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Synthesis and Characterization of Chitosan Encapsulated Zinc Oxide Nanoparticles and its Application in Maize under Zinc Deficit Soil

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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 deficiency is a growing concern among the global population, and it can be attributed to the depletion of significant portions of agricultural land and the production of foods that are low in zinc. In the current study, an efficiency-centric method of maize cultivation with zinc oxide nanoparticles encapsulated in chitosan (Cs-ZnO NPs) is proposed and evaluated. In order to investigate, NPs

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were chemically synthesized using process improvement for better size and shape and characterized using Scanning electron microscope (SEM), Energy-dispersive X-ray spectroscopy (EDS) and Transmission electron microscope (TEM). The NPs under characterization showed less than 100 nm diametric globular particles. A field experiment was conducted in randomized block design with six different treatments to study the impact of foliar applied Zn at various concentrations (50, 100, and 150 mg L⁻¹) through Cs-ZnO NPs on plant growth, SPAD and grain yield in maize plant, with respect to foliar ZnSO₄ @0.5 %, chitosan @150 mg L⁻¹ and the control. The highest grain yield of 52.5 q ha⁻¹ in maize was recorded with Zn supplied through Cs-ZnO NPs at 100 mg L⁻¹, compare to other treatments. Similarly significant improvements in plant height and chlorophyll content were noted at 100 mg L⁻¹ Zn through Cs-ZnO NPs. These results indicates that Cs-ZnO NPs can improve grain yield and plant growth performance of maize in a sustainable and efficiency centric manner.

Keywords: Maize; nanoparticles; zinc; chitosan.

1. INTRODUCTION

Nanotechnology is an exciting new technology that has the potential to completely change farming and food production in a way that will last. The controlled delivery of essential nutrients, pesticides, and fertilizers is one area where nanoparticles may find utility in agricultural systems [1,2]. Rapid absorption by plants is possible with nano-based fertilizers because of their active metabolic and water/mineral conduction. The negative impacts of chemical fertilizers are reduced by using nano-fertilizers, which also enhance soil health and nutrient delivery [3,4]. However, the composition of nanoparticles (NPs) has a considerable impact on their applicability. About 40% of the nutrients in traditional fertilizers are absorbed by plants, whereas the rest is leached away, creating an imbalance in soil nutrients[5,6]. To combat these problems, scientists are focusing on creating new nano fertilizers with enhanced nutrient use efficiency. This might lead to higher crop yields through enhanced photosynthesis, reduced water usage, delayed senescence, and increased tolerance to both biotic and abiotic challenges.

Zinc (Zn), is a key micronutrient that regulates a multitude of physiological and molecular processes that are fundamental to crop resistance to environmental stress [7]. In addition to being an important transitional metal, zinc is found in all six classes of enzymes and serves as a component of several transcription factors. In plants, zinc affects several life cycle processes. It regulates several processes, including nucleic acids, saccharides, and lipids and plays an important role in carbohydrate and protein synthesis[8]. Researchers have shown that zinc plays a significant role in plant growth,

development, and yield. It assists plants in overcoming biotic and abiotic stresses by participating in many physiological processes, including resistance to pathogens, drought, or heat [9]. Despite the presence of adequate quantities of zinc in the majority of soils, its absorption may be hindered by various chemical and physicochemical factors within the soil [10–12]. Zinc, on the other hand, is the most commonly deficient trace element in agriculture, and a deficit can result in a 40% loss in crop productivity [13,14]. Zinc deficits are common in alkaline soils, light sandy soils, and soils with relatively high levels of accessible phosphorus. In comparison to other micronutrients, maize (*Zea mays L.*) has a notably high zinc requirement. The ideal zinc concentrations in maize tissue fall within the range of 20 to 60 mg kg⁻¹. Foliar zinc spray can be utilized as a supplement to maize nutrition, particularly in conditions where soil qualities may restrict Zn bioavailability to plants [7,10].

Maize is considered the "Queen of Cereals" due to its extensible application and adaptability. It is the most widely cultivated grain in the world, belonging to the Poaceae family, and is the main source of nourishment in many developing countries. In recent years, maize has garnered scholarly interest due to its status as the most vital food source for food security and its status as an unrivaled food source for millions of humans and animals worldwide [15,16]. Maize is currently experiencing a decline in nutritional value and production potential due to a number of factors, including micronutrient deficiencies; maize is particularly vulnerable to zinc deficiency [17].

