Zinc deficiency and toxicity-induced alterations of chloroplast pigments in acid lime [*Citrus aurantiifolia* (Christm.) Swingle]

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ABSTRACT

Zinc is an essential micronutrient involved in various physiological processes in plants, particularly in chloroplast development and pigment biosynthesis. Although application of zinc have been widely studied in citrus crops, limited information exists on the chloroplast pigment response of acid lime to graded zinc levels applied through substrate under controlled greenhouse conditions. This study aimed to fill that gap by evaluating how zinc deficiency and toxicity affect chlorophyll a, chlorophyll b, total chlorophyll, chlorophyll a/b ratio, and carotenoid contents over a two-year period.A pot experiment was conducted using acid lime seedlings grown in acid-washed river sand under greenhouse conditions. Seven zinc concentrations (0.0 to 15.0 mM/l$ZnSO_4$) were applied at regular intervals, and pigment contents were quantified at six growth stages using spectrophotometry. The results demonstrated a biphasic zinc response, where moderate zinc application (particularly at 7.5 mM/l) significantly enhanced chlorophyll a, b, total chlorophyll, and maintained a balanced chlorophyll a/b ratio, indicative of optimal chloroplast function. Both zinc deficiency (0.0 mM/l) and toxicity (≥12.5 mM/l) led to pigment degradation, reduced photosynthetic efficiency, and signs of physiological stress. Carotenoid accumulation was elevated under Zinc-deficient and Zinc-toxic treatments, suggesting a photoprotective response against oxidative damage.In conclusion, the study identifies 7.5 mM/l zinc as the optimal concentration for maximizing chloroplast pigment stability and minimizing stress-induced degradation in acid lime.

Keywords:carotenoids, chloroplast pigments, *Citrus aurantiifolia*, deficiency, nutrient stress, toxicity, zinc,

INTRODUCTION:

Acid lime [*Citrus aurantiifolia* (Christm.) Swingle], commonly known as key lime or Mexican lime, holds significant economic importance, particularly for small and marginal farmers, because of its year-round flowering and fruiting habit, which ensures regular income (Ladaniya *et al*., 2020). The fruits are rich in vitamin C, organic acids, flavonoids, and essential oils, which confer antimicrobial, antioxidant, and anti-inflamatory properties (Abirami *et al*., 2022) like as other citrus (Deb *et al*., 2024, Deb *et al*., 2025). These attributes make acid lime a valuable crop not only in the fresh market but also in processed products like beverages, pickles, and nutraceuticals. Furthermore, with the increasing global demand for natural, functional foods and the growing emphasis on climate-resilient crops, acid lime contributes meaningfully to nutritional security, agro-industry

diversification, and sustainable horticulture (Kumar and Jain, 2018).

Micronutrients play a fundamental role in plant growth, and among them, zinc is especially crucial for acid lime cultivation. Zinc is involved in numerous physiological functions, including enzyme activation, protein metabolism, and hormone regulation, all of which are essential for overall plant development and fruit production (Fageria *et al*., 2002). Zinc also plays a direct role in the synthesis of chlorophyll and maintenance of chloroplast structure, thereby influencing the efficiency of photosynthesis (Cakmak, 2000). In many tropical fruit-growing regions, zinc deficiency is common due to calcareous soils or high pH conditions, which limit zinc availability to roots (Alloway, 2008).Deficiency symptoms in acid lime include Interveinal chlorosis, small and narrow leaves, shortened internodes, and poor flowering and fruit set (Mousavi *et al*., 2013). These symptoms lead to a noticeable decline in tree vigour, productivity, and fruit quality (Tisdale *et al*., 1993). On the other hand, excessive application of zinc can cause toxicity, which is expressed as leaf necrosis, chlorosis, and restricted root and shoot growth, ultimately inhibiting photosynthetic function and plant development (Zhao and McGrath, 2009).

A well-balanced zinc supply supports better chlorophyll retention, enhances photosystem activity, and improves plant resilience under environmental stress (Zhao and McGrath, 2009).While numerous studies have examined the impact of foliar-applied zinc on citrus crops, there remains a clear knowledge gap in understanding how different concentrations of media-applied zin**c** under controlled (greenhouse) conditions affect chloroplast pigment dynamics in acid lime. Most existing research focuses on field applications or nutrient deficiencies, with limited emphasis on the physiological pigment responses to graded zinc levels in a protected environment. Furthermore, the relationship between zinc concentration and its threshold for toxicity, particularly its inhibitory effects on carotenoid accumulation has not been sufficiently explored. In this context, the present study was conducted to investigate how varying concentrations of zinc applied through the growing medium affect the biosynthesis of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids in acid lime under greenhouse conditions. The objective was to determine the optimal zinc concentration that maximizes photosynthetic pigment content without triggering toxicity symptoms, and to assess whether pigment trends are consistent across seasonal growth cycles and years.

