Research Article

EFFECT OF COPPER EXPOSURE ON GROWTH IN HYACINTH BEAN (*Lablab purpureus* (L.) Sweet)

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ABSTRACT

"Heavy metals" is a general collective term, which applies to the group of metals and metalloids with atomic density greater than 4 g/cm^3 or more, greater than water. Copper (Cu) is an essential element for all living organisms. Cu is released both naturally and through human activities; it is extensively spread into the environment and often causes environmental pollution. *Lablab purpureus* is a species of bean in the family Fabaceae. The present study was conducted to understand the effects of different concentration of copper metal stress on the morphology and physiology of Hyacinth bean. Copper stress was induced in 14-day old seedlings of uniform size with half-strength Hoagland media containing $CuSO_4$ (10mM, 50mM, 100mM, 500mM, 1000mM, 1500mM) for a period of 14 days. Growth parameters such shoot length, root length petiole length, leaf area, plant biomass and chlorophyll estimation were carried out. All the growth parameters showed significant reduction when Cu concentration was increased. Concentration of chlorophyll a and b reduced considerably with increase in concentration of copper.

Keywords: Lablab purpureus, Hoagland medium, Heavy metal, Copper

1. INTRODUCTION

The term "heavy metals" refers to any metallic element that has a relatively high density and is toxic or poisonous even at low concentration (Lenntech, 2004). "Heavy metals" is a general collective term, which applies to the group of metals and metalloids with atomic density greater than 4 g/cm3 or more, greater than water (Hawkes, 1997). However, being a heavy metal has little to do with density but concerns chemical properties. Heavy metals include lead (Pb), cadmium (Cd), zinc (Zn), mercury (Hg), arsenic (As), silver (Ag) chromium (Cr), copper (Cu) iron (Fe), and the platinum group elements (Farlex, 2005). Soil management can also change its physical, chemical, and biological characteristics, and as a result, different responses by biological activities

to heavy metal toxicity can be observed. Also, the activities of microorganisms that promote plant growth can be altered by high concentrations of metals (Wani *et al.,* 2007). At high concentrations, all heavy metals have strong toxic effects and are regarded as environmental pollutants (Chehregani *et al.,* 2005). Soil pollution with heavy metals will lead to losses in agricultural yield and hazardous health effects as they enter into the food chain (Schickler and Caspi, 1999). In heavy-metal polluted soils, plant growth can be inhibited by metal absorption. Heavy metal uptake by crops growing in contaminated soil is a potential hazard to human health because of transmission in the food chain (Fries *et al.,* 2006).

Copper (Cu) is an essential element for all living organisms. Cu is released both naturally and through human activities; it is extensively spread into the environment and often causes environmental pollution. Anthropogenic inputs include those from industrial and urban activities (sludge from sewage treatment and urban waste) and uncontrolled use of Cu-containing fungicides (Marschner, 2012). Normally, Cu concentration in non-polluted soils is $10-30$ mg kg^{-1} dry weight (Baryla *et al.,* 2000), but higher concentrations can be found near Cu production facilities. It is well known that Cu, as an essential micronutrient, is necessary for plant growth and development, and has many biological functions (Marschner, 2012).

Lablab purpureus is a species of bean in the family Fabaceae. It is native to Africa and it is cultivated throughout the tropics for food. Common names include hyacinth bean, bonavist bean/pea, dolichos bean, seimbean, lablab bean, Egyptian kidney bean, Indian bean. *Lablab* is remarkably adaptable to wide areas under diverse climatic conditions such as arid, semi-arid, sub-tropical and humid regions. Being a legume, it can fix atmospheric nitrogen to the extent of 170 kg/ha besides leaving enough crop residues to enrich the soils with organic matter. It is a drought tolerant crop and grows well in dry lands with limited rainfall. It prefers relatively cool seasons (temperature ranging from 14-28 °C) with the sowing done in July-August.

The present study was conducted to understand the effects of different concentration of copper on the growth of Hyacinth bean.

2. MATERIALS AND METHODS

Plant Materials and Germination

Seeds of *Lablab purpureus* were collected from Kerala Agricultural University, Mannuthy and were surface sterilized with 0.1% (w/v) mercuric chloride for 1 min, rinsed immediately with large volume of sterile distil water and imbibed overnight with distilled water. The overnight-soaked seeds were sown in trays containing autoclaved sand and irrigated daily with distilled water. The germination was carried out under natural greenhouse conditions; day/night temperature and relative humidity were: 30/25°C and 75/70% respectively. The average photoperiod was 12 h light/12 h dark.

