Synthesis and characterization of Zinc-Salicylic acid Chitosan Nanomaterial and its invitro bioactivity in wheat

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Abstract- **In this work zinc, salicylic acid chitosan nanomaterials (NMs) has been synthesized and characterized for its application in wheat seedling grown invitro in microglass house experiment, Zn-SA chitosan NM was prepared by using ionic gelation method. Further the characterization is done by DLS, FTIR and TEM analysis. Synthesized Zn-SA chitosan NM then used for the seed priming of wheat seedling and further for foliar application inside the microglass house experiment. Zn-SA chitosan NM (0.01 to 0.16%) treated wheat plant showed significant growth promotery effect in terms of germination %, shoot length, root length, Seed vigour index (SVI) compared to control. This is an important NM development in agriculture sector as chitosan-based NM are biocompatible and biodegradable and also mimic the natural elicitation activity in plant for biotic and abiotic stress management.**

***Keywords*: Chitosan, Microglass house, Nanomaterial, Zinc, Salicylic acid, Biocompatible**

I. INTRODUCTION:

Chitosan, an amino polysaccharide, is derived from the biowaste produced by the seafood industry. It is a high-value bio-polymer for medicine, pharmaceutics, and agriculture because to its remarkable biocompatibility, biodegradability, and non-toxic characteristics [1, 4, 6]. Chitosan has been utilised extensively in agriculture over the past decade for plant development and protection [7]. With the development in nanotechnology, research and development (R&D) of chitosan-based nanomaterials (NMs) in the study of plants has significantly increased. In recent years, a significant improvement in the synthesis, characterization, and evaluation of various chitosan-based NMs in plants has highlighted the potential of the nanotechnology in agriculture. Numerous NMs are undertaking rigorous laboratory testing in preparation for field experiments in a variety of crops.

Studies on chitosan nanoparticles are now being undertaken, and they deliver remarkable outcomes in nutritional supplementation. The intrinsic properties of chitosan biopolymer and its NMs as a potential source of nutrients for plants should be considered. In addition, chitosan NMs has special qualities that distinguish them from other polymers and their byproducts, such as their cationic character, which is absent in chitin. Chitosan has remarkable reactivity to pH and other physiological changes because of the presence of amino (-NH_2) and hydroxyl (-OH) functional groups, which act as binding sites for enhanced functionality [2, 3, 5]. Different stress conditions in farming environments might adversely affect different crops. The stresses may be biotic (caused by pests and pathogens) or abiotic (the result of environmental factors like salinity or nutrient deficiency). Utilising micronutrients to improve plant development and production potential can help with these problems. One such micronutrient is zinc (Zn), which is recognised for fostering strong plant growth, increasing emergence, accelerating establishment, supporting healthier root growth, and enhancing yield potential. likewise, it has been discovered that salicylic acid can help crops adapt to harsh climatic circumstances by reducing the damaging effects of salt stress. We presented in a hypothesis based on previous studies that zinc (Zn), salicylic acid, and chitosan together in order will have a synergistic impact, enhancing the bioactivity of crops. This means that the combination Zn-SA chitosan could potentially lead to even more significant improvements in plant vigour, stress resistance, and overall crop productivity. By harnessing the beneficial properties of zinc, salicylic acid, and chitosan, we are looking to offer a promising solution for optimizing crop performance and robustness against various stressors in agriculture. Further field studies and experiments will be necessary to validate and explore the full potential of Zn-SA chitosan NM in crops here in this research paper we are focused on synthesis and characterization of proposed Zn-SA chitosan NM.

II. MATERIAL AND METHODS:

The present research study entitled “Synthesis and characterization of Zinc-Salicylic acid-based Chitosan Nanomaterial” was conducted at Department of Molecular Biology and Biotechnology, Rajasthan College of Agriculture (RCA), Maharana Pratap University of Agriculture and Technology (MPUAT), Udaipur in 2022-23.

MATERIAL:

Chitosan [Low Mol. Weight Deacetylated chitin, Poly (D-glucosamine)], sodium tripolyphosphate (TPP) [Technical grade, 85%], salicylic acid (SA) [ReagentPlus®, ≥99%] and $ZnSO_4$ [ACS reagent, ≥99%] were purchased from Sigma-Aldrich, St. Louis, MO, USA and all other chemicals used in present study were procured from HiMedia, Mumbai, India.