MATERIALS AND METHODS:

The present investigation was conducted at the Instructional Farm of the Department of Horticulture & Postharvest Technology, Institute of Agriculture, Visva-Bharati, Sriniketan, West Bengal over two consecutive years (2022–2023 and 2023–2024) in a controlled greenhouse environment to evaluate the effect of varying levels of zinc application through the substrate (acid washed river sand) on chloroplast pigment synthesis in acid lime seedlings. The experiment was designed as a pot (20cm diameter) trial using seedlings grown of seeds collected from uniform sized fresh ripe fruits. Only the nuclear seedlings were considered for the present experiment. The each pot as fed with Modified Hoagland solution (without zinc) @ 100ml in every week and the watering was done with distilled water @ 500 ml per pot in every 5 days interval. Standard plant protection measures were followed throughout the period of experimentation. The zinc treatments were applied as zinc sulfate ($ZnSO_4,7H_2O$) mixed thoroughly into the potting medium at seven concentrations (T_1: Control or 0.0 mM/l (milli mole per litre), T_2: 2.5 mM/l, T_3: 5.0 mM/l, T_4: 7.5 mM/l, T_5: 10.0 mM/l, T_6: 12.5 mM/l and T_7: 15.0 mM/l) at 15 days interval starting from 3 months age of the plants @ 100 ml per pot.

These treatments were arranged in a CompletelyRandomized Design (CRD) with three replications per treatment and keeping 10 plants under each replication. Uniform irrigation and cultural practices were maintained across all treatments.

To assess the influence of zinc on chloroplast pigment synthesis, the following pigments like chlorophyll a, chlorophyll b, total chlorophyll (sum of a + b) and carotenoids were quantified at six growth stages viz.3, 6, 9, 12, 15, and 24 months after planting, spanning the full experimental period. Fully expanded, healthy leaves were sampled from each treatment and replication at each interval. For consistency, the same relative leaf position was maintained across plants and time points.Pigments were estimated using the method as described by Lichtenthaler and Wellburn (1983), which remains a widely accepted protocol for chlorophyll and carotenoid quantification. For this, 0.5 g of fresh leaf tissue was homogenized in 80% acetone and centrifuged. The clear supernatant was collected, and absorbance readings were taken using a UV-Visible spectrophotometer at specific wavelengths (663 nm for chlorophyll a; 645 nm for chlorophyll b and 480 nm for Carotenoids).The equations used for quantificationare as follows:

Chlorophyll *a* = $12.7(A_{663}) - 2.69(A_{645})$
Chlorophyll *b* = $22.9(A_{645}) - 4.68(A_{663})$
Total chlorophyll = $20.2(A_{645}) + 8.02(A_{663})$
Carotenoids = $(1000 \times A_{470} - 1.82 \times$ Chl *a* – $85.02 \times$ Chl *b*) / 198

The experiment followed a Completely Randomized Design (CRD) with seven treatments and three replications. The pooled data from two years were statistically analyzed using Analysis of Variance (ANOVA) as per standard procedure. Treatment means were compared using the Critical difference (CD) test at 5% level of significance. Standard error of the Mean (SEm ±) was also calculated for each parameter.Statistical analyses were carried out using IBM SPSS Statistics (Version 27.0). Prior to conducting ANOVA, assumptions such as normal distribution and homogeneity of variance were verified.

RESULTS AND DISCUSSION:

To assess the impact of zinc on pigment composition, the greenhouse pot experiment was conducted to evaluate how zinc deficiency and toxicity influence chloroplast pigments in acid lime, focusing on chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, and the chlorophyll a/b ratio over a 24-month period.

