Zinc loaded chitosan nanoparticles:
A promising nanocarrier for plant growth of Proso Millet (*Panicum miliaceum* L.)

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CERTIFICATE

This is to certify that the project report entitled “**Zinc loaded chitosan nanoparticles: A promising nanocarrier for plant growth”** is bonafide work carried out by Merin Johnson, B.Sc Chemistry student, under the joint supervision and guidance of Ms. Jaiby Cyriac, Assistant Professor in Botany, Newman College, Thodupuzha and Dr. Sindhu Joseph, Assistant Professor in Chemistry, Bharata Mata College, Thrikkakara, Kerala in partial fulfillment of award of the degree of Bachelor of Science in Chemistry at BHARATA MATA COLLEGE, THRIKKAKARA affiliated to Mahatma Gandhi University, Kottayam, during the period 2020-2023. The results embodied in the dissertation work have not been submitted to any other Institution or University for the award of any Degree.

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DECLARATION

I **Ann Mary Varghese** hereby declare that this project report entitled “**Zinc loaded chitosan nanoparticles: A promising nanocarrier for plant growth of Proso Millet”**, is record of bonafide work carried out under the joint supervision and guidance of Ms. Jaiby Cyriac, Assistant Professor in Botany, Newman College, Thodupuzha and Dr. Sindhu Joseph, Assistant Professor in Chemistry, Bharata Mata College, Thrikkakara, and the same has not been submitted elsewhere for any degree or diploma earlier.

Ms.Ann Mary Varghese

Place : Thrikkakara

Date :

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ABSTRACT

The necessity to treat nutrient shortages in soil has driven the development of sophisticated nutrient delivery systems, particularly in nutrient-deficient areas, in order to increase crop yields. One interesting strategy is to utilize nanocarriers that can effectively release nutrients slowly and in a regulated way, resulting in increased availability of nutrients in plant roots and minimizing loss of nutrients. In this work, we focus on mitigating zinc (Zn) deficiency in plants, which occurs when soils are insufficient of zinc. We wanted to see how well chitosan, an ecologically safe and biodegradable substance, worked as a possible nanocarrier for zinc nutrition delivery.The purpose of this study was to assess the effectiveness of chitosan, which is a bio-polymer obtained from chitin and an ecologically beneficial and biodegradable substance, as a prospective nanocarrier for the delivery of zinc nutrient.The development and characterisation of nano chitosan-zinc composites are presented in this paper.In this study, chitosan nanoparticles were synthesized using an innovative approach based on ionic gelation, with sodium tripolyphosphate (TPP) functioning as a cross-linking agent and to produce chitosan zinc nanocomposite (CSZNC). The effect of CSZNC as a possible nanocarrier for regulated zinc administration was assessed by examining the growth characteristics of Proso Millet ( ) as well as numerous biochemical reactions.Through this research, our aim is to assisting farmers in increasing their incomes and achieving higher crop yields. By utilizing chitosan-based nanocarriers, we hope to enhance nutrient delivery efficiency and contribute to sustainable agricultural practices.

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CHAPTER 1

INTRODUCTION

Human beings began to look for methods to reduce environmental pollution in the context of a return to nature, leveraging scientific and technological advances to enhance living conditions. Researchers want to develop safe, low-cost solutions that are also effective.(Faqir et al., 2021) Micronutrients are essential for both human health and global agriculture. Worldwide, micronutrient deficiencies affect more than a billion people. "Hidden hunger" is the term used to describe micronutrient deficiencies, which mostly affect iron and zinc. Farmers in the agricultural sector deal with a variety of micronutrient deficiency symptoms in crops, including zinc, copper, manganese, and iron.Among these, zinc is one of the micronutrients that has the greatest impact on agricultural productivity, and a lack of it affects crops all around the world.It is one of the most prevalent elements in the crust of the earth and is essential for the healthy growth and development of both plants and animals. It is a crucial part of enzyme/coenzyme molecules, which are involved in a number of physiological processes, including DNA replication, protein synthesis, and lipid metabolism.Numerous micronutrients are necessary for agricultural plants like wheat, cereals, and rice, and zinc is a crucial micronutrient that is absorbed by the plants' root hair cells.Lack of this essential element is linked to stunted growth, increased susceptibility to infectious illnesses, skin rashes, and diarrhoea.(Kalra et al., 2020)

Applying fertilisers containing zinc, such as zinc sulphate, zinc oxysulfates, artificial chelates, and organic natural complexes, can remedy a zinc shortage.Zn-fertilizers can improve the quality and productivity of a variety of crops, including wheat, rice, and peas.The brand-new, rapidly developing sector of research and technology known as "Nanotechnology" must create the revolutionary Zn fertiliser compositions. These Zn nano fertilisers are helpful for longer-lasting micronutrient releases and higher absorption efficiencies.Encapsulations (micro and nano), nanomaterials, nanodevices, and nanoparticles are all components of the new fertiliser formulations. All required features, including high stability, solubility, efficiency, controlled release with regard to time, enhanced targeted activity, and lower eco-toxicity, should be present in nano fertiliser, along with a simple, safe method of delivery.The bioavailability and absorption of nutrients can be improved by nanofertilizers.(Tondey et al., 2019)

Global agriculture is desperately looking for solutions to reduce the environmental impact of traditional chemical fertilizers. Next-generation fertilizers that are biodegradable, eco-friendly, and powered by renewable energy might be the answer, allowing for greater nutrient usage efficiency and a reduced environmental imprint. The agriculture sector study regarding chitosan nanoparticles (NMs) has grown over the last decade, revealing their use in increasing agricultural production not just as plant immune boosters, but also through gradual, regulated, and targeted nutrient delivery to plants. Chitosan NMs are a rich supply of carbon, nitrogen, oxygen, and phosphorus for plants. Furthermore, chitosan NMs can be further functionalized by additional nutritional payloads via their functional groups. (Prajapati et al., 2022)

In order to administer agrochemicals, a variety of biopolymers, including chitosan and alginate, are now being researched for use in medicinal and pharmacological purposes. Chitin is a precursor of chitosan. The primary method for preparing chitin in both industrial settings and academic research labs is deacetylation.After cellulose, chitin is one of the most prevalent polymers in nature. Chitin-rich trash from the seafood industry and crab shells are used to make this polymer's raw components.It is a homopolysaccharide that is linear.Biocompatible, biodegradable, and non-toxic polymers include chitin and chitosan.These characteristics allow for a variety of uses, including medication delivery and biomaterials for tissue engineering. Chitosan's main drawback is that it can only dissolve in acidic environments. The chemical change can overcome chitosan's poor solubility in water. Thus, chitin/chitosan has undergone chemical changes to change some of its features, including solubility, antibacterial activity, and the capacity to interact with other substances, as well as to improve the polymer's ability to process.The nanoparticles of chitosan have been widely researched for medication and gene delivery applications.The loading of metal ions on chitosan nanoparticles increases their antibacterial activity because the structure of chitosan is suitable for metal ion binding via ion exchange, complexation, physical sorption, and other methods (Anitha et al., 2009). There haven't been any reports yet on the usage of chitosan nanoparticles as a "nanocarrier" for the delivery of micronutrients in millets.In order to achieve agronomic biofortification, it was hypothesised that the zinc complexed chitosan may be employed to create nanoparticles [Zn-CNP] suitable for foliar distribution of zinc. Under zinc-deficient conditions, Zn-CNP's potential as a "nanocarrier" for the delivery of micronutrients was evaluated.(Deshpande et al., 2017)

Zinc deficiency

One of the 17 important minerals for healthy plant growth and development is zinc. It is one of the eight micronutrients that plants require. With enzymes and proteins involved in carbohydrate metabolism, protein synthesis, gene expression, auxin (growth regulator) metabolism, pollen formation, maintenance of biological membranes, defence against heat stress and photo-oxidative damage, and resistance to infection by specific pathogens, zinc plays a significant role in plants (Alloway,2008).A lack of zinc in plants slows down photosynthesis, nitrogen metabolism, fruit development, flowering, and growth phases, lowers yield and quality, and leads to less efficient nutrient usage. Some of the typical signs of zinc deficiency in plants include light green, yellow, or bleached spots in the interveinal areas of older leaves; emerging leaves are smaller and frequently called "little leaves," and they show rosetting, which is when the internodal distance gets so small that all the leaves appear to emerge from the same point.

Similar to other nutrients, zinc is important for human health. Numerous biological processes in the human body depend on it. The average adult's body has 2–3 g of zinc. Every component of the body, including the organs, tissues, bones, fluids, and cells, contain it. More than 300 enzymes in the human body depend on it for growth (height, weight, and bone development), cell division, immune system, fertility, taste, smell, and appetite, as well as for the development of the skin, hair, and nails, as well as vision.(Soumitra et al. 2013).

In soils and crops all across the world, zinc insufficiency has emerged as the most common micronutrient deficiency, leading to significant production losses and a decline in nutritional quality (Sillanpää, 1982). Nearly half of the world's soils are thought to be zinc deficient. Cereal grains already have low zinc concentrations, therefore cultivating them on these possibly zinc-deficient soils lowers grain zinc concentration even more.