Treatment and experimental design:

Copper stress was induced in 14-day old seedlings of uniform size with half-strength Hoagland media containing $CuSO_4$ (10mM [C2], 50mM [C3], 100mM [C4], 500mM [C5], 1000mM [C6] and 1500mM [C7]) for a period of 14 days. Samples of leaves and roots were collected by uprooting the plant after 14-day treatment. Plants grown on half-strength Hoagland media served as control [C1]. Samples used for determination of fresh and dry weight were used immediately after collection. After determining the dry weight, the plant material was grinded and homogenized. The experimental design used was done in triplicate.

Determination of growth parameters, leaf area and Plant Biomass

Growth measurements for the plants exposed to heavy metal treatments and control were taken at times mentioned previously, namely after 14 days of treatment. The three replicates taken for each treatment were used to calculate the mean of each measurement. The measurements taken were the length of the shoot, root and petiole, leaf length, leaf breadth, whole plant weight. The plant parts were stored in paper bags and oven dried at 80 °C until constant weight was reached to calculate dry weight.

Dermal morphology

Qualitative features of foliar epidermal cells were observed. Measurement of the guard cells, subsidiary cells and trichomes were taken with micrometer.

Stomatal index was calculated by using the following formula, $S.I = S/E+S \times 100$

Where, SI =Stomatal index, S= number of stomata per unit area, E= number of epidermal cells in the same unit area

Chlorophyll Estimation

For chlorophyll analysis one gram freshly cut leaves were taken and ground with 40ml of 80% acetone and it was centrifuged at 10000 rpm for 5 minutes. The supernatant was transferred and the procedure was repeated till the residue becomes colorless. The source of the solution was read at 645nm and 663nm against the solvent (Arnon, 1949).

3. RESULTS AND DISCUSSION

Growth parameters, leaf area and plant biomass

The effects of copper exposure on growth parameters like shoot length, root length, petiole length, leaf area and fresh-dry weight was assessed in the seven treatments (Table 1; Fig. 1).

Shoot length ranged from 53.5±0.64 cm (treatment-C1) to 36.7±3.09 cm (treatment-C7). Root length was highest in treatment-C1 (10.5±0.3 cm) and lowest in treatment-C7 (7.04±0.43 cm). Petiole length ranged from 3.5±0.51 cm (treatment-C1) to 2.66±0.16 cm (treatment-C7). Leaf length and breadth were noted highest in treatment-C1 (5.96±0.35 cm; 5.63±0.03 cm) and lowest in treatment-C7 (4.33±0.06 cm; 4.26±0.06 cm). Leaf area ranged from 14.88 cm^2 (treatment-C6) to 5.22 cm^2 (treatment-C4). Fresh weight was highest in treatment-C2 and treatment-C4 (24±0.40 gm) and lowest in treatment-C7 (20±0.40 gm). Similarly dry weight was highest in treatment-C4 (2.7±0.05 gm) and lowest in treatment-C7 (1.36±0.03 gm) (Fig. 2).

Shortening of the stem due to Cu toxicity has been observed in other plants (Karataglis and Babalonas, 1985; Lidon and Henriques, 1992; Moustakas *et al*., 1997). The toxic effect of Cu on the root system is of particular importance for the whole plant. Since the root is the main site of entry into the plant of water and nutrients, any defect or malformation of the root creates problems for plant growth and development. Application of higher Cu (10^{-3}mM and above) in nutrient solution decreased the roots, shoots and leaf length of maize (*Zea mays* L. cv. *Cargill* 350 Hybrid) plants compared to control (Benimali *et al*., 2010). Application of higher Cu (10^{-3}mM and above) concentration in nutrient solution decreased the fresh and dry weights of maize (Cargill 350 Hybrid) plants as compared to control (Benimali *et al.,* 2010). Copper induced significant

reduction in biomass of soybean (>100 ppm Cu) and of chickpea (>60 ppm Cu) (Adhikari *et al*., 2012).

Dermal morphology

The effects of copper exposure on dermal morphology were analysed by studying the number and size of stomata and trichomes (Table 2).

Stomatal index ranged from 28 (treatment-C1) to 14.63 (treatment-C7). Guard cell length is highest in treatment-C2 (28.30±0.96 µm) and lowest in treatment-C7 (17.48±0.83 µm). Breadth of guard cells is highest in treatment-C1 (6.24±0.41 µm) and lowest in treatment-C7 (2.91±0.41 µm). Trichome length and breadth are highest in treatment-C1 (1.78±0.31 µm; 0.15±0.01 µm) and lowest in treatment-C7 (0.09±0.005 µm; 0.01±0.0006 µm).

