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Zn Check: Revolutionizing Plant Nutrition with Amino Acid-based Zinc Chelation

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Zinc plays a vital role in plant growth and development, impacting essential physiological processes such as cell division, nitrogen, carbohydrate metabolism and chloroplast development. However, zinc deficiency poses a significant challenge in agriculture, particularly in crops grown on calcareous soils. Traditional zinc fertilizers, like Zn EDTA, face limitations in alkaline soils, prompting the need for innovative solutions. This study introduces an amino acid-based chelating agent, Zinc Check, synthesized to address these challenges effectively. The research demonstrates the stability and efficacy of Zinc Check across diverse soil conditions, ensuring zinc availability to plants. Through various analytical techniques and pot trials with Pak choi plants, the study confirms the effectiveness of Zinc Check in promoting plant growth and chlorophyll synthesis, surpassing the performance of conventional zinc fertilizers. Moreover, the investigation explores

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the pH tolerance of Zinc Check, highlighting its stability across a wide pH range. Overall, this study underscores the importance of innovative solutions like Zinc Check in addressing zinc deficiency and enhancing agricultural productivity, contributing to global food security efforts.

Keywords: Zinc EDTA; Pak choi; Alkaline soils; pH tolerance; amino acids; FTIR spectral analysis.

1. INTRODUCTION

Zinc holds a pivotal role as an essential micronutrient crucial for the optimal growth and development of plants. Its multifaceted functions encompass various physiological processes vital for plant health. Zinc's significance lies in its involvement in fundamental cellular activities such as cell division, nitrogen metabolism, carbohydrate metabolism, and regulation of water relations during plant growth, as highlighted by Brady (1990). Notably, its presence is indispensable for chloroplast development and efficient photosynthesis, the cornerstone of plant productivity. The global agricultural landscape faces a prevalent challenge in the form of zinc deficiency, particularly in crops thriving on calcareous soils, as underscored by Liu et al. [1]. This deficiency phenomenon is not confined to specific regions but is a pressing nutritional constraint worldwide, as articulated by Sillanpaa (1982), Rastija et al. (2011), and specifically acknowledged in Pakistan by Khattak (1991), Rashid (2006), and Tariq et al. (2008). The escalating prevalence of zinc deficiency underscores its status as one of the most significant nutritional predicaments globally. The application of zinc fertilizers emerges as a critical strategy to alleviate zinc deficiency and promote robust plant growth and development. The indispensable role of zinc in enzymatic activation, protein synthesis, chlorophyll formation, and carbohydrate metabolism underscores its irreplaceable function in sustaining plant vitality, as noted by Degryse (2015) and Vikash et al. (2017).

Chelators such as ethylenediaminetetraacetic acid (EDTA) chelate different heavy metals in the soil. In cause of, most heavy metals with low soil bioavailability and various chelating agents, such as EDTA, have been applied to plants to improve the bioavailability of metals [2]. Organic chelating agents such as EDTA are more efficient, environmentally friendly and biodegradable compared to inorganic chelating agents [3]. EDTA is a scientifically accepted chelating agent for improving the solubility, absorption and stability of metals [4].

The drawback of Zn EDTA lies in its vulnerability to breakdown and loss of function when applied to alkaline soils, presenting a significant challenge for agricultural practices in such regions [5]. However, the advent of an amino acid-based chelating agent offers a breakthrough solution, providing enhanced stability and efficacy across a wider spectrum of soil conditions [6]. This new chelating agent, synthesized and formulated in the present study, offers a promising solution that remains effective across diverse soil types and agro-climatic conditions. The versatility and effectiveness of the amino acid-based chelating agent make it a crucial asset in modern agriculture, addressing a critical need by accommodating various soil types and agro climatic conditions [7].

Pak choi (*Brassica campestris* L.), an Asian leafy vegetable belonging to the Brassicaceae family, is now cultivated globally. Glucosinolates (GSLs), sulfur- and nitrogen-containing glycosides, are prevalent in these plants, with around 200 types identified [8]. These plants are crucial for human health due to their content of folate, vitamin C, carotenoids, phenolic compounds, and glucosinolates [Hanson et al., 2009; Zhu et al., 2013]. Several studies, including those on pak choi, have reported the salt tolerance of brassica plants, indicating that a concentration of 50 mM NaCl can affect their growth [Keling et al., 2010]. However, there is limited information on the effect of zinc on brassica plants such as pak-choi, particularly as a biostimulant against salt stress and an inducer of GSL synthesis.