The same is true of India. According to an analysis of over 256,000 soil samples from all over India, zinc deficiency affected agricultural yields the most frequently in India, accounting for nearly 50% of soils. Large zinc removals resulting from high crop yields and intensive cropping systems, less application of organic manures, increased use of phosphatic fertilisers, which causes phosphorus-induced zinc deficiency, and the use of subpar irrigation water are some of the causes of the rise in zinc deficiency incidences.. Low total zinc concentrations, like those found in sandy soils, highly weathered parent materials with low total zinc contents, like those found in tropical soils, high calcium carbonate contents, like those found in calcareous soils, neutral or alkaline pH, like those found in heavily limed soils or calcareous soils, high salt concentrations, like those found in saline soils, peat and muck, like those found in organic soils, and high phosphate status are protracted flooding or waterlogging, as in soils used for rice.(Soumitra et al. 2013).

If the current trend continues, zinc deficiency in India is predicted to rise from its current level of about 50% to 63% in 2025. Additionally, more marginal lands are being placed under intense cultivation without providing enough nutritional supplemental input.A significant link exists between soil zinc deficiency and human zinc insufficiency. According to estimates, zinc insufficiency affects around one-third of the world's population.(Soumitra et al. 2013).

Nanofertilizer

In order to increase production per unit area, global agricultural cropping systems use a lot of fertilisers, pesticides, and herbicides. However, using higher doses than necessary of these chemicals and fertilisers causes a number of issues, including environmental pollution (soil, water, and air pollution), low input consumption efficiency, decreased food quality, development of resistance in various weeds, diseases, and insects, decreased income from the production, degraded soil, and deficiency.Due to their increased surface area, surface area, and use efficiency, nanomaterials such as fertilisers, insecticides, and herbicides can be efficient tools utilised by agriculture for efficient pest and nutrient management and prevent leaving residues in the environment.(Ghaly, 2009, Quasem 2009) .Nanoparticles with a size of under 100 nm can be used as fertiliser for effective nutrient management, making them more environmentally friendly and lowering environmental contamination.These days, nanotechnology offers a variety of nanomaterials and nanodevices that play a specific role in agriculture, such as nano biosensors that can measure moisture in the soil content as well as nutrient status and also be used for site-specific water and nutrient management. With regard to agricultural production, nanotechnology plays a bigger role thanks to its environmental safety, ecological sustainability, and financial stability. The value chain of the global agriculture production system can utilise the nanoparticles created by nanotechnology.(Joseph ,2006)

Nano-fertilizers "Nano fertilizers are synthesised or modified versions of conventional fertilisers, fertilisers bulk materials, or extracted from various vegetative or reproductive components of the plant by various chemical, physical, mechanical, or biological methods with the aid of nanotechnology. They are used to improve soil fertility, productivity, and the quality of agricultural outputs. Materials in their whole can be used to create nanoparticles.(Brunnert et al. 2006)

In order to promote penetration into the plant from the applied surface and increase uptake and utilisation of nutrients, nano-fertilizers have high surface areas and particles that are smaller than the pores of plant roots and leaves.Nanoparticle-encased fertilisers will boost the accessibility and uptake of nutrients by crop plants.Numerous studies have shown that nano fertilisers increase the growth, yield, and quality characteristics of the crop, resulting in a higher yield and higher-quality crop product for human and animal use.(Tarafdar et al. 2012). Fertilisers with nanoparticles in them will increase the availability and uptake of vitamins and minerals by crop plants.Nano fertilisers boost the development, yield, and quality features of the crop, giving rise to a larger yield and improved-quality crop product for both human and animal use, according to numerous research. Numerous studies found that nano fertilisers had a considerable impact on seed germination and seedling development, demonstrating their impact on seed and seed vigour. Nano fertilisers can easily penetrate seeds and increase nutrient availability to growing seedlings, resulting in healthy plants with longer shoots and roots. However, if concentration is higher than the optimum level, it may inhibit plant germination and seedling growth.Nano fertilisers improve seed germination, vigour, growth parameters production of dry matter, chlorophyll, and photosynthesis rate, leading to increased making and transfer of photosynthets to various parts of the plant.(Meena,2017)

The use of various nano-fertilizers has a bigger impact on improving agricultural output since it lowers the cost of fertiliser for the cultivation of crops and reduces pollution risk. The use of nano-fertilizers for agricultural purposes need to be of higher social concern. By using nano-fertilizers effectively, fertiliser nutrient consumption efficiency in crop production can be increased. Up to the optimum applicable doses and concentration, nano fertilisers increase crop growth and yield, but if the concentration is higher than the optimum, they also have an inhibitory impact on the crop plant, which inhibits crop growth and yield. (Meena,2017)

Nanofertilizers play a vital role in crop biochemical and physiological processes by boosting nutrient availability, which aids in strengthening metabolic processes and encouraging meristematic activities, resulting in increased apical growth with photosynthetic area. Nano-zinc treatment increases growth indices such as plant height, leaf number, and both dry and fresh weight of savory plants (Vafa et al., 2015). The use of nanoscale Zn fertilizer increases the plant growth boosting hormone content.The use of nanofertilizers improved the antioxidant potential of rice. Antioxidants include secondary metabolites generated by plants in response to adverse situations such as water stress, salt, and nutrient deficiency.(Mahil et al., 2019).In recent years, several researchers have attempted to investigate the potential benefits of nanofertilizers to boost agricultural productivity. Foliar use of a nanofertilizer improved yield metrics in wheat crops (Abdel-Aziz et al., 2018). According to Janmohammadi et al. (2016), foliar administration of nTiO2 hardly manipulates growth, leading to favorable alterations in the components of yield.

Types of Nanofertilizers

Nanofertilizers are nanometer-scale materials, often in the form of nanoparticles, that contain macro and micronutrients and are supplied to crops in a regulated manner (Adisa et al., 2019; Shang et al., 2019).Nanofertilizers are categorized into three types based on their formulation: 1) the nanoscale fertilizer, which is a conventional fertilizer that has been reduced in size, usually in the form of nanoparticles; 2) the nanoscale additive fertilizer, which is a traditional fertilizer that contains a supplement nanomaterial; 3) the nanoscale coating fertilizers, which is nutrients that have been encased by nanofilms or incorporated into nanoscale pore size of a host material (Mastronardi et al., 2015).

Nutrients encapsulated by films or retained in nanopores with a carrier material such as clays have been employed to create nanocomposite structures for regulating release of nutrients (Golbashy et al., 2016; Tarafder et al., 2020).Three separate elements stimulate the release of nutrients that have been immobilized or encapsulated in a specific nanocarrier (biologic, chemical, and physical). Bacteria, fungus, and other microorganisms biodegrade the coating, which is made of a biodegradable and synthetic polymeric substance, allowing nutrients to be released and fixed in the soil. Moisture, solubilization, pH fluctuation, type of soil (Weeks and Hettiarachchi, 2019), are examples of chemical-triggered mechanisms. Ultrasound, magnetic field, heat, and diffusion-controlled release are the physical components (Mikkelsen, 2018).

Application of Zinc in Plants

Zinc is essential for plant development and crop nutrition due to the fact that it is required in several enzymatic activities, processes of metabolism, and oxidation-reduction reactions.Zn plays a vital role in plant metabolism through its impact on the functions of hydrogenase and carbonic anhydrase, as well as the stability of ribosomal fractions, and cytochrome synthesis (Hafeez et al., 2013). Furthermore, it is essential for the production of proteins, carbohydrates, growth hormones, and the maintenance of cell membrane integrity (Tondey et al., 2019).Broadley et al. (2007) proved the relevance of Zn in plants first in maize, subsequently in barley as well as dwarf sunflower.

Zn-activated plant enzymes are required for the metabolism of glucose, membrane integrity maintenance, synthesis of proteins, auxin production regulation, and pollen formation (Hafeez et al., 2013).Furthermore, the formation of proteins, gene expression along with regulation, the photosynthesis process, lipid as well as nucleic acid metabolism, chlorophyll production, phytohormone action, membrane structure, and disease and drought resistance (Noulas et al., 2018).Its lack causes plant abnormalities which include delayed development, chlorosis, and decreased leaves, as well as spikelet sterility. Zinc deficiency also results in stunted growth among the plants. There is a considerable association between crop development and production and Zn concentration in tissues, according to the findings (Hafeez et al., 2013).Zn can be transported as Zn^{2+} in plants or, as is commonly assumed, bonded to organic acids. At high soil pH values, it is likely to be absorbed as a monovalent cation ($ZnOH^{+}$). Zinc accumulates in root tissues before being transferred to the shoot via the xylem (Noulas et al., 2018). Zn's great propensity for developing tetrahedral structures with N-, O-, and also S-donor ligands underpins its metabolic roles, and Zn appears to influence plant water transport and absorption (Tsonev et al., 2012).Zinc is an enzymatic and protein-structural component in many enzymes, as well as a mental activator for many enzymes and a structural component within protein domains that may interact with other molecules. Transcriptional factor DNA binding together with protein-protein interactions are mediated by "Zn finger" proteins. Using sequenced metal-binding motifs, bioinformatic approaches may currently predict Zn-binding sites (Cabot et al., 2019).

