions. Beyond 0.100 mg/l concentration of Cu, sprouting decreased and after 1 mg/l concentration, no sprouting was observed. The results are indicative of the requirement of certain low quantities of Cu for sprouting in mulberry.

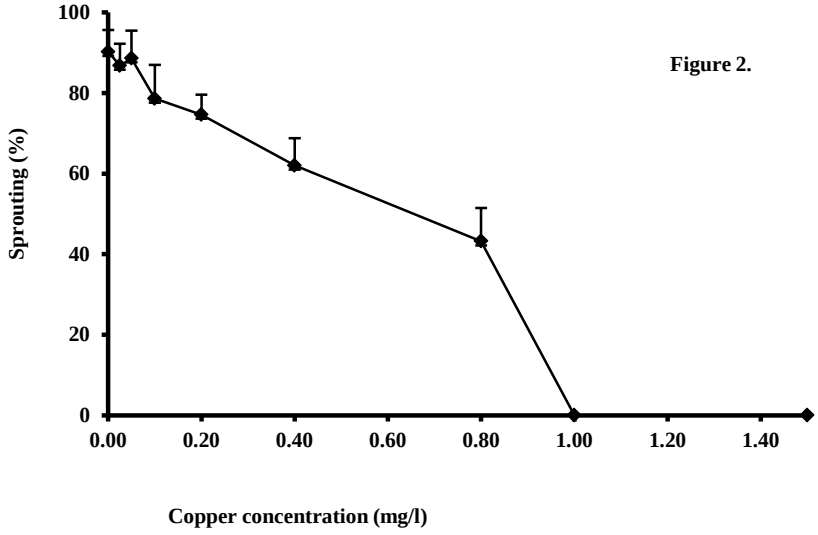

Figure 2: Sprouting percentage of mulberry cuttings under control and experimental concentrations of copper (mg of $CuCl_2$/l). Values are mean of 5 replications (± SD).

c. Time taken for initiation of rooting (days): The time, in days, taken for initiation of rooting, from the initiation of experimentation under control and experimental conditions are depicted in Figure 3. It is generally observed that mulberry cuttings initiate rooting on 17^{th} day and beyond. In the case of the time (days) taken for mulberry cutting, mulberry cuttings under control conditions initiated their rooting on 16^{th} day. Under imposed experimental conditions of Cu concentrations of 0.025 and 0.05 mg/l, mulberry cuttings initiated rooting one day earlier. Thus, under Cu concentration of 0.025 mg/l, rooting occurred on day 14 and for Cu concentration of 0.050 mg/l, it was on 16^{th} day. Under Cu concentrations from 0.10 mg/l onwards (up to 0.8mg/l), time taken for initiation of rooting in mulberry cuttings ranged

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from 15 to 16 days only. Beyond 0.80 mg/l (1.0 and 1.5 mg/l) of Cu concentrations, no sprouting was observed in experimental mulberry, indicating that these Cu concentrations are not suitable for rooting in mulberry.

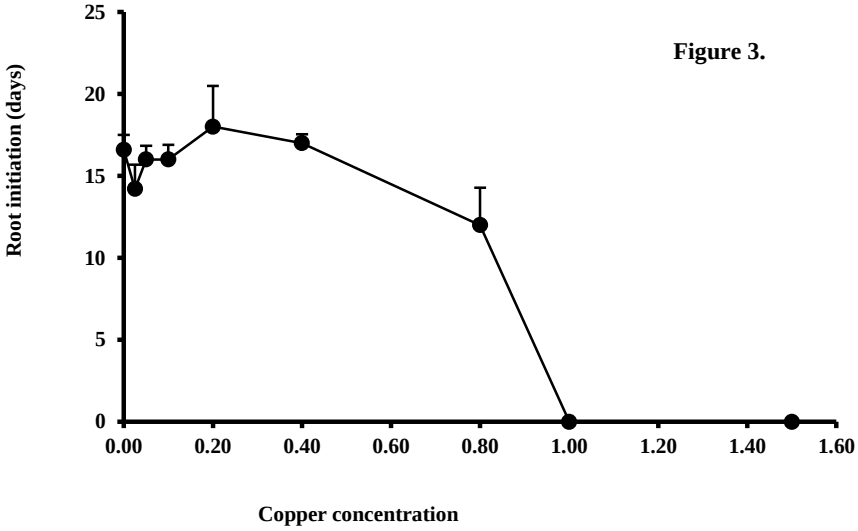

Figure 3: Time taken for initiation of rooting in mulberry cuttings under control and experimental concentrations of copper under different concentrations (mg of $CuCl_2$/l). Values are mean of 5 replications (± SD).

d. Time taken for initiation of shoot (days): Data on time taken for initiation of shoot (sprouting) in mulberry cuttings under control and different Cu concentration are shown in Figure 4. Shoot initiation was observed much earlier compared to root initiation in mulberry. Under control condition, shoot initiation in mulberry was observed on day 8 of experiment initiation. However, low concentration of Cu induced sprouting at least by one day earlier; the shoot initiation in Cu concentrations of 0.025, 0.050 and 0.10 mg/l, sprouting appeared between day 7 and day 8. The differences are not significant. Shoot initiation time in days, was comparable to control under Cu concentrations up to 0.80 mg/l. Higher Cu concentrations (1.00 and 1.50 mg/l) induced no sprouting/shoot initiation at all (Figure 4).