Dermal morphological characters reveal that the exposure to copper affects the plant by characteristic reduction in the number and size of stomata as well as in the size of the trichomes. Stomatal index also results in reduction in accordance with increase in concentration of copper treatment. Similar phenomena have been described in other plants grown in substrates rich in heavy metals (Barcelo and Poschenrieder, 1990).

Chlorophyll estimation

The effects of copper in chlorophyll concentration were studied (Table 3; Fig. 3). Chlorophyll a content was more in control plant (22.08µg/ml) and least content in treatment-C6 (6.43µg/ml), chlorophyll b content was higher in treatment-C5 (27.11µg/ml) and least in treatment-C1 (14.68µg/ml). The study also showed that chlorophyll a/b ratio was highest in treatment-C1 (1.50 µg/ml) and lowest (0.24 µg/ml) in treatment-C6. Total chlorophyll content in control is 36.76µg/ml and that of treatment-C7 was 29.43µg/ml. From the analysis a significant decrease in total chlorophyll content in accordance with increase in concentration of copper was observed.

Panou-Filotheou *et al*., (2001) studied effects of copper toxicity on leaves of Oregano (*Origanum vulgare* subsp. *hirtum*). The chlorophyll content of chlorotic leaves of Cu-treated plants was investigated. Total chlorophyll (a + b) was found to decline with increasing concentrations of soil Cu. In plants, Cu deficiency altered root and leaf construction, as well as significant reduction in chlorophyll pigments and photosynthesis (Yruela 2005, 2009) in spinach (*Spinacia oleracea* L.), 160 µM Cu in the solution culture decreased chlorophyll content by 45 % over control

treatment (Ouzounidou *et al.*,1998). Higher Cu (7 and 10 μM) in the nutrient solution decreased photosynthetic pigments in maize plants (Mocquot *et al.*,1996) Exposure of Chinese cabbage to enhanced Cu concentrations (≥2 μM) decreased photosynthetic pigments (Shahbaz *et al.,* 2010), leaves of cucumber plants exhibited a significant decline in photosynthesis under Cu stress (Vinit-Dunand *et al.,* 2002). Leaves of sunflower treated with 0.4, 0.5 and 0.6 mM Cu decreased chlorophyll (a + b) contents by 19.2, 26.3 and 31.6 %, respectively, as compared to control treatment (Zengin and Kirbag 2007) There is strong evidence that reduction in chlorophyll biosynthesis is related to structural damages of the photosynthetic apparatus at the thylakoid level under Cu stress (Cisato *et al.,* 1997) and interference of Cu with chlorophyll organization (Caspi *et al.,* 1999).

4. CONCLUSION

Copper metal stress shows characteristic effect on growth parameters (shoot & root length, petiole length, leaf length and leaf breadth), dermal morphology (stomatal index, length & breadth of guard cells and trichomes) and chlorophyll content of *Lablab purpureus.*

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APPENDIX

Table 1: Growth parameters, leaf area and plant biomass of *Lablab purpureus* in different copper treatments

Character studied	C1	C2	C3	C4	C5	C6	C7
Shoot length (cm)	53.5±0.64	48.5±0.64	40.8±0.42	39±2.73	41±2.04	41.2±1.97	36.7±3.09
Root length (cm)	10.5±0.3	7.43±0.6	8.2±0.35	9.43±0.49	8.2±0.35	7.34±0.37	7.04±0.43
Petiole length (cm)	3.5±0.51	3.16±0.31	3.13±0.34	3.13±0.34	3.00±0.28	2.83±0.16	2.66±0.16
Leaf length (cm)	5.96±0.35	5.5±0.17	5.4±0.2	4.86±0.06	4.60±0.20	4.63±0.03	4.33±0.06
Leaf breadth (cm)	5.63±0.03	5.53±0.03	5.3±0.25	5.03±0.14	4.93±0.06	4.86±0.06	4.26±0.06
Leaf area (cm^2)	6.97	6.73	5.97	5.22	13.95	14.88	11.35
Fresh weight (gm)	22±1.15	24±0.40	23±0.57	24±0.40	22±1.15	20.6±0.12	20±0.40
Dry weight (gm)	2.48±0.01	2.5±0.05	2.43±0.03	2.7±0.05	1.93±0.06	1.56±0.03	1.36±0.03

Table 2: Characterization of dermal morphology of *Lablab purpureus* in different copper treatments