Nevertheless, elevated levels of zinc have been noted to be toxic to plants; thus, it is imperative

to examine both the threshold level and the potential benefits that zinc oxide nanoparticles may offer in comparison to the diverse types of zinc fertilizers presently employed. The purpose of this study was to investigate the potential of chitosan encapsulated ZnONPs as a novel nanofertilizer that may increase crop health and yield by enriching maize with the right amount of zinc. The experiment aimed to determine the relationship between a certain concentration of nanoparticles (NPs) and the alterations in the plant's morphological and physiological characteristics compared to the control.

2. MATERIALS AND METHODS

2.1 Synthesis of Nanoparticles

The chitosan encapsulated nanoparticles were synthesized using the method outlined by [18] with little modifications. To synthesize 1 gm of chitosan was dissolved in 1 percent acetic acid and stirred for 30 minutes till complete dissolution of chitosan powder occurs. Further, 1 gm of Zinc oxide were added to the mixture and the stirring was continued for another 2 hours at 60 °C. Then 2M NaOH was added to attain a pH of 10 and the mixture precipitates. Following this, the mixture was again agitated for another 6 hour and then it was centrifuged at 10,000 rpm to separate chitosan encapsulated zinc oxide nanoparticles which further washed with double distilled water and final wash with ethanol. Finally, these nanoparticles were dried in oven at 100 °C for 48 hours and kept in an airtight vessel for further characterization and field application.

2.2 Characterization of Nanoparticles

The nanoparticles characterization was done at Indian institute of technology, Kharagpur and IARI, New Delhi. Synthesized nanoparticles were characterized for their surface morphology and size using advanced Fe-scanning electron microscopy (Carl ZEISS SMT, Germany). The

elemental composition of nanoparticles was assessed using energy-dispersive X-ray spectroscopy (INCA PentaFET x3, Oxford Instrument UK). Transmission Electron Microscopy (TEM) was also employed to determine the size of synthesized nanoparticles using (JEOL TEM model JEM-110, Tokyo, Japan).

2.3 Experimental Details

A field experiment was conducted in July 2022 at N.E Borlaug crop research centre, G.B Pant university of agriculture and technology, Pantnagar, Uttarakhand, India. A total of six treatment were laid in randomized block design. Recommended dose of N:P:K at the rate of 120:60:40 kg ha⁻¹ was applied in all the treatments equally at the time of sowing. Nanoparticles were applied foliarly using the hand sprayer (Capacity 2 L). Treatment details for nanoparticles are present in Table 1.

2.4 Observation Recorded

The SPAD (model-at LEAF CHL STD meter) chlorophyll meter was used to record the SPAD reading (Chlorophyll index) at 30 DAS and 60 DAS. A fully matured young leaves were selected to record the reading. The plant height of the maize plants was taken using the wooden measuring scale at 30 DAS, 60 DAS and harvest. The Grain yield was taken in quintals per hectare (q ha⁻¹) after threshing and drying of grains at optimum moisture level.

2.5 Statistical Analysis

The data were collected from the experiment, while the investigation was processed with Windows Excel, and statistical analysis was carried out with software SPSS v23.0. ANOVA was used to examine the significance of differences between treatments at 5% significance level.

Table 1. Treatment details

Treatments	Treatment Details
T ₁	Zinc Sulphate 0.5%
T ₂	Cs-ZnO NPs 50 mg L ⁻¹
T ₃	Cs-ZnO NPs100 mg L ⁻¹
T ₄	Cs-ZnO NPs150 mg L ⁻¹
T ₅	Bulk Chitosan 150 mg L ⁻¹
T ₆	Control

*Cs-ZnO-Chitosan encapsulated Zinc oxide nanoparticles

3. RESULTS AND DISCUSSION

3.1 Characterization of Nanoparticles

3.1.1 Morphological analysis

Surface characterization was done using the scanning electron microscope. The result of the SEM is illustrated in Fig 1. The investigation using electron microscopy confirmed that the chitosan encapsulated zinc oxide nanoparticles possesses the spherical morphology Fig 1(b,c). The existence of a white matrix encircling ZnO NPs provides conclusive evidence for the presence of chitosan biopolymer and the development of nanocomposites. Similar findings have been documented by [18,19].