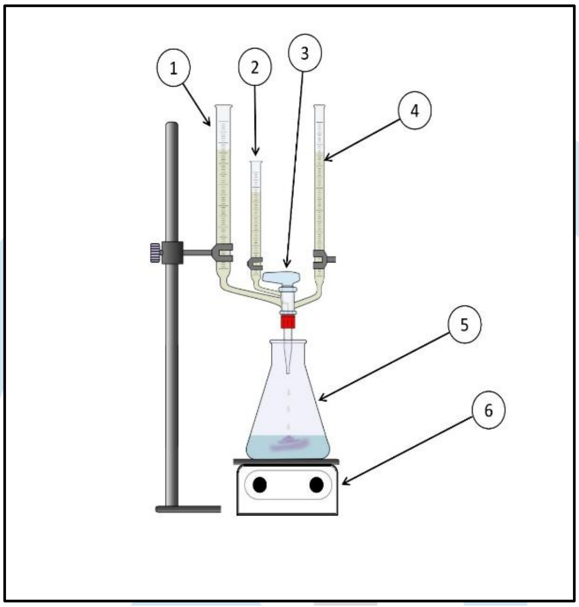

Figure 1: Setup having (1) Sodium-tripolyphosphate (TPP); (2) $ZnSO_4$; (3) Regulator; (4) Salicylic acid (SA); (5) Chitosan; (6) Magnetic stirrer

SYNTHHESIS OF Zn-SA CHITOSAN NM:

Zn-SA chitosan NM were prepared by ionic gelation method [8]. In which the ratio of chitosan:TPP (1:1) and SA (0.01% w/v) and Zn (0.02% w/v) were standardized in a number of preliminary experiments. In brief, chitosan (0.5%, w/v) was dissolved in acetic acid (0.5%) in 100 ml of ultrapure water, under continuous stirring at 180 rpm on magnetic stirrer (Remi Laboratory Instruments, Mumbai, India) and filtered through Whatman filter papers (12.5 cms). Similarly, TPP (0.5%) was dissolved in ultrapure water and filtered. TPP solution was added to chitosan solution along with salicylic acid (0.01%) and $ZnSO_4$ under magnetic stirrer (180 rpm). The resulting NMs were purified through centrifugation at 10,000 ×g for 10 min at 4 °C and washed with ultrapure water. [figure.1] After that the drying was done by lyophilizing the obtained semisolid material.

CHARACTERIZATION OF Zn-SA CHITOSAN NM:

The particle size, zeta-potential and polydispersity index (PDI) of the Zn-SA chitosan NM were measured using dynamic light scattering zetasizer (Zetasizer Nano, ZS90, Malvern, U.K) at a scattering angle of 90° with refractive index 1.59 used as reference. Fourier transform infrared spectroscopy (FTIR) analysis was done for presence of functional group of Zn-SA chitosan NM using FTIR spectrophotometer (Alpha, Bruker, Germany) between the range of 600 and 3800 cm^{-1} with 1cm^{-1} resolution. TEM micrographs were obtained using JEOL JEM-1011 TEM (JEOL, USA) operated at 80-100 kV using the 400-mesh.

EFFECT OF Zn-SA CHITOSAN NM ON WHEAT SEEDLING IN MICROGLASS HOSUE:

Wheat seedling were firstly surface sterilized using sodium hypochlorite (0.1%) and carbendazim (0.1%) to deal with fungal and bacterial contamination [8]. After that the seeds are treated with Zn-SA chitosan NM (0.1-0.16%) along with water and SA (0.01%) as control for 2hrs. After treatment, seeds were kept in indoor microglass house equipped with pot trays containing mixture of vermicompost and cocopeat with controlled light period for incubation of seedlings. The data like germination %, root length, shoot length and SVI were analysed 15 Days after sowing (DAS).