Chlorophyll a content: The chlorophyll a (Chl a) content of acid lime leaves responded significantly to varying concentrations of zinc over time under greenhouse conditions (Table 1). At 3 months after treatments, Chla content ranged from 0.94 mg/g FW in T_7 (15.0 mM/l zinc) to 1.08 mg/g FW in T_4 (7.5 mM/l zinc). Although differences at 3 months were not statistically significant, a trend emerged indicating enhanced Chla in plants treated with moderate zinc doses. By 6 months and onwards, treatment differences became statistically significant ($p < 0.05$), confirming the role of zinc concentration on Chl a dynamics. At 6 months, maximum Chla (1.12 mg/g) was observed in T_4, while the lowest (0.94 mg/g) occurred in T_6 (12.5 mM/l zinc). Similarly, at 9 months and 12 months, T_4 consistently maintained the highest Chl a levels (1.18 and 1.21 mg/g, respectively), whereas T_7 declined sharply (0.79 and 0.71 mg/g). This pattern persisted through 15 and 24 months, with T_4 showing optimal Chl a content (1.23 and 1.28 mg/g), suggesting long-term enhancement of chlorophyll biosynthesis under moderate zinc application. In contrast, both zinc deficiency (T_1, 0.0 mM/l) and toxicity (T_6, 12.5 mM/l; T_7, 15.0 mM/l) significantly reduced Chl a content over time, with T_7 recording the lowest levels at 24 month (0.63 mg/g). This highlights a biphasic zinc response, wherein chlorophyll synthesis is promoted by adequate zinc levels but

inhibited under both deficient and toxic concentrations.

Zinc is a vital cofactor in chlorophyll biosynthesis, involved in the structure and activation of enzymes such as carbonic anhydrase and RNA polymerase (Marschner, 2012). Moderate zinc supplementation likely facilitated enzyme activity and protein synthesis necessary for chlorophyll formation, thus explaining the higher Chla in T_3 and T_4 treatments. Moreover, zinc plays a structural role in maintaining membrane integrity, which is crucial for thylakoid function and chlorophyll stabilization (Alloway, 2008). Zinc deficiency, as observed in T_1, likely impaired the synthesis of δ-aminolevulinic acid, a precursor of chlorophyll, thus reducing Chl a concentration. Deficient zinc also leads to increased oxidative stress due to impaired antioxidant enzyme systems, causing chlorophyll degradation (Broadley *et al*., 2007). Conversely, excessive zinc in T_6 and T_7 may have induced phytotoxic effects by generating reactive oxygen species (ROS), leading to lipid peroxidation and chloroplast damage, ultimately lowering chlorophyll content (Cakmak, 2000). The temporary increase in Chl a observed in T_7 at 6 months (1.05 mg/g) followed by a sharp decline suggests an initial physiological acclimation phase before toxicity symptoms manifested. This aligns with earlier findings where high zinc doses initially stimulated pigment production before causing oxidative damage (Prasad *et al*., 1999). Overall, the data suggests that zinc at 7.5 mM/l (T_4) optimally supports chlorophyll a biosynthesis in acid lime, while both deficiency and excess zinc result in deleterious effects on chloroplast pigment levels.

Chlorophyll b content: The chlorophyll b (Chl b) content in acid lime showed variable responses to different zinc concentrations over time under greenhouse conditions (Table 2). Although treatment differences during the initial growth phases (3, 6, and 9 months after treatment) were statistically non-significant, a clear pattern emerged from 12 months onward, where significant variations in Chl b were observed due to the contrasting zinc treatments. At 3 months after treatment, Chl b content ranged narrowly from 0.34 mg/g fresh weight (FW) in T_7 (15.0 mM/l zinc) to 0.45 mg/g in T_4 (7.5 mM/l zinc), indicating early mild responsiveness of pigment synthesis to Zinc. By 9 months, plants under T_4 maintained the highest Chl b (0.58 mg/g), suggesting that moderate zinc supplementation promoted chlorophyll accumulation. From 12 month onwards, significant treatment effects were evident (CD at 5% = 0.11 at 12 months). The maximum Chl b was recorded under T_4 (0.60 mg/g), followed by T_3 (5.0 mM/l zinc, 0.43 mg/g), while zinc-deficient (T_1) and zinc-toxic treatments (T_6 and T_7) consistently showed lower values. At 24 months, Chl b content in T_4 remained relatively high (0.48 mg/g), whereas, T_6 (12.5 mM/l) and T_7 (15.0 mM/l) recorded the lowest values of 0.36 and 0.40 mg/g, respectively. Interestingly, while T_3 (5.0 mM/lz inc) showed a late increase to 0.53 mg/g, it was still slightly lower than the earlier peak seen under T_4, indicating a sustained but plateaued effect under moderately optimal Zinc conditions.

