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Effects of Copper and Zinc Application on the Oil Palm Root Morphology and Epidermis Cell Size

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ABSTRACT

The growth of oil palm is highly dependent on the availability of nutrients, supplied through fertilizers. This is especially true for peat soils where there is a lack of Zn and Cu due to the soil being deficient of mineral and clay content. The deficiency of Zn and Cu has been found to inhibit oil palm plant and root growth. The objective of this study is to determine the effect of different concentration of Zn and Cu chelates on the root development of oil palm roots cell walls. The oil palm seedlings were harvested after two different growing periods; the first harvest was conducted after three months of planting while the second harvest was after 6 months of planting. The harvested oil palm seedlings were measured for root length, root thickness and root surface area. The best root growth for the copper treatments was with concentrations of 8.00 mg Cu/palm. The cell epidermis size and cortex cells of the oil palm seedlings in this treatment was the smallest, meaning that it did not experience any Cu toxicity, while it had the longest root length, root surface area, root fresh weight and root dry weight. For the zinc treatments, the best root growth was at 5.00 mg/palm. This is because in this treatment, the oil palm seedlings achieved the highest root length, root surface area, root fresh weight and root dry weight. This means that the best concentration of Cu to promote root growth of oil palm seedlings is 8.00 mg Cu/palm while the best concentration of Zn was 5.00 mg Zn/palm. Further research needs to also be conducted using a combination of both Zn and Cu applications at the same time, to determine if the combined micronutrients had different effects on the oil palm seedlings roots growth.

1. Introduction

Oil palm (*Elaeis guineensis* Jacq.) is a perennial crop vital to produce edible oils; Malaysia is currently the 2nd world's largest producer and exporter of palm oil. Malaysia produces about 47% of the world's supply of palm oil and is a major agriculture crop in Malaysia, besides natural rubber. Oil palm is a monoecious plant, meaning that it has male and female flowers on the same tree. The oil palm fruit is dark in colour and the colour will change to reddish orange during maturity. *Tenera* is a hybrid species resulting from a cross between *pisifera* and *dura*. *Tenera* hybrid can produce about 4 to 5 tonnes of crude palm oil (CPO) per hectare. Wakisaka et al. [1] stated that the oil palm industry in Malaysia alone produces more than 12 million tonnes of CPO per year, while empty fruit bunch (EFB) produced is more than 14 million tonnes together with 25 million tonnes of palm oil mill effluent (POME). The oil palm originates from the tropical region of West Africa, but oil palm grows well in Southeast Asian countries, especially Malaysian and Indonesia due to the suitable climatic condition and soil fertility. Rieger [2] summarized that the world production of oil palm is about 153,578,600 million tonnes, higher than any other agriculture crops in the world.

The growth of oil palm depends on the fertility of the soil and the availability of nutrients supplied through fertilizer applications. Nutrients needed by oil palm includes nitrogen (N), potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg) and sulphur (S) while micronutrients needed for oil palm are manganese (Mn), boron (B), zinc (Zn), copper (Cu), iron (Fe), molybdenum (Mo) and chlorine (Cl) which are essential in promoting oil palm tree growth. In Malaysia, cultivation of oil palm takes place mainly on highly weathered and acidic soils. The ability of oil palm trees to grow well on acidic soil has made Malaysia a major producer of oil palm products. Fertilizer is applied below the oil palm fronds due to the presence of roots that can absorb the nutrients or in holes around the plant in order to avoid the loss of fertilizer by the leaching of nutrients. For peat,

leaching of zinc (Zn) and copper (Cu) is a problem due to the lack of mineral and clay content in peat soil to hold these nutrients. Their deficiency can reduce plant and root growth and also cause chlorosis symptoms.

The biggest constraint in oil palm production is low soil fertility. Obi and Udoh [3] summarized that more than 95% of oil palms grown in Southeast Asia are on acid, low fertility and highly weathered soils. Soil physical properties like depths, texture and aggregate stability are essential factors in determining the suitability to establish a large oil palm plantation. Furthermore, suitable types of soil will encourage root elongation and give a strong anchorage to the tree.