This innovation not only tackles the challenges associated with zinc deficiency but also underscores the importance of continual innovation in meeting the dynamic demands of the agricultural sector. Moreover, zinc's pivotal role in starch-to-sugar conversion and its contribution to bolstering plant resilience against cold temperatures further emphasize its indispensability in plant physiology [9]. In essence, zinc lysinate (Commercial product of this study) serves as a cornerstone micronutrient vital for catalyzing metabolic reactions crucial for optimal plant growth and performance, thereby underscoring the necessity of ensuring adequate zinc supply for agricultural output [10]. Understanding and addressing zinc deficiency remain pivotal endeavors in advancing agricultural sustainability and global food security (Johnson & Smith, 2019). By leveraging innovative solutions like amino acid-based chelating agents, the agricultural sector can strive towards achieving enhanced productivity and resilience in the face of evolving challenges.

2. MATERIALS AND METHODS

2.1 Preparation/synthesis of Zinc Lysinate

The preparation of zinc organic acid chelates using amino acids such as Lysine monohydrochloride involves a meticulous procedure aimed at achieving high-quality chelates with enhanced properties. In a controlled laboratory setting, approximately 0.1 mole each of Zinc sulphate heptahydrate ($ZnSO_4.7H_2O$) and the chosen amino acid or specified organic acid are meticulously weighed and added to a beaker containing 100 ml of ethanol. The use of ethanol as a solvent facilitates the dissolution and reaction of the components. To ensure thorough mixing and reaction, the mixture is stirred continuously while being subjected to heat, typically under reflux conditions, employing a reflux condenser apparatus. The duration of the reflux process, lasting between 5 to 10 hours, is carefully monitored and may vary depending on the specific chelates desired. During this period, the zinc ions from the zinc sulphate react with the amino acid or organic acid, forming stable chelates through coordination bonding. Following the completion of the reflux process, the mixture is allowed to cool to room temperature. Subsequently, the cooled mixture undergoes filtration to separate the solid chelates from the solvent. The resulting zinc chelates, appearing as a fine white powder, are meticulously collected and stored under appropriate conditions for further analysis and utilization. The depicted flow chart, as represented in Fig.1, visually outlines the sequential steps involved in this formulation methodology, providing a comprehensive overview of the chelate synthesis process and the outcome of this study in a product form is represented in Fig. 6. This method ensures the successful isolation of high-quality zinc chelates, poised for subsequent evaluation and application across various domains, including agriculture and nutrition. Here after the synthesized product Zinc lysinate is commercially named as Zinc Check and the same is communicated throughout the study.

2.2 Quality Analysis

According to the Fertilizers Control Order (FCO) of 1985, a standardized protocol was adhered for estimating the nutrient content present in fertilizers. Additionally, the chemical composition of the product was identified through Fourier Transform Infrared (FTIR) analysis (Agilent Cary 630 FTIR). Performing FT-IR analysis of samples provides useful indicators to characterize changes in organic matters, without the need of extraction procedures [11].

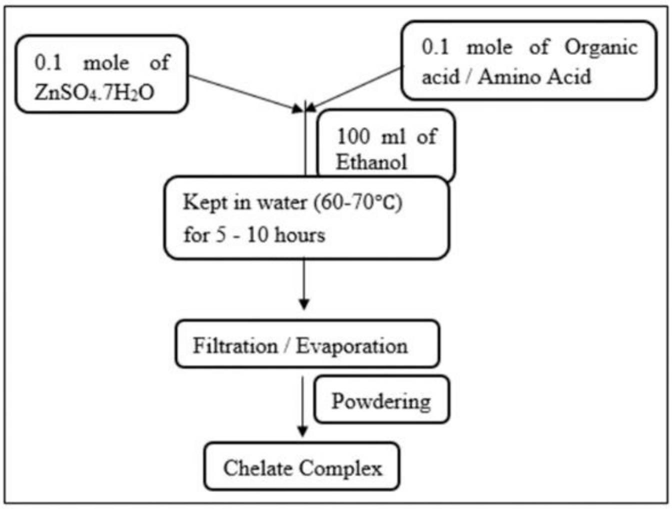

Fig. 1. Schematics for the synthetic methodology of Zinc-Chelate complex formulation

