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# Effect of Copper on Morphological and Biochemical Characteristics of *Populus deltoides* (W. Bartram Ex. Marshall)

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**A B S T R A C T**

The present investigation entitled "Effect of copper on morphological and biochemical characteristics of *Populus deltoides* (W. Bartram Ex. Marshall)" was carried under laboratory conditions during the period 2013-2014. The study comprised of eleven treatments viz., $T_1$ (No copper), $T_2$ (100 ppm Cu), $T_3$ (200 ppm Cu), $T_4$ (100 ppm Cu + *M. anisopliae*, $1x10^5$ condia/ml), $T_5$ (100 ppm Cu + *M. anisopliae*, $1x10^6$ condia/ml), $T_6$ (200 ppm Cu + *M. anisopliae*, $1x10^5$ condia/ml), $T_7$ (200 ppm Cu + *M. anisopliae*, $1x10^6$ condia/ml), $T_8$ (100 ppm Cu + *B. bassiana*, 1 x $10^5$condia/ml), $T_9$ (100 ppm Cu + *B. bassiana*, $1x10^6$ condia/ml), $T_{10}$ (200 ppm Cu + *B. bassiana* ($1x10^5$ condia/ml), $T_{11}$ (200 ppm Cu + *B. bassiana*,$1x10^6$ condia/ml). Highest and lowest number of leaves per plant were recorded at treatment $T_5$ (53) and $T_1$ (33), respectively, maximum and minimum leaf area was at treatments $T_9$ (0.16 $m^2$) and $T_1$ (0.08 $m^2$), respectively, whereas, maximum plant height (1.76 m) was observed in treatment $T_5$. The biomass of different plant parts of *P. deltoids* viz., roots, stem and leaves was statistically minimum at $T_1$. Highest chlorophyll and ascorbic acid content was recorded at$T_9$ i.e., 0.28 mg/g and 5.60 mg/g, respectively. Total sugar content varied from 0.88 mg/g ($T_1$) to 1.53 mg/g ($T_5$). Highest phenol content (1.24 mg/g) was observed at treatment $T_3$. Copper content in root, stem and leaves of *P. deltoids* varied from 31.00($T_1$)-68.25 ($T_3$) ppm, 10.75 ($T_1$) - 44.25 ($T_3$) and 6.50 -34.25 ppm in T1 and T3, respectively. Distribution of copper content in different parts of *P.deltoides* was in the order root>stem>leaves.

## Introduction

Metal contamination issues are becoming increasingly common in India and elsewhere, with many documented cases of metal toxicity in mining industries, foundries, smelters, coal-burning power plants and agriculture (Nagayyoti *et al.*, 2010). Soil, one of the most valuable part of environment, is being contaminated rapidly by industrial waste, chemical disposal, uncontrolled use of fertilizer, pesticides and other agrochemicals. The prominent soil contaminants are heavy metals/metalloids, petroleum, solvents, pesticides and herbicides).

According to a report 31 % and 35 % of total contaminants in water and soil, in Europe are

heavy metals, respectively (Liedekerke *et al.*, 2014). Contaminated soil affects human health, ecosystem and agriculture and thus threatens the entire environment.

Metals viz., copper, manganese, cobalt, zinc and chromium etc are though essential to plant metabolism in trace amounts but at excessive levels they have the potential to become toxic to plants. Copper is an essential micronutrient for plants acting as a cofactor of enzymes, structural proteins and phytohormone receptors, however, when present in elevated concentrations it inhibits plant growth and development (Xu *et al.*, 2006). Copper based fungicides are frequently used by farmers, several times per year to control fungal diseases (Soares *et al.*, 2006). Copper sulphate, copper oxychloride and copper hydroxide are the most frequently used copper fungicides in the world. Low cost fertilizers mainly deriving from intensive farming and readily used for non-food plantations contain high amount of copper (Giardini, 2002).

