Original Research Article

Investigating the Impact of Zinc on Chlorophyll Content and Leaf Area in Arecanut Seedlings

ABSTRACT

A sand culture experiment was carried out at ICAR-CPCRI, Regional Station, Vittal in the year 2021, to evaluate the impact of different concentrations of Zinc (Zn) on chlorophyll content and leaf area in arecanut seedlings. Eight varieties of arecanut seedlings (Mangala, Swarnamangala, Madhuramangala, Shatamangala, SouthKanara local (S K local), Thirthahalli, Sirsi arecanut selection-1 (SAS -1), Hirehalli dwarf) were cultivated in a naturally ventilated glasshouse using sand culture provided with 0.031, 0.093 and 0.156 ppm of Zn. After six months of growth, the seedlings were assessed for chlorophyll a, chlorophyll b, total chlorophyll content, and total leaf area. The results indicated that the chlorophyll content and total leaf area of arecanut seedlings were significantly influenced by different varieties and varying levels of zinc supplementation.Maximum values for both chlorophyll content and total leaf area were observed at a Zn concentration of 0.093 ppm (Z_2 level). This study suggests that among the different levels of Zn, a concentration of 0.093 ppm (medium level) is optimal for promoting the growth of arecanut seedlings.

*Keywords:*Arecanut, Varieties, Zinc concentrations, Chlorophyll content, Leaf area.

1. INTRODUCTION

Chlorophyll, the green pigment found in the chloroplasts of plant cells, is instrumental in facilitating photosynthesis, the process by which plants harness light energy to synthesize organic compounds. It stands as a cornerstone of plant physiology, governing the plant's ability to convert solar energy into vital chemical resources (Kosesakal, et al., 2009).

Arecanut (Areca catechu L.), a significant cash crop, is valued for its economic importance and cultural significance. It holds a particular prominence in many tropical regions as a key contributor to agro-economies(Bavappa, 2004).Optimizing the growth and yield of arecanut necessitates a comprehensive understanding of the factors that influence its physiological processes, including chlorophyll biosynthesis.

Among the essential micronutrients that play a pivotal role in plant growth and development, Zn emerges as a critical determinant. As an indispensable cofactor for enzymes, Zn participating in chlorophyll synthesis holds sway over the green pigmentation crucial to photosynthetic efficacy. It also plays a crucial role in influencing the leaf area of plants. The availability of Zn is known to significantly impact the synthesis and activity of auxins, particularly indole acetic acid (Cakmak et al., 1989).When zinc availability is limited, it can lead to a decrease in auxin synthesis or an increase in their degradation. Since auxins are involved in promoting cell elongation, a deficiency in zinc can hinder the elongation of cells,

ultimately affecting leaf expansion and development. As a result, the overall leaf area of plants may be restricted, impacting their ability to capture sunlight for photosynthesis and, consequently, influencing their overall growth and productivity (Salami and Kenefick, 1970).Studies conducted in various arecanut growing regions have reported that Zn was found to be one of the most deficient and critical micronutrients. The availability of Zn in the soil, therefore, becomes a factor of paramount importance for the well-being and productivity of arecanut plantations(Kumar et al., 2018).

Balancing Zn levels presents a delicate challenge, as both deficiency and excess can lead to detrimental effects. Zn deficiency manifests in chlorosis(a condition marked by the yellowing of leaves due to reduced chlorophyll production) and Leaf expansion. Conversely, an overabundance of Zn can incite toxicity, disrupting the intricate metabolic processes underlying chlorophyll formation (Rudani et al., 2018). The equilibrium between deficiency and excess is fundamental, underlining the necessity for a nuanced approach to Zn supplementation in arecanut cultivation.

Despite the critical role that Zn plays in chlorophyll metabolism and leaf area development, a comprehensive understanding of its impact on arecanut seedlings remains an area ripe for exploration. This study embarks on a systematic investigation into the influence of different levels of Zn on chlorophyll content and leaf area of arecanut seedlings.

