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Evaluation of nitrate-based chitosan nanofertilizer on the morpho-physiological responses of maize under semi-field condition

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***Abstract*— *Global agriculture is frantically seeking for ways to mitigate the detrimental environmental effect of standard chemical fertilizers. Next-generation fertilizers made from biodegradable, eco-friendly, renewable energy sources may be the answer, allowing for greater nutrient efficiency and a lower environmental footprint. Chitosan nanoparticles (NPs) have recently been characterized as a viable way to improve plant immune systems through slow, controlled, and targeted nutrient delivery. Chitosan is a linear amino polysaccharide having a rigid structure, hydrophilic properties, and crystal structures. Chitosan, due to its amino group content, can combine with other chemicals, penetrate the plant's vascular system, and activate plant metabolic-physiological pathways. Surface-functionalization of chitosan with additional active compounds improves the bio-functionalities of the structure. In the current study, a nitrate-based chitosan nanofertilizer was formulated by ionic gelation, and its foliar efficacy on maize was comprehensively examined in a semi-field condition. The manufactured nanofertilizer, at 2% (w/v), had a substantial influence on root dynamics as compared to control groups. The current study strongly anticipates the potential of novel nitrate-based chitosan nanofertilizer to be a very valuable and novel bio-stimulant, as well as a good supply of macronutrients for agricultural plants. It may also be thoroughly researched for other crops in order to further develop the technique.*

***Keywords*— Chitosan, Surface-functionalization, ionic-gelation, bio-stimulant**

I. INTRODUCTION

Maize (Zea mays L.) is the third most important cereal for global food security after wheat and rice. Maize belongs to the family Poaceae and is believed to be originated from Mexico and Central America (**Nawaz *et al.*, 2020**). The word "maize" is thought to be derived from the ancient word "mahiz" and it was brought to India in 17th century (**Messias *et al.*, 2015**; **Tanumihardjo *et al.*, 2020**). Maize is monoecious plant with tassel, pistillate flowers on axillary shoots; ear shoot consists of a shank or cob, florets with long filamentous stigmas, silk. The total global production of maize in the year 2022 was 1163.9 million metric tonnes (MMT). For optimum maize production, loam, clay loam, sandy clay and pH 5.6 are considered as the best combination (**Pilevar *et al.*, 2020**; **Muhammad *et al.*, 2022**). To achieve higher production nutrient management is crucial at every developmental stage. An inadequate and imbalanced supply of nutrients to the crop results in yield diminution. In addition, the continuous practice of cultivating maize-wheat cropping sequence deterioration soil exhaustively; thus, it is difficult to sustain both grain quality and crop productivity. Therefore, it is necessary to provide balanced nutrition to the crop which may sustain soil fertility and lead to higher productivity. Eventually, in order to higher productivity, farmers are compelled to use an excess quantity of inefficient traditional fertilizers, which may lead to soil corrosion, deprivation of agroecosystems, deposition of excess chemicals, heavy metal accumulation, eutrophication, nitrate accumulation, global warming and increased resistivity in pathogens towards chemicals (**Subramanian *et al*., 2015**; **Singh *et al*., 2017**; **Sharma and Bhende, 2024**). Apart from them, only half of the applied supplements are taken up by plants; the rest is discharged into the environment as noxious vapor or harmful leachates, causing environmental nuisance (**Chouhan and Mandal, 2021**). The structure and functionality of conventional fertilizers and their applications are therefore needed to be redefined in order to maintain sustainable agricultural output without endangering the ecosystem. In this alarming scenario, nano-enabled next-generation fertilizers are anticipated as substitutes to, or even full alternatives for, traditional chemical fertilizers. Moreover, a smart delivery system of potential agrochemicals should be developed to allow immobilization, protection, and controlled release of active compounds (**Kumaraswamy *et al*., 2021**). In this scenario, nanobiotechnology can be a promising way for achieving a revolution in agriculture by providing agrochemicals according to crop demand. Nanotechnology has many applications in all stages of production, processing, storing, packaging and transport of agricultural products and other marketing facilities (**Kumaraswamy *et al*., 2019**). Despite of having various benefits, accumulation of metal-based NMs in plants and subsequent possible deposition in food and soil can pose a great threat to human health. The possible interaction of metallic NMs with soil irreversibly impact on chemical-biological and physical structure of soil (**Simonin and Richaume, 2015**). In the current scenario, biopolymer-based nanofertilizers have currently been reported as promising alternatives of commercially available chemical fertilizers due to its biodegradable nature and potential to maintain food security throughout the globe. Biogenic materials can be explored as matrices for nutrient supply to plants with inbuilt character of slow/control release (**Prajapati *et al*., 2022**). Keeping all this in mind there are several delivery systems were developed by scientist such as flexible biopolymer capable of forming films, hydrogels, scaffolds, fibers, microspheres, and nanoparticles; used as a good delivery system for the controlled release of active compounds (**Rani *et al*., 2024**). The first report on chitin traces back to 1811 by French Professor of natural history Henri Braconnot. He found out the alkaline-insoluble fraction from mushrooms and named it "fungine" (**Haghighi *et al*., 2020**).