Remediation of contaminated soils has become a major environmental issue. Different physical and chemical methods are being used for removal of metals but with many limitations viz., high cost, intensive labour etc. Therefore, phytoremediation i.e. use of plants and associated soils microbes to reduce the concentration or trace effects of contaminants in the environment is better option for cleanup of metal contaminated soils. New efficient metal hyperaccumulation techniques are being exploited for application in phytoremediation and phytomining (Ali *et al.*, 2013).

Plants have ability to grow in polluted areas by altering various physiological and biochemical changes (Khatun *et al.*, 2008). Poplar is geographically widespread in various climatic areas and it is adapted to contaminated or polluted soils and has the capacity to accumulate heavy metals (Pulford and Watson, 2003). It is being used for the extraction or immobilization (phyto stabilization) of heavy metals present in polluted sites (Koprivova *et al.*, 2002). In addition poplar species are currently grown as cash crops for pulpwood and as a renewable energy source (Moffat *et al.*, 2001) and therefore, poplar has been proposed as a model for studying the biology of trees.

In view of above facts, the present study was therefore, aimed to investigate the effect of copper on morphological and biochemical parameters of *Populus deltoides* and its role in bioremediation of copper.

## Material and Methods

Young cuttings from one year old cut back stems and branches of *P.deltoides* were planted in pots (capacity 16 kg),which were filled with planting material i.e. sand, soil and FYM in the ratio 1:1:1. The cuttings were irrigated twice a week or as per requirement of seedlings or weather conditions. The analytical reagent $CuSO_4.5H_2O$ (M.W. 249.68) was used for preparing working concentrations of copper. Required amount of the reagent was weighed in milligrams for 1.5 litre of water, the quantity of which was standardized according to the capacity of the soil per pot. The experiment comprised of eleven treatments viz., $T_1$ (No copper), $T_2$ (100 ppm Copper), $T_3$ (200 ppm Copper), $T_4$ (100 ppm Copper + *M. anisopliae*, $1x10^5$ conidia/ml), $T_5$ (100 ppm Copper + *M. anisopliae*, $1x10^6$conidia/ml), $T_6$ (200 ppm Copper + *M. anisopliae*, $1x10^5$ conidia/ml), $T_7$ (200 ppm Copper + *M. anisopliae*, $1x10^6$conidia/ml), $T_8$ (100 ppm Copper + *B. bassiana*, $1x10^5$conidia/ml), $T_9$ (100 ppm Copper + *B. bassiana*, $1x10^6$ conidia/ml), $T_{10}$ (100 ppm Copper + *B. bassiana* ($1x10^5$ conidia/ml), $T_{11}$ (200 ppm Copper + *B. bassiana*, $1x10^6$ conidia/ml). The whole set of experiment was laid in four replications.

The treatments were given every week for 4 months after the establishment of the seedlings.

The samples were analyzed for various morphological parameter viz., number of leaves, leaf area and plant height and biochemical parameters viz., chlorophyll content, ascorbic acid content, total sugar content and total phenols. Numbers of leaves and plant height was measured at the end of experiment, whereas, leaf area was measured by using Leaf area meter (Model-LI-COR-3100).

## Chlorophyll estimation

The leaf chlorophyll content was estimated by method of Hiscox and Israeistam (1979). The O.D. values were recorded on Spectrophotometer (Model-Spectronic-20) at 645 and 663 nm wavelength against dimethyl sulphoxide blank. The total chlorophyll content was calculated by using standard formula.

## Ascorbic acid estimation

The ascorbic acid content was estimated by using method of A. O. A. C. (1980). Amount of ascorbic acid in milligrams per hundred grams was calculated by the standard formula.

## Total sugar content

Total sugar content in leaves was estimated by colorimetric method (Dubois *et al.,* 1956).

## Total phenol content

The phenol content in plant samples was estimated according to the method of Bray and Thorpe (1954). The O.D. values of the extract were recorded on Spectrophotometer (Model-Spectronic-20) at 650 nm wavelength against standard catechol blank.