The cell wall of plant roots has a great reactivity towards accumulation of metals and also has the capability to act as barrier to inhibits the passage of non-hyperaccumulating metals. Straczek et al. [4] summarized the affinity of cations for exchangeable sites on root cell walls decreases in the order of H>Cu>Ca>Zn and H>Cu>Zn. Even though Zn has a high affinity for cell walls, there is still no concrete evidence of the Zn-roots associated complexes stability.

Copper (Cu) is an important micronutrient, which is responsible for the formation of cell walls, plants metabolism, synthesis of protein, electron transport in photosynthesis, mitochondrial respiration and hormone secretion in plants [5]. It exists in Cu⁺ and Cu²⁺ state, and it has the potential to generate reactive oxygen in the metal catalyzed by the Haber-Weiss reaction [5,6]. In addition, copper is important for plant growth and makes the plant resistant to diseases [7,8]. However, excess of Cu can affect the number of lateral root and root length. Marschner [9] reported that accumulation of excess Cu in plants leads to growth inhibition, discoloration of leaf and necrotic conditions. Apart from playing significant roles as a redox component participating in the electron transfer reactions in photosynthetic processes in plants, the excess free intracellular Cu ions produce an auto-oxidation and Fenton reaction of radical hydroxyls [10,11].

The deficiency of zinc will reduce the grain yield and also the nutritional quality of grain in crop plants. Environmental effects on the soil such as highly weathered soil profiles (Ultisols and Oxisols) will reduce the availability of zinc for plants because of low Zn concentrations in the

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parent materials [12]. Zinc plays a role as a regulatory co-factor process in plants. For the leaf, zinc is important for the carbohydrate formation and essential in the production of chlorophyll of the oil palm leaves. Furthermore, the absence of zinc will affect the efficiency of water uptake by the plants. Besides that, zinc can help the plant in the growth of roots. There are abundance of research focusing on the yield of oil palm, fungus infection and nutrient disorders. However, research on the effects of micronutrients on the development of the cell walls of oil palm roots is still not well understood. As Zn and Cu play essential roles in the formation and growth of the roots, it is vital that we better understand the optimal concentrations required for positive root growth. Besides, too much Zn and Cu concentrations in the soil can lead to toxicity in the plants. Therefore, the objective of this study is to determine the effects of different concentrations of Zn and Cu on the root growth and development of oil palm roots cells.

2. Experimental Methods

2.1 Seedlings and Study Location

Oil palm seedling (ML 161) obtained from the Federal Land Development Authority (FELDA), Malaysia was selected for this study. The fertilizers used were copper and zinc chelates. This experiment was conducted at the shed-house in Ladang 10, University of Putra Malaysia (latitude 2.99° N, longitude 101.71° E).

2.2 Experimental Design

The experimental design used for this study was completely randomized design (CRD). There were five treatments respectively for copper chelates and zinc chelates at varying concentrations of fertilizer applications, with four replicates for each treatment. The treatment levels are shown in Table 1. The oil palm seedlings were planted on *Typic hapludox* soil, which is very fine, kaolinitic and isohyperthermic, pH 5.0 to 6.4 and consists of 1.00% organic matter. For macronutrient level, total nitrogen, available P and exchangeable K, the levels were 0.09%, more than 4.0 mgPkg⁻¹ and more than 0.1 cmolkg⁻¹ respectively. The CEC value was 6.35 cmolkg⁻¹. The oil palm seedlings were planted in 120 poly bags. Each polybag contained 20-25 kg of Munchong series soil. During the planting period, different concentrations of copper chelate and zinc chelate treatments were applied and harvested at two different periods. The first harvest was conducted after three months of planting while the second harvest was after 6 months of planting. Each sampling involved 40 oil palm seedlings. The 80 polybags planted with oil palm seedlings in this study was divided into two main groups. The main reason for this was because each group will be harvested at 2 different periods. The first 40 experimental units were harvested on 2nd August 2012. The harvested oil palm seedlings were measured for root length, root thickness and root surface area. The second harvest for the remaining 40 oil palm seedlings was done on 2nd November 2012, and the same analyses were conducted.