2. MATERIAL AND METHODS

The experiment was conducted at ICAR-Central Plantation Crops Research Institute, Regional station, Vittal, Karanataka, India between February to August 2021. A complete randomized design was adopted with a factorial arrangement consisting of eight arecanut varieties*viz.*,Mangala, Swarnamangala, Madhuramangala, Shatamangala, South Kanara local, Thirthahalli, SAS-1 and Hirehalli Dwarf (Plate 1), three Zn levels*viz.*,low (0.031 ppm), medium (0.093 ppm) and high (0.156 ppm) and three replications (8 × 3 × 3).The seed nuts of respective varieties were sown in sterile media containing sterile silica sand. After 3 months, the germinated seedlings were transferred to 10 L capacity plastic pots containing 8 kg of sterile silica sand. The modified Hoagland nutrient solution was used as source of nutrients. It contained 80 N, 80 K, 50.08 Ca, 20 P, 15.19 Mg, 4.68 Fe, 0.3 Mn, 0.125 B, 0.012 Mo, 0.0079 Cu, (ppm). These nutrients were provided using salts of NH_4NO_3, $Ca(NO_3)_2 \cdot 4H_2O$, KNO_3, KH_2PO_4, K_2SO_4, $MgSO_4 \cdot 7H_2O$, Sodium salts of Fe EDTA, H_3BO_3, $CuSO_4 \cdot 5H_2O$, $MnSO_4 \cdot H_2O$, and $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$. From this nutrient solution, three working solutions containing varied levels of zinc i.e., low (0.031 ppm), medium (0.093 ppm) and high (0.156 ppm) were prepared using $ZnSO_4 \cdot 7H_2O$. Every day 100 ml deionized water was added to each pot, to meet the water requirement of the plants, and 100 ml of Modified Hoagland's nutrient solution was added to each pot as per the treatments at weekly intervals with a total of 25 applications. Experiment was carried out under naturally ventilated glasshouse condition for six months. During the experimental period, glasshouse temperature varied from 28–37^{o}C and relative humidity varied from 70–98 per cent. Shade net was used to provide 50 per cent of shade to the seedlings. No serious occurrences of pests and diseases were observed during the experimental period. Hence fungicides or insecticides were not applied to the seedlings.

PLATE. 1. Seednuts of arecanut varieties used in the study

2.1 MEASUREMENT OF CHLOROPHYLL CONTENT AND TOTAL LEAF AREA:

A known weight of the leaf sample (25-30 mg) was incubated in 2.5 ml of DMSO at 65°C for 10 hours (i.e., till complete discoloration of discs). After incubation, the supernatant was collected by decanting and leaf tissue was discarded. The absorbance of the extract was measured at 645 nm and 663 nm using DMSO as a blank in a spectrophotometer (Blanke et al., 1992). The chlorophyll a, chlorophyll b and total chlorophyll content was calculated using the formula,

Chlorophyll a (mg g^{-1}) = $\frac{12.7*(A663) - 2.69*(A645) \times V}{1000* W*a}$

Chlorophyll b (mg g^{-1}) = $\frac{22.9*(A645) - 4.68*(A663) \times V}{1000* W*a}$

Total chlorophyll (mg g^{-1}) = Chlorophyll a + Chlorophyll b

Where, A=Absorbance at a specific wavelength (645nmand663nm)

V=Volume of the extract (2.5ml)

W=Fresh weight of the leaf tissue (g)

2.1.1 Total leaf area (cm^2)

The total leaf area of the plant was calculated by using the dry weight method. From three leaves of each plant, three discs of 10 cm^3were collected, the area of three discs was calculated and dried in a hot air oven, and the dry weight was recorded. On the other hand, leaves from the entire plant were dried and dry weight was recorded (Pacheco at al., 2020). The total leaf area was calculated using the following formula

Total leaf area(cm^2)= $\frac{\text{Area of 3 discs} \times \text{dry weight of leaves}}{\text{Dry weight of 3 discs}}$

2.2 STATISTICAL ANALYSIS:

A two –way factorial completely randomized design was used for the statistical analysis of the data. Analysis of variance was performed to compare the effects of different varieties, Zn levels and their interactions on chlorophyll content and total leaf area. The significance was calculated based on results of F-tests and treatment means were compared by determining the least significant difference (LSD) at 5% level of probability (P = 0.05).