2.3 pH Tolerance

The experiment began with the manipulation of aqueous solutions initially at a neutral pH of 7. These solutions were subsequently adjusted to different pH levels to explore the impact on the stability of metal chelates, particularly those of iron (Fe) and zinc (Zn). To achieve acidic conditions, two approaches were employed: the addition of concentrated hydrochloric acid (HCl), resulting in a pH range of 2.5 to 3, or the use of a dilute solution of acetic acid (20% Acetic Acid), yielding a pH range of 4.6 to 5. Conversely, to create alkaline environments, the neutral solutions were treated with either a 10% sodium hydroxide (NaOH) solution, leading to a pH range of 8.7 to 9, or an aqueous ammonia (NH_3) solution, resulting in a pH range of 11.2 to 11.8. Following the adjustment of pH levels, the desired metal chelates were dissolved in these varied solutions. Subsequently, the relative stability of the metal chelates was assessed, alongside the determination of the total soluble iron (Fe) and zinc (Zn) content. This experimental approach allowed for the examination of how pH alterations influence the stability of metal chelates and the solubility of iron and zinc, providing valuable insights into their behavior under different environmental conditions.

2.4 Pot Trials

The pot mixture used in the experiment underwent sterilization prior to its utilization for the study. The experimental setup followed a completely randomized design, comprising three treatments and eight replications. In this study, Pak choi, a leafy vegetable, was employed as the test subject. Plastic pots of the capacity of 5 liters were filled with 3.5 kg soil whose chemical properties are shown in Table 1. The soil was mixed with sand in ratio 1:1. Seeds were sown in each pot at 7 cm depth. Three series, each consisting of eight pots seeded with Pak-choi seeds, were established and designated as T_1, T_2 and T_3. The treatments were delineated as follows: - T_1: Control setup- T_2: A commercially available Fe/Zn EDTA complex, representing the market standard- T_3: An in-house product developed specifically for this study (Zn Check). The imposition of treatments occurred only once, coinciding with the sowing of the crop. The entire experiment was conducted in the commercial green house during 2022-23, at Multiplex Research and Development, unit Peenya, Bangalore, Karnataka (13˚02'12.1"N 77 ˚31'16.0"E).

2.5 Estimation of Chlorophyll and Plant Biomass Content

Sample Preparation: Leaves from various treatments were crushed to release chlorophyll pigments. Acetone was used as a solvent to extract chlorophyll from the crushed leaves. The protocol of Lichtenthaler et al. 1983 with slight modifications was followed for the extraction process: The crushed leaf samples were mixed with acetone and then centrifuged to separate the soluble chlorophyll from the leaf debris. The supernatant containing chlorophyll was carefully filtered to remove any remaining solid particles. The volume of the chlorophyll extract was adjusted to 100 ml using acetone to standardize the concentration for analysis. Analysis by UV-Visible Spectrophotometer: The chlorophyll extract was analyzed using a UV-Visible spectrophotometer. Absorbance readings were taken in the range of 400 to 800 nm. The wavelengths corresponding to the maximum absorbance peaks for chlorophyll A and chlorophyll B were identified at 662 nm and 617 nm, respectively. Calculation of Chlorophyll Content: Chlorophyll A, chlorophyll B, and total chlorophyll content were calculated using the following formulas:

$$\text{Chlorophyll A } (\mu g/ml) = (13.95 \times A662) - (6.88 \times A646)$$

$$\text{Chlorophyll B } (\mu g/ml) = (24.96 \times A646) - (7.32 \times A662)$$

$$\text{Total Chlorophyll } (\mu g/ml) = \text{Chlorophyll A} + \text{Chlorophyll B}$$

The analysis report is preliminary prepared based on two parameters that were examined after 45days of treatment

The two parameters are

1. Total chlorophyll content present in the leaves of pak-choi
2. Total biomass weight after harvest.

2.6 Specification of Zinc Check

This substance exhibits a crystalline texture and presents a pale white coloration. It demonstrates solubility in both aqueous and alcoholic solvents. With a molar mass of 355.7492 g/mol and a specific gravity of 1.2383, it maintains a pH of 6.77 and the final product of the study is depicted in Fig.7.