3.1.2 Elemental Composition

Energy dispersive X-ray spectroscopy (EDS) was employed to ascertain the existence of elements within the Cs-ZnO nanoparticles. Fig. 2 illustrates the imaged distribution of different elements within the synthesized nanoparticle. The EDS

spectrum (Fig. 2) demonstrates that the synthesized nanoparticles contain, carbon, zinc, oxygen and nitrogen components. The formation of ZnO particles is confirmed by the detection of Zn and O, and the presence of Cs in the Cs-ZnO nanoparticles is supported by the detection of other elements N. One possible explanation for the presence of nitrogen in the synthesized Cs-ZnO nanoparticles is that the amine groups (-NH₂) of chitosan excited the X-rays. Similar results were recorded by [20], who observed that the Cs-ZnO nanoparticle contained nitrogen.

3.1.3 Size distribution of nanoparticles

The transmission electron microscopy (TEM) micrograph of the synthesized Cs-ZnO nanoparticles revealed a uniform spherical nanostructure with an average diameter of less than 100 nm (Fig. 3). The existence of spherical fragments in the Cs-ZnO nanoparticles confirms the presence of Chitosan. Similarly, [21,22] documented that the electron microscopy of Cs-ZnO NPs revealed agglomerated grains arranged in the shape of rods.

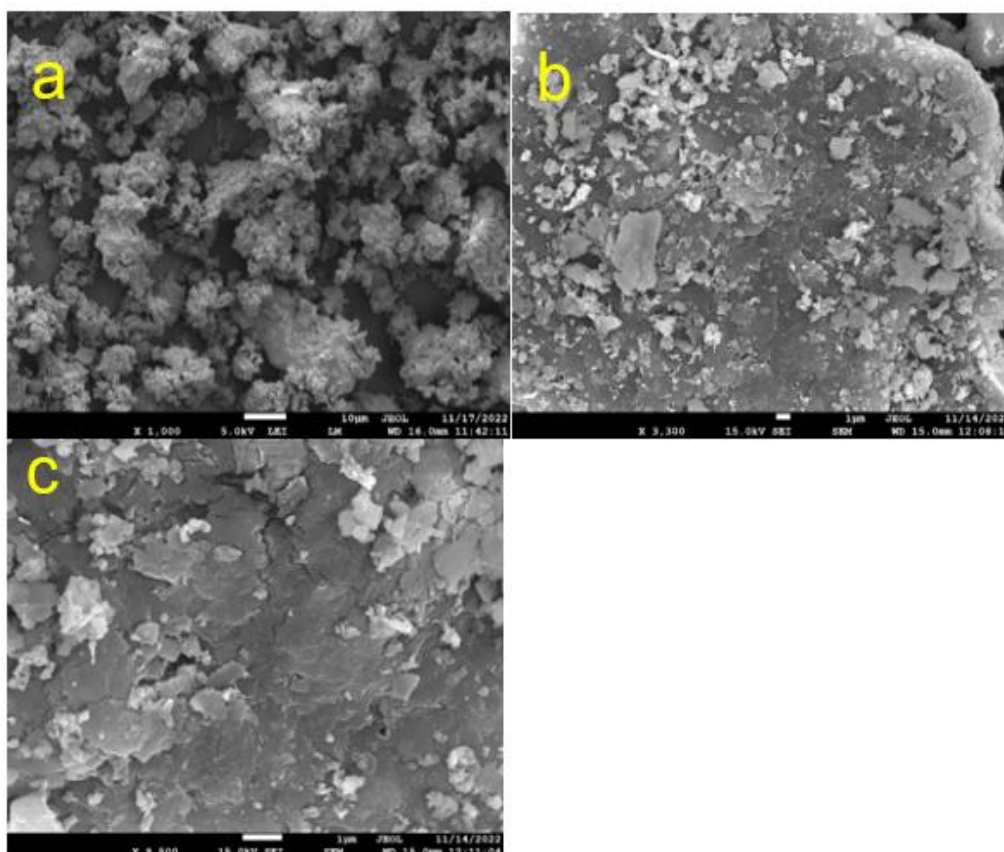


Fig 1. SEM images a) ZnO; b and c) Chitosan encapsulated Zinc Oxide nanoparticles

