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Research article

INFLUENCE OF COPPER STRESS ON THE GROWTH PERFORMANCE OF *EUCALYPTUS CAMALDULENSIS* SEEDLINGS

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ABSTRACT: Heavy metals are a group of elements to which belong micronutrients that are essential for adequate plant growth and development. In the present study our model example is copper (Cu). The accumulation of Cu salts in the environment causes toxicity to plants and ultimately Cu may find its way to human via food chain constituting a real threat to human life. The aim of this investigation was to analyze the response of *Eucalyptus camaldulensis*, an important tree species to Cu stress. To address this goal *E. camaldulensis* seedlings were maintained in long soil columns under nursery conditions and exposed to a wide range of Cu treatments: 0 (control), 10, 30, 50 and 80 µM Cu (supplemented as $CuSO_4.5H_2O$ for 4 weeks). Growth performance was monitored before and after Cu exposure. Growth data were obtained by measuring plant shoot height, stem diameter and leaf formation regularly once a week and the growth rates were determined. At harvest, each plant was separated into root and shoot systems to evaluate Cu effect on biomass production, accumulation and partitioning. Dry mass was subsequently determined for all plant fractions. Leaf formation rate was drastically reduced and the effect was most pronounced in plants which received the highest Cu level. Similar patterns were observed for both shoot height and stem diameter growth rates. Total plant dry mass as well as root-to-shoot were significantly reduced in response to Cu treatments, especially in seedlings exposed to the highest Cu concentrations. These findings might suggest that *E. camaldulensis* is sensitive to Cu stress. Further experiments to validate this speculation are required to elucidate the physiological events responsible for the poor performance of *E. camaldulensis* under Cu-polluted soils.
Keywords: *Eucalyptus camaldulensis*, growth, dry mass, copper stress, Sudan**.**

INTRODUCTION

The modern world is faced by several environmental hazards constituting a serious threat to human and other biological communities [1]. Among these environmental problems is the accumulation of heavy metals in plants and hence in the food chain as a result of the pollution of the soils and water with these elements. During the recent past the varied anthropogenic factors such as industrial activities, mining, sewage disposal, electroplating, ore refining and the uncontrolled development of cities have led to increasing the contamination of soil, water and air [2]. The heavy metal loads have been increasing over time due to atmospheric inputs. Such accumulation in the upper layers of the forest soil affects the establishment of seedlings and forest regeneration [3].

Some heavy metals play significant roles in plant growth and development. However, their accumulation in the natural ecosystems has been increasing and often far beyond the required limit for normal plant growth. Here, we present copper Cu^{2+}, a redox-active transition metal and an essential micro-nutrient for plant growth [4], but it can also be a potentially toxic element when tissue concentrations exceed only slightly the optimal demand [5, 6, 7]. The biological significance of copper can be understood through its contribution in various physiological processes in the plants. In these processes copper plays a role as a structural element in regulatory proteins besides its participation in photosynthetic electron transport, mitochondrial respiration, oxidative stress responses, cell wall metabolism and hormone signalling [4, 8].

The beneficial effect of copper on plant growth and development has been observed long ago when using fungicides which contain copper salts [9]. However, too frequent and prolonged fungicides application in agricultural soils results in over-accumulation of copper rendering both environmental quality and soil fertility for crop growth at great risk [10]. Plant species vary widely in their responses to copper exposure, particularly when available in high concentrations [11, 12, 13]. Generally for adequate plant growth, the concentration of a nutrient element should be adjusted at an optimum. To maintain metal homeostasis, plants – like other living organisms have different developed strategies involving the fine regulation of uptake, transport and distribution of a metal within the plant [5, 14, 15]. However, when plants receive extremely low or high dosage from such nutrients, their metabolic activities are disturbed and the growth is impaired [7]. Copper is one of the extremely toxic metals due to its high redox properties, therefore its cellular concentration must be at low levels to prevent phytotoxicity or plant death [6, 16]. In one report, [17] showed that when plants are grown at copper concentration above the optimal level, growth is inhibited and important cellular processes such as photosynthesis and respiration are disturbed. Previous studies showed that presence of heavy metals in excessive amounts significantly affected plant water status, causing water deficit and subsequent changes in the plants [18]. This is particularly important for seedlings because they are more sensitive to environmental stresses than mature trees. Growth analysis studies in poplar (*P. tremula* L. x *P. alba* L.) by [19] showed that low concentrations of copper had no inhibitory effect on culture quality (i.e., degree of chlorosis and browning) and shoot development. However, high concentrations especially of copper and lead, inhibited shoot and root development. On the other side, nutrient deficiency can critically limit plant growth. [20] showed that copper deficiency may have a particularly adverse effect by reducing root growth more than the shoot growth in young *Populus trichocarpa* hybrid.