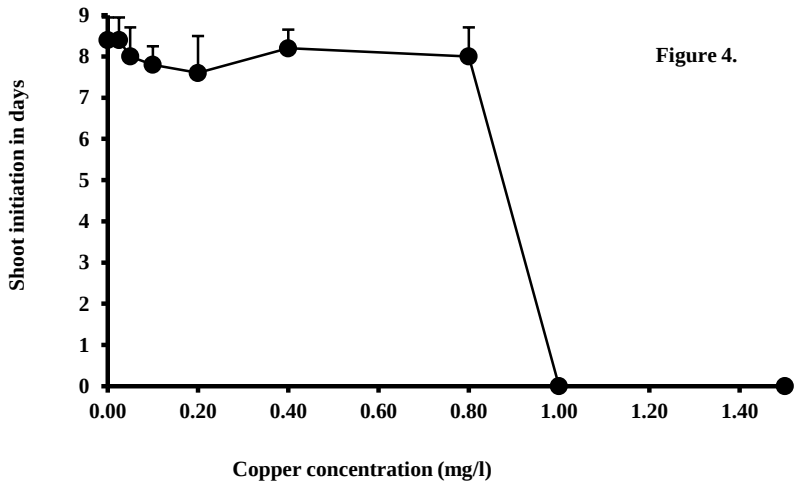

Figure 4: Time taken for initiation of shooting (sprouting) in mulberry cuttings under control and experimental concentrations of copper under different concentrations (mg of $CuCl_2$/l). Values are mean of 5 replications (± SD). Note insignificant difference in time taken for initiation of sprouting between control and up to 0.80 mg Cu concentration.

II. Root growth attributes: Root developmental attributes such as root length, number of roots and root weight are recorded on 60^{th} day of experimentation. Analyzed data from the recorded data on root developmental attributes, root length, number of roots and root weight are figuring in Table 2. In general, the root developmental attributes were high for control (distilled water alone) and treatments up 0.5 mg of Cu/l of distilled water. Thereafter, all the root developmental attributes studied (root length, number of roots and root weight) drastically reduced to zero level. Rooting growth

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attributes recorded less readings for control and increased values in root length, number of roots and root weight for initial treatments (from 0.025 to 0.500 mg of $CuCl_2$/l). Thereafter, the treated mulberry cuttings showed a decreased pattern and reached zero level from Cu concentrations of 1 mg/l and above; indicating no root development or growth in experimental mulberry cuttings and thus, 0% rootgrowth attributes(Table 2.).

Table 2: Root growth attributes of mulberry cuttings of control (distilled water) and experimental conditions (mg of $CuCl_2$/l). Values of individual treatment are mean 5 replications (± SD).

Treatment (mg of CuCl2/l)	Root length (cm)		No. of roots		Root weight (g)	
	Average	± SD	Average	± SD	Average	± SD
Control	14.090	2.291	37.000	4.848	0.565	0.150
0.025	16.472	0.981	36.000	5.568	0.624	0.202
0.050	15.820	1.512	29.000	4.690	0.752	0.153
0.100	9.500	2.117	24.200	4.147	0.642	0.146
0.200	8.020	1.245	22.400	5.595	0.552	0.106
0.400	7.580	1.938	19.800	3.701	0.440	0.253
0.800	5.480	1.341	8.400	3.209	0.236	0.116
1.000	0	0	0	0	0	0
1.500	0	0	0	0	0	0
Regression	-0.892016234		-0.942256388		-0.958656766	

a. Root length: One of the important root growth attribute is the root length. Root length in mulberry cuttings under control (distilled water) condition recorded 14 cm under controlled condition. Thereafter, the root length increased for experimental Cu concentrations of 0.025and 0.050 mg of $CuCl_2$/l, recording around 16 cm in both the cases. However, higher concentrations of Cu from 0.10 to 0.80 mg/l implicated reduction in root length and under further high concentration of Cu (1.0 and 1.5 mg/l), there was no rooting and therefore no measurements of root length.