The involvement of Zn in the proper functioning and integrity of genetic material is the most significant influence of Zn upon protein metabolism. Zn is known to be needed for a minimum of two chromatic proteins, in addition to its influence on both DNA and RNA structural stability, and Zn is expected to serve a critical physiological contribution to the structure and function of the biomembrane (Brown et al., 1993).Plant species have a wide variety of Zn needs and concentrations due to variances in Zn absorption coming from soil, transportation from roots and shoots, and also sequestration in plant organs (Umair Hassan et al., 2020).Plant species absorb Zn in different ways, which is determined by the nature and quantity of the development media. Zn is absorbed as divalent cations or as complexes containing organic substances, and it follows a linear pattern in relation to its concentrations within the soil's nutrients. The roots then transport it through the xylem to the shoot tissues. Zn is transported to roots and xylem through symplast and apoplast, although considerable amounts of this metal was also detected in phloem, showing that it is transported by xylem as well as phloem tissues.

Zinc efficiency refers to plants' ability to maintain high yields in soils with limited Zn availability (Hafeez et al., 2013).Zinc typically exists within the symplasts in mesophyll cells in plants as nicotianamine complexes, while it can also exist in various forms depending on the kind of cell (Sturikova et al., 2018). Zn proteins can aid and impede both the crop and its assailants in plant defense. Zn also helps to build phenolic compounds and other plant-defense mechanisms, therefore micronutrient participation in plant defense has been extensively documented..Zinc is required for the plant's response to pests and diseases. Despite this, Zn defensive mechanisms in plants vary significantly. The outcomes of plant-pest/pathogen interactions differ depending on the efficiency of Zn-related responses in limiting the invader's attack, the enemy's ability to overcome plant defenses, and other environmental conditions that might favor either host or invader.However, a protective Zn level against some illnesses may enhance vulnerability to a separate pathogen on the same plant. Zn has specific functions in controlling pests and diseases in systems of plants, and these activities include mechanisms that generate high- or low-Zn environments. In addition to their role in growth and development, zinc finger proteins regulate biotic reactions to stress in plants. (Cabot et al., 2019)

Introducing zinc fertilizers to plants is a rapid and efficient way to address a zinc shortage in soils, crops, and humans. There are numerous zinc fertilizers and commodities available on the market.In a majority of agroclimatic zones and also soil types, all crops have demonstrated zinc sensitivity.The amount of zinc within the soil impacts how strongly the crop reacts. Crop response to sprayed zinc might rise as soil zinc levels decreased (Das et al., 2013).Zinc-containing fertilizers, which include zinc sulphate ($ZnSO_4$), zinc oxysulfates (ZnO), inorganic Zn complexes, synthesized chelates, and also naturally generated organic complexes, can help to alleviate a zinc deficit. Zinc fertilizers have shown promising results in enhancing the yield and quality of several crops, such as wheat, rice, and peas. However, their effectiveness is hampered by various factors like leaching, the formation of organic ligand complexes with soil components, and precipitation leading to the formation of insoluble Zinc salts. As a result, water-soluble $ZnSO_4$ applied to soils tends to experience loss and fixation in an unusable form, thereby reducing the nutrient uptake efficiency of plants.(Tondey et al., 2019). Zinc oxide nanoparticles, which possess unique optical, chemical, semiconducting, catalytic, photochemical, and antibacterial properties, have emerged as an alternative to conventional Zn fertilizers. These nanoparticles offer potential benefits as a source of zinc micronutrients, presenting a promising solution to improve crop growth and quality.

The use of chemical fertilizers has been beneficial in increasing food grain production. However, prolonged reliance on conventional synthetic fertilizers has led to adverse effects on crop plants, including reduced fertilizer response rates and declining productivity and yield. To address these challenges and align with sustainable agriculture practices, there is a need to develop innovative fertilizers known as "nanofertilizers" or novel fertilizer application techniques, as proposed by Tondey et al. (2019).Studies like the one by Pathak et al. (2012) have indicated that the reproductive efficiency of chickpeas can be improved through foliar application of Zinc. Therefore, the emerging field of "Nanotechnology" must explore the creation of Zn fertilizer compositions. By utilizing nanoscale or nanostructured Zn nanofertilizers, it is possible to enhance the effectiveness of Zn nutrient delivery, leading to improved crop output and reduced soil component toxicity, thereby contributing to environmental protection.Nanofertilizers can be formulated as nano-sized nutrients, particularly micronutrients like ZnO NP fertilizers, or as soluble nutrients loaded on or encapsulated with nanoparticles to efficiently deliver nutrients to crops. These newly developed Zn nanofertilizers should be evaluated for qualities such as higher absorption rates and sustained, controlled micronutrient release in an accessible form over extended periods (Tondey et al. 2019).

Chitosan

Chitosan is a biogenic polymer based on amino-polysaccharides that is mass-produced from sustainable resources such as seafood waste.Chitosan is a N-acetyl derivative of chitin that is formed by N-deacetylationChitosan is used extensively in the food and bioengineering sectors for encapsulating active food components, immobilizing enzymes, as a carrier for controlled medicine administration, and as a plant growth booster in agriculture. Chitosan is also an antibacterial agent and a defense elicitor. Chitosan possesses a number of intriguing features, including biodegradability, biocompatibility, bioactivity, nontoxicity, and polycationic nature (Divya & Jisha, 2018). Because of its superior biodegradability, biocompatibility, and non-toxicity, it is a highly valuable biopolymer for medicine, pharmaceutics, and agriculture (Angelo et al., 2021; Chouhan & Mandal, 2021).

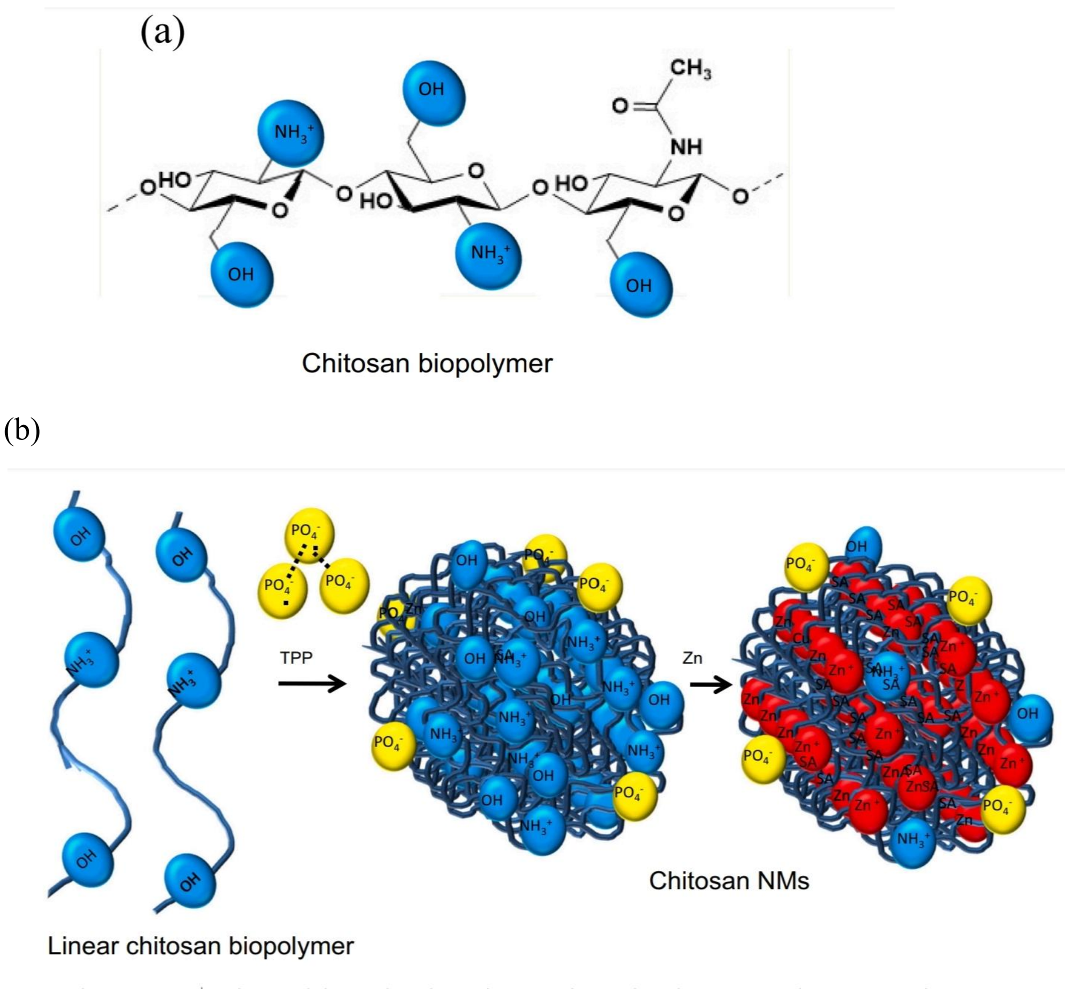
Chitin, a biological waste from seafood, is deacetylated for commercial chitosan manufacture. Because of the growing need for chitosan in new industries such as agriculture, the generation of chitosan along with its basic ingredient, chitin, has increased dramatically over the previous decade and is likely to continue in the years ahead (Azmana et al., 2021).The favorable biological attributes of chitosan, including its relatively non-toxic, biocompatible, and biodegradable nature, as well as its cationic properties, bioadhesive features, and ability to enhance permeability, have sparked significant interest in exploring chitosan-based particles for delivering various substances like anti-cancer agents, therapeutic proteins, genes, antigens, and more. Low molecular weight (LMW) nanoparticles of chitosan exhibited considerable promise in the delivery of drugs and non-viral vector gene delivery applications in recent years.This is because LMW chitosan has greater solubility, biocompatibility, bioactivity, biodegradability, and even less toxicity when compared to HMW chitosan. Furthermore, several studies have highlighted the importance of size and demonstrated the benefits of nanoparticles over microspheres.(Fan et al., 2012).The obvious features of chitosan biopolymer as well as its NMs as a potential plant nutrition source should be explored. Chitosan-based NMs are chitosan compounds that maintain innate properties such as elicitation, antibacterial activity, and affinities for biological membranes (Jogaiah et al., 2020).