Despite the significance of copper in maintaining adequate plant growth and development, knowledge on the copper requirements and sensitivity of trees is still in-sufficient. *Eucalyptus camaldulensis* is economically considered one of the most important forest tree species for its high productivity in a relatively short rotation. The current information with respect to the responses of *Eucalyptus camaldulensis* to copper is fairly understood, yet our knowledge to the physiological mechanisms behind these responses to copper stress is rather poor. Therefore, this experiment was carried out under nursery conditions and the principal objective was to determine the copper effect on the physiology and growth of young *Eucalyptus camaldulensis* seedlings. To address this goal, growth performance and the patterns of dry matter production, accumulation and partitioning were evaluated.

MATERIALS AND METHODS

Plant materials and experimental conditions

The current investigation was conducted on copper stress treatments employing young *Eucalyptus camaldulensis* seedlings. Germination and early establishment of the seedlings were performed in a nursery under partial shade. Freshly collected *Eucalyptus camaldulensis* seeds were directly sown in black non-transparent polythene bags (15 cm diameter X 40 cm height), five seeds per bag. The polythene bags were filled with a soil mixture containing silt: sand in a 2: 1 ratio (by volume), leaving the top 5 cm as a margin for irrigation. The bags were also perforated (6 holes/ bag) to ensure good aeration and also to facilitate easy drainage of excess water. Thereafter, the sown bags were placed under partial shade in a nursery. Watering was applied on daily basis by flood irrigation to field capacity for the first two months, then every other day for one month. During this phase of seedlings establishment singling and weeding were timely carried out. A total of 40 seedlings were chosen to run the experiment on the basis of vigour and uniformity in shoot height (mean shoot height 38.6 ± 1.05 cm). These candidate seedlings were grown for two more months for hardening outside the nursery. Watering was similarly maintained close to field capacity every second day.

Experimental design and copper treatments

The seedlings for the experiment were distributed into five groups of eight seedlings each. Each group was assigned for each of the following Cu treatments: 0 (control), 10, 30, 50 and 80 μM Cu) supplied as $CuSO_4.5H_2O$ to detect the range of sub-optimum, optimum and excess copper supply. Copper treatments were applied every week by adding a fresh copper solution to the seedlings at the same time with irrigation. The copper treatments lasted for four weeks.

Growth performance

To monitor growth, some growth variables (plant shoot height, number of leaves and stem diameter at the stem-root interface) were regularly determined once a week throughout the entire experimental period. At harvest, the final measurements of these growth parameters were also taken. During copper treatment, the growth rates of these growth variables were calculated for the last two weeks of the Cu treatment.

Dry mass production and partitioning

After four weeks exposure to copper treatments, the experiment was terminated by destructive harvesting of the whole plants. Each seedling was separated into shoot and root. The shoot was further divided into leaves and stem. Similarly, the root system was carefully rinsed with distilled water immediately after harvest then divided into coarse and fine roots. The fresh mass of each plant fraction was determined and for dry mass determination all plant materials were oven-dried to a constant weight at 60°C for seven days.

Statistical analysis

Results were statistically treated using the statistical programme JMP 5.1 Start Statistics, 3rd edition (SAS Institute, Inc., Cary, North Carolina, USA). The experiment was established in a completely randomised design. Data were expressed as means of eight replicates for each copper treatment. Analysis of variance was performed as One-Way-ANOVA and the separation of the means was performed by Tukey-test. A probability level of $P \leq 0.05$ was chosen to show the statistically significant variations among the means. Means followed by same letters are not significantly different from each other.

RESULTS AND DISCUSSION

In this study growth performance and dry mass production were evaluated using young seedlings of *Eucalyptus camaldulensis* to characterise the response of the seedlings under a four weeks exposure period to a relatively wide range of copper stress conditions (0 – 80 µM Cu).

Growth analysis

To evaluate the response patterns of *Eucalyptus camaldulensis* seedlings exposed to different levels of copper stress, the growth was assessed by taking regular measurements of some growth variables; plant shoot height, stem diameter and number of leaves. Growth analysis showed that plant shoot height was not affected by low doses of copper. However, growth rates were drastically reduced in plants that received the highest levels of copper treatment (50 and 80 µM Cu). Similarly, it was observed that leaf formation was significantly decreased in seedlings supplied with the highest levels of copper, relative to the controls (Table 1). The diameter growth was affected only at the highest copper concentration similar to the patterns observed in shoot elongation growth and leaf formation.