Chlorophyll b, primarily associated with the light-harvesting complex II (LHCII), depends on adequate zinc levels for structural integrity of thylakoid membranes and efficient protein synthesis (Barickman *et al*., 2014). Moderate zinc (T_3 and T_4) thus promoted pigment accumulation by facilitating biosynthetic pathways and reducing oxidative damage. Deficient zinc levels (T_1, 0.0 mM/l) likely disrupted protein synthesis and impaired the formation of δ-aminolevulinic acid, a precursor of chlorophyll molecules, leading to reduced Chl b (Broadley *et al*., 2012). On the other hand, elevated zinc concentrations (T_6 and T_7) induced toxicity, possibly through enhanced generation of reactive oxygen species (ROS), causing peroxidation of lipids and degradation of pigments (Feng *et al*., 2010). The observed late-stage reduction in

Chl b under zinc toxicity may also be attributed to antagonistic effects on nutrient uptake, particularly magnesium and iron—key elements in chlorophyll synthesis (Kabata-Pendias, 2010). The transient increase in Chl b at 9 month in T_7 (0.51 mg/g) followed by a subsequent decline indicates a short-term adaptive response, after which toxic effects overwhelmed the plant's metabolic balance. Similar biphasic responses to micronutrients have been observed in citrus and leafy crops exposed to varying metal stress conditions (Ranjbar and Bahmaniar, 2007). In conclusion, chlorophyll b synthesis in acid lime is maximally promoted under moderate zinc application (especially 7.5 mM/l), while deficiency and excessive zinc concentrations reduce pigment levels due to biosynthetic limitations or toxicity-induced degradation.

Total chlorophyll content: The total chlorophyll content in acid lime plants varied considerably with different zinc concentrations across the growth period (Table 3). While early intervals (3 and 6 months after treatment) exhibited non-significant differences among treatments, clear and statistically significant trends emerged from 9 months onward, indicating the progressive impact of zinc on chloroplast pigment synthesis under greenhouse conditions. At 3 month, total chlorophyll ranged from 1.49 mg/g fresh weight (FW) in T_7 (15.0 mM/l zinc) to 1.59 mg/g in T_4 (7.5 mM/l zinc), with all values being statistically non-significant. A similar trend was observed at 6 month, where zinc levels had not yet exerted a strong differentiating effect on pigment development. However, as the experiment progressed, substantial differences in chlorophyll content were recorded. By 9 months, T_4 (7.5 mM/l zinc) showed the highest total chlorophyll content (1.76 mg/g FW), followed by T_3 (5.0 mM/l zinc) at 1.62 mg/g, both significantly higher than zinc-deficient (T_1, 1.40 mg/g) and zinc-toxic treatments (T_6 and T_7 at 1.36 and 1.30 mg/g, respectively). The same pattern persisted through 12, 15, and 24 months. At 24 months, T_4 maintained the highest chlorophyll level (1.86 mg/g FW), while T_7 (zinc toxicity) recorded the lowest (1.08 mg/g).

This data clearly indicates that moderate zinc supplementation (particularly at 7.5 mM/l) enhances chlorophyll synthesis and retention, whereas, both zinc deficiency and excess lead to progressive pigment degradation. Zinc plays a crucial role in chlorophyll biosynthesis and structural stabilization of chloroplast membranes (Rehman *et al*., 2012). It is a cofactor for carbonic anhydrase and other enzymes involved in chlorophyll formation and photosynthetic carbon fixation (Yusuf *et al*., 2011). Under zinc-deficient conditions (T_1), limited enzyme activity and impaired protein synthesis likely hampered chlorophyll production and led to premature senescence. Zinc deficiency also induces oxidative stress, which degrades chlorophyll molecules due to the accumulation of reactive oxygen species (ROS) (Dang *et al*., 2024). This explains the consistent decline in chlorophyll content in T_1 across all intervals. On the other hand, zinc toxicity at high concentrations (T_6 and T_7) likely disrupted cellular homeostasis, leading to chloroplast damage, reduced pigment biosynthesis, and enhanced chlorophyll degradation. High zinc concentrations can interfere with iron (Fe) and magnesium (Mg) uptake—both essential for chlorophyll synthesis—resulting in impaired pigment metabolism (Kalayci *et al*., 2008). Moreover, excessive zinc is known to displace essential metals from enzymatic sites, destabilize protein structures, and generate oxidative stress in plant tissues (Roosta *et al*., 2018). The optimal performance under T_4 and T_3 treatments supports the idea that zinc enhances photosynthetic efficiency when applied at proper doses. A similar improvement in total chlorophyll content under moderate zinc levels has been reported in citrus and other crops (Sharma *et al*., 2010).