The positively charged nature of chitosan NMs, which is not found in the chitin starch, gelatin, cellulose, and glucans polymers and also their products, along with the availability of amino

($-NH_2$) and hydroxyl (-OH) functional groups that serve as binding sites for improved functionalities, and also its high responsiveness to the pH level and other physiological changes, constitute the main distinguishing characteristics of chitosan NMs when in comparison to other polymers and their products. As a result, agricultural nanotechnology research is focusing on the precise administration of nutrients to plants using chitosan NMs and the creation of next-generation fertilizers (Prajapati et al., 2022).Furthermore, because of their biodegradability and biocompatibility, as well as their production from natural resources (sea food bio-waste), chitosan NMs are a feasible solution for next-generation fertilizers that reduce a range of environmental consequences (Iftime et al., 2019).The presence of $-NH_2$ and C6-OH groups is the backbone of chitosan biopolymer is an intrinsic characteristic that makes it extremely bioactive.

Chitosan has a net positive charge due to the $-NH_2$ group, which allows it for interaction with anionic molecules such as cell membrane phospholipids and both functional and structural anionic proteins. By interacting with the receptors in cells and speeding up metabolic activities, the -OH functional group works as an electron acceptor thus speeds up signal transmission. Cross-linking linear chitosan biopolymer into nanoscale structures using tripolyphosphate (TPP; $Na_5P_3O_{10}$), on the other hand, can boost the functionality of the chitosan polymer.The anionic TPP binds to the cationic $-NH_2$ groups found in linear chitosan components and converts them into chitosan NMs. The resulting chitosan NMs had a larger surface area compared to volume ratio with a greater number of functional groups on the surface, implying a better possibility of interaction with plant cells. Particle size, dispersity, and also surface charge (zeta potential) are three important properties of chitosan NM's biological activity in plants. Size is critical for better contact with biological surface and intra-tissue penetrability.

To maintain product homogeneity, stability, and bioactivity consistency, a reduced dispersity index is necessary.Zeta potential influences both surface interactions between biomolecules and the ability of NMs to permeate intracellular areas. Changing the quantity of the starting material, in this example, chitosan, as well as other physical factors in the process of synthesis, such as pH and temperature, might result in alterations to the physicochemical properties of chitosan NMs. (Prajapati et al., 2022)



[Fig.1.1 (a) Functional groups (–NH_3^+ and –OH) of chitosan biopolymer (b)The conversion of linear chitosan biopolymer into compact chitosan NMs occurs through cross-linking of anionic tripolyphosphate (TPP) with cationic –NH_2 groups present in chitosan. This transformation results in NMs with a higher surface area to volume ratio, showcasing an increased number of functional groups on their surface.]

Among the several ways created to manufacture chitosan nanoparticles, the ionic gelation approach has received a lot of interest since it is non-toxic, devoid of organic solvents, easy to use, and controlled (Agnihotri et al., 2004) Ionic gelation is based on ionic interactions between positively charged chitosan primary amino groups and negatively charged polyanion groups, including sodium tripolyphosphate (TPP), and it is the most commonly employed ion cross-linking agent simply because of its non-toxic as well multivalent properties.(Shu,2002) This physical cross-linking procedure not only removes the utilization of chemical cross-linking and emulsifying agents, which are frequently hazardous to organisms, but it also eliminates the risk of drug damage, particularly biological agent(Berger et al., 2004)

Traditional chitosan/TPP nanoparticles often have a broad particle size range and low stability, limiting their applicability in some applications. It is still difficult to manufacture chitosan/TPP nanoparticles having high levels of monodispersity along with stability in a simple and efficient manner. Thus, in this study, we chose to use LMW chitosan with a high degree of deacetylation and concentrate on the reproducible manufacturing of chitosan/TPP nanoparticles that with those desirable properties, with the goal of promoting the research and development of chitosan/TPP nanoparticles in plant growth applications(Fan et al., 2012)

Proso Millet

Millets are seasonal small-seeded grain crops produced for food, feed, forage, and fuel all over the world. Millets come in around 20 distinct varieties. Proso millet (Panicum miliaceum L.), finger millet, pearl millet, foxtail millet, barnyard millet, little millet, and kodo millet are all commonly cultivated varieties (Habiyaremye et al., 2017). Millet is the sixth most important cereal grain in the world, providing a substantial source of energy and protein to millions of people in India, Africa, and China, particularly those living in dry and semiarid regions (Amadou, et al., 2013). Millet is one of the most ideal crops for agricultural sustainability and the future supply of food due to its short duration and extensive adaptability under varied climatic circumstances (Habiyaremye et al., 2017). Millets are a key source of both protein as well as energy for millions of humans in Japan, China, Africa, as well as India, particularly in hot and arid climates (Rachie, 1975; Amadou et al., 2013).

Proso Millet (*Panicum miliaceum* L.) is a resistant to drought crop that has long been utilized as human nourishment.Proso millet (Panicum miliaceum) is also known as common millet. This millet is currently grown mostly in China, India, Russia, the United States, Pakistan, European countries, and so on.When taken in by human beings, proso millet offers several advantages. It is free from gluten, making it excellent for gluten-intolerant persons. Proso millet includes a lot of lecithin, which helps the nervous system. Proso millet is high in fiber and provides vital vitamins and minerals that are required for a balanced diet. It is gluten-free and abundant in carbohydrates, protein, and essential amino acids.Proso millet is a food crop that also has substantial health advantages. Its nutritious value makes it a suitable diet for people with diabetes due to its low glycemic index. It is also thought to be advantageous to heart as well as liver health. Proso millet provides excellent environmental advantages in addition to human health benefits. Proso millet has numerous distinguishing traits, including drought tolerance and a short growth season, making it a suitable rotating crop for wintertime wheat-based dryland agricultural systems. Proso millet is a climate-smart, free from gluten, and also tiny grain cereal that is beneficial to both human beings as well as the environment (Das et al., 2019).

Figure 1.2 : Proso Millet (*Panicum miliaceum* L.)

CHAPTER 2

OBJECTIVES OF THE STUDY

MATERIALS USED AND EXPERIMENTAL METHODS

2.1 Objectives of the study :

- Synthesis of nanochitosan zinc composite.
- Efficacy of a newly created composite in promoting plant growth and plant growth parameters of Proso Millet.
- Development of environmentally safe nano- carriers to encapsulate Zn nutrients and their characterization
- Morphological responses of chitosan slow- release Zn nanocomposite in proso millet

2.2 Materials

India Sea Foods in Cochin, Kerala, is where we got our chitosan (poly [-2-amino-2-deoxy-(1-4)-D glucopyranose)]. It is 90% deacetylated and has a molecular weight of 60,000 daltons. The chitosan (CS) granules were dissolved in acetic acid and then re-precipitated with sodium hydroxide (NaOH). Acetic acid, sodium tripolyphosphate, and zinc sulphate ($ZnSO4.7H2O$) were among the Merck compounds available for analytical use. The ATL-1 type of proso millet was supplied by Rajagiri College's Department of Biosciences in Cochin, Kerala, India.

2.3 Instrumentation

2.3.1 Electronic Weighing Balance: Sartorius model was used for accurate weighing

FIGURE 2.1: Electronic Weighing Balance

2.3.2 Air oven: LABLINE oven used for drying the sample

FIGURE 2.2: LABLINE air oven

2.3.3 Homogeniser

FIGURE 2.3 : Homogeniser

2.3.4 Magnetic Stirrer

FIGURE 2.4 : Magnetic Stirrer

2.3.5 Atomic absorption spectrometer (pinnacle 900H, Perkin Elmer) : Used for the analysis of Zn^{2+}

FIGURE 2.5: : Pinnacle 900H Atomic Absorption Spectrometer

Figure 2.6 : Field Emission Scanning Electron Microscopy (FESEM)

Figure 2.7 : Energy-dispersive X-ray analysis (EDAX).