Chitosan, a seaweed product, is biodegradable, biocompatible, and ecologically benign, making it a viable alternative to standard chemical fertilizers (**Prajapati *et al*., 2022**). It has several biological activities, including antifungal, antibacterial, antiviral, and plant growth regulator (**Saharan and Pal, 2016**). Furthermore, chitosan is known to enhance plant defence responses (**Kumaraswamy *et al*., 2018**). Although chitosan is a biopolymer with high added value, and its properties are related to its molecular weight. Thus, high molecular weight values provide low solubility of chitosan, presenting limitation in its use. Based on this, several studies have developed for different hydrolysis methods to reduce the molecular weight of chitosan and also to enhance its activity. There is a great deal of potential to increase agricultural productivity. When chitosan is factionalized with additional compounds having potential relevance in agriculture.

Maize production is critical for global food and nutritional security, with its versatile uses making it a vital component of the agri-food system. Maize production heavily relies on adequate nutrient management, with nitrogen, phosphorus, and potassium being the most critical nutrients. Among the essential nutrients, nitrogen (N) is the most vital nutrient element, performing as a component of many organic compounds viz., proteins, amino acids, nucleic acids and nucleotides, enzymes, vitamins, hormones, alkaloids etc. Empirically, a ton of grain maize requires almost 9-11 kg of N (**Kumar *et al*., 2021**). Potassium (K) fertilization on the other hand plays a significant role in enhancing the yield, water use efficiency and nutrient utilization proficiency in maize. K has vital role in photosynthesis, water storage control and stomata opening in leaves (**Kandil *et al*., 2020**). Application of KNO_3, have differential response in terms of growth and development of a crop. The cob girth increased progressively with the foliar applications of either water or KNO_3, during drought, stressed plants uptake more K to regulate the internal mechanisms and ameliorate water deficiency. The use of K as a compatible solute can osmo-regulate the cellular environments in water stress conditions. The effects of K deficiency stress on root morphology and function are not significant, but nevertheless observable (**Lacerda *et al*., 2018**).

II. MATERIALS & METHODS

2.1 Synthesis and characterization

In the current study, we used the ionic-gelation approach to develop the nitrate-based chitosan nanofertilizer and evaluated its foliar efficacy on the morpho-physiological responses of maize. Initially, 1% (w/v) chitosan was dissolved in 0.5% (v/v) acetic acid *via* electrospinning at 320 rpm until clear, homogenous solutions were obtained, followed by cross-linking with

0.5% TPP (Sodium tripolyphosphate) solution (w/v). Following that, surface functionalization of chitosan was achieved by manually introducing a potassium-nitrate (0.2g/100 ml) to the chitosan-TPP complex. At the end of the process, 5% surfactant (NP 40) was added drop-by-drop to obtain a stable formulation that was suitable for characterization.