III. RESULT AND DISCUSSION

Synthesis and Characterization of Zn-Sa Chitosan Nm

DLS study of Zn-SA chitosan NM revelled the mean size of 495.4 ± 0.05 nm, 0.458 PDI and zeta potential of 28.3 (Figure.2). Stability and monodispersity is of synthesized NM is confirmed by high zeta potential and low PDI value. In an FTIR investigation, the peak at 3357 cm^{-1} in bulk chitosan moved to 3394 cm^{-1} in Zn-SA Chitosan NMs, correlating to the $–NH_2$ and –OH groups in Zn-SA Chitosan NM (Figure.3). The interaction of chitosan, TPP, Zn, and SA in Zn-SA Chitosan NMs shown by the peak at 1640, 895, and 3342 cm^{-1} are corresponding to amide ($CO–NH_2$), anhydrous glycosidic and primary amide ($–NH_2$) groups, the peaks at 1553 and 1640 cm^{-} shows $−OCOCH_3$ (acetoxy group) of SA and interaction of SA and chitosan is represented at 1307 cm^{-1} where carboxyl group (-COOH) of SA associate with primary amide of ($-NH_2$) of chitosan. Further the microscopic analysis of Zn-SA chitosan NM by TEM revealed found to have aggregated spherical shapes of (10-20 nm size) which are linked together to form a chain network structure consisting pores in synthesized NM. (Figure.4)

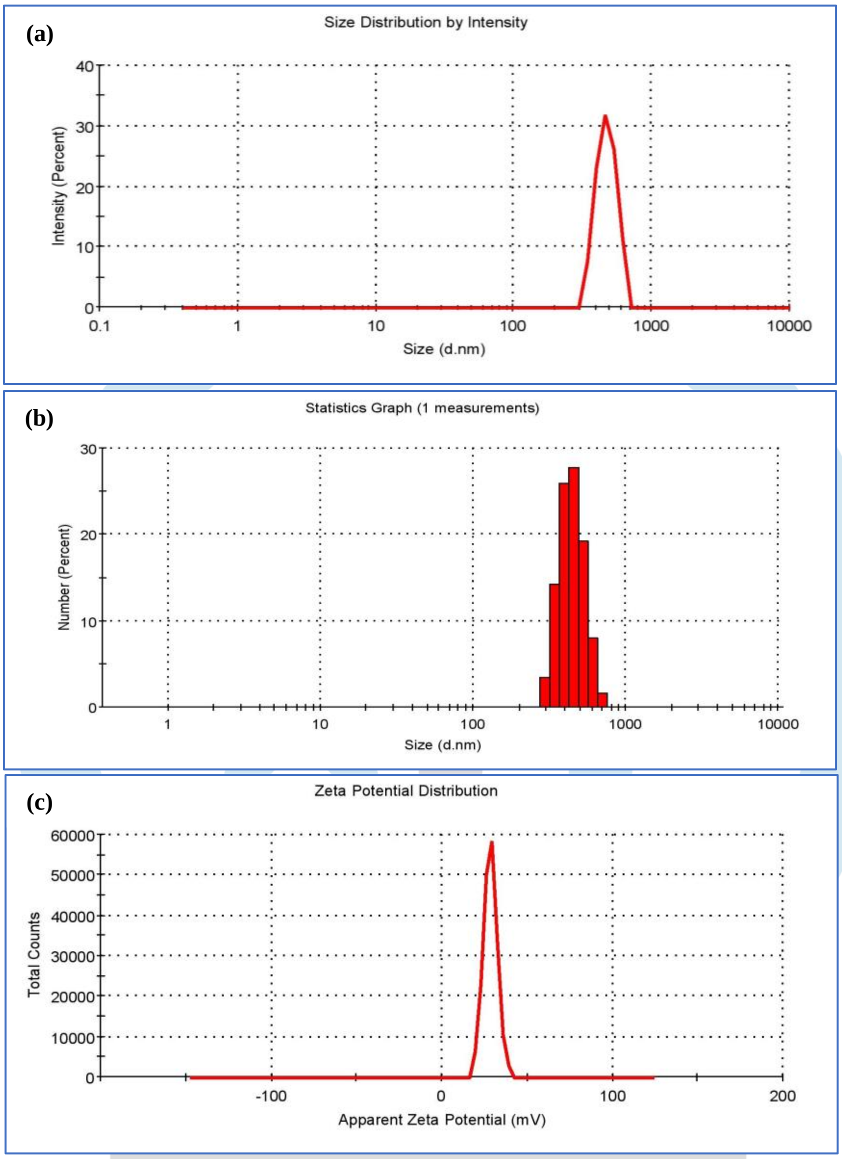

Figure 2: DLS analysis of Zn-SA chitosan NM for (a) size distribution by intensity (b) number distribution (C) zeta potential.