Table 1: Effects of different Cu treatments [0 (control), 10, 30, 50 and 80 µM Cu] on the growth rate of plant shoot height, stem diameter and leaf formation of *Eucalyptus camaldulensis.* The copper treatments lasted for four weeks. Growth measurements were carried out every week before and after copper addition. Values presented are mean growth rate of eight plants per treatment (n = 8, ± SE). Means separation was performed by Tukey test at $P \leq 0.05$. Means connected by similar letters are not significantly different from each other.

Copper treatment (µM)	Plant shoot height (cm/week)	Leaf formation (number of leaves/week)	Stem diameter (cm/week)
0 (control)	13.8 ± 1.05 a	8 ± 0.74 a	1.4 ± 0.79 a
10	11.5 ± 0.94 a	7 ± 0.51 a	1.2 ± 0.98 a
30	9.7 ± 0.88 a	5 ± 0.32 ab	0.8 ± 0.78 a
50	6.5 ± 0.75 b	3 ± 0.11 bc	0.5 ± 0.35 b
80	4.2 ± 0.53 bc	1 ± 0.23 c	0.2 ± 0.29 bc

Dry mass production and partitioning

To evaluate the influence of copper treatments on the growth of *Eucalyptus camaldulensis* seedlings total dry mass as well as root-to-shoot ratio were determined. Dry mass was found to be reduced across all copper treatments; however, significant reductions were observed only in plants supplied with the highest copper concentration when compared with the un-treated control seedlings (Table 2). Root-to-shoot ratios displayed typical fashions where statistically significant reductions were detected in plants with the highest copper concentration. These findings are in agreement with [21] who reported significant reductions in the biomass of Cu-treated roots of *Brassica junea* L. The observed reduction in plant dry mass is also in confirmity with similar investigations on *Pinus pinea* and *Pinus pinaster* seedlings exposed to copper stress [22]. Elevated copper supply has also been reported to reduce both shoot and root growth in creeping Bentgrass, *Agrostis palustris* Huds. Penncross [23], which adds aditional supporting evidence to our present findings.

Table 2: Effects of different Cu treatments [0 (control), 10, 30, 50 and 80 µM Cu] on total plant dry mass and root-to-shoot ratio of *Eucalyptus camaldulensis.* The plants were exposed to copper treatments for four weeks. Values presented are the mean of eight plants per treatment (n = 8, ± SE). Means separation was performed by Tukey-test at $P \leq 0.05$. Means connected by similar letters are not significantly different from each other.

Copper treatment (µM)	Total plant dry mass (g)	Root-to-shoot ratio
0 (control)	14.859 ± 0.39 a	0.17 ± 0.03 a
10	11.541 ± 0.48 a	0.16 ± 0.08 a
30	8.074 ± 0.81 b	0.11 ± 0.04 a
50	6.112 ± 0.77 bc	0.07 ± 0.02 b
80	4.233 ± 0.42 c	0.05 ± 0.01 b

Plant responses to high levels of heavy metals have been investigated in trees as well as other plant species [24, 25]. In this study, the growth data analysis indicated that the growth of *Eucalyptus camaldulensis* seedlings was in-sensitive to Cu treatments below 50 µM. However, the severity of the stress was most substantiated in plants treated with the highest levels of Cu concentrations (50 and 80 µM). It might be speculated that the inhibiting effect of copper on growth is a direct effect of the metal on both cell division and cell elongation. This speculation might be in accordance with [15] who noted that in hydroponic experiments the inhibition of root growth is a consequence of the adverse effect of heavy metals on root cell elongation and mitotic activity.

CONCLUSIONS

In conclusion and based on the present findings, the drastic effect of copper on growth and dry mass of *Eucalyptus camaldulensis* obviously indicates its sensitivity to copper stress, particularly at high concentration levels. Further research work is needed to elucidate the mechanisms responsible for the observed responses in *Eucalyptus camaldulensis* present in environments with elevated levels of copper. Improving our understanding of the physiology of *Eucalyptus camaldulensis* under heavy metal-contaminated sites would pave way for rational long-term silvicultural as well as management plans to maximize the multiple benefits from this valuable forest tree species. Currently, the rapid development of biotechnology might bring forward possibilities to make use of genetic engineering techniques for modifying the genetic make-up of *Eucalyptus camaldulensis* towards better tolerance ability to heavy metals, which would be then employed as candidate tree species in the process of phytoremediation to alleviate the horrible environmental pollution in heavy metal-polluted areas.

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