The structural characteristics and stability parameters of the newly synthesized nanostructure were studied and confirmed using dynamic light scattering (DLS), viscosity, and TEM (Transmission Electron Microscopy) analysis. The Z-average size (hydrodynamic size) of the component particles, poly dispersive index (PDI), and zeta potential were determined using a Zetasizer (ZS90, Malvern) at a constant temperature of 30°C and a scattering angle of 90° during the DLS investigation. Viscosity of the formulation was tested at 30°C in a rotating viscometer (ViscoQC 100, Anton Paar) using an L1 spindle at 100 rpm. TEM was performed in accordance with the standard protocol (Ottaviani *et al.*, 2000) to better understand the structural features and morphology of the components in the sample under consideration.

2.2 Experimental design and treatment details

The current study was carried out under the supervision of Department of Molecular Biology and Biotechnology, Rajasthan College of Agriculture (RCA), Maharana Pratap University of Agriculture and Technology (MPUAT), Udaipur. The seeds of maize cultivar PHM-3 were procured from the Department of Soil Science and Agriculture Chemistry, RCA, MPUAT, Udaipur. A pot experiment was carried out in a cage-house, where pots were arranged in CRD (Completely Randomized Design) way with three replications. Each pot contained the same amount of soil and recommended dose of fertilizer for maize growth. A total of 6 treatments were considered under the study (**Table 1**) and in each pot, 5 healthy seeds were sown and watered regularly.

Table 1 Experimental outline

Treatments, replication	6, 3
Experimental design	CRD
T1	Control (water)
T2	KNO_3 (2%)
T3	Nitrate-CS NF* (2%)
T4	Nitrate-CS NF (1%)
T5	Nitrate-CS NF (2%)
T6	Nitrate-CS NF (3%)
Mode of experiment	In pots
Pot size, number of seed per plot	25cm x 25cm, 05
Plant protection	As per recommendation
Stage of application	1st foliar at 25 DAS, 2nd foliar at 55 DAS

*Surfactant free

2.3 Foliar application

The maize cultivar PHM-3 seeds underwent the treatment outlined in **Table 1** and were subsequently planted in plastic pots, with five seeds per pot, filled with soil collected from a maize field. Foliar application of nanofertilizer was performed with three different concentrations (1, 2 and 3% v/v) along with 2% KNO_3 and water as control. One treatment of 2% nanofertilizer was also performed without surfactant. 1st foliar was performed at 25 DAS and 2nd at 55 DAS. Growth parameters including shoot length (cm), root length (cm), pre-embryonic root number, lateral root number, stem diameter (cm), leaf area (cm^2) was recorded at the stage of physiological maturity.

2.4 Statistical analysis

Statistical analysis was conducted utilizing JMP software, specifically version 12, to rigorously analyse the data. A significance level of $P=0.05$ was employed to ascertain significant differences among the various treatment groups. It is important to note that all experiments were carried out with a minimum of three replicates, and each experiment was conducted three times for robustness and reliability. This approach ensured the consistency and credibility of the experimental results.

III. RESULTS AND DISCUSSION

This study was undertaken to synthesize, characterize and investigate the impact of foliar nitrate-based chitosan nanofertilizer on morpho-physiological characteristics of maize (*Zea mays* L.) under the semi-field condition (in pots). First of all, nitrate-based chitosan nanofertilizer was prepared with the concept of ionic-gelation (**Choudhary *et al.*, 2019**). Initially, a constructive chemical interaction among the positively charged amino groups ($-NH_2$) of chitosan and negatively charged sodium tripolyphosphate (TPP) yielded a nano-matrix (milky white in colour), in which potassium nitrate solution (0.2g in 100ml) was plunged to obtain a stable nano-formulation. Next, 5% surfactant (NP 40) was mixed to the solution very carefully to make it stable. Afterwards, the newly synthesized nano-formulation was characterized and employed for subsequent applications in maize, aligning with the research objectives and experimental design.