Chlorophyll a/b Ratio: The chlorophyll a to b (Chl a/b) ratio is a crucial physiological

indicator reflecting the balance between light-harvesting complex proteins and core photosynthetic pigments. In this study, the Chl a/b ratio of acid lime showed significant variation under different zinc concentrations at various growth stages, especially from 6 months after treatment onwards (Table 4). At 3 months, the Chl a/b ratio ranged from 2.41 (T_4) to 2.76 (T_7), but the differences were statistically non-significant. This suggests that early-stage photosynthetic pigment balance was not yet strongly influenced by zinc treatments. However, at 6 months, notable shifts began to emerge. The ratio was highest in T_7 (2.69) and lowest in T_1 (1.79), with T_4, T_3, and T_5 showing intermediate and more stable ratios (2.32-2.38). From 9 months onward, significant differences became more evident. The Chl a/b ratio in the optimal zinc range (T_3 and T_4: 5.0–7.5 mM/l) remained relatively balanced, maintaining values around 2.01–2.74, suggesting a harmonious development of both chlorophyll forms. The T_4 treatment at 12 months showed a ratio of 2.01, indicating efficient chlorophyll b synthesis relative to chlorophyll a. Meanwhile, plants under zinc deficiency (T_1: 0.0 mM/l) or toxicity (T_6 and T_7: 12.5 and 15.0 mM/l, respectively) showed irregular patterns. Notably, T_7 exhibited a sharp drop in the ratio at 9 months (1.55), which continued to decline to 1.57 at 24 month indicating a disproportionate degradation of chlorophyll a or increased accumulation of chlorophyll b.

These findings suggest that both zinc deficiency and excess disrupt the balance of chlorophyll synthesis. Zinc is known to be vital for protein synthesis, enzyme activation, and membrane integrity factors that collectively influence chloroplast structure and function (Broadley *et al*., 2007). Disruption in these processes can lead to oxidative degradation or impaired synthesis of chlorophyll a, skewing the Chl a/b ratio. Higher Chl a/b ratios generally indicate dominance of reaction centre chlorophylls, while lower ratios suggest expansion of light-harvesting complexes (LHCs), often as an acclimation response to stress (Anderson *et al*., 1995). In zinc-deficient and zinc-toxic treatments, the observed fluctuations may result from stress-induced overexpression or degradation of LHC proteins, affecting chlorophyll b content and consequently altering the ratio. The optimal balance found in T_3 and T_4 treatments supports previous research where moderate zinc application enhanced pigment biosynthesis, maintained chloroplast ultrastructure, and supported balanced growth (Fathi*et al*., 2009). Conversely, extreme zinc concentrations either too low or too high likely led to reduced enzymatic activity for chlorophyll biosynthesis, disrupted plastid development, and oxidative damage to photosynthetic proteins (Shao *et al*., 2008). The elevated Chl a/b ratio in zinc-toxic treatments (T_7 and T_6) during early stages might reflect an initial suppression of chlorophyll b synthesis or an adaptive thinning of LHCs to limit light absorption under oxidative stress. Over time, however, such treatments led to pigment degradation and structural damage, explaining the final low ratios at 24 month.

Carotenoid content: Carotenoids are essential accessory pigments that play a crucial role in light harvesting and photo-protection by quenching excess energy and reactive oxygen species (ROS). In this study, carotenoid content in acid lime exhibited significant variation in response to different zinc concentrations across six time intervals under greenhouse conditions (Table 5). At the early stage (3 months after treatment), carotenoid content was highest in T_6 (12.5 mM/l zinc; 0.69 mg/g FW) and T_1 (0.0 mM/l zinc; 0.67 mg/g FW), while the lowest values were observed in T_4 (7.5 mM/l; 0.187 mg/g FW). Similar trends continued at 6 month, with carotenoid levels declining across the optimal zinc treatments (T_3 and T_4) and remaining elevated under zinc deficiency (T_1) and zinc toxicity (T_6). As time progressed, carotenoid content declined gradually in T_1 and T_6, reaching 0.39 and 0.41 mg/g FW respectively by 24 month.