3. RESULTS AND DISCUSSION

3.1 Quality Analysis of Zinc Check

The study investigated whether an in-house synthesized Zn chelate reacts with phosphorus salts. Through 1:1 stoichiometric ratio reactions, visual precipitation observations, and infrared spectroscopy, it was determined that no significant secondary displacement reaction occurred. Soil analysis of samples revealed that the Zn chelate retained its zinc content at 14-15%, indicating stability in the presence of phosphorus salts. This suggests that insoluble Zinc-Phosphate formation did not take place, as evidenced by the absence of an increase in relative zinc content to 50-51%, which would correspond to zinc phosphate. The FTIR spectra analysis revealed key peaks indicating the presence of functional groups in solution states of the Zn Check compound i.e. In the solution state, the carbonyl group stretching frequency remained consistent at 1589 and 1663 cm-1, with a slight peak shift observed due to the presence of water as a solvent. However, the significant observation was the absence of additional peaks upon reacting with phosphorus salts (Fig.2,3 & 4). Notably, there were no additional peaks observed after reacting with phosphorus salts (Mono-potassium phosphate and Ammonium phosphate). For instance, equi-molar stoichiometric reactions with mono-potassium salt yielded identical spectra to native Zn Check, indicating no secondary reactions occurred. This consistency across spectra suggests that phosphorus salts do not interfere with Zn availability from the Zn Check fertilizer. Thus, the study concludes that phosphorus presence in soil does not impede zinc ion mobility, ensuring ready availability of zinc to plants when Zn Check is applied. Majee et al., 2020 also used FTIR as a tool for qualitative analysis of in-house synthesized organic fertilizers. Similar experiment concerning analysis of the FT-IR spectra of activated sludge treating wastewater containing phenols, the appearance of absorption peaks in range 2124 2082 cm−1 was observed [12].

3.2 pH Tolerance

The study's results indicate consistent Zn content maintenance across various pH conditions: At highly Acidic pH (2.5-3), Moderately Acidic pH (4.6-5), Neutral pH (7), and Moderately Alkaline pH (8.5-9.0), Zn content remained stable at 15-16%, 15.2%, 15-16%, and 15.6%, respectively. However, at Highly Alkaline pH (11.2-11.8), dissociation occurred due to leaching and reaction with base, leading to a notable drop in Zn content to 3-4% as depicted in Table 3.