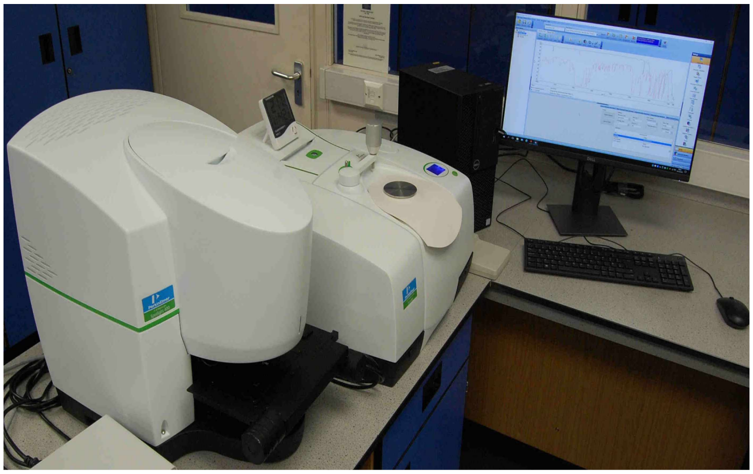


Figure 2.8 : Fourier transform infrared spectroscopy (FTIR)

2.4 Characterization

2.4.1 Physical and chemical characterization of CSZC

The newly synthesized composites underwent characterization using various equipment, including X-ray Diffraction (XRD), Particle Size Analyzer (PSA), Fourier transform infrared spectroscopy (FTIR), Zeta Potential, Field Emission Scanning Electron Microscopy (FESEM), Atomic Absorption Spectrometry (AAS) and Energy-dispersive X-ray analysis (EDAX).

2.4.2 Fourier transform infrared (FTIR) analysis

For FTIR, each sample had been prepared in potassium bromide (KBr) as a pellet in a 1:99 ratio of sample to KBr, and it was recorded using an ABB FTLA 2000-100 (Quebec, Canada) with resolution limit of 16 cm-1.

2.4.3 Field Emission Scanning Electron Microscopy (FESEM)

For the morphological analysis, the sample was placed onto vitreous carbon stubs and allowed to dry under ambient conditions. Subsequently, the samples were coated with a layer of gold and subjected to imaging in high vacuum mode using a Zeiss EVO MA10 scanning electron microscope (Carl Zeiss Promenade, Germany) at an acceleration voltage of 20 kV.

2.4.4 Energy Dispersive X-Ray Analysis (EDS or EDAX)

To conduct elemental analysis of the Chitosan Zinc Nanocomposite, Energy Dispersive X-ray Spectroscopy (EDS) was carried out in combination with Field Emission Scanning Electron Microscopy (FESEM).

2.4.5 (Atomic Absorption Spectroscopy) AAS

The metallic element concentrations were determined using Atomic Absorption Spectroscopy (AAS). The impact of zinc concentration in the loading process was thereby investigated.

2.5 Synthesis of chitosan nanoparticles

Chitosan nanoparticles were synthesized through the ionic gelation method using TPP anions, as described by Saharan in 2013, with some slight modifications. Initially, 0.3g of Chitosan was dissolved in 1% (v/v) acetic acid and stirred for three hours at 250 rpm using a magnetic stirrer (Remi Laboratory Instruments, Mumbai, India). The solution was then filtered through Whatman's paper to remove any debris. Separately, TPP was dissolved at a 1% (w/v) concentration in ultrapure water and filtered as well.

The cross-linking process was carried out by gradually adding 1 ml of TPP to each 25 ml of the Chitosan solution using a disposable plastic syringe, while stirring at 750 rpm on a magnetic stirrer. The pH of the mixture was adjusted to an optimized level of 6.5 using 2N NaOH. The resulting formulation underwent centrifugation three times for 10 minutes at 10,000 rpm, and the residue was subsequently re-suspended in the ultrapure water.

2.5.1 Synthesis of chitosan Nano fertilizer

Through the combination of the ionic gelation method and co-encapsulation of zinc nutrient, a chitosan zinc nanocomposite (CSZC) was synthesized (Deshpande in 2017). The process involved incorporating Zinc sulphate solution into a nano chitosan solution with constant magnetic stirring around 750 rpm and maintaining an optimal pH of 6 before completing the cross-linking reaction as previously mentioned. To assess the impact of different ZnSO4 concentrations on the complexation efficacy, zinc sulphate was added in ratios of 1:5, 1:10, 1:20, and 1:30 (Zinc:CS proportion). The resulting chitosan zinc nanocomposite was isolated and also purified by subjecting the colloidal solution to centrifugation (10,000 x g) followed by multiple washes with the deionized water.

To ascertain the zinc content within the newly formed nanocomposite, a known quantity of the sample was extracted from the CSZC solution using 5 ml of 0.1M HCl. The extraction process was carried out for 24 hours at room temperature. Subsequently, nanoparticles (NPs) were separated through centrifugation (10,000 x g) and the supernatant containing zinc was then quantified using an atomic absorption spectrophotometer (AAS, Aanalyst 400 Perkin Elmer, USA).

To calculate the actual zinc content in the new composite and its entrapment efficiency, the following equations were employed.

Entrapment efficiency (EE) = $W_1/W_2 \times 100$

where, W_2 represents the initial total weight of zinc added to the chitosan solution, while W_1 corresponds to the measured actual zinc content.

2.5.2 In vitro pot study experiments

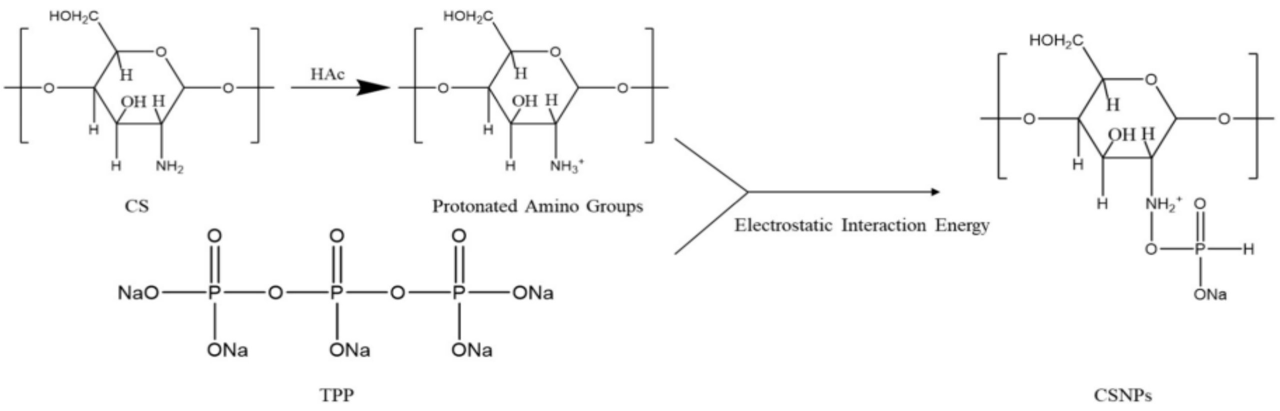
Proso millet seeds were subjected to a surface sterilization process using 10% sodium hypochlorite for 10 minutes. Subsequently, the seeds were treated with various concentrations of nanoparticles (as optimized in the in vitro antifungal assay) for a duration of 4 hours. After treatment, the seeds were dried, placed on germinating paper in Petri plates, and then 50 ml of water was added. The Petri dishes were covered and placed in an incubator at 28-31°C, with periodic replenishment of fresh water. This investigation was conducted following the recommendations of the International Seed Testing Association. After 9 days of incubation, data were collected for seed germination percentage, shoot length (cm), root length (cm), root number, and also the fresh-dry weight of seedlings (g).

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Synthesis of zinc-loaded nanoparticles of chitosan

The CSZC was synthesized through the ionotropic gelation method (as depicted in Fig. 1) utilizing TPP as the cross-linking agent. In this process, the -NH_2 groups on CS undergo protonation in an acidic environment. The negatively charged phosphate groups of TPP are then electrostatically attracted to the NH_3^+ groups on CS, resulting in a self-assembly of the particles (illustrated in Fig. 3.1).



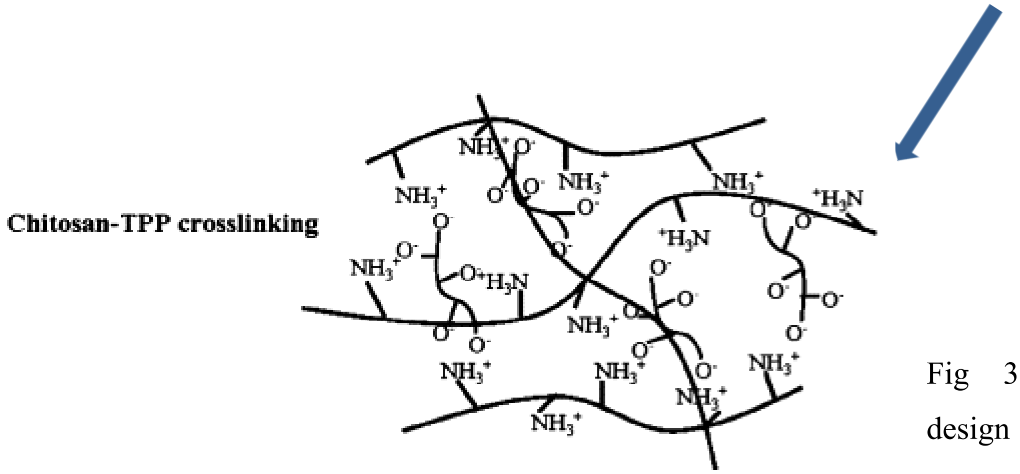


Fig 3.1 Schematic design of chitosan nanoparticles.