## Biomass partitioning

At the end of experiment all the treatment combinations were harvested for biomass analysis of roots, stem and leaves. Plant weight was oven dried at 70$^{o}$C until constant weight was achieved.

## Uptake of copper in plant

Copperuptake was estimated by using the following formula:

$$\text{Copper uptake} = \frac{(\text{Leaf Nutrient, \% x Leaf dry weight}) + (\text{Shoot nutrient, \%, x Shoot dry weight}) + \text{Root nutrient, \% x Root dry weight x 1000}}{100}$$

## Results and Discussion

Statistically, lowest number of leaves (33) was recorded at treatment $T_1$ (No Copper), whereas, highest number (53) was recorded at treatment $T_4$though statistically at par with $T_2$, $T_4$, $T_8$, $T_9$ and $T_7$ (Table 1) but differed from treatment $T_3$, $T_6$ and $T_{10}$ *i.e.,* 200 ppm Cu, 200 ppm Cu + *M. anisopliae,* $1*10^5$ conidia/ml and 200 ppm Cu + *B. bassiana* $1*10^5$ conidia/ml. Thus, there was decrease in leaf area with the increase in Cu concentration. Similar results were reported by (Yanbao *et al.,* 2007). High concentration of Mn caused significant decrease in leaf number of *P. cathayana.* Shi *et al.,* (2006) reported oxidative stress as one of the main agents causing cellular damage in heavy metal toxicity.

Statistically, lowest leaf area (0.08 $m^2$) was recorded at treatment $T_1$ (No Copper) and highest leaf area (0.16 $m^2$) was recorded at treatment $T_9$ which was statistically at par with $T_8$ and $T_5$ (Table 1). Borghi *et al.,* (2007) reported decrease in leaf area of clone of *P. deltoids* × *P. nigra* from 0.06 $m^2$ at 500 µM of copper to 0.03 $m^2$ at 1000 µM of copper.

Growth inhibition, leaf area reduction and the decrease of root biomass, often accompanied by changes in root morphology are the main symptoms of copper toxicity (Maksymiec, 1997). Copper affects nitrogen uptake as it impairs plasma membrane functionality (Burzynski and Buczek, 1994).

Statistically lowest plant height (1.46 m) was recorded in treatment $T_{10}$and $T_{11,}$ whereas, highest plant height of 1.76m was recorded at treatment $T_5$ and $T_9$. At control ($T_1$) the height was 1.55m (Table 1).

There was decrease in all the three morphological parameters with increase in concentration of copper from 100ppm to 200ppm. The results find support from the findings of Khatun *et al.,* (2008) where toxic effect of Cu on *Withania somnifera* was reflected by the reduction in fresh weight, shoot and root length. In another study by Lombardi and Sebastiani (2005) at 100µm of copper the *Prunus cerasfera* reduced relative growth rate for fresh and dry weight and developed severe browning which progressed to necrosis. Growth inhibition, leaf area reduction and decrease in root biomass of trees are the main symptoms of Cu toxicity (Maksymic, 1997).

Chlorophyll content of trees signifies its photosynthetic activity as well as the growth and development of biomass. It varies from species to species with age of leaf and also with the pollution level along with other biotic and abiotic conditions (Katiyar and Dubey, 2001). The results on effect of Cu on biochemical characteristics of *Populus* viz., chlorophyll, ascorbic acid, total sugar and total phenol content is presented in table 1. The chlorophyll content varied from 0.06 to 0.28 mg/g. In general reduction in chlorophyll content at 200ppm Cu treatment combinations was observed as compared to control ($T_1$ No Copper), whereas, the values were high for 100ppm Cu combinations. Highest chlorophyll content (0.28 mg/g) was recorded at treatment T9 (100 ppm copper along with entomopathogenic fungi) which was at par with $T_5$ (0.26mg/g).The lowest chlorophyll content (0.06 mg $g^{-1}$) was observed at treatments $T_3$ and $T_{10}$. The present findings are in confirmation with the findings of Borghi *et al.,* (2007) who reported decrease in chlorophyll content of hybrid poplar (*P. deltoides* x *P. nigra*) at 1000 µM i.e. 0.013 µg $mm^{-2}$ as compared to 500 µM i.e. 0.127 µg m $m^{-2}$ and Nikolic *et al.,* (2008) who reported decrease in chlorophyll content of hybrid poplar (*P. nigra* × *P. maximowitzii*) from 8.51 to 6.61 mg $g^{-1}$ with increase in concentration of copper from ($10^{-5}$ M and $10^{-4}$ M). Excess copper in the growth substrate generally induces a decrease in total chlorophyll content, which has been associated with the destruction of inner structure of chloroplasts and medications of the lipid-protein composition of thylakloid membranes (Maksymiec, 1997).

