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REVIEW

Chitosan Based Nanoformulation for Sustainable Agriculture with Special Reference to Abiotic Stress: A Review

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Abstract

Chitosan is a naturally occurring biological macromolecule and second most abundant polysaccharide next to cellulose, derived from deacetylation of chitin. Due to its biocompatibility, biodegradability, nontoxic and broad spectrum of antimicrobial activity, it has become an important field of drug delivery system study. With the advancement in nanotechnology, chitosan based nanoformulations have sought considerable attention in agricultural sciences. The first part of this review focuses on the overview of chitosan and its nanoparticles, its different mode of synthesis and challenges, and controlled release mechanism of encapsulated molecules. The subsequent section focuses on the uptake and translocation of chitosan based nanoformulation including plant growth, nutrition and special focus on abiotic stress mitigation strategies. We conclude that chitosan based nanoformulation holds great promises in encapsulating bioactive molecules for controlled release thus reduces environmental hazard, and improves plant growth, yield and subsequently mitigates various biotic and abiotic stresses. Chitosan based nanoformulations have good controlled release behaviour and long stability of bioactive compounds encapsulated inside chitosan nanoparticle, and have prosperous future for improving agricultural productivity in the era of climate change.

Keywords Abiotic stress mitigation · Chitosan nanoparticle · Controlled release · Plant growth

Introduction

Agricultural research has been confronted by many factors such as incident of diseases, pests, nutrient losses, and low productivity due to varying environmental stress such as drought, salinity, temperature and heavy metal stress. Also, demand for food is increasing due to increasing global population coupled with global climate change, urbanization and depletion of arable land. These issues have challenged agricultural researchers to look for option for more natural and environmental friendly material for use in agriculture. It has been projected that the global food production would increase by 70–100% by 2050 [1]. In this context, chitosan has emerged as the promising alternatives to mitigate these challenges without compromising soil and agro-ecosystem. Chitosan has proven to have fascinating properties such as broad spectrum of antimicrobial properties, anti-inflamatory, bio-adhesion, biocompatibility with other compounds, etc. Chitosan is the deacetylated form of chitin which is the second most abundant polymer found next to cellulose. Chitin is present in marine organisms such as shells of crustaceans, insect cuticles and fungal cell walls [2]. Chitosan is obtained from chitin through alkaline deacetylation of chitin which is composed of linear chain consisting of two subunits, D-glucosamine and N-acetyl-D-glucosamine which are linked by glycosidic bonds. Compared to chitin, chitosan has amine group which facilitate functional derivatives formation and structural modification. In plants, chitosan elicits numerous defense responses and improves plant growth and yield [3].

Chitosan application in agriculture is gaining worldwide attention due to its beneficial characteristics such as biodegradability, biocompatibility, non-toxic and stimulate plant growth, yield and induced resistance against myriad of biotic and abiotic stresses. There are considerable reports on chitosan stimulating growth and yield on various crops such as in rice [4], soybean [5], maize [6], potato [7], tomato [8]. The ability may be linked to improved physiological mechanism, higher nutrient absorption, cell division and synthesis of protein [9, 10]. Chitosan

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application suppresses many fungal, bacterial, viral and nematodes. For examples, in fungal namely, *Alternaria, Rhizophus, Bortrytis, Fusarium*, etc. have been reported to significantly suppressed by chitosan treatment [10]. Also, antibacterial activities have been reported against different plant pathogenic bacteria such as *Xanthomonas* spp., *Pseudomonas* spp. [10] and resistance against powdery mildew as well as promote growth in tomato [11]. In tomato, chitosan conferred resistance against tomato mosaic virus and improved growth and yield [12]. Chitosan is effective in controlling many plant pathogenic nematodes such as *Meloidogyne spp.* in tomato [13]. The mechanism of inducing resistance to insect, pest and diseases is primarily based on the biosynthesis of protective biomolecules and up-regulation of defense related genes [10, 14].

The rapid advancement in nanotechnology has brought considerable attention in agricultural science. Encapsulating active ingredient such as fertilizer, pesticides and other bioactive agents into chitosan domain has proved to be effective owing to its slow release mechanism and reduced environmental contamination on account of reduced evaporation and leaching. Nanotechnology offers a wide range of application in agricultural field such as nano-based formulation of agrochemicals, elicitors and fertilizers and nanosensor for smart monitoring of plants' activities. Nanoparticles can penetrate through plant surface membrane through cuticle, stomata, trichomes, hydathodes, wounds, stigma and root junction [15, 16]. These characteristics of nanoparticles could be the reasons behind growing interest in the field of agricultural nanotechnology. Controlled release of fertilizers with natural biodegradable component such as chitosan based nanoformulation plays a crucial role in achieving sustainable agricultural practices [17]. Nanosized chitosan has emerged as an effective polymer for use in agriculture due to its higher rate of efficacy with increased mobility, large surface area owing to its nano size, and lower toxicity as compared to conventional pesticides [18]. Also, fascinating properties such as biocompatibility, biodegradability, and film forming properties of chitosan based nanoparticles have prosperous future for agricultural application [19].The ability of chitosan to combine with other bioactive compounds has unravelled certain drawbacks of nanocomposites preparation such as hydrophilicity, low encapsulation efficiency, controlled release mechanism and weak mechanical strength [20]. Recently, foliar application of chitosan nanoparticle has shown to increase yield, mineral content and enhance innate immunity in finger millet [21]. Similarly, chitosan nanoparticle incorporated with salicylic acid improved wheat growth and grain weight with higher photosynthetic ability [22]. Moreover, chitosan nanoparticle incorporated with NPK observed higher yield and harvest index in wheat [23, 24].

Chitosan application in plant disease protection has been studied and comprehensively been reviewed elsewhere, however, there are dearth of published literatures indicating chitosan's role in abiotic stress mitigation. Chitosan and its derivatives have been applied and observed to confer abiotic stress such as drought, salinity, temperature and heavy metal toxicity [25]. They influence plant physiological aspect and gene associated with stress protective metabolite. Also, with their ability to scavenge ROS, elicit defense responses and ultimately increase plant growth and development. With the advancement in nanotechnology, chitosan polymer has exhibited to be more reliable candidate for synthesizing nanoparticles which have proven to be more efficient and more efficacious than bulk chitosan. Also, as a carrier polymer of various plant beneficial bioactive compounds and metal encapsulation approaches, chitosan nanoparticles have great prospect in meeting sustainable agricultural goal.

Contributing to zero waste economy in food industry is imperative to sustainable development benefiting both the economy and the environment. Chitosan production from marine crustacean is another effective way to utilize marine biowaste. It is noteworthy that about 6–8 million tonnes of marine biowaste is produced annually [26] and production of 1 kg of chitosan utilizes over 1 tonne of water, however, its positive impact such as nano formulations in crop management overweight the negative implication of huge water uses. The slow release mechanism and high bioavailability along with low toxicity could be beneficial for sustainable agriculture with lower impact to environmental and health issues. In agriculture, chitosan has evolved to be most promising growth enhancer and strong anti-fungal and bacterial antimicrobial agents [3]. Also, with the advancement in nanotechnology, chitosan based nanoparticle containing agriculturally important bioactive compounds that have biostimulant ability, sustained release mechanism and defense inducer in plants have promising results to be used in agriculture[27].

Overview of Chitosan/Chitosan Nanoparticle