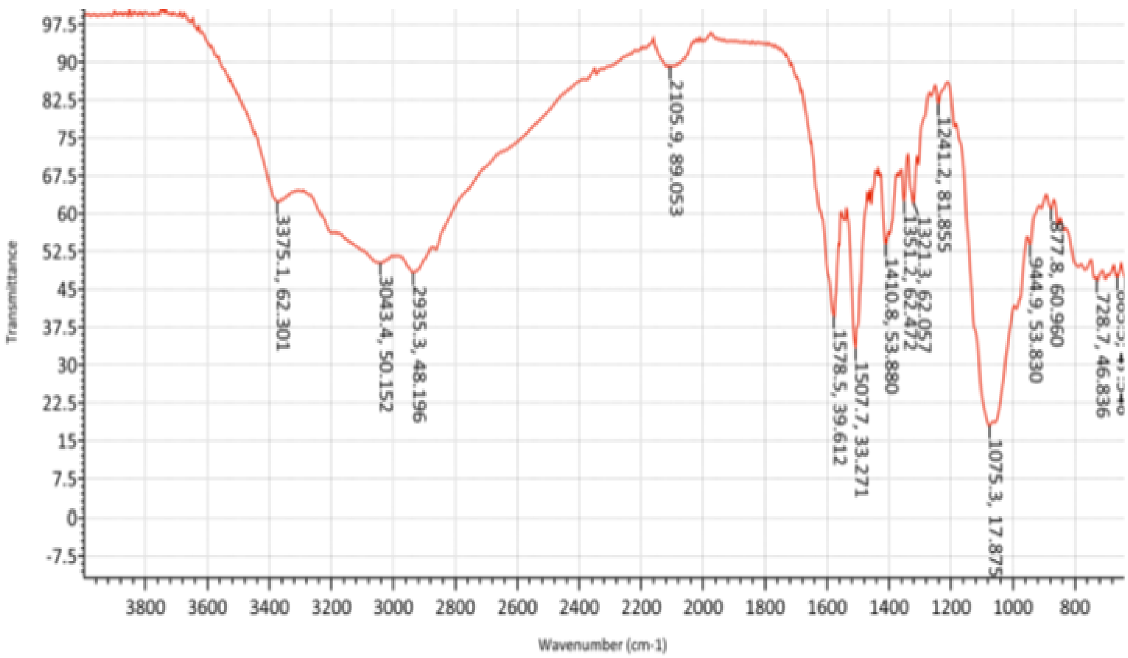

Fig. 2. FT-IR spectra for native Zn Check product

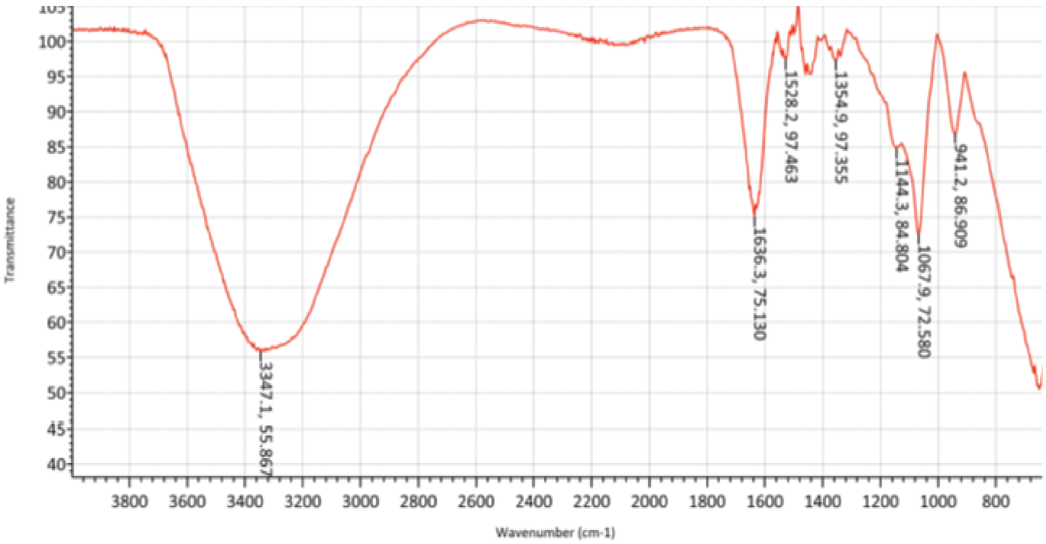

Fig. 3. FT-IR spectra for native Zn Check product with ammonium phosphate in liquid state