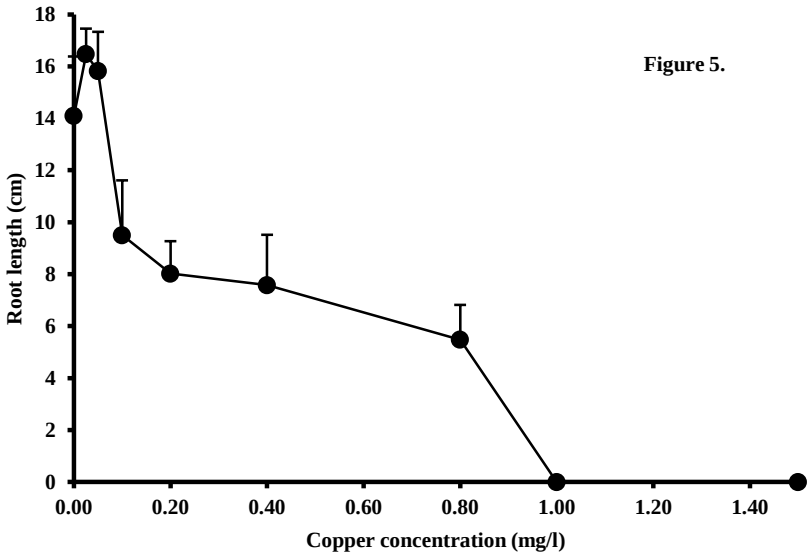

Figure 5.

Figure 5: Graph showing rooting development attribute, root length in mulberry cuttings under control and experimental concentrations of copper under different concentrations (mg of $CuCl_2$/l). Values are mean of 5 replications (± SD).

b. Number of root: Number of roots in mulberry indicates healthiness of developing plant for optimum intake of water and nutrients from soil and thereafter transport of these materials to shoot system on the surface of the soil. Number of roots recorded per mulberry plant (Figure 6) under control condition was around 37. Interestingly, the number of roots per plant decreased from the lowest Cu concentration (0.025 mg/l) itself and reached gradually to the 0 level at 1.0 mg/l, indicating direct influence of Cu on the number of roots in mulberry cuttings under study.

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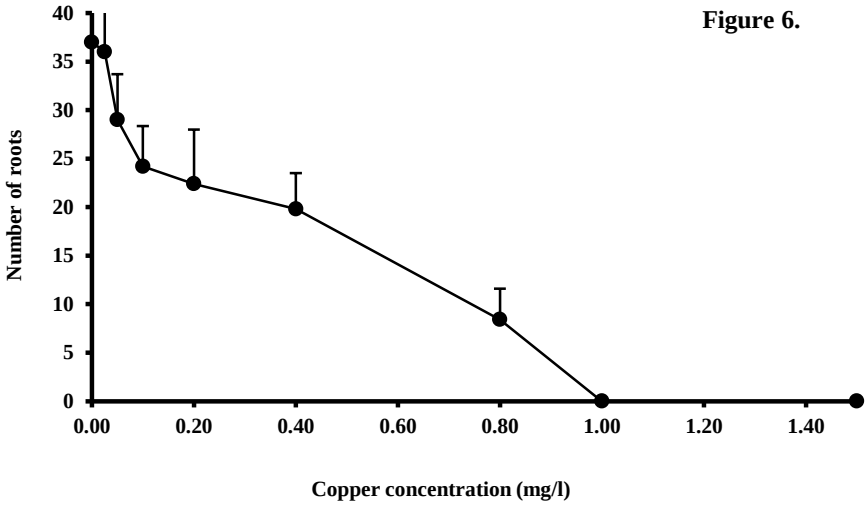

Figure 6.

Figure 6: Graphic representation of rooting development attribute, number of roots per plant in mulberry cuttings under control and experimental concentrations of copper under different concentrations (mg of $CuCl_2$/l). Values are mean of 5 replications (± SD).

c. Weight of root: Weight of mulberry roots indicates robustness of root-system. For mulberry cuttings under control condition, weight of roots per plant (Figure 7) recorded was around 0.6 g. As in other case, except in number of roots per plant, the weight of roots per plant increased in low concentrations of Cu (0.025, 0.5 and 0.10 mg/l), the weight of roots per plant increased. From Cu concentration above 1.00 g/l, root weight showed decreasing trend and from 1.00 mg/l onwards, no roots were observed and therefore no measurements of root weight recorded.