Meanwhile, T_4 and T_5 treatments (7.5 and 10.0 mM/l zinc) showed a gradual and consistent increase in carotenoid content from 3 month to 24 month, ending with values of 0.28 and 0.35 mg/g FW respectively. This progressive increase may indicate the activation of carotenoid biosynthesis as a photoprotective response under moderately favourable zinc concentrations. Interestingly, T_2 (2.5 mM/l zinc) showed a steady increase in carotenoids over time, peaking at 0.38 mg/g FW at 24 month, which surpassed some of the higher zinc levels. On the other hand, T_3 (5.0 mM/l zinc), which showed optimal chlorophyll content in earlier observations, had relatively moderate carotenoid levels, peaking at 0.447 mg/g FW at 3 month and declining to 0.26 mg/g FW at 24 month.

These trends suggest a complex interaction between zinc nutrition and carotenoid metabolism. High carotenoid content under zinc deficiency and toxicity conditions (T_1 and T_6) might represent a defensive strategy to counteract oxidative stress caused by impaired chloroplast function and excess ROS generation (Singh and Prasad, 2014). Zinc plays a pivotal role in maintaining membrane stability and activating antioxidant enzymes. In its absence or excess, ROS accumulation may trigger carotenoid accumulation as part of the plant's adaptive stress response (Hasanuzzaman *et al*., 2020). Moreover, the moderate zinc levels in T_4 and T_5 may have provided optimal conditions for maintaining balanced growth, improving photosystem stability, and enhancing non-photochemical quenching, reflected by a consistent rise in carotenoids (Cakmak, 2000). This aligns with findings where carotenoid biosynthesis was enhanced under conditions of improved zinc-mediated stress mitigation. Conversely, a decline in carotenoids over time in T_3 and T_6 may suggest pigment degradation due to long-term zinc imbalance, despite initial adaptation. The early high values in these treatments could indicate an initial oxidative response, followed by pigment breakdown due to prolonged stress, especially under zinc toxicity (Rout and Das, 2003). Overall, the results indicate that carotenoid content in acid lime is not linearly related to zinc concentration, but rather modulated by the plant's oxidative and metabolic status. Treatments with moderate zinc concentrations (especially T_4 and T_5) favoured a healthy and sustained accumulation of carotenoids, likely supporting both light harvesting and photo-protection under greenhouse conditions.

CONCLUSION

Zinc application significantly influenced chloroplast pigment dynamics in acid lime under greenhouse conditions. Moderate zinc levels, particularly at 7.5 mM/l (T_4), consistently enhanced chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents across all time points, supporting optimal pigment biosynthesis and chloroplast function. In contrast, both zinc deficiency (0.0 mM/l) and toxicity (≥12.5 mM/l) reduced pigment concentrations, likely due to impaired enzymatic activity, oxidative stress, and disrupted nutrient uptake. The chlorophyll a/b ratio remained balanced under moderate zinc but deviated under stress conditions, indicating pigment imbalance and structural damage. Carotenoid trends revealed a defensive role under zinc-induced stress, with accumulation under both deficiency and toxicity. Overall, the results underscore a biphasic zinc response, where only moderate concentrations sustain photosynthetic pigment stability and promote physiological resilience in acid lime.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1: Effect of varied doses of zinc on changes in chlorophyll a content of acid lime seedlings:

Treatments (zinc conc.) milli mole per litre (mM/l)	3 month	6 month	9 month	12 month	15 month	24 month
T_1 (0.0 mM/l/l)	1.01	0.97	0.95	0.93	0.86	0.79
T_2 (2.5mM/l/l)	1.03	1.07	1.01	0.99	0.93	0.87
T_3 (5.0 mM/l/l)	1.06	1.10	1.13	1.18	1.20	1.23
T_4 (7.5mM/l/l)	1.08	1.12	1.18	1.21	1.23	1.28
T_5 (10.0 mM/l/l)	1.04	1.09	1.10	0.97	1.01	1.03
T_6 (12.5mM/l/l)	0.99	0.94	0.90	0.85	0.80	0.75
T_7 (15.0 mM/l/l)	0.94	1.05	0.79	0.71	0.67	0.63
SE±m	**NS**	**0.04**	**0.03**	**0.03**	**0.02**	**0.04**
CD (0.05)	**NS**	**0.13**	**0.09**	**0.09**	**0.08**	**0.13**