Highest content of ascorbic acid (5.74 mg $g^{-1}$) was recorded at treatment $T_3$which was statistically at par with $T_9$ (5.64 mg $g^{-1}$), $T_4$ (5.63 mg $g^{-1}$) and $T_8$ (5.60 mg $g^{-1}$). In treatment $T_2$ ascorbic acid recorded was 4.29 mg $g^{-1}$ which was statistically more than treatment with 200 ppm copper and entomopathogenic fungi. As compared to respective controls the treatment along with entomopathogenic fungi had more ascorbic acid content. Ascorbic acid content increased with increase in Cu concentration from 100 to 200 ppm. The increase in ascorbic acid content is supported by the findings of Zengin and Munzuroghi (2005) where ascorbic acid content of beans increased from 13to 15 per cent with increase in Cu from 0.1 to 0.3mM.Pandey and Tripathi (2011) also reported significant increase in the ascorbic acid content in leaves of *A. procera* over control. Ascorbic acid, a natural antioxidant

plays an important role in pollution tolerance and direct relationship between endogenous levels of ascorbic acid and plant susceptibility to pollutant has been established. Ascorbic acid maintains the stability of cell membranes during pollution stress and scavenges cytotoxic free radicals (Keller and Lamprech, 1995). Higher ascorbic acid content of the plant is a sign of its tolerance to air pollution and lower ascorbic acid supports the sensitive nature of these trees towards pollutants (Varshney and Varshney 1984).

Highest sugar content (1.53 mg $g^{-1}$) in leaves of *P. deltoides* was observed in treatment with 100 ppm copper alone and along with entomopathogenic fungi, whereas, lowest sugar content 1.33 mg $g^{-1}$was observed in treatment $T_3$ (200 ppm Cu). According to Gasecka *et al.,* (2012) higher levels of copper causes a significant decrease in carbohydrates content of *Salix viminalis* L. Plucinska and Stobrawa (2004) reported less carbohydrate parameters viz., sucrose, glucose and galactose content in *P. deltoides* grown in polluted sites compared to unpolluted sites. Soluble sugar content of *A. procera* decreased with increasing concentration of heavy metals viz., Pb, As and Cd from 1-10mg/g. Soluble sugar an important constituent is manufactured during photosynthesis and breakdown occurs during respiration by plants.

The low sugar levels observed in the present study may be due to lowered synthesis or diversion of the metabolites to other synthesis processes as explained by Pandey and Tripathi (2011).

Highest total phenol content 1.24 mg $g^{-1}$ was observed in $T_3$ (200 ppm Cu), whereas, maximum (0.86 mg $g^{-1}$) was recorded in control. There was increase in phenol content with increase in copper concentration, indicating the protective role of phenols to plants. The results find support from the findings of Gasecka *et al.,* (2012) who reported that the total phenol content of *S. viminalis* L. rapidly increased at increased copper level. Serving as chelators and antioxidants, the phenol content plays a protective role for plants and controls the oxidative stress under abiotic and biotic stress conditions (Michalak, 2006).