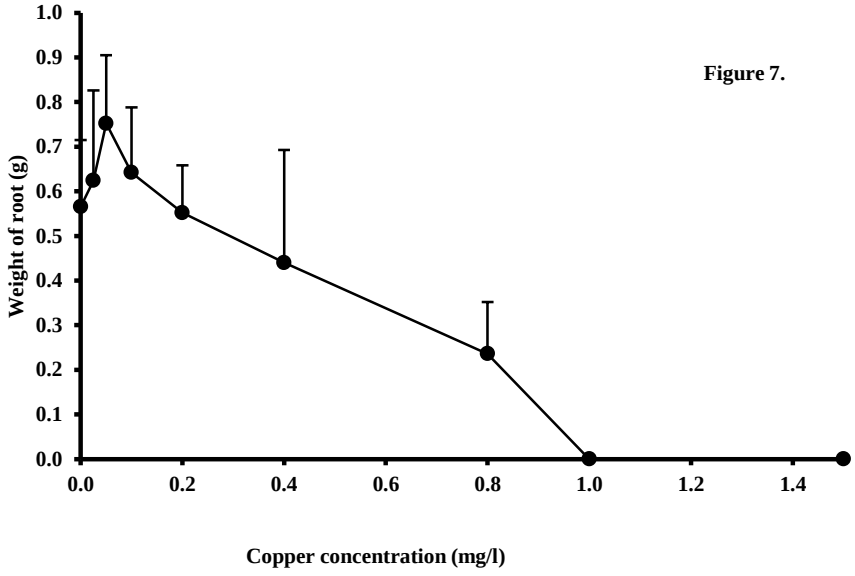

Figure 7.

Figure 7: Graphic representation of weight of roots per mulberry plant under control and experimental concentrations of copper under different concentrations (mg of $CuCl_2$/l). Values are mean of 5 replications (± SD).

III. Shoot growth attributes: In the case of shoot developmental attributes in the mulberry cuttings under experimental conditions, four developmental attributes were studied *viz*., shoot length, number of leaves per plant, leaf weight and leaf area per leaf. The data are presented in Table 3. In general, readings of shoot developmental attributes were high for control (distilled water alone) and treatments up to 0.500 mg of Cu/l. Thereafter, all shoot developmental attributes studied reduced to zero level at 1.0 mg/l and beyond. Shoot growth attributes recorded less reading for control and

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increased values in leaf weight alone per plant for initial treatments (from 0.025 to 0.050 mg of copper chloride/l), however, with no statistical significance. Thereafter, the tread mulberry cuttings showed a decreased pattern and reached zero level from Cu concentrations of 1.0 and above, indicating no shoot formation in experimental mulberry cuttings and thus, 0% shoot development (Table 3.).

Table 3: Shoot growth attributes of mulberry cuttings of control (distilled water) and experimental conditions (mg of $CuCl_2$/l). Values of individual treatment are mean 5 replications (± SD).

Treatment (mg of CuCl2/l)	Shoot length (cm)		No. of leaves		Leaf weight (g)		Leaf area (cm^2)	
	Average	± SD	Average	± SD	Average	± SD	Average	± SD
Control	11.126	2.866	4.200	0.748	1.096	0.173	35.560	2.608
0.025	10.426	2.541	2.800	0.748	1.520	0.279	34.778	2.033
0.050	9.354	3.411	3.000	0.632	1.196	0.117	35.752	2.603
0.100	8.816	2.833	2.000	0.632	1.032	0.111	33.824	1.591
0.200	8.424	3.674	2.000	0.632	1.080	0.194	35.092	1.641
0.400	7.904	2.883	1.400	0.490	1.020	0.306	33.696	1.740
0.800	5.446	1.982	1.600	0.800	0.950	0.205	31.778	2.630
1.000	0	0	0	0	0	0	0	0
1.500	0	0	0	0	0	0	0	0
Regression	-0.95217679		-0.909722126		-0.901784428		-0.877718992	

a. Shoot length (cm): Length of shoot indicates importance of photosynthetic parts in mulberry plant. The trend recorded in shoot length of mulberry cuttings under experimental conditions (Figure 8) is different from that of other parameters. Mulberry shoot length observed under control condition was 11 cm. The shoot length gradually reduced from 10.4 cm for 0.025 mg/l of Cu concentration to 5.0 Cm for Cu concentration of 0.80 mg of Cu. From. For Cu concentrations of 1.00 and 1.50 mg/l, the shoot formed was zero and no shoot lengths (Figure 8) were recorded. The differences however are non-significant.