Table 1 Copper and zinc treatment levels for each oil palm tree

Treatments	Replications
CuT ₀	4
CuT ₁	4
CuT ₂	4
CuT ₃	4
CuT ₄	4
ZnT ₀	4
ZnT ₁	4
ZnT ₂	4
ZnT ₃	4
ZnT ₄	4
Total of replications	40

Note: CuT₀ had Cu 0 mg/palm; CuT₁ had Cu 2 mg/palm; CuT₂ had Cu 4 mg/palm; CuT₃ had Cu 6 mg/palm; CuT₄ had Cu 8 mg/palm; ZnT₀ had 0 Zn mg/palm; ZnT₁ had 5 Zn mg/palm; ZnT₂ had Zn 10 mg/palm; ZnT₃ had 15 Zn mg/palm; ZnT₄ had 20 Zn mg/palm

2.3 Sampling and Analyses

Cross section of oil palm roots was examined using scanning electron microscope at the Institute of Biosciences, UPM. Length, surface area, fresh and dry weights of oil palm roots were also measure during the first and second harvest.

2.4 Statistical Analyses

Statistical analysis was conducted for all the data collected. Analysis of variance (one-way ANOVA) was used to determine the treatments effects, followed by Tukey post-hoc test to compare the significant difference

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among the means of treatments. Correlation analyses were carried out to determine the relationship between Zn and Cu treatments with the root length, surface area, fresh weight and dry weight. All of the data analyses were done using statistical analysis system (SAS) software.

3. Results and Discussion

3.1 Effect of Cu and Zn Treatment on Oil Palm Root Cell Size after 1st Harvest

The 1st harvest was conducted three months after planting the oil palm seedlings. As shown in Figs. 1 and 2; and Table 2, the largest size of root epidermis cell in CuT₁ is 87.5 µm, while the cortex cell size is 17.5 µm. Treatment CuT₄ exhibits the smallest epidermis (41.12 µm) and cortex (8.5 µm) cells size. This result indicates that the size of oil palm root epidermis and cortex cells decreased with increasing rates of copper chelates applied. Tisdale et al. [10] found that the transport of electron is facilitated by the presence of copper which is essential during cell wall formation. Furthermore, Marschner [9] stated that lignification of the cell wall will be distorted when the plant is experiencing Cu deficiency. An increase in length of the root epidermis and cortex cells is an indication of the plant experiencing toxicity in the soil, as it is the frontier tissue in direct contact with the toxic element [13,14]. In this study, as CuT₄ had the shortest root epidermis, it is a clear indication that the oil palm seedlings were not experiencing any Cu toxicity despite having the highest concentration of Cu in the growth medium.