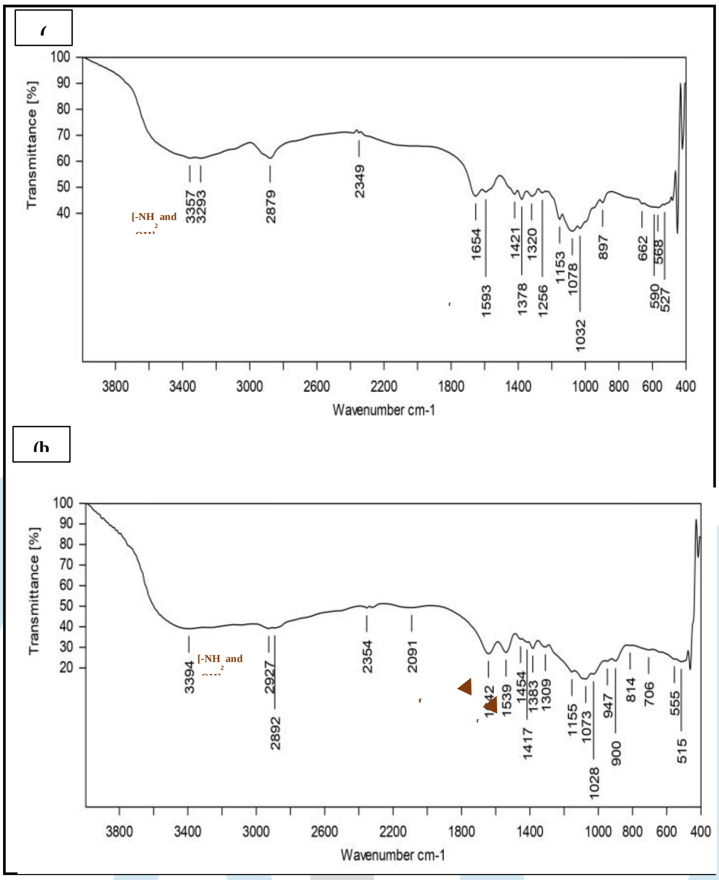

Figure 3: FTIR spectra of (a) bulk chitosan (b) Zn-SA

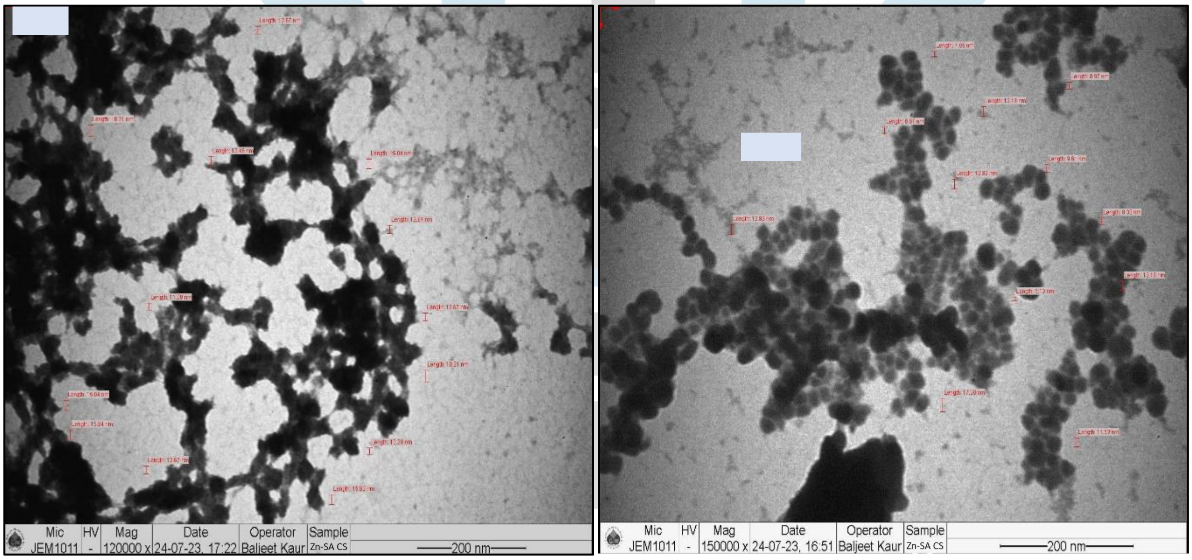

Figure 4: TEM micrographs of Zn-SA chitosan NM (a) 120000x (b)150000x Magnification.