Although not all amino groups were neutralized by TPP due to polymer conformation and steric hindrances, the remaining NH_3^+ groups gave rise to more soluble complexes with metal ions, particularly zinc and copper (Ribeiro et al.,2020). Because of its high concentration of amine and hydroxyl groups, chitosan is a well-known bio-absorbent. As a result, it may be readily functionalized to produce metal complexes.(Ramchandra et al., 2011)

3.2 Characterization study

In FTIR analysis the spectra showed a shift from 3486 cm^{-1} (bulk chitosan) to 3437 cm^{-1} (Zn-chitosan NPs), were sharper, and corresponded to $-NH_2$ and -OH groups in NPs (Fig. 3.2). The peaks in CSZC have been shifted to 1639 ($-CONH_2$), 1430 ($-NH_2$), 907 cm -1 (anhydrous glycosidic), and 582 cm^{-1} (-ZnO), showing the interaction of chitosan, TPP, and Zn (Fig. 3.2).The rough spherical symmetric porous character of CSZN is revealed by FESEM measurements (Fig. 3.4). The interaction among zinc and chitosan during the synthesis of CSZC alters the chitosan structure. Furthermore, the Edax study validated the existence of carbon, oxygen, and nitrogen, indicating that these elements are essential components of chitosan, however phosphorus and Zn were due to the presence of TPP and ZnSO4, respectively.

AAS studies indicated a progressive increase in the amount of Zn intercalated on nano chitosan, ranging from 15% to 35%, as the molar concentration of loaded zinc increased. This highlights the efficiency of chitosan as an effective Zn adsorbent. The interaction between the amino group of chitosan and the hydroxyl groups of the chitosan chain plays a significant role in binding zinc ions and forming ionic cross-links with TPP molecules (Table 1). Based on the composite with the highest percentage of Zn intercalation, further applications were pursued.

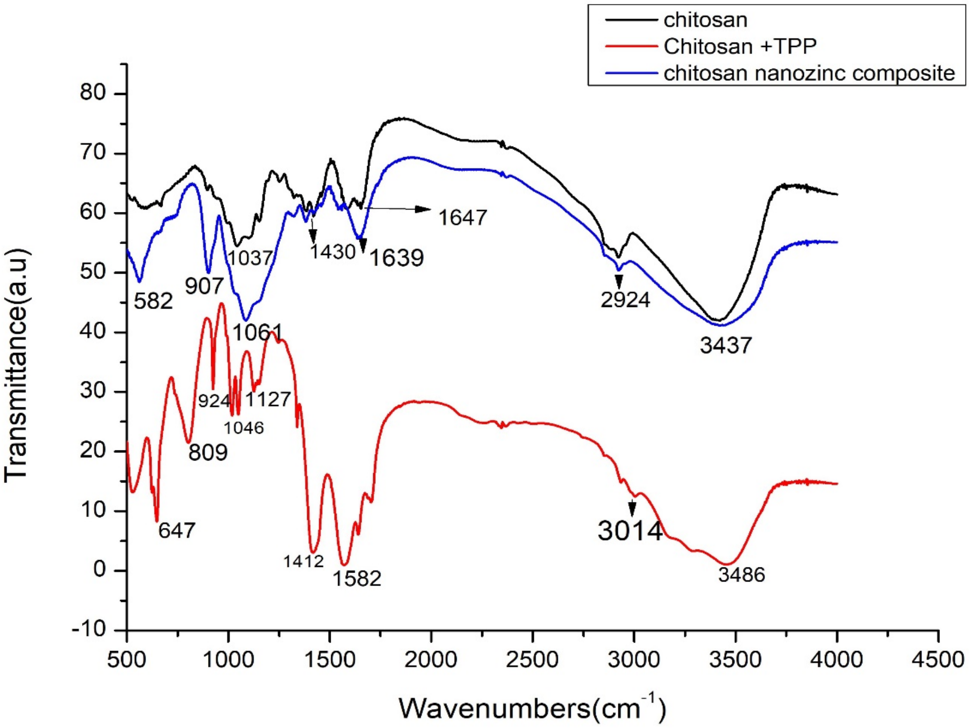


Figure 3.2 : FTIR Analysis

The mineral composition of the chitosan proved that there was an efficient loading of zinc in the composite.

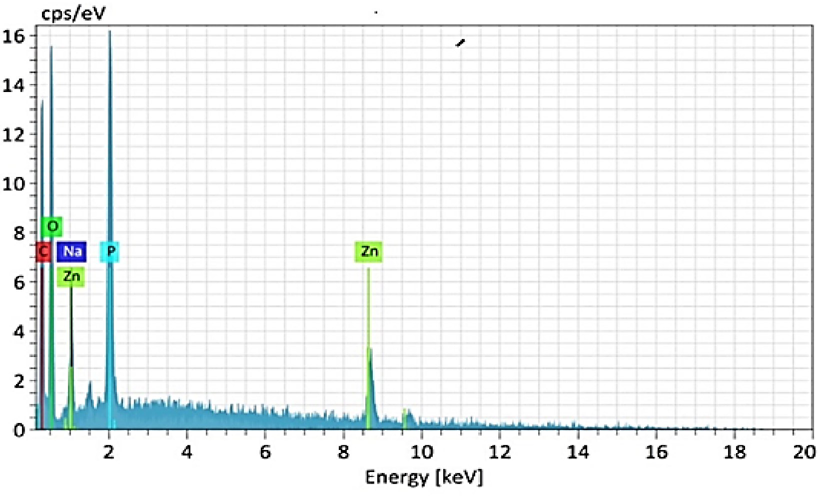


FIGURE 3.3 EDAX recorded in the binding energy region of 0-20 keV

Name of the elements	Atomic No.	Netto	Mass (%)	Mass Norm (%)	Atom (%)	Abs. erro (%) (1 sigma)	Rel. erro (%) (1 sigma)
Carbon	6	5994	27.39	43.67	55.46	1.48	5.41
Oxygen	8	6755	23.91	38.13	36.35	1.26	5.28
Sodium	11	1498	2.58	4.11	2.73	0.31	11.97
Phosphorus	15	9204	5.25	8.36	4.12	0.26	5.04
Zinc	30	2119	3.58	5.72	1.3	0.21	5.72

TABLE 3.1: EDAX showing the atomic percentages of various elements contained in the produced sample.

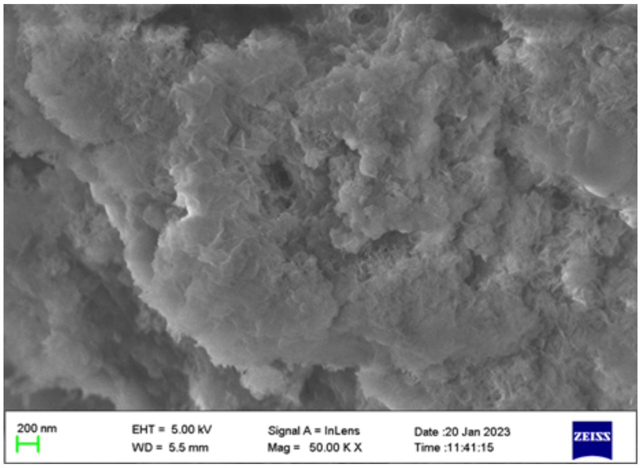


FIGURE 3.4 : SEM Image of Chitosan

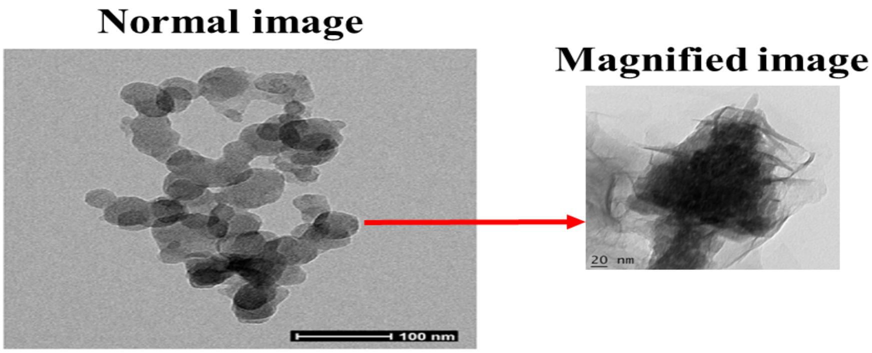


FIGURE 3.5: TEM Image of Chitosan Zn Nanocomposite

Table 3.2 : Effect of concentration of zinc in loading (from AAS)

Chitosan(%) (CS)	ZnSO4 ratio with CS	TPP(%)	Adsorption efficiency(%)
0.3	1:30	1	15
0.3	1:20	1	22
0.3	1.10	1	28
0.3	1:5	1	35

Effect of CSZC on growth enhancement in Proso millet

Under laboratory conditions, the effect of seed treatment on proso millet seed germination were studied. For 3 hours, seeds were soaked in chitosan concentrations of 0.5, 1.5, 2.5, and 3.0 g kg^{-1} seed. According to the International Seed Health Testing Rules, the treated seeds were put on wetted paper towels. As controls, 15 seeds were treated with distilled water alone for the same amount of time.