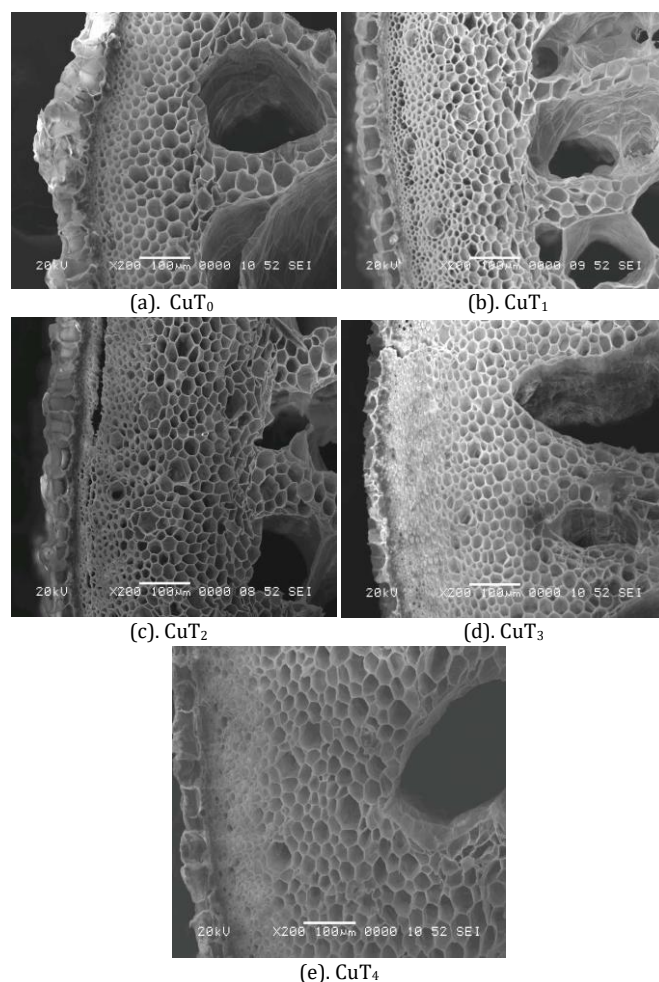


Fig. 1 Cross section of oil palm roots epidermis and cortex cells treated with Cu (1st harvest)(Magnification: 200x) Note: CuT₀, 0 mg Cu/palm; CuT₁, 2 mg Cu/palm; CuT₂, 4 mg Cu/palm; CuT₃, 6 mg Cu/palm; CuT₄, 8 mg Cu/palm)

The largest oil palm roots epidermis and cortex cells size for Zn treatments was seen in ZnT₀ which are 67.39 µm and 9.78 µm respectively (Table 2). In contrast, ZnT₄ showed the smallest epidermis and cortex cells size of 35.96 µm and 5.96 µm. The treatment results showed that the epidermis and cortex cell size of oil palm roots decreased with increasing applications of Zn. Fageria [11] stated that Zn aids in plant growth, which is essential in carbohydrate and enzyme formation. Similar to Cu, the oil palm seedlings in the treatment with the highest Zn concentrations (ZnT₄) had the smallest root epidermis and cortex cells size. This means that the oil palm seedlings were not experiencing Zn toxicity.

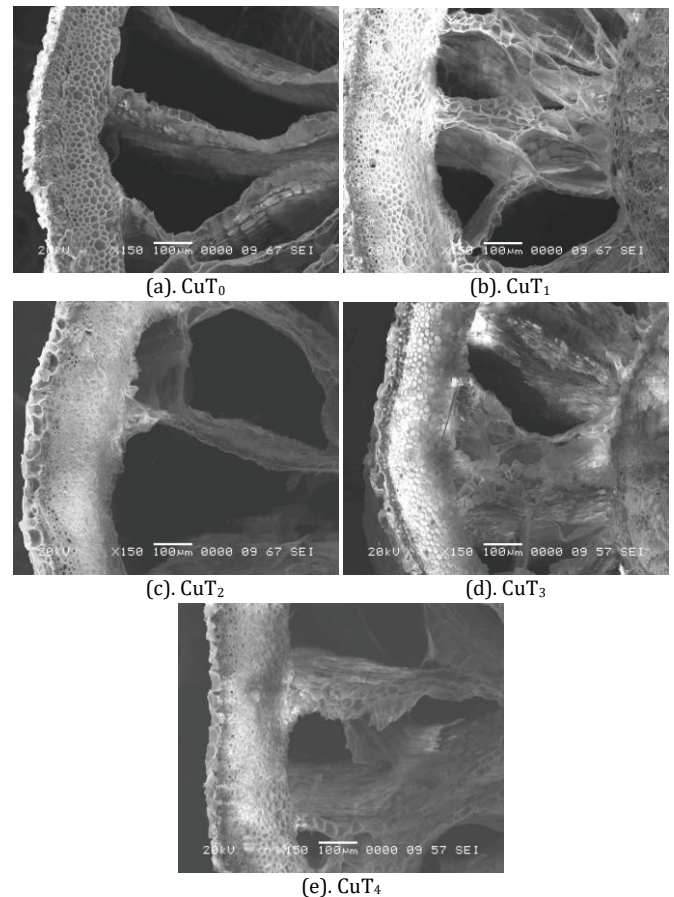
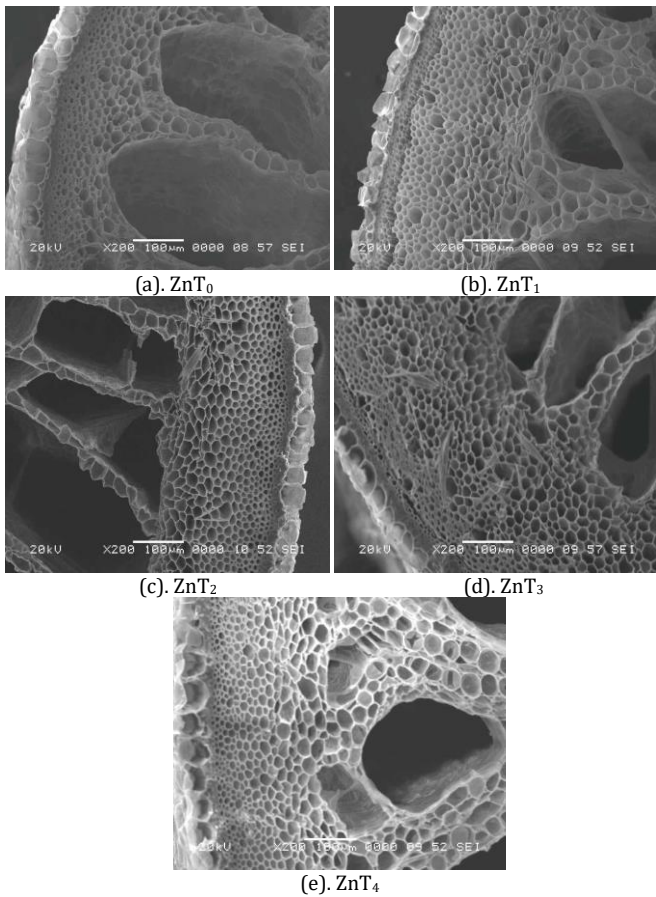