Characters	C1	C2	C3	C4	C5	C6	C7
Stomatal index (µm)	28	20.68	18.57	19.75	18.57	16.66	14.63
Guard cell length (µm)	27.47±0.83	28.30±0.96	27.47±0.83	24.14±0.83	22.47±0.83	21.64±0.96	17.48±0.83
Guard cell breadth	6.24±0.41	5.41±0.41	5.41±0.41	4.16±0.48	3.74±0.41	3.74±0.41	2.91±0.41
Length of trichome (µm)	1.78±0.31	0.16±0.03	0.16±0.03	0.15±0.04	0.13±0.01	0.13±0.03	0.09±0.005
Breadth of trichome (µm)	0.15±0.01	0.04±0.03	0.01±0.001	0.01±0.002	0.03±0.02	0.03±0.02	0.01±0.006

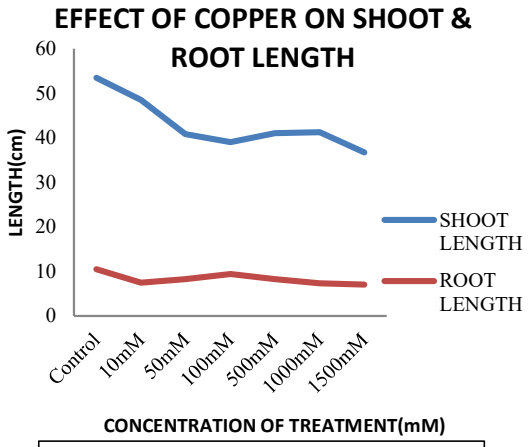

Fig. 1 Graph showing the effect of copper on shoot and root length of *Lablab purpureus*

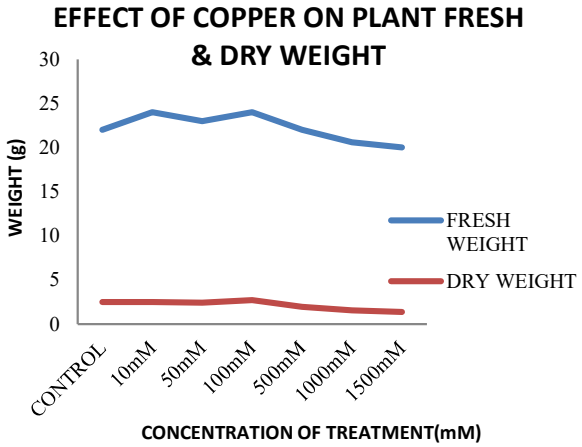

Fig. 2 Graph showing the effect of copper on fresh and dry weight of *Lablab purpureus*

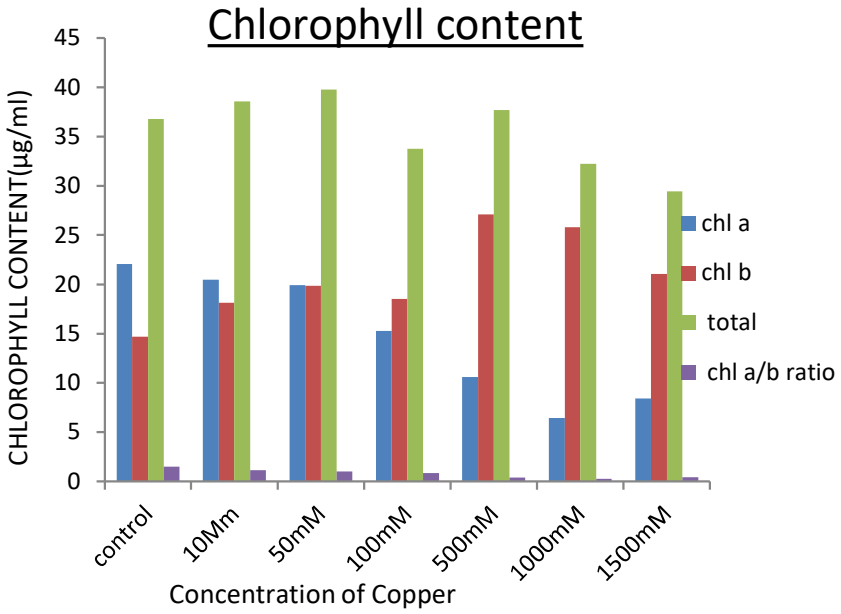

Fig. 2 Graph showing the effect of copper on chlorophyll content of *Lablab purpureus*