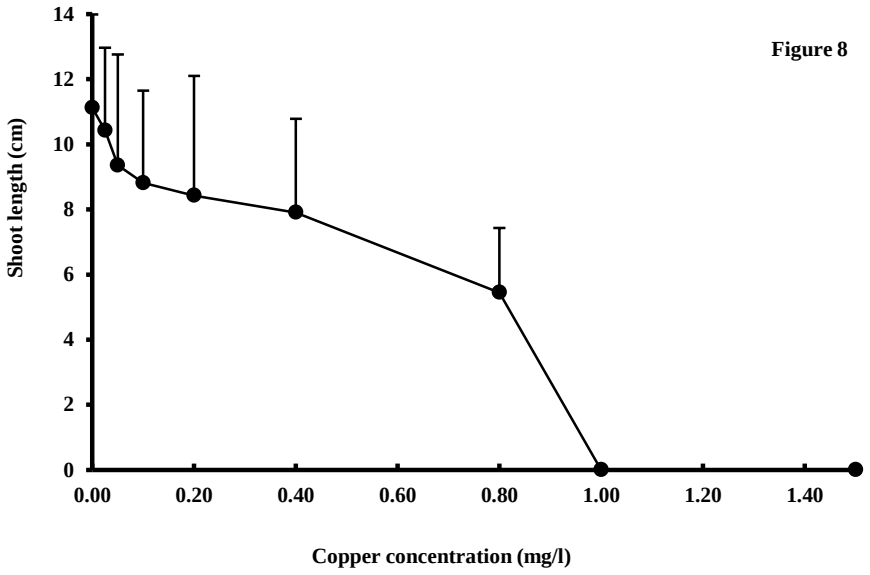

Figure 8: Graphic representation of shoot length (cm) of shoot per mulberry plant under control and experimental concentrations of copper under different concentrations (mg of $CuCl_2$/l). Values are mean of 5 replications (± SD).

b. Number of leaves per plant: In the case of number of leaves per plant, the number recorded for control mulberry plants was only 4 on an average. The subsequent treatments from 0.025 to 0.80 mg/l of Cu concentration resulted in

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gradual reduction in number of mulberry leaves per treated plants. As usual, there was no sprouting at all under 1.00 and 1.50 mg/l Cu concentrations of treatment and no leaves formation recorded (Figure 9).

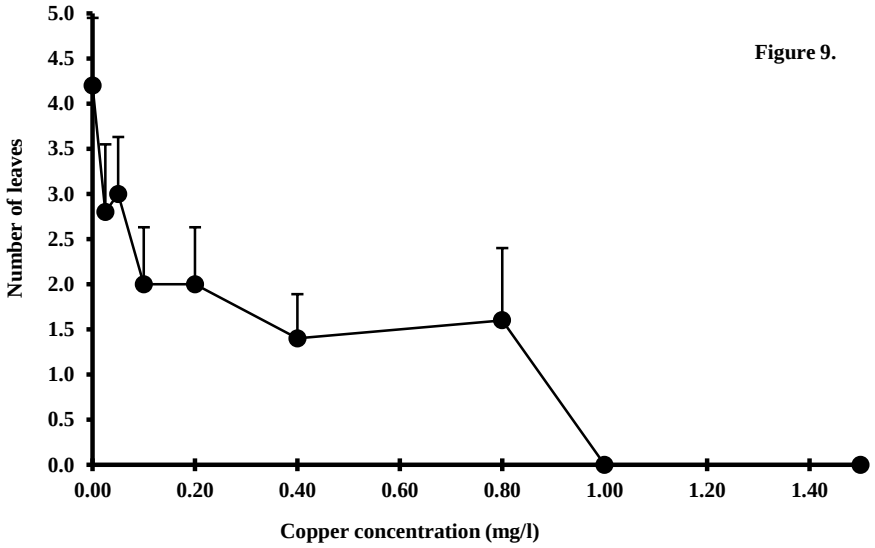

Figure 9: Graphic representation of number of leaves per plant of mulberry under control and experimental concentrations of copper under different concentrations (mg of $CuCl_2$/l). Values are mean of 5 replications (± SD).

c. Leaf weight (g): Leaf weight in control plants of mulberry recorded only 1.09 g. For the experimental concentration of 0.025 mg/l Cu, the leaf weight increased, reading at 1.5 g and the same for Cu concentration of 0.050 mg/l was 1.2 g. From the next experimental concentration of Cu (0.40 mg/l), the leaf weight decreased to reach zero level from 1.0 to 1.5 mg/l of Cu concentration (Figure 10). The differences however are non-significant.

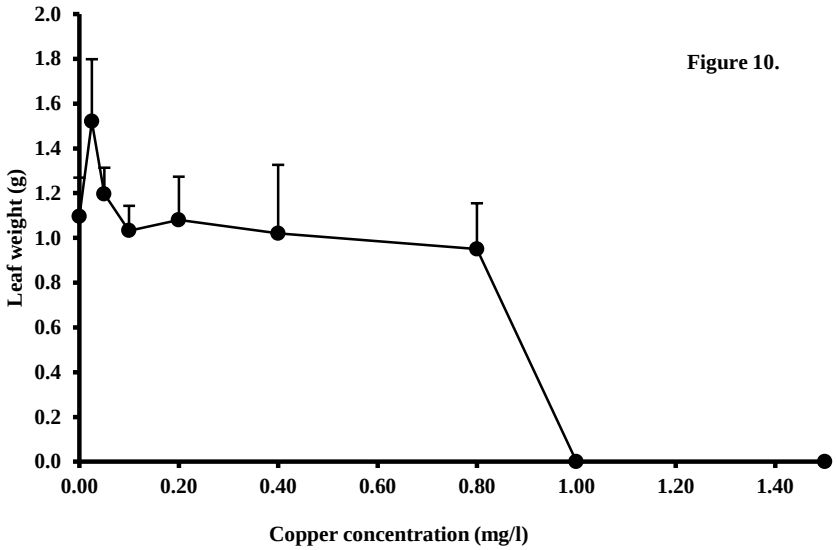

Figure 10: Graphic representation of leaf weight (g) per mulberry plant under control and experimental concentrations of copper under different concentrations (mg of $CuCl_2$/l). Values are mean of 5 replications (± SD).