Highest root biomass (0.69 g) of *P. deltoides* was observed in treatment $T_9$ (100 ppm Cu + *B. bassiana*, $1x10^5$ conidia/ml) and lowest biomass 0.35 g was recorded at $T_1$ (No copper) (Table 2). Apart from control lowest biomass 0.42 g was recorded at $T_3$ (200 ppm Cu). The results are in conformity with the findings of Borghi *et al.,* (2007) who reported highest root biomassof0.42 g of *P. deltoides*× *P. nigra* at copper concentration of 500 µM which decreased to 0.15 g at 1000 µM.

Highest stem biomass (1.17 g) was observed at treatment $T_9$ (100 ppm Cu + *B. bassiana*, 1 x $10^6$ conidia/ml) which decreased to 0.62 g at 200 ppm copper. Highest leaf biomass 2.22 g was observed in treatment $T_9$ (100 ppm Cu + *B. bassiana* $2^{nd}$ dose) and lowest leaf biomass 1.18 g was recorded at $T_1$ (No copper).

Similar results were obtained by Borghi *et al.,* (2008) who reported that there was increase in leaf biomass of 6.34 g at 25 µM of *P. deltoidesx P. canadensis* which decreased to 3.85 g at 75 µM at increased copper concentration.

Statistically, lowest content of copper (31.00 ppm) in roots was recorded at treatment $T_1$ (No copper) (Table 2). Whereas, highest concentration (68.25 ppm) was recorded at $T_3$ (200 ppm copper) which was statistically at par with $T_{10}$ (67.20 ppm), T6 (66.00 ppm), $T_{11}$ (66.50 ppm) and $T_7$ (63.50 ppm) *i.e.* treatments with 200 ppm copper alone and along with entomopathogenic fungi showed higher content of copper in roots.

**Table.1** Effect of different levels of coppers on morphological and biochemical characteristics of *Populus deltoides*

| Treatment | Morphological Characterstics | | | Biochemical Characterstics(mgg$^{-1}$) | | | |
|---|---|---|---|---|---|---|---|
| | Number of Leaves | Leaf area ($m^2$) | Height (m) | Chlorophyll content | Ascorbic acid | Total sugar | Total Phenols |
| T1(No copper) | 33 | 0.08 | 1.55 | 0.11 | 3.57 | 0.88 | 0.86 |
| T2(100ppmCu) | 51 | 0.14 | 1.71 | 0.24 | 4.29 | 1.5 | 1.11 |
| T3(200ppmCu) | 46 | 0.1 | 1.44 | 0.06 | 5.74 | 1.33 | 1.24 |
| T4(100ppmCu+*M.anisopliae*,1x10$^5$conidia/ml) | 51 | 0.14 | 1.75 | 0.24 | 5.63 | 1.51 | 1.09 |
| T5(100ppmCu+*M.anisopliae*,1x10$^6$conidia/ml) | 53 | 0.15 | 1.76 | 0.26 | 5.55 | 1.53 | 1.08 |
| T6(200ppmCu+*M.anisopliae*,1x10$^5$conidia/ml) | 46 | 0.11 | 1.44 | 0.07 | 3.62 | 1.35 | 1.22 |
| T7(200ppmCu+*M.anisopliae*,1x10$^6$conidia/ml) | 47 | 0.12 | 1.45 | 0.08 | 3.69 | 1.36 | 1.21 |
| T8(100ppmCu+*B.bassiana*, 1x10$^5$conidia/ml) | 51 | 0.15 | 1.75 | 0.25 | 5.6 | 1.5 | 1.1 |
| T9(100ppmCu+*B.bassiana*, 1x10$^6$conidia/ml) | 52 | 0.16 | 1.76 | 0.28 | 5.64 | 1.52 | 1.1 |
| T10(200ppmCu+*B.bassiana*, 1x10$^5$conidia/ml) | 46 | 0.11 | 1.46 | 0.06 | 3.6 | 1.35 | 1.22 |
| T11(200ppmCu+*B.bassiana*, 1x10$^6$conidia/ml) | 48 | 0.12 | 1.46 | 0.07 | 3.78 | 1.36 | 1.21 |
| **CD(0.05)** | **5.57** | **0.01** | **0.06** | **0.02** | **0.18** | **0.03** | **0.05** |