DLS analysis of the synthesized nitrate-based chitosan nanofertilizer revealed an average hydrodynamic diameter (Z-average) of 303.5 nm with a polydispersity index (PDI) of 0.248 (**Figure 1a**) and zeta potential of +22.3 mV (**Figure 1b**). The obtained particle size confirms the formation of nanoparticles within the acceptable nano-range for chitosan-based delivery systems. The relatively low PDI value (<0.3) indicates narrow and uniform particle size distribution, suggesting controlled nanoparticle formation through ionic gelation. The positive zeta potential is attributed to the protonated amino groups ($-NH_3^+$) of chitosan, which contribute to moderate colloidal stability of the formulation. Furthermore, the number distribution data indicated that the majority of nanoparticles were present in the size range of 50–70 nm, with the highest population observed at 58.77 nm (25.8%) (**Figure 1c**). Structural morphology of nitrate-based chitosan nanofertilizer was studied by Transmission Electron Microscopy (TEM), which revealed the synthesized formulation under investigation containing discrete spherical aggregates, typically falling within the size range of 40 to 80 nm (**Figure 2**). The presence of uniformly distributed nanoparticles with positive surface charge suggests improved interaction with plant surfaces and enhanced nitrate delivery efficiency in maize. Viscosity analysis indicated that the encapsulation of potassium nitrate with in chitosan matrix influenced flow properties of the synthesized nitrate-based chitosan nanofertilizer. The formulation exhibited a low viscosity value of 7.4 cP, which is suitable for agricultural spray applications and may facilitate better interaction with plant surfaces. The particles appeared as dark, discrete nanostructures forming moderate aggregates of 40-80 nm. Such aggregation may result from intermolecular hydrogen bonding and electrostatic interactions during sample preparation. The nanoscale morphology supports successful formation of nitrate-based chitosan nanofertilizer through ionic gelation.