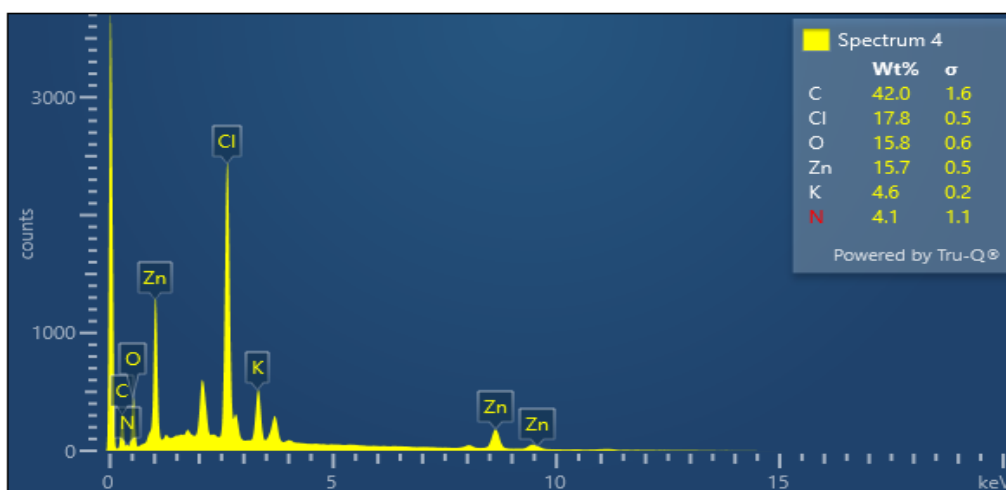


Fig. 2. EDS Spectrum of Chitosan encapsulated zinc oxide nanoparticle

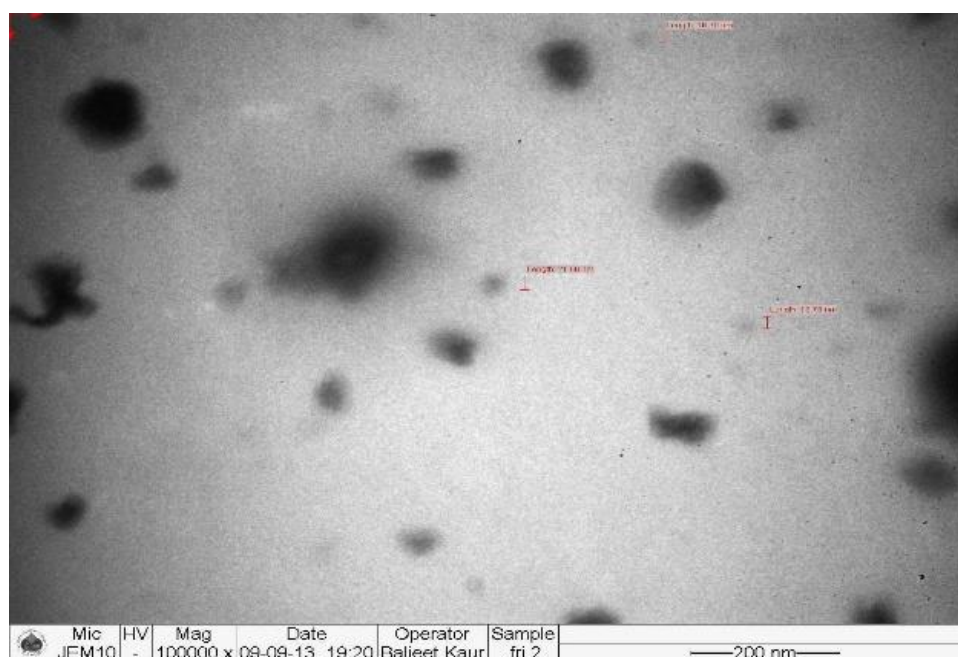


Fig. 3. TEM image of Chitosan encapsulated zinc oxide nanoparticle

3.2 Crop Studies

3.2.1 Grain yield

Application of Zinc substantially increased the grain yield in all the treatment irrespective of sources (Fig 4). The significantly higher grain yield of 52.53 q ha⁻¹ was recorded with 100 mg L⁻¹ Zn supplied through Cs-ZnO nanoparticles which was statistically at par with treatment of Cs-ZnO @50 mg L⁻¹ and Cs-ZnO @150 mg L⁻¹. The yield increment was 23.4 % higher in Cs-ZnO @100 mg L⁻¹ as compared to control and 15 % more than the conventional Zinc Sulphate