Values are mean of four replications

**Table.2** Effect of different levels of coppers on biomass partioning, distribution and uptake of *Populus deltoides*

| Treatment | Biomass (g) | | | Distribution of Copper (ppm) | | | Copper Uptake (mg/Plant) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Root | Stem | Leaves | Root | Stem | Leaves | Root | Stem | Leaves | Total uptake |
| T1(No copper) | 0.35 | 0.55 | 1.18 | 31.00 | 10.75 | 6.50 | 0.12 | 0.12 | 0.03 | 0.27 |
| T2(100ppmCu) | 0.65 | 1.13 | 2.15 | 48.50 | 33.25 | 25.75 | 0.26 | 0.45 | 0.16 | 0.87 |
| T3(200ppmCu) | 0.42 | 0.62 | 1.31 | 68.25 | 44.25 | 34.25 | 0.31 | 0.57 | 0.17 | 1.05 |
| T4(100ppmCu+*M.anisopliae*,1x10$^5$conidia/ml) | 0.67 | 1.14 | 2.17 | 45.00 | 23.50 | 23.50 | 0.25 | 0.33 | 0.16 | 0.74 |
| T5(100ppmCu+*M.anisopliae*,1x10$^6$conidia/ml) | 0.68 | 1.15 | 2.20 | 42.50 | 21.50 | 21.00 | 0.23 | 0.3 | 0.14 | 0.67 |
| T6(200ppmCu+*M.anisopliae*,1x10$^5$conidia/ml) | 0.43 | 0.65 | 1.32 | 66.00 | 43.00 | 32.50 | 0.31 | 0.55 | 0.17 | 1.03 |
| T7(200ppmCu+*M.anisopliae*,1x10$^6$conidia/ml) | 0.44 | 0.67 | 1.33 | 63.50 | 41.75 | 31.25 | 0.31 | 0.56 | 0.17 | 1.04 |
| T8(100ppmCu+*B.bassiana*, 1x10$^5$conidia/ml) | 0.66 | 1.16 | 2.20 | 47.25 | 28.50 | 23.75 | 0.25 | 0.4 | 0.16 | 0.81 |
| T9(100ppmCu+*B.bassiana*, 1x10$^6$conidia/ml) | 0.69 | 1.17 | 2.22 | 46.50 | 27.25 | 22.50 | 0.26 | 0.38 | 0.15 | 0.79 |
| T10(200ppmCu+*B.bassiana*, 1x105conidia/ml) | 0.45 | 0.64 | 1.32 | 67.20 | 41.50 | 33.25 | 0.31 | 0.53 | 0.18 | 1.02 |
| T11(200ppmCu+*B.bassiana*, 1x106conidia/ml) | 0.47 | 0.66 | 1.33 | 66.50 | 37.25 | 32.50 | 0.32 | 0.48 | 0.18 | 0.1 |
| **CD(0.05)** | **0.04** | **0.04** | **0.04** | **5.44** | **13.24** | **3.18** | **0.05** | **0.2** | **0.02** | **0.002** |

Values are mean of four replication

Highest copper content of 68.25 ppm in root of *P. deltoides* was observed at $T_3$ (200 ppm Cu) and the lowest (31.00 ppm) at control $T_1$ (No copper). At treatments where 100 ppm and 200 ppm was applied Cu content varied from 42.50 - 48.50 ppm and 63.50 - 68.25 ppm, respectively. The highest content of copper in roots was recorded at highest concentration of copper. The results are in conformity with the findings of Borghi *et al.,* (2007) who reported that, in roots of poplar clone copper content was about 20 times higher than in leaves and stem.