d. Leaf area (cm^2): Leaf area (cm^2) was almost statistic (statistically no difference) for control and experimental Cu concentrations up to 0.8 mg/l of $CuCl_2$, from which point, there was no sprouting and hence, no leaf area recorded (Figure 11). The leaf area ranged from 36 cm^2 (control – distilled water) to 32 cm^2 for Cu concentration of 0.800 mg/l. The differences however are non-significant.

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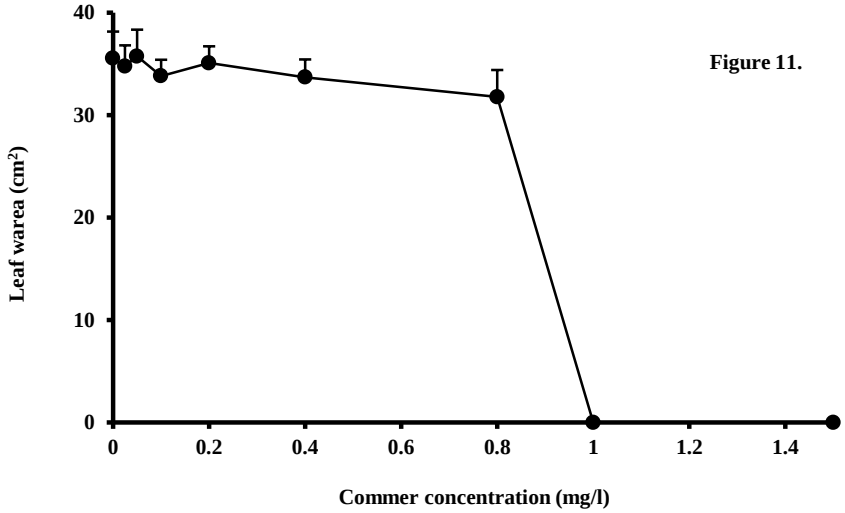

Figure 11: Graphic representation of leaf area (cm^2) for mulberry plant cuttings under control and experimental concentrations of copper under different concentrations (mg of $CuCl_2$/l). Note no differences in leaf area from control to Cu concentration of 0.800 mg of $CuCl_2$/l. Values are mean of 5 replications (± SD).

IV. Tolerance Index (TI) for rooting and sprouting in mulberry under experimental Cu concentrations: Estimates of Tolerance Index (TI) are the most important measurements, indicating intensity of toxicity to plant or an organism. In the present study, Cu was taken as essential micronutrient and heavy metal as well. The results on TI derived from concerned formula for rooting and sprouting are presented in Figure 12. The tolerance in rooting of mulberry cuttings for the heavy metal, Cu was almost 100% for $CuCl_2$ concentrations of 0.025 and 0.050 mg/l. For later Cu concentrations up to 0.080 mg/l, TI gradually reduced to near 40% and for Cu concentrations of 1.000 and 1.500 mg/l, the same was 0%. Notably, the TI was more for sprouting (statistically significant at 1% level) over that for rooting for Cu concentrations from 0.050 to 0.80 mg/l indicating that rooting is more sensitive to Cu rather than sprouting.