@0.5%. This might be due to Cs-ZnO nanoparticles enhanced crop yields by improving yield-attributing characteristics in the treated plants. Apart from promoting photosynthesis, the yield increase is due to improvements in physiological and biochemical processes. This improvement may be attributed to Zinc's role as a co-factor for various crucial enzymes. Additionally, ZnO NPs have the ability to penetrate leaf surfaces and release Zn ions through their cuticles due to their nano-sized dimensions, diffusible nature, and higher solubility [23,24].

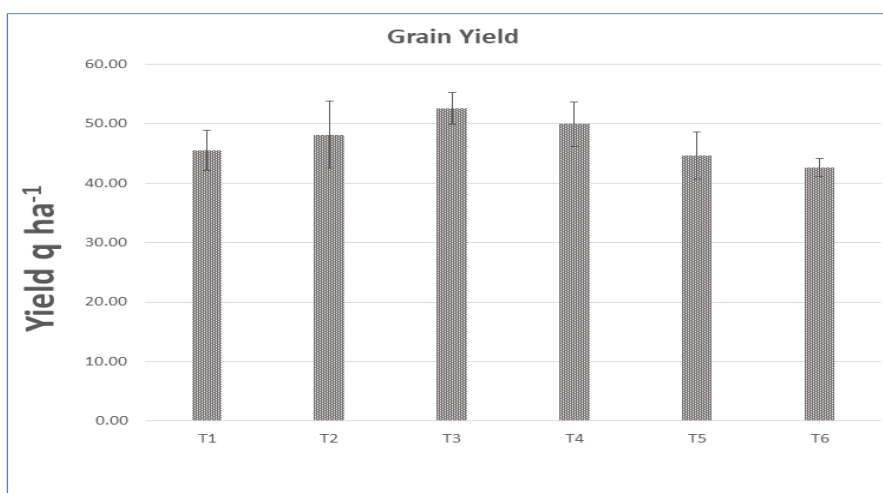


Fig. 4. Effect of different Zinc treatments on grain yield in q ha⁻¹

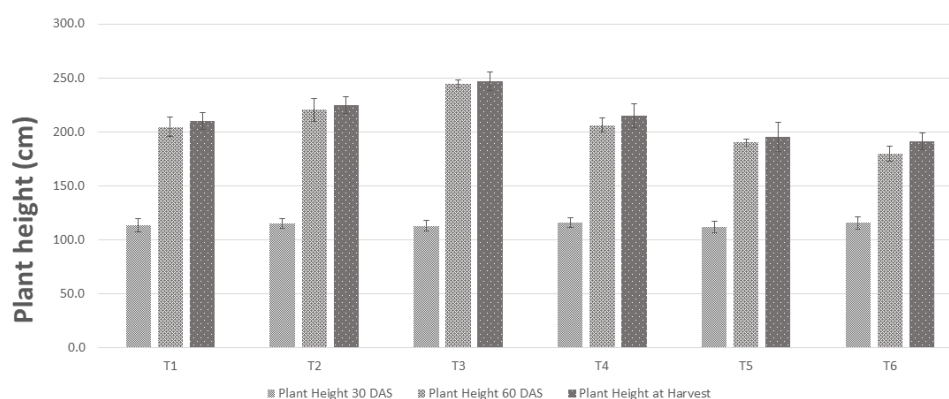


Fig. 5. Effect of different zinc treatments on plant height in cm

3.2.3 Plant height

The plant height of maize plants was taken at different crop growth period Viz. 30 DAS, 60 DAS and at Harvest (Fig 5). At 30 DAS there were no significance difference observed between within different treatments which were due to the reason that foliar application was done at this stage i.e at 30 DAS. However, at 60 DAS the significant difference can be seen between the treatments. The higher plant height was recorded, 244.4 cm in Cs-ZnO @100 mg L⁻¹ over the conventional ZnSO₄ @0.5% and control. Similar trend was seen at harvest where the Cs-ZnO @100 mg L⁻¹ recorded the significantly higher plant height (17%) over conventional ZnSO₄. An increasing trend in plant height was observed when the plant was supplied with zinc. Application ZnO NPs to the leaves leads to an increase in shoot biomass which might be