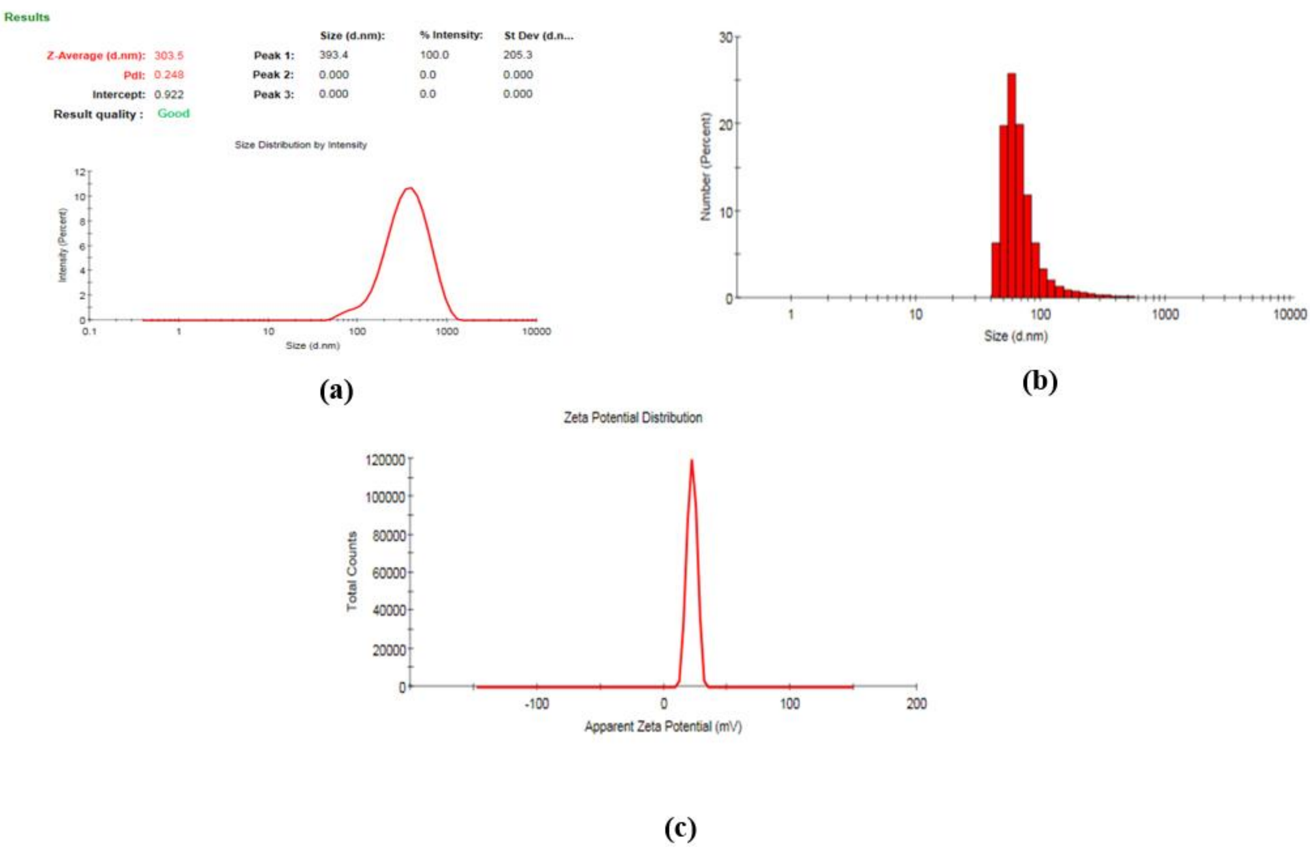

Fig.1 DLS analysis of nitrate-based chitosan nanofertilizer (a) Z-average and size distribution by intensity (b) number distribution by per cent (c) Zeta potential

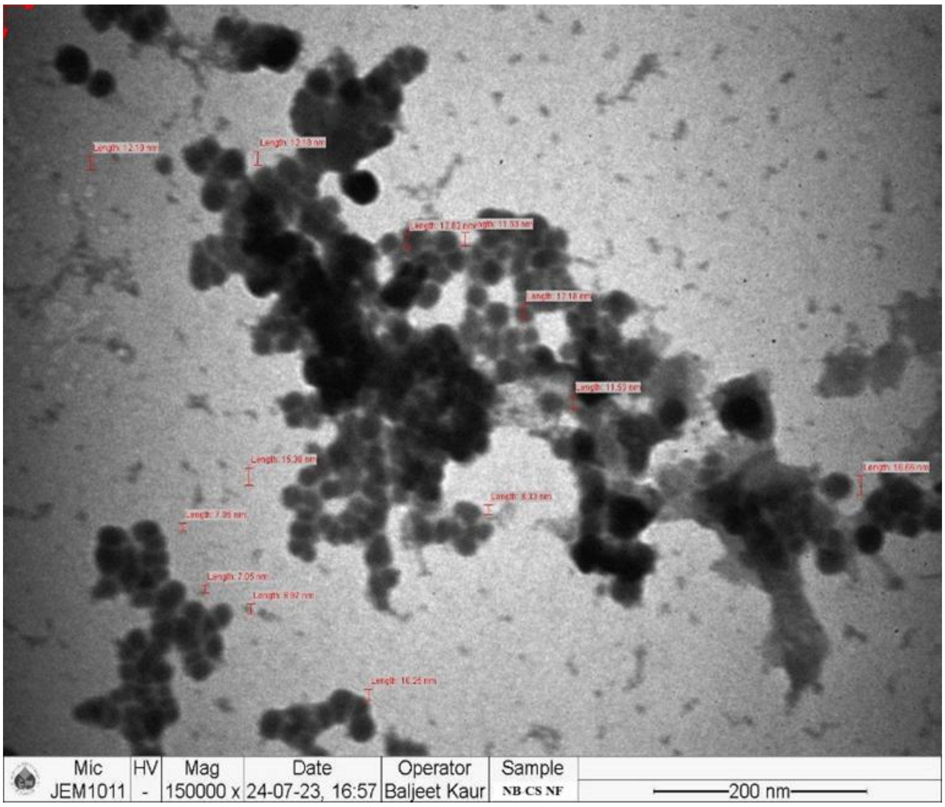

Fig.2 TEM micrograph of nitrate-based chitosan nanofertilizer at 150Kx magnification

Table 2 Morphological responses of maize towards nitrate-based chitosan nanofertilizer