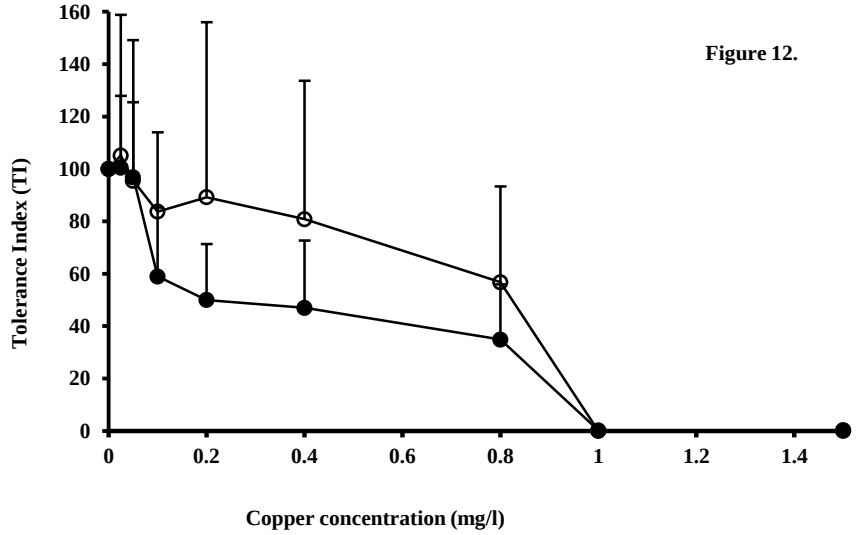

Figure 12: Graphic representation of Tolerance Index (TI) of experimental mulberry cuttings for rooting (closed circles) and sprouting (open circles) towards induced Cu toxicity under control and different concentrations of copper (mg of $CuCl_2$/l). Values are mean of 5 replications (± SD). Note significantly more TI values ($p< 0.01$) for sprouting over rooting for Cu concentrations from 0.050 to 0.80 mg/l indicating that rooting is more sensitive to Cu over sprouting.

V. Phytotoxicity of rooting and sprouting: Phytotoxicity of mulberry plant is an indicator towards understanding the level of toxicity that mulberry can withstand in an induced experimental condition. The phytotoxicity of rooting and sprouting in experimental mulberry cuttings exposed to control (distilled water) and different Cu concentrations (0.025,

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0.050, 0.100, 0.400, 0.800, 1.000 and 1.500 mg of $Cucl_2$/l) are depicted in Figure 13. Phytotoxicity of rooting in mulberry is comparatively high, compared to that of sprouting. Phytotoxicity was below 0% for lower experimental concentrations of Cu (0.025, 0.050 and 0.100 mg/l) for rooting. However, this trend in registering negative phytotoxicity for sprouting was restricted to two lower Cu concentrations (0.025 and 0.050 mg/l). For other experimental Cu concentrations, up to 0.800 mg/l, the phytotoxicity ranged from40 to 50% for rooting and 5 to 30% for sprouting (Figure 13). The negative phytotoxicity in lower Cu experimental concentrations (0.025, 0.050 and 0.100 mg/l) is indicative of both the parameters (rooting and sprouting) are much tolerant to those low Cu concentrations. Higher Cu concentrations resulted in increased phytotoxicity, indicating that these Cu concentrations are not tolerable to mulberry rooting and sprouting.

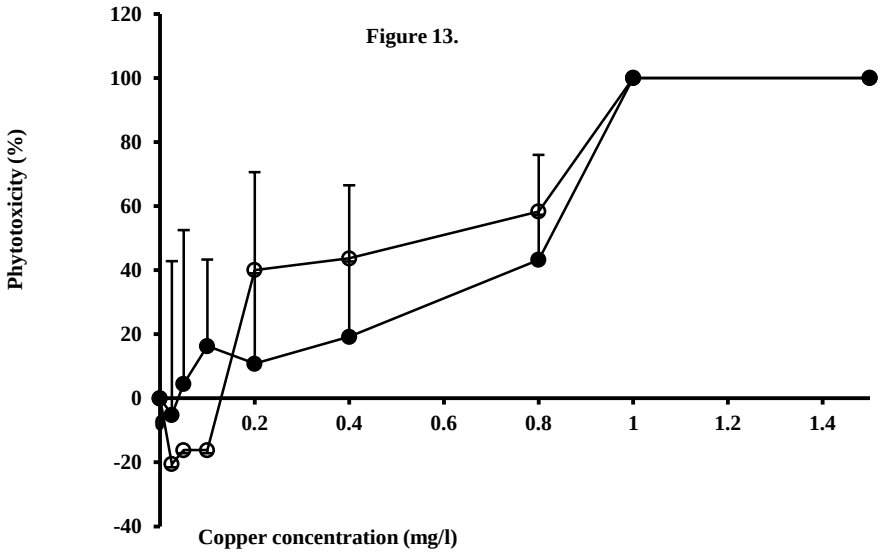

Figure 13: Graphic representation of Phytotoxicity of experimental mulberry cuttings for rooting (open circles) and sprouting (closed circles) towards induced Cu toxicity under control and different concentrations of copper (mg of $CuCl_2$/l). Values are mean of 5 replications (± SD). Note negative phytotoxicity for lower Cu concentrations and positive phytotoxicity for other experimental conditions. Also, note significantly less phytotoxicity values ($p< 0.01$) for sprouting over rooting for Cu concentrations from 0.050 to 0.80 mg/l indicating.