Fig. 2 Cross section of oil palm roots epidermis and cortex cells treated with Zn (1st harvest)(Magnification: 200x) Note: ZnT₀, 0 mg Zn/palm; ZnT₁, 5 mg Zn/palm; ZnT₂, 10 mg Zn/palm; ZnT₃, 15 mg/palm; ZnT₄, 20 mg Zn/palm

Fig. 3 Cross section of oil palm roots epidermis and cortex cells treated with Cu (2nd harvest)(Magnification: 200x) Note: CuT₀, 0 mg Cu/palm; CuT₁, 2 mg Cu/palm; CuT₂, 4 mg Cu/palm; CuT₃, 6 mg Cu/palm; CuT₄, 8 mg Cu/palm

Table 2 Effects of Cu and Zn treatments on oil palm roots cell size (1st harvest)

Treatment	Copper treatments (Cu)		Zinc treatment (Zn)	
	Epidermis (µm)	Cortex (µm)	Epidermis (µm)	Cortex (µm)
T ₀	87.5	17.5	67.39	9.78
T ₁	57.58	16.67	43.62	8.72
T ₂	50.40	15.09	41.49	7.66
T ₃	43.08	9.3	36.81	6.81
T ₄	41.12	8.5	35.96	5.96

Note: CuT₀, 0 mg Cu/palm; CuT₁, 2 mg Cu/palm; CuT₂, 4 mg Cu/palm; CuT₃, 6 mg Cu/palm; CuT₄, 8 mg Cu/palm; ZnT₀, 0 mg Zn/palm; ZnT₁, 5 mg Zn/palm; ZnT₂, 10 mg Zn/palm; ZnT₃, 15 mg/palm; ZnT₄, 20 mg Zn/palm

Table 3 Effects of Cu and Zn treatments on oil palm roots cell size (2nd harvest)

Treatment	Copper treatments (Cu)		Zinc treatment (Zn)	
	Epidermis (µm)	Cortex (µm)	Epidermis (µm)	Cortex (µm)
T ₀	40.26	12.31	47.27	7.68
T ₁	37.95	10.77	38.38	7.27
T ₂	34.62	10.00	33.33	6.87
T ₃	33.08	7.44	39.00	6.26
T ₄	25.39	6.15	31.72	5.66

Note: CuT₀, 0 mg Cu/palm; CuT₁, 2 mg Cu/palm; CuT₂, 4 mg Cu/palm; CuT₃, 6 mg Cu/palm; CuT₄, 8 mg Cu/palm; ZnT₀, 0 mg Zn/palm; ZnT₁, 5 mg Zn/palm; ZnT₂, 10 mg Zn/palm; ZnT₃, 15 mg/palm; ZnT₄, 20 mg Zn/palm