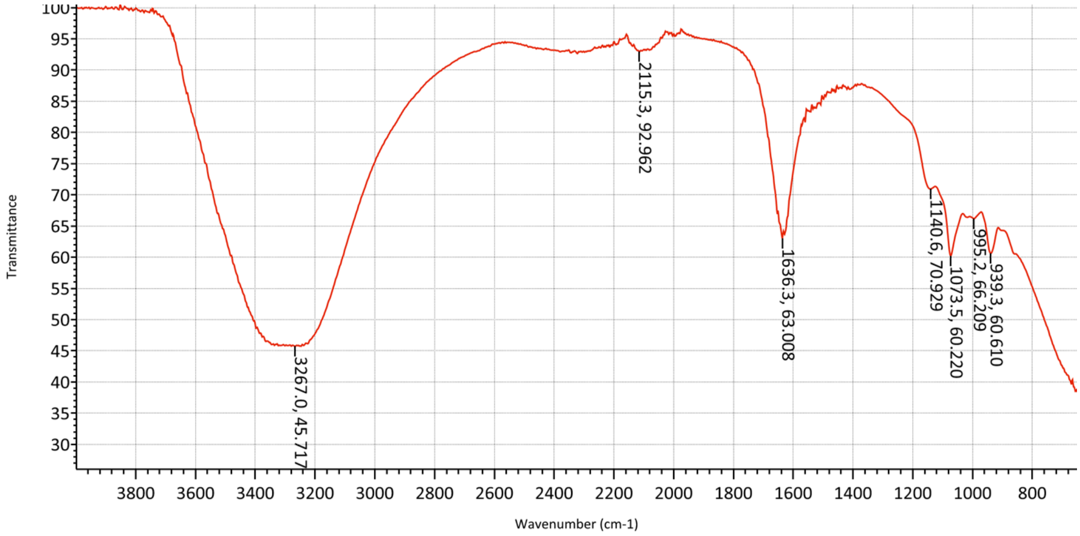

Fig. 4. FT-IR spectra for native Zn Check product with mono-potassium phosphate in liquid state

Fig. 5. Effect of Zn Check on plant growth and yield parameters

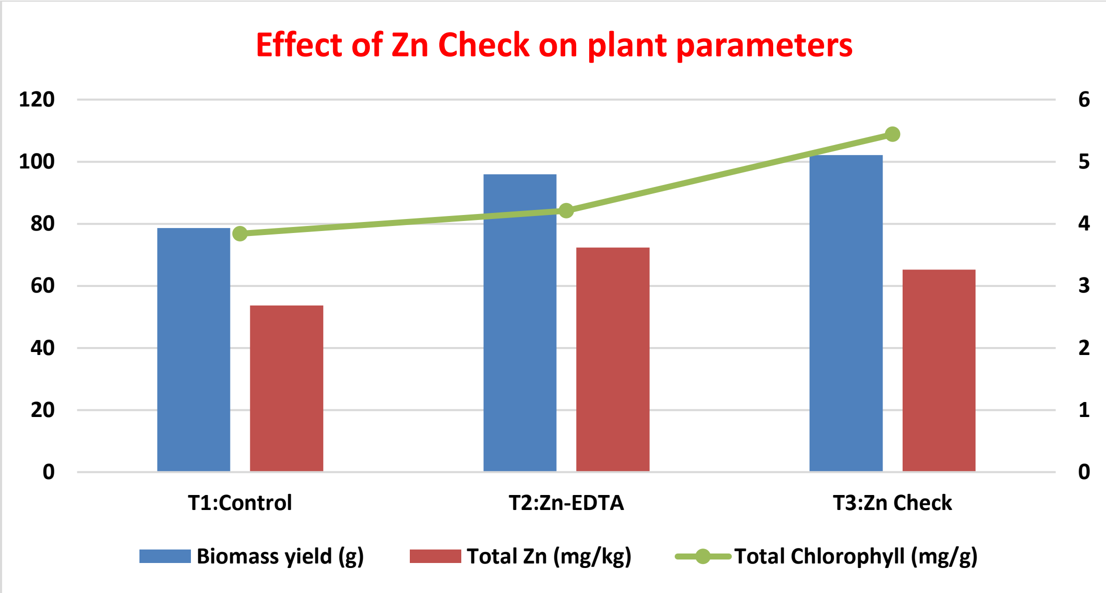

Fig. 6. Effect of Zn Check on plant growth and yield parameters

Fig. 7. The commercialized product of this study

Table 1. Experimental details of the study

Treatment details	Dosages
T_1:Control	0
T_2:Zn EDTA	64.1mg/1.4kg
T_3:Zn Check (15-17% Zn content)	44.3mg/1.4kg