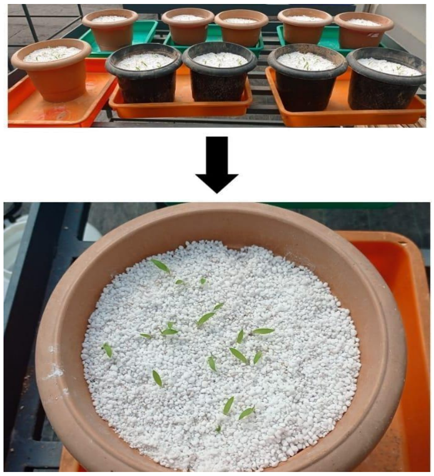


Figure 3.5 : Plant growth experiment using synthesised Zn chitosan Nanocomposite of Proso Millet.

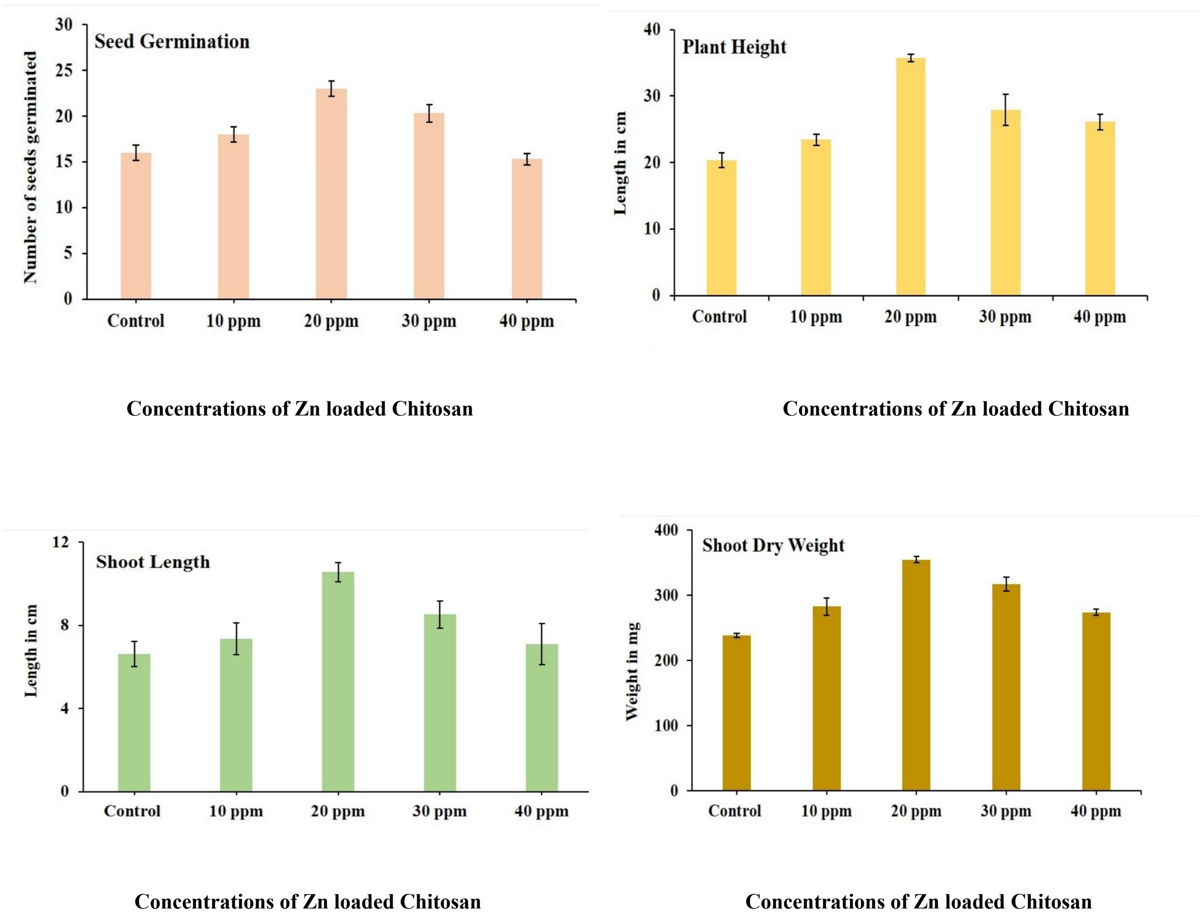


Figure 3.6 : Effects of Zn loaded Chitosan nanoparticles in seed germination, shoot length, plant height, and shoot dry weight of Proso millet.

Under different concentrations of Zn loaded chitosan Nanoparticles the seed germination, shoot length, plant height, and shoot dry weight of Proso millet has been improved and the optimum concentration is found to be 20 ppm .

CHAPTER 4

CONCLUSION

Chitosan nano fertilizer has been found to effectively deliver essential nutrients to plants. One significant advantage of chitosan is its biodegradability and biocompatibility, ensuring no risks after application.As a result, research into the application of chitosan in agriculture has recently increased. Acting as a proficient adsorbent, chitosan captures nutrients like zinc and forms complexes, enabling slow and sustained release of encapsulated nutrients, leading to long-lasting effects and reduced fertilizer wastage. This dual benefit positively impacts both plant health and financial gains. The current study involves a quantitative characterization of newly prepared Chitosan nanomaterials, evaluating their efficacy. Notably, chitosan nanoparticles have already demonstrated success in drug delivery and preclinical studies, highlighting the versatility of modifying the chitosan backbone to suit specific needs.

Additionally, SEM images of the Chitosan provide a detailed insight into the nanomorphology of the fertilizer, allowing for better understanding of its structure, persisting period, flow dynamics, functioning duration, and surface area. And in this study, we observed that Zn-loaded Chitosan nanoparticles promoted germination and seedlings growth of Proso millet.

FUTURE SCOPE

Nanofertilizers have been shown to significantly boost crop growth, quality, and quantity. It enhances nutritional availability in the plant body. Because of nutritional deficiencies in the plant body, cultivation destruction might be greatly minimized. The future scope of our study is to collect different agro-morphological traits (particularly yield) under different concentrations of Chitosan Zn Nanoparticles and to analyze zinc contents in shoot, root, seeds and tissues of proso millet under different concentrations of Chitosan Zn Nanoparticles and also to Analyze the expression pattern of ZIP family transporters in shoot and root tissues of proso millet under different concentrations of Chitosan Zn Nanoparticles.

REFERENCES

1. Kalra, Tanya, Pushpa C. Tomar, and Komal Arora. "Micronutrient encapsulation using nanotechnology: Nanofertilizers." Plant Arch 20, no. 2 (2020): 1748-1753
2. Faqir, Yahya, Jiahua Ma, and Yunlong Chai. "Chitosan in modern agriculture production." Plant, Soil and Environment 67, no. 12 (2021): 679-699.
3. Anitha, A., VV Divya Rani, R. Krishna, V. Sreeja, N. Selvamurugan, S. V. Nair, H. Tamura, and R. Jayakumar. "Synthesis, characterization, cytotoxicity and antibacterial studies of chitosan, O-carboxymethyl and N, O-carboxymethyl chitosan nanoparticles." Carbohydrate polymers 78, no. 4 (2009): 672-677.
4. Deshpande, Paresh, Ashwin Dapkekar, Manoj D. Oak, Kishore M. Paknikar, and Jyutika M. Rajwade. "Zinc complexed chitosan/TPP nanoparticles: A promising micronutrient nanocarrier suited for foliar application." Carbohydrate polymers 165 (2017): 394-401
5. Prajapati, Damyanti, Ajay Pal, Christian Dimkpa, Upendra Singh, Khaidem Aruna Devi, Jagdish Lal Choudhary, and Vinod Saharan. "Chitosan nanomaterials: A prelim of next-generation fertilizers; existing and future prospects." Carbohydrate Polymers 288 (2022): 119356..
6. Soumitra, Das, and A. Green. "Importance of zinc in crops and human health." Journal of SAT Agricultural Research 11 (tosan/TPP nanoparticles: A promising micronutrient nanocarrier suited for foliar 2013).
7. Alloway BJ. 2008. Zinc in soils and crop nutrition. Paris, France: IFA; and Brussels, Belgium: IZA.
8. Sillanpää M. 1982. Micronutrients and the nutrient status of soils: A global study. FAO Soil Bulletin No. 48. Rome, Italy: Food and Agriculture Organization
9. Meena, D. Singh, C. Gautam, O. P. Patidar, H. M. Meena, G. Prakasha, and J. Vlshwa. "Nano-fertilizers is a new way to increase nutrients use efficiency in crop production." Int. J. Agric. Sci 9, no. 7 (2017): 3831-3833.
10. Ghaly A. E. (2009) American J. Biochem. Biotechnol, 5, 210-220.
11. Quasem J. M., Mazahreh A. S. and Abu-alruz K. (2009) American J. Applied Sci., 6, 888-896.
12. Braun H. and Roy R. N. (1983) Proc. Symp. Efficient use of fertilizers in agriculture development in Plant and Soil Science, 10, 251-270.
13. Brunnert I., Wick P., Manserp., Spohnp., Grass R. N., Limbach L. K.,Bruinink A. and Stark W. J. (2006) Environmental Science & Technology,40, 4374-4381
14. Liscano J. F., Wilson C. E., Norman R. J. and Slaton N. A. (2000) AAES Res Bulletin, 963, 1–31.