Treatments	Shoot length (cm)	Root length (cm)	Pre-embryonic root number	Lateral root number	Stem diameter (cm)	Leaf area (cm^2)
Control (Water)	75.00 ± 1.73^{c}	16.00 ± 1.53^{b}	19.00 ± 1.53^{a}	222.67 ± 4.85^{d}	2.610 ± 0.14^{a}	85.06 ± 2.61^{b}
KNO_3 (2%)	78.67 ± 2.96^{bc}	19.67 ± 0.88^{a}	19.00 ± 0.58^{a}	327.33 ± 7.42^{ab}	2.92 ± 0.32^{a}	104.52 ± 1.10^{a}
Nitrate-based chitosan nanofertilizer						
2%*	87.67 ± 2.60^{ab}	20.33 ± 1.20^{ab}	20.67 ± 1.76^{a}	322.33 ± 4.81^{b}	2.70 ± 0.13^{a}	97.82 ± 4.11^{ab}
1%	87.33 ± 1.45^{ab}	16.67 ± 0.88^{ab}	18.33 ± 0.67^{a}	311.67 ± 3.76^{b}	2.60 ± 0.13^{a}	95.58 ± 3.53^{ab}
2%	89.67 ± 1.20^{a}	23.33 ± 1.20^{a}	22.00 ± 2.31^{a}	361.00 ± 10.97^{a}	3.29 ± 0.17^{a}	106.49 ± 1.94^{a}
3%	83.33 ± 2.33^{abc}	20.00 ± 1.54^{ab}	20.00 ± 2.64^{a}	261.00 ± 9.54^{c}	2.72 ± 0.11^{a}	93.38 ± 3.05^{ab}

*Surfactant-free

Various growth parameters were recorded at physiological maturity of the plants. Each value is the mean of triplicate with 5 plants in each replication. Mean ± SE values not connected by same letter are significantly different at *p=0.05* as determined by Turkey-Karmer HSD.

The combined characterization results collectively validate the successful synthesis of a stable, nitrate-based chitosan nanofertilizer system with desirable physicochemical properties. In the current study, impact of nitrate-based chitosan nanofertilizer has been comprehensively explored in terms of growth and root dynamics in maize at a semi-field condition. Foliar application of nitrate-based chitosan nanofertilizer at a lower dose (2%) significantly increased root length, pre-embryonic root number and lateral root number as compared to KNO_3 (2%) and control (water). But in contrary, shoot length, stem diameter and leaf area have not been significantly influenced. Higher dose (3%) of crafted material negatively impacted the root system (root length, pre-embryonic root number and lateral root number), indicating the plant's sensibility of critical limit to respond towards the foliar feeding of nitrate-based chitosan nanofertilizer (**Table 2**).

These findings collectively suggest that the foliar application of nitrate-based chitosan nanofertilizer (2%) attributed an impactful nutrition to the plant, resulting in better maize growth and development. Moreover, chitosan as nanocarrier is thought to play significant role in delivering the active ingredients into the plant cells as the promising affinity of natural chitin and/or chitosan receptors on the plant surfaces towards chitosan nanomaterials persuades (Parodi *et al*., 2015). Altogether, the fine-tuned physicochemical properties and availability of nitrogen and intrinsic bioactivity of chitosan in nitrate-based chitosan nanofertilizer, have contributed to enhance biochemical and physiological responses of maize plant. We recommend the formulation to evaluate its potential in other crops in field condition.

IV. CONCLUSION

Together, these thorough results highlight the many facets of the research, from material characterisation to real-world uses in enhancing plant health and development. With foliar application of nitrate-based chitosan nanofertilizer, maize crop growth and yield are increased, which is explained by its special physiochemical properties: larger surface area, higher number of nanoparticles, and lower PDI value with low viscosity. Compared to potassium nitrate (2% w/v) and water, foliar application of nitrate-based chitosan nanofertilizer at an optimal dose (2% w/v) significantly increased shoot length, root length, pre-embryonic root number, lateral root number, and leaf area. According to the study, laboratory-synthesized nitrate-based chitosan nanofertilizer can be a highly beneficial and innovative bio-stimulant, as well as a good source of macronutrients for agricultural plants, and should be investigated on a large scale for various crops to further mature the technology.

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