3.3 Chlorophyll and Biomass Estimation

Several studies report a decrease in chlorophyll content caused by zinc toxicity [13]. The study revealed significant differences among all the treatments administered. Treatment T_3, involving the Zn-Lysinate Complex with a Zn content of 15-17%, exhibited the highest total chlorophyll content at 5.44 mg/g, followed closely by T_2, which utilized Zn-EDTA (96), compared to the untreated control at 4.21 mg/g. Hisamitsu et al [14] highlighted that zinc deficiency disrupts chlorophyll synthesis. They emphasized that increased chlorophyll content is attributed to zinc, which serves as a structural and catalytic component in proteins and enzymes, essential for normal pigment biosynthesis, as noted by Balashouri in [15]. Broadley et al. [16] further elucidated zinc's role as necessary for the structural and catalytic components of proteins and enzymes crucial for normal growth and development. The reduction in chlorophyll content at low Zn may be associated with low zin/magnesium because Zn does not directly affect chlorophyll formation or which are part of the chlorophyll molecule. Regarding plant biomass, Treatment T_3 demonstrated the highest biomass yield of 102.12 g, followed by T_2-96.00 g, while the untreated control, T_1, yielded 78.60 g. Whereas, the total Zin content of the composite plant sample has revealed that the treatment T_3 has shown highest Zinc content (72.53 mg/kg) of the dried plant biomass, followed by T_2 (65.26 mg/kg) and least in the treatment T_1 (53.71 mg/kg). Notably, Treatments T_2 and T_3 displayed comparable results, showing no significant difference between them (Fig.5 &6). In summary, the findings underscored the efficacy of both T_2 and T_3 treatments in enhancing chlorophyll content and promoting plant growth compared to the untreated control, with T_3 showing a slight advantage in chlorophyll content and biomass production. Our findings are also in confirmation with (Chakmak and Marschner, [17-19], Liu et al., [1] who reported that dry matter of wheat and cotton plant increased with increasing zinc from deficient levels to sufficient level and the complied details is depicted in Table 2 [20-24].

Table 2. Experimental details on effect of Zn Check on total chlorophyll and plant biomass

Treatment details	Dosage	Total Chlorophyll (mg/g)	Biomass (g)
T_1:Control	0	3.84^{c} (0.00)	78.60^{b} (0.00)
T_2:Zn-EDTA	64.10 mg/1.40kg	4.21^{b} (9.63)	96.00^{a} (22.13)
T_3:Zn Check (15-17% Zn content)	44.30 mg/1.40kg	5.44^{a} (41.66)	102.12^{a} (29.92)
CD at (0.05)		0.21	8.64
CV		4.53	9.01
S.Em.		0.07	2.93

**Values are the mean of 8 replications. Mean ratings a column that are followed by the same letter are not significantly different according to LSD at P ≤0.05. Values within the parenthesis are the per cent increase over control*

Table 3. pH adaptability data for Zn Check at acidic-neutral and alkaline conditions

Metal Chelates	Highly Acidic pH (2.5-3)	Moderately Acidic pH (4.6-5)	Neutral pH (7)	Moderately Alkaline pH (8.5-9.0)	Highly Alkaline pH (11.2-11.8)
Zn Check (Solid Compound)	Stable, Zn Content 15-16%	Stable, Zn Content 15.2%	Stable, Zn Content 15-16%	Stable, Zn Content 15.6%	Dissociation is observed due to leaching and reaction with base, Zn Content 3-4%

Table 4. Details of total Zinc content in plant sample

Treatment details	Total Zinc content in plant sample(mg/kg)
T_1:Control	53.71
T_2:Zn-EDTA	65.23
T_3: Zn Check (15-17% Zn content)	72.37

4. CONCLUSION

In conclusion, the study found that an in-house synthesized Zn chelate did not react significantly with phosphorus salts, as confirmed through 1:1 stoichiometric ratio reactions, visual precipitation observations, and infrared spectroscopy. Soil analysis indicated that the Zn chelate retained its zinc content at 14-15%, demonstrating stability in the presence of phosphorus salts and suggesting the absence of insoluble zinc-phosphate formation. FTIR spectra analysis revealed consistent peaks indicating the presence of functional groups in the Zn Check compound, with no additional peaks observed after reacting with phosphorus salts. This consistent behavior across spectra suggests that phosphorus salts do not interfere with zinc availability from the Zn Check fertilizer. Therefore, the study concludes that the presence of phosphorus in soil does not hinder zinc ion mobility, ensuring the ready availability of zinc to plants when Zn Check is applied even at alkaline soil conditions.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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