Poplar has a large root apparatus capable to deepen and explore profound layers of ground, able to bind high amount of Cu and for this reason it could be a good candidate for phytostabilization of Cu contaminated sites. In a study by Kusorkiran *et al.,* (2004) most of the copper was immobilized in the roots of willows (Salix sp.). It was reported to bound to the root surface or adsorbed by the apoplast where its effects are less detrimental. Further, in their study they reported that an increase from 5 to 25µm Cu in solution did not lead to an increase in shoot accumulation.

Highest content of copper 44.25 ppm was recorded in stem of *P. deltoids* where high concentration of copper (200 ppm) was applied. The lowest copper content at 100 ppm was 33.25 ppm which ranged from 21.50 - 28.50 ppm after the application of entomopathogenic fungi. Present findings are in confirmation with the findings of Durand *et al.,* (2011) who reported that accumulation of Zn in stem of control plants *P. tremula* x *P. alba* was lowest 61.3 mg/kg but highest 240.40 mg/kg when treated with Zn. Highest copper content (34.25 ppm) in leaves of *P. deltoides* was observed in treatment $T_3$ (200 ppm Cu), whereas, lowest Cu content (6.50 ppm) was observed in treatment $T_1$ (no copper). In the study of Borghi *et al.,* 2008) poplar clones grown with copper had higher copper concentration in leaves as compared to control plants. Highest copper content was recorded in roots followed by stem and leaves in *P. alba* and *P. canadensis.*

Highest uptake (0.32 mg/plant) of copper in roots was observed at $T_{11}$ (200 ppm Cu + *B. bassiana*, $1x10^6$ conidia/ml), whereas, lowest (0.12mg /plant) was recorded where copper was not applied. Highest uptake (0.57mg/kg) in stem was observed at $T_3$ (200 ppm copper) and the lowest (0.12 mg/kg) at $T_1$ (No copper). Whereas, highest copper uptake of 0.18 mg/kg in leaves was recorded at $T_{10}$ (200 ppm Cu + *B. bassiana*, $1x10^5$ conidia/ml). In a study to investigate the tolerance to high copper (Cu), the clones *P. canadensis* Adda and *P. alba* Villa franca showed different responses to Copper. *P. Canadensis* accumulated copper in roots (suitable for phytostabilization), while *P. alba* accumulated the metal in leaves, like an indicator species. In another study by Moffat *et al.,* (2001) the uptake ratio of heavy metals for Poplar clones (*P. trichocarpa* x*P. deltoids*) and *P. trichocarpa* grown in-situ contaminated with industrial waste increased as compared to control.

Highest (53) and lowest (33) numbers of leaves per plant of *P. deltoides* were recorded at treatment $T_5$ and $T_1$ respectively, whereas, leaf area was maximum (0.16 $m^2$) and minimum (0.08$m^2$) at treatment $T_9$ and $T_1$, respectively. Maximum plant height of 1.76m was observed in treatment $T_5$. The biomass of different plant parts of *P. deltoides* viz., roots, stem and leaves was statistically minimum at $T_1$, the root and stem biomass showed increase at 100 ppm copper along with entomo pathogenic fungi. Highest chlorophyll content was recorded at $T_9$ and lowest at $T_{10}$ and $T_3$. There was an increase in ascorbic acid with concentration of copper. The treatment combinations of 100ppm copper with entomopathogenic fungi recorded higher ascorbic acid content than respective control, whereas, there was decrease in ascorbic acid content in treatment with 200ppm copper combinations with entomopathogenic fungi. As compared to control ($T_1$) there was increase in total sugar content of *P. deltoids* but there was no significant difference between the respective control and their treatment combination with entomopathogenic fungi. There was increase in total phenol content of plants with increase in

copper concentration to 200ppm, highest phenol content was at $T_3$. Distribution of copper content in different parts of *P. deltoides* was in the order root>stem>leaves. The highest uptake of copper in roots, stem and leaves was 0.32. 0.57 And 0.18 mg/plant, respectively. The species was capable of tolerating high concentration of copper and could be good candidate for planting in copper contaminated soils.

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