because zinc is involved in membrane function, cell elongation and protein synthesis [25,26]. Enhancements in vegetative parameters were observed in maize plants cultivated in soil deficient in zinc, following foliar application. Foliar applications of micronutrients are known to be more effective than soil applications for crop plants because the nutrients are able to reach the cells and contribute to plant growth more effectively. In groundnut, foliar zinc oxide nanoparticles were found to be more effective than soil application for improving plant growth and development [26].

3.2.4 SPAD

The SPAD (chlorophyll index) is a quick and non-destructive way to detect chlorophyll levels in plants and determine their nutritional status. The result of the study showed no significant

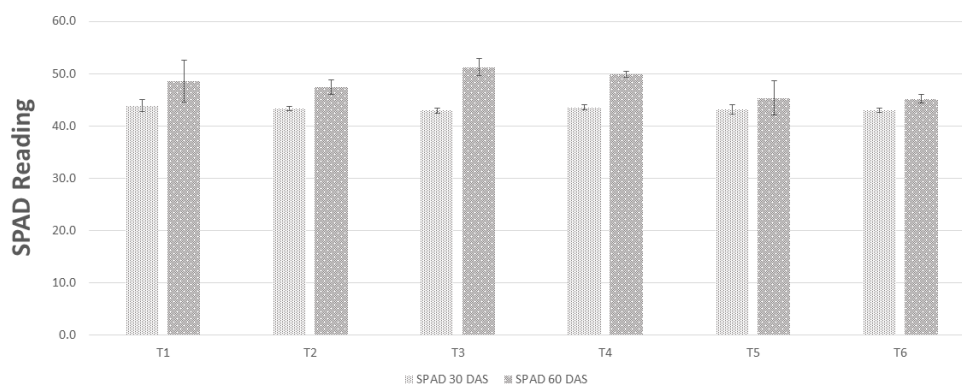


Fig. 6. Effect of different Zinc treatments on SPAD reading

difference at 30 das, this is because of the reason that the foliar treatment of Zn was given at 30 DAS (Fig 6). However, at 60 DAS a significant difference in the chlorophyll index were observed in comparison to control between the treatments. The highest value 51.2 (13 % higher) were observed in Cs-ZnO @100 mg L⁻¹ in with respect to control i.e. 45.2. A positive correlation is anticipated between a higher SPAD value and enhanced dry matter production in plants. Additionally, the presence of micronutrients, such as zinc, in the meristematic area can promote chlorophyll biosynthesis by being involved in photosynthesis pigments [27]. Zinc foliar application, on the other hand, maintains leaf erectness, which improves light interception properties, decreases transpiration rate, and maintain nutrient imbalances; furthermore, zinc improves chloroplast structure, which increases chlorophyll concentration [28].

4. CONCLUSION

Based on the findings, it can be concluded that chitosan-encapsulated zinc oxide nanoparticles exhibited distinct morphological characteristics, emphasizing their 'nano dimension' and suitability for use as a nano fertilization material. Foliar application of Cs-ZnO NPs proved to be effective in increasing the grain yield and plant growth. Maximum grain yield, plant height, and SPAD (Chlorophyll index) were recorded when foliar spray of nanoparticles was applied at 100 mg L⁻¹ Zn through Cs-ZnO NPs. The positive effect of nanoparticles on crop can be attributed to improved chlorophyll content, increased enzyme activity, and stress resistance. Similarly, smaller particle size, higher surface area, and surface charge density make Cs-ZnO NPs more reactive and enhance their uptake ability through the leaf surface. It is evident that ZnO

nanoparticles, particularly when combined with chitosan, can be used as a transformational agronomic management tool and yield improvement agent for maize due to the synergistic effects of chitosan and ZnO nanoparticles that pave the way for sustainable and efficiency-driven crop production.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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