4. DISCUSSIONS

Data presented in the current communication clearly indicated that Cu affected rooting and sprouting attribute of V1 mulberry cuttings to various levels, the level of effects are Cu concentration dependent. Further, Cu affected differently on regeneration and growth parameters differently. The effect on root and shoot development properties ranged from change in their number, length, weight, area to their complete inhibition. Comparatively, implications of Cu on various root parameters studied were larger than those on shoot. Further, effects of Cu are on higher side for regeneration characteristics than on growth characteristics. It is obvious that roots are in direct contact with the metal solution (growth medium) whereas the bio-available concentration in the stem and leaves is determined by the rate and amount of heavy metals transferred from the medium through the cut-open end of the stem and roots after their development. Although root and shoot initiation occurred in most of the treatment concentration of Cu tested, their further growth (number of roots, length, weight) was affected indicating the inhibitory effect of Cu (Baker and Walker, 1989; Barcelo and Poschenrieder, l990; Breckle, 1991). Such inhibitory effects have resulted in total inhibition of the root and shoot formation above 0.80 mg/l of Cu. Prince (1999) reported root initiation was arrested above 0.4 mg/l while shoot initiation occurred up to 0.8 mg/l. Early initiation of rooting was apparent in all the initial low Cu concentration and the same was delayed in later higher Cu concentrations. Prince (1999) reported that Cu increased the size of root and probably resulted in weight of the roots. Increase in root tip enlargement as a consequence of exposure to toxic metals has been reported (Smith and Brennan, 1984). However, such experiments were not considered in the present study. Apart from the effect of Cu on various root development parameters it also affected the growth of the shoot, number of leaves, weight as well as leaf area. At lower concentrations of Cu, the effect was positive; increasing the measurements of parameters. The higher concentrations, on the other side reduced the measurements of these characteristics. Very low concentrations of heavy

metals have been reported to stimulate root growth (Sahu*et al*, 1988; Shaw *et al*., 1988; Narwal *et al*., 1990; Gussarsson, 1994). The results of this study were in accordance with the studies reported by Shaikh *et al*. (2013), the negative effects of copper on shoot and root have a direct relationship to their toxicity on shoots and roots. Further, there was no appearance of sprouting or rooting at higher concentrations of 1.000 and 1.500 mg/l. Significant increase was observed in the leaf area above 0.2 mg/l of Cu indicating the requirement of Cu for leaf especially, may be, for chlorophyll increase. Prince, (1999) working on toxicity of three heavy metals, Cd, Cu and Hg ranked the order of their relative effect on root development as Cd > Cu > Hg and that on shoot development as Cd > Hg > Cu, indicating the relative importance of Cu for root generation.

The tolerance index of the species decreased as the concentration of copper increased, into which the mulberry cuttings were immersed with replacement of copper solution each alternate day. Studying the copper toxicity on germination and growth *Triticum aestivum* and *Lactuca sativa* Effects on germination and growth, Jelea *et al*. (2016) reported that the non-tolerant plants exhibited decreased growth while the tolerant ones were stimulated by the elevated copper concentrations for optimal growth. However, only one species of mulberry (*M. alba*) was used in the present study. The tolerance index of the species decreased as the concentration of copper increased, into which the mulberry cuttings were immersed.

Phytotoxicity of shoot and root increased as the concentration of copper increased. The highest copper phytotoxicity on root and shoot were observed at 0.8 g/l and above in the present study. Interestingly, the phytotoxicity was more for shooting attributes compared to rooting characteristic. Negative phytotoxicity was recorded for cuttings exposed to lower concentrations of Cu (0.025 and 0.050 mg/l), indicating the promontory action of copper at these lower concentrations (0.025 and 0.050 mg/l) only and the same increased to positive side from 0.10 mg/l concentrations and above, again indicating initiation of toxicity at 0.01 mg/l level and increased further on higher Cu concentrations. This observation hints at the consideration of critical tolerant concentrations of Cu for mulberry cuttings rooting and sprouting. From the data, it is apparent that both the rooting and sprouting attributes of mulberry at lower concentration of 0.025 and 0.050 mg/l are more than control conditions and the attributes decreased beyond 0.050 mg/l concentration levels. Further, Cu concentrations of 1.0 and 1.5 g/l resulted in no rooting and sprouting. Therefore, it can safely be inferred that the effective concentration of Cu must be between 0.025 and 0.050 mg/l. Prince (1999) reported that effective concentration (the concentration which causes significant changes compared to the control) for rooting and sprouting in mulberry under solution culture is between 0.025 and 0.050 mg/l of Cu.

From the data presented in the present communication it is concluded that rooting in mulberry cuttings was delayed under experimental conditions, increased for initial low concentrations and drastically reduced thereafter. No change in shooting initiation time was observed for most of Cu concentrations and all root growth attributes recorded high values for initial two Cu concentrations over control mulberry cuttings and there after the attributes decreased. For shoot development attributes, all the four parameters studied did not show any statistical variations up to 0.25 mg/l Cu concentration compared to control. The last two Cu higher concentrations (1.0 and 1.5 mg/l) induced neither rooting nor shooting in mulberry cuttings. Tolerance Index (TI) was more for mulberry cutting shooting character while it was low for mulberry cutting rooting. On the contrary, phytotoxicity was less for mulberry cutting sprouting compared to mulberry cutting rooting. The effective concentration for Cu seems to be between 0.025 to 0.050 mg/l of Cu.

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