Invitro Seedling Growth in Microglass House Experiment

Different concentration of Zn-SA chitosan NM (0.01, 0.04, 0.08, 0.12 and 0.16%, w/v) along with control water and SA (0.01%, w/v) were used to treat wheat seedlings of Raj-4079 for 2hrs and foliar application of same treatment was given 7 DAS. Germination %, shoot length and root length, seed vigour index was measured 15 DAS. (Table.1) (Figure.5)

Table.1 Effect of Zn-SA chitosan NM on seedling growth in microglass house experiment.

Treatment (%)	Seed germination (%)	Shoot length (cm)	Root length (cm)	Seed vigour index (SVI)
Control (water)	100±0[a]	19.22±0.57[a]	7.64±0.52[b]	2686.5±4.87[b]
SA (0.01)	95±2.88[a]	19.65±0.36[a]	9.94±0.59[ab]	2814.92±153.24[ab]
Zn-SA chitosan NM				
0.01	100±0[a]	18.97±0.56[a]	9.61±0.55[ab]	2858.66±112.59[ab]
0.04	100±0[a]	20.1±0.51[a]	11.22±0.571[a]	3132.33±5.36[ab]
0.08	100±0[a]	21.56±0.53[a]	10.66±0.62[a]	3222.66±9.33[a]
0.12	100±0[a]	20.50±0.56[a]	9.24±0.528[ab]	2974.33±109.21[ab]
0.16	100±0[a]	19.72±0.64[a]	9.19±0.56[ab]	2891.13±120.11[ab]

*Each value is the mean of triplicates and each replication of 20 seedlings. Mean ± SE followed by the same latter is not significantly different at $p = 0.05$ as determined by Tukey-Kramer HSD. Growth data were recorded after 15 Days after sowing (DAS).

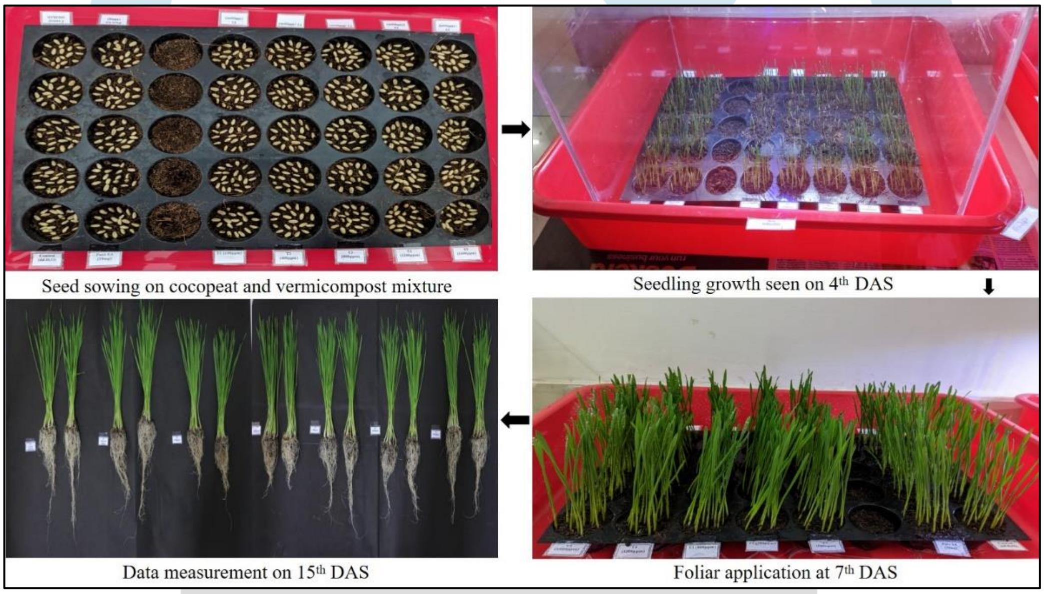

Figure 5: Microglass houe experiment of *invitro* grown wheat seedling

IV. CONCLUSION

Here synthesized NM consisting Zinc, salicylic acid, chitosan along with TPP as crosslinker was confirmed by using DLS, FTIR analysis for its characterization. TEM analysis data confirmed the spherical shape of NM at 120000x and 150000x magnification. Further, Synthesized Zn-SA chitosan NM treatment has significantly amending response in wheat plant growth compared to control and sole SA in *in vitro* microglass house experiment. However, field tests must be carried out to validate and adapt technology from laboratory to field conditions with the goal to accomplish the promises made in the present research.

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