15. Joseph T. and Morrisson M. (2006) Eur. Nanotechnol. Gateway.
16. Tarafdar J. C., Xiang Y., Wang W. N., Dong Q. and Biswas P. (2012c) Applied Biological Research, 14, 138-144.
17. Vafa Z N, Sirousmehr A R, Ghanbari A, Khammari E and Falahi N, 2015, Effect of nano-zinc and humic acid in quantitative and qualitative characteristics of savory (Satureja hortensis L.). International Journal of Biosciences, 6: 124-136.
18. Mahil, E. IYARIN THANKA, and BN ARAVINDA Kumar. "Foliar application of nanofertilizers in agricultural crops–A review." J. Farm Sci 32, no. 3 (2019): 239-249.
19. Adisa, I. O., Pullagurala, V. L. R., Peralta-Videa, J. R., Dimkpa, C. O., Elmer, W. H., Gardea-Torresdey, J. L., et al. (2019). Recent advances in nano-enabled fertilizers and pesticides: a critical review of mechanisms of action. Environ. Sci. Nano 6, 2002–2030
20. Mastronardi, E., Tsae, P., Zhang, X., Monreal, C., and Derosa, M. (2015). Strategic role of nanotechnology in fertilizers: potential and limitations. Berlin, Germany: Springer. 25–67.
21. Shang, Y., Hasan, M. K., Ahammed, G. J., Li, M., Yin, H., and Zhou, J. (2019). Applications of nanotechnology in plant growth and crop protection: a review. Molecules 24, 2558–2580.
22. Golbashy, M., Sabahi, H., Allahdadi, I., Nazokdast, H., and Hosseini, M. (2016). Synthesis of highly intercalated urea-clay nanocomposite via domestic montmorillonite as eco-friendly slow-release fertilizer. Arch. Agron. Soil Sci. 63, 84.
23. Tarafder, C., Daizy, M., Alam, M. M., Ali, M. R., Islam, M. J., Islam, R., et al. (2020). Formulation of a hybrid nanofertilizer for slow and sustainable release of micronutrients. Acs Omega 5, 23960–23966.
24. Weeks, J. J., and Hettiarachchi, G. M. (2019). A review of the latest in phosphorus fertilizer technology: possibilities and pragmatism. J. Environ. Qual. 48, 1300–1313
25. Mikkelsen, R. (2018). Nanofertilizer and Nanotechnology: a quick look. Better Crops 102, 18–19.
26. Hafeez, B. M. K. Y., Y. M. Khanif, and Muhammad Saleem. "Role of zinc in plant nutrition-a review." American journal of experimental Agriculture 3, no. 2 (2013): 374.
27. Broadley, Martin R., Philip J. White, John P. Hammond, Ivan Zelko, and Alexander Lux. "Zinc in plants." New phytologist 173, no. 4 (2007): 677-702.

28. Cabot, Catalina, Soledad Martos, Mercè Llugany, Berta Gallego, Roser Tolrà, and Charlotte Poschenrieder. "A role for zinc in plant defense against pathogens and herbivores." Frontiers in plant science 10 (2019): 1171.
29. Das, S., and A. Green. "Importance of zinc in crops and human health." J SAT Agric Res 11 (2013): 1-7.
30. Tondey, Manisha & Kalia, Anu. "Biotechnological Prospects of Green Synthesis of Zinc Oxide Nanoparticles and their Application as Zn Fertilizer for Improving Crop Productivity and Quality" (2019): 219-233
31. Noulas, Christos, Miltiadis Tziouvalekas, and Theodore Karyotis. "Zinc in soils, water and food crops." Journal of Trace Elements in Medicine and Biology 49 (2018): 252-260.
32. Sturikova, Helena, Olga Krystofova, Dalibor Huska, and Vojtech Adam. "Zinc, zinc nanoparticles and plants." Journal of hazardous materials 349 (2018): 101-110.
33. Tsonev, Tsonko, and Fernando Jose Cebola Lidon. "Zinc in plants-an overview." Emirates Journal of Food & Agriculture (EJFA) 24, no. 4 (2012).
34. Brown, Patrick H., Ismael Cakmak, and Qinglong Zhang. "Form and function of zinc plants." In *Zinc in Soils and Plants: Proceedings of the International Symposium on 'Zinc in Soils and Plants' held at The University of Western Australia, 27–28 September, 1993*, pp. 93-106. Springer Netherlands, 1993.
35. Pathak, Girish Chandra, Bhavana Gupta, and Nalini Pandey. "Improving reproductive efficiency of chickpea by foliar application of zinc." *Brazilian Journal of Plant Physiology* 24 (2012): 173-180.
36. Umair Hassan, Muhammad, Muhammad Aamer, Muhammad Umer Chattha, Tang Haiying, Babar Shahzad, Lorenzo Barbanti, Muhammad Nawaz et al. "The critical role of zinc in plants facing the drought stress." Agriculture 10, no. 9 (2020): 396.
37. Fan, Wen, Wei Yan, Zushun Xu, and Hong Ni. "Formation mechanism of monodisperse, low molecular weight chitosan nanoparticles by ionic gelation technique." Colloids and surfaces B: Biointerfaces 90 (2012): 21-27.
38. S.A. Agnihotri, N.N. Mallikarjuna, T.M. Aminabhavi, Recent advances on chitosan-based micro- and nanoparticles in drug delivery, Journal of Controlled Release 100 (2004) 5–28.
39. X.Z. Shu, K.J. Zhu, The influence of multivalent phosphate structure on the properties of ionically cross-linked chitosan films for controlled drug release, European Journal of Pharmaceutics and Biopharmaceutics 54 (2002) 235–243. [13] J. Berger, M. Reist, J.M. Mayer, O. Felt, N.A. Peppas, R. Gurny, Structure and interactions in covalently and ionically crosslinked chitosan hydrogels for biomedical applications, European Journal of Pharmaceutics and Biopharmaceutics 57 (2004) 19–34.

40. Angelo, L. M., Franca, D., & Faez, R. (2021). Biodegradation and viability of chitosan-based microencapsulated fertilizers. Carbohydrate Polymers, 257, Article 117635.
41. Azmana, M., Mahmood, S., Hilles, A. R., Rahman, A., Arifin, M. A.b., & Ahmed, S. (2021). A review on chitosan and chitosan-based bionanocomposites: Promising material for combating global issues and its applications. International Journal of Biological Macromolecules, 185, 832–848.
42. Divya, K., and M. S. Jisha. "Chitosan nanoparticles preparation and applications." Environmental chemistry letters 16 (2018): 101-112.
43. Chouhan, D., & Mandal, P. (2021). Applications of chitosan and chitosan based metallic nanoparticles in agrosciences-a review. International Journal of Biological Macromolecules, 166, 1554–1569.
44. Iftime, M. M., Ailiesei, G. L., Ungureanu, E., & Marin, L. (2019). Designing chitosan based eco-friendly multifunctional soil conditioner systems with urea controlled release and water retention. Carbohydrate Polymers, 223, Article 115040.
45. Jogaiah, S., Satapute, P., De Britto, S., Konappa, N., & Udayashankar, A. C. (2020). Exogenous priming of chitosan induces upregulation of phytohormones and resistance against cucumber powdery mildew disease is correlated with localized biosynthesis of defense enzymes. International Journal of Biological Macromolecules, 162, 1825–1838.
46. J. Berger, M. Reist, J.M. Mayer, O. Felt, N.A. Peppas, R. Gurny, Structure and interactions in covalently and ionically crosslinked chitosan hydrogels for biomedical applications, European Journal of Pharmaceutics and Biopharmaceutics 57 (2004) 19–34.
47. Amadou, I., Gounga, M. E., and Le, G. W. (2013). Millets: nutritional composition, some health benefits and processing-A review. Emirates J. Food Agric. 25, 501–508.
48. Habiyaremye, C.; Matanguihan, J.B.; D'Alpoim Guedes, J.; Ganjyal, G.M.; Whiteman, M.R.; Kidwell, K.K.; Murphy, K.M. Proso Millet (Panicum miliaceum L.) and Its Potential for Cultivation in the Pacific Northwest, U.S.: A Review. Front. Plant Sci. 2017, 7.
49. Rachie, K. O. (1975). The Millets. Importance, Utilization and Outlook. Hyderabad: International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)
50. Das, Saurav, Rituraj Khound, Meenakshi Santra, and Dipak K. Santra. "Beyond bird feed: Proso millet for human health and environment." Agriculture 9, no. 3 (2019): 64.

51. Ribeiro, Elisa Franco, Taís Téo de Barros-Alexandrino, Odilio Benedito Garrido Assis, Américo Cruz Junior, Amparo Quiles, Isabel Hernando, and Vânia Regina Nicoletti. "Chitosan and crosslinked chitosan nanoparticles: Synthesis, characterization and their role as Pickering emulsifiers." Carbohydrate Polymers 250 (2020): 116878.
52. Patale, Ramchandra L., and Vandana B. Patravale. "O, N-carboxymethyl chitosan–zinc complex: a novel chitosan complex with enhanced antimicrobial activity." Carbohydrate Polymers 85, no. 1 (2011): 105-110.