3. RESULTS AND DISCUSSION

3.1 Chlorophyll content:

Different varieties have significantly influenced the chlorophyll content (Table 1). The variety Hirehalli Dwarf recorded maximum chlorophyll a and total chlorophyll content (2.08 mg g^{-1}, 3.42 mg g^{-1} respectively) and the variety Shatamangala recorded maximum chlorophyll b content (1.51 mg g^{-1}). Varied levels of Zn also differed significantly for chlorophyll b and total chlorophyll contents. chlorophyll b and total chlorophyll contents were found to be increased significantly with increase in Zn levels up to a certain level (Z_2 level) later it decreased (Fig.1). Highest chlorophyll b and total chlorophyll content was reported in Z_2 level of Zn (1.41 mg g^{-1}, 3.36 mg g^{-1} respectively). Increase in chlorophyll 'b' content was rather higher than chlorophyll 'a'content due to increase in Zn levels. Among interactions, the treatment V_8Z_2 (variety Hirehalli Dwarf treated with 0.093 ppm of Zn) recorded maximum chlorophyll a, chlorophyll b and total chlorophyll content (2.20, 1.63 and 3.83 mg g^{-1}, respectively). In general, Zn application increases chlorophyll content in plants as it acts as a structural and catalytic component of proteins, enzymes and as co-factor for the normal development of pigment biosynthesis (Balashouri, 1995).

The excess Zn treatment brought about a marked decrease in photosynthetic pigment in plants (Waghmare et al., 2018). It might be due to excess supply of Zn resulting in interference with the synthesis of chlorophyll. The formation of chlorophyll pigment depends on the adequate supply of iron. Granick (1951) has suggested protoporphyrin is a precursor for chlorophyll synthesis. The excess supply of Zn seems to prevent the incorporation of iron in protoporphyrin molecule resulting in the reduction of chlorophyll pigment. Similar findings were also reported by Tayyaba et al., (2013) in hydroponically grown mung bean, Munirah et al. (2015) in Maize, Adil et al. (2022) in Wheat, Grewal et al. (1997) in rape seed.

3.2 Total leaf area (cm2)

As shown in the table 1, among different varieties, a significantly maximum total leaf area (1263.8 cm^2) was observed in the variety Madhuramangala. Among varied Zn levels, the Z_2 level showed a significant maximum total leaf area (940. 6 cm^2). In case of interaction, the variety Madhuramangala grown at a Zn concentration of 0.093 ppm recorded a maximum total leaf area (1445.5 cm^2). This may be due to increased leaf dry weight of this variety which results in greater leaf area. In case of Zn levels, the total leaf area was maximum at medium Zn level. This might be due to adequate Zn levels promoting auxin production which in turn stimulates leaf primodia formation and subsequent leaf expansion. It is also a crucial component of enzymes involved in chlorophyll synthesis that helps in efficient photosynthesis. Similar results were observed by Khalid et al. (2006) in their study on wheat.

Table 1.Independent and interactive effects of Zn levels and varieties on chlorophyll content and total leaf area of different arecanut varieties recorded after six months of planting

Treatment	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Total chlorophyll (mg g^{-1})	Total leaf area (cm^2)
Variety				
V_1	1.94	1.42	3.36	893.90
V_2	1.99	1.39	3.39	1111.42
V_3	1.88	1.27	3.15	1263.78
V_4	1.79	1.51	3.30	872.02
V_5	2.02	1.39	3.41	959.93
V_6	1.87	1.16	3.01	752.53
V_7	1.89	0.99	2.88	733.74
V_8	2.08	1.34	3.42	483.34
SEM	**0.04**	**0.02**	**0.05**	**28.88**
LSD @ 5%	**0.10**	**0.06**	**0.14**	**82.48**
Zinc Level				
Z_1	1.93	1.32	3.25	909.09
Z_2	1.94	1.41	3.35	940.56
Z_3	1.92	1.19	3.11	801.85
SEM	**0.02**	**0.01**	**0.03**	**17.69**
LSD @ 5%	**NS**	**0.04**	**0.09**	**50.51**
Interactions				
V_1Z_1	2.03	1.27	3.30	741.73
V_1Z_2	1.87	1.53	3.40	1035.60
V_1Z_3	1.93	1.47	3.40	904.37
V_2Z_1	2.00	1.37	3.37	1221.53
V_2Z_2	2.07	1.53	3.60	1032.73
V_2Z_3	1.90	1.00	2.90	1080.00
V_3Z_1	1.93	1.47	3.40	1139.40
V_3Z_2	1.83	1.37	3.20	1445.47
V_3Z_3	1.87	0.97	2.84	1206.47
V_4Z_1	1.80	1.63	3.43	856.83
V_4Z_2	1.80	1.60	3.40	1018.20
V_4Z_3	1.77	1.30	3.07	741.03
V_5Z_1	1.97	1.40	3.37	1060.00
V_5Z_2	2.00	1.37	3.37	1004.63
V_5Z_3	2.10	1.40	3.50	815.17
V_6Z_1	1.93	1.10	3.03	933.33
V_6Z_2	1.87	1.33	3.20	743.63
V_6Z_3	1.80	1.03	2.83	580.63
V_7Z_1	1.87	0.87	2.74	813.37
V_7Z_2	1.90	1.20	3.10	701.80
V_7Z_3	1.90	0.90	2.80	686.07
V_8Z_1	1.93	1.17	3.10	506.50
V_8Z_2	2.20	1.63	3.83	542.43
V_8Z_3	2.10	1.43	3.53	401.10
SEM	**0.06**	**0.04**	**0.09**	**50.03**
LSD @ 5%	**NS**	**0.11**	**0.24**	**142.87**