3.2 Effect of Cu and Zn Treatment on Cell Size after 2nd Harvest

The second harvest was done 6 months after planting. Treatment CuT₀ showed the largest epidermis and cortex cell size of each 40.62 µm and 12.31 µm, respectively (Figs. 3 and 4; and Table 3). Treatment CuT₄ showed the smallest size of oil palm roots epidermis and cortex cells size. The size of the epidermis and cortex cells for the copper treatments decreased with increasing rates of copper chelates. As for Zn treatment, the largest oil palm root epidermis (47.27 µm) and cortex (7.68 µm) cells was found in ZnT₀, while the smallest roots epidermis (31.72 µm) and cortex (5.66 µm) was observed in ZnT₄ (Table 3). The treatment results show that the epidermis and cortex cells size of oil palm roots decreased with increasing applications of Zn. These results were similar to the 1st harvest oil palm seedlings. This means that the oil palm seedlings did not experience Cu or Zn toxicity, as the epidermis and cortex cell size was the smallest in the treatment with the highest concentrations of Cu and Zn.

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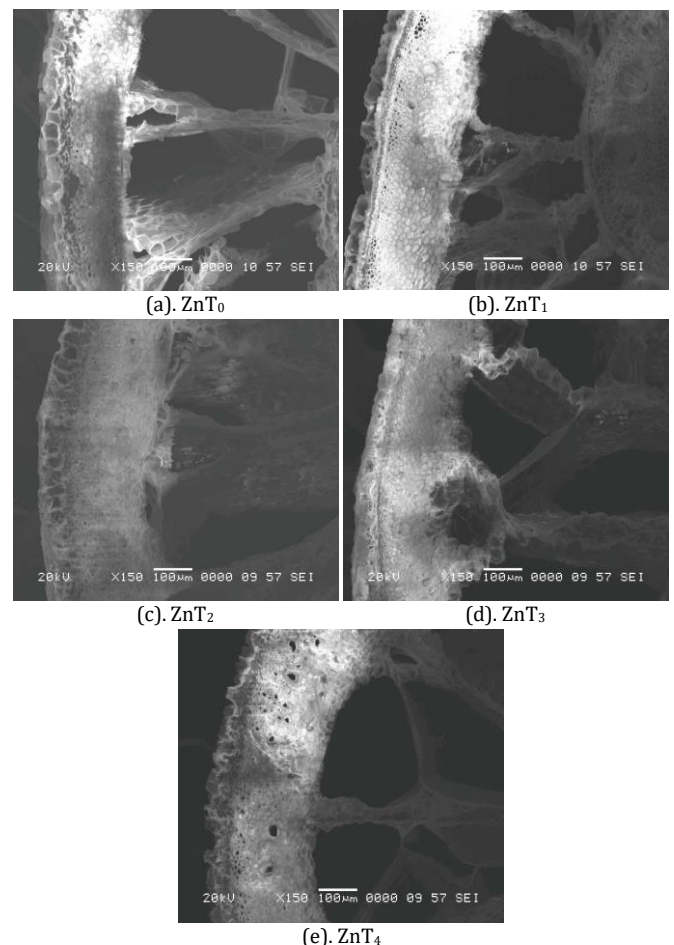


Fig. 4 Cross section of oil palm roots epidermis and cortex cells treated with Zn (2nd harvest). ZnT₀, 0 mg Zn/palm; ZnT₁, 5 mg Zn/palm; ZnT₂, 10 mg Zn/palm; ZnT₃, 15 mg/palm; ZnT₄, 20 mg Zn/palm

3.3 The characteristic of Oil Palm Roots in Cu and Zn Treatments - 1st Harvest

Table 4 below summarizes the effects of different Cu and Zn treatment level analyzed after the 1st and 2nd harvest on selected characteristics of oil palm roots. From the results, there are significant differences detected between treatment levels for dry weight under Cu treatment for the 1st harvest phase.