Table 2: Effect of varied doses of zinc on changes in chlorophyll b content of acid lime seedlings:

Treatments (zinc conc.)	3 month	6 month	9 month	12 month	15 month	24 month
T_1 (0.0 mM/l/l)	0.40	0.54	0.45	0.41	0.39	0.38
T_2 (2.5mM/l/l)	0.41	0.46	0.41	0.37	0.41	0.43
T_3 (5.0 mM/l/l)	0.43	0.47	0.49	0.43	0.48	0.53
T_4 (7.5mM/l/l)	0.45	0.47	0.58	0.6	0.54	0.48
T_5 (10.0 mM/l/l)	0.42	0.47	0.4	0.39	0.42	0.45
T_6 (12.5mM/l/l)	0.38	0.43	0.46	0.42	0.39	0.36
T_7 (15.0 mM/l/l)	0.34	0.39	0.51	0.36	0.38	0.4
SE±m	**NS**	**NS**	**NS**	**0.03**	**0.03**	**0.02**
CD (0.05)	**NS**	**NS**	**NS**	**0.11**	**0.09**	**0.08**

Table 3: Effect of varied doses of zinc on changes in total chlorophyll a content of acid lime seedlings:

Treatments (Zinc conc.)	3 month	6 month	9 month	12 month	15 month	24 month
T_1 (0.0 mM/l/l)	1.51	1.49	1.40	1.34	1.26	1.18
T_2 (2.5mM/l/l)	1.53	1.51	1.42	1.27	1.35	1.43
T_3 (5.0 mM/l/l)	1.57	1.58	1.62	1.54	1.59	1.66
T_4 (7.5mM/l/l)	1.59	1.64	1.76	1.70	1.78	1.86
T_5 (10.0 mM/l/l)	1.55	1.52	1.50	1.49	1.41	1.37
T_6 (12.5mM/l/l)	1.50	1.46	1.36	1.28	1.22	1.14
T_7 (15.0 mM/l/l)	1.49	1.44	1.30	1.18	1.11	1.08
SE±m	**NS**	**NS**	**0.04**	**0.04**	**0.03**	**0.06**
CD (0.05)	**NS**	**NS**	**0.12**	**0.13**	**0.10**	**0.15**

Table 4: Effect of varied doses of zinc on changes in the ratio of chlorophyll a and chlorophyll b content of acid lime seedlings:

Treatments (Zinc conc.)	3 month	6 month	9 month	12 month	15 month	24 month
T_1 (0.0 mM/l/l)	2.52	1.79	2.11	2.27	2.20	2.08
T_2 (2.5mM/l/l)	2.51	2.32	2.46	2.67	2.26	2.02
T_3 (5.0 mM/l/l)	2.46	2.34	2.30	2.74	2.50	2.32
T_4 (7.5mM/l/l)	2.41	2.38	2.03	2.01	2.27	2.67
T_5 (10.0 mM/l/l)	2.47	2.32	2.75	2.48	2.40	2.29
T_6 (12.5mM/l/l)	2.60	2.18	1.95	2.02	2.05	2.08
T_7 (15.0 mM/l/l)	2.76	2.69	1.55	1.97	1.76	1.57
SE±m	**NS**	**0.06**	**0.07**	**0.06**	**0.05**	**0.06**
CD(0.05)	**NS**	**0.18**	**0.21**	**0.18**	**0.16**	**0.19**

Table 5: Effect of varied doses of zinc on changes in carotenoid content of acid lime seedlings:

Treatments (zinc conc.)	3 month	6 month	9 month	12 month	15 month	24 month
T_1 (0.0 mM/l/l)	0.67	0.63	0.59	0.55	0.51	0.39
T_2 (2.5mM/l/l)	0.287	0.30	0.313	0.327	0.34	0.38
T_3 (5.0 mM/l/l)	0.447	0.42	0.393	0.367	0.34	0.26
T_4 (7.5mM/l/l)	0.187	0.20	0.213	0.227	0.24	0.28
T_5 (10.0 mM/l/l)	0.21	0.23	0.25	0.27	0.29	0.35
T_6 (12.5mM/l/l)	0.69	0.65	0.61	0.57	0.53	0.41
T_7 (15.0 mM/l/l)	0.38	0.40	0.42	0.44	0.46	0.52
SE±m	**NS**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**
CD (0.05)	**NS**	**0.06**	**0.05**	**0.06**	**0.05**	**0.05**