NS – Non - significant, V_1 - Mangala, V_2 - Swarnamangala, V_3 - Madhuramangala, V_4 -Shatamangala, V_5 - SK Local, V_6 - Thirthahalli, V_7 - SAS-1, V_8 - Hirehalli Dwarf, Z_1- Zn @ 0.031ppm, Z_2- Zn @ 0.093 ppm, Z_3 - Zn @ 0.156 ppm.

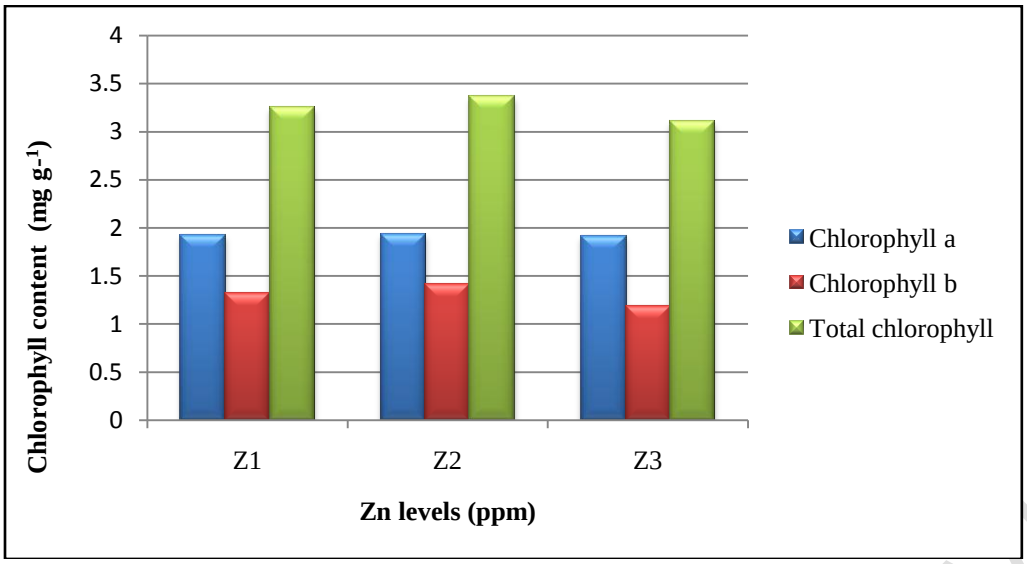

Fig. 1. Influence of Zn levels on chlorophyll content

4. CONCLUSION

In conclusion, the experiment demonstrated that varied Zn supplementation significantly influenced both chlorophyll content and total leaf area in arecanut seedlings, with the most favorable outcomes observed at a Zn concentration of 0.093 ppm (Medium concentration). This implies that maintaining a Zn level of 0.093 ppm is optimal for fostering the growth of arecanut seedlings. Nonetheless, it is imperative to highlight the necessity for additional research, specifically targeted at adult palms, to refine and offer precise recommendations for effective Zn supplementation in arecanut cultivation, ensuring a comprehensive and informed approach to sustainable cultivation practices.

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