As for Zn treatment, root length and dry weight of oil palm roots shows comparative differences between levels. Oil palm roots in CuT₄ treatment showed the highest dry weight of 14.1 g followed by CuT₂ (13.7 g), CuT₃ (13.5 g), CuT₁ (11.6 g) and CuT₀ (10.7 g) has the lowest amount of oil palm roots dry weight. As CuT₄ had the highest root dry weight, it shows that the oil palm seedlings in this treatment had the best root growth. This is a further proven with its lowest length in root epidermis and cortex cells, which shows that the plant is not experiencing Cu toxicity. Under Zn treatment (1st harvest), ZnT₁ shows the longest root length of 7789.9 cm while T₂ show the shortest root length of 3275.6 cm. For the dry weight, ZnT₁ shows the highest value of 11.3 g, while ZnT₀ shows the lowest dry weight of 6.2 g. The analyzed results for the 2nd harvest for both Cu and Zn treatments exhibits no significant differences for root length, surface area, fresh weight and dry weight of oil palm seedling roots.

The treatment results show that the epidermis and cortex cells size of oil palm roots decreased with increasing applications of Zn. ZnT₁ had the longest root length, indicating that the oil palm seedlings in this treatment had the best root growth. It means that the ideal Zn concentration of Zn is 5 mg palm⁻¹.

Table 4 Length, surface area, fresh and dry weight of oil palm roots for in 1st and 2nd harvest

Treatment	First (1 st) harvest		Second (2 nd) harvest	
	Copper (Cu)	Zinc (Zn)	Copper (Cu)	Zinc (Zn)
Root length (cm)				
T ₀	4809.2a	3521.7b	14570a	17625a
T ₁	4878.6a	7789.9a	17653a	19130a
T ₂	5150.4a	3275.6b	18927a	18028a
T ₃	5083.4a	4237.2ab	18826a	18824a
T ₄	5392.1a	4325.0ab	19987a	18957a
Surface area (cm²)				
T ₀	1875.2a	1002.0a	3282a	4029a
T ₁	1921.1a	1992.6a	4790a	6935a
T ₂	2065.6a	1231.3a	4825a	4892a
T ₃	1984.2a	1456.5a	4810a	5000a
T ₄	2103.2a	1275.8a	5001a	5633a
Fresh weight (g)				
T ₀	53.4a	38.2a	72.4a	149.2a
T ₁	54.9a	47.6a	152.2a	160.5a
T ₂	60.7a	39.4a	176.7a	143.4a
T ₃	57.2a	42.2a	176.0a	142.8a
T ₄	61.2a	43.8a	176.9a	146.0a
Dry weight (g)				
T ₀	10.7b	6.2b	24.3a	41.4a
T ₁	11.6ab	11.3a	40.8a	42.3a
T ₂	13.7ab	6.8b	50.1a	37.8a
T ₃	13.5ab	7.4ab	48.2a	38.6a
T ₄	14.1a	8.0ab	51.6a	40.4a

Note: CuT₀, 0 mg Cu/palm; CuT₁, 2 mg Cu/palm; CuT₂, 4 mg Cu/palm; CuT₃, 6 mg Cu/palm; CuT₄, 8 mg Cu/palm; ZnT₀, 0 mg Zn/palm; ZnT₁, 5 mg Zn/palm; ZnT₂, 10 mg Zn/palm; ZnT₃, 15 mg/palm; ZnT₄, 20 mg Zn/palm; Means within column with different letters are significantly different at p≤0.05

4. Conclusion

From this study, the best root growth for oil palm seedlings was in CuT₄ (8.00 mg Cu/palm). The cross section of root of oil palm seedling for the 2nd harvest in copper chelates treatment was almost similar to that of first harvest. For the root characteristics, the rates of copper chelate at treatment 5 (8.00 mg/palm) showed the highest root length, root surface area, root fresh weight and root dry weight. This means that the best concentration of Cu to promote root growth of oil palm seedlings is 8.00 mg Cu/palm. For zinc chelates, the best root growth was in ZnT₁ (5.00 mg/palm). Increasing the application of zinc chelates to the oil palm seedling causes the root cells to be smaller and become more compact (in harvest 1). For second harvest in zinc chelates treatment, the cross section of root of oil palm seedling was slightly different from first harvest. The aerenchyma of the root became larger while the cortex became more compacted. Therefore, the best rates zinc chelates was treatment 1 (5.00 mg Zn/palm). More research needs to be conducted for longer growing durations to determine the optimum concentration of Zn and Cu for optimum oil palm root growth. Besides that, this research only observed the effects of Zn and Cu as separate applications. Hence, applications using a combination of both Zn and Cu at the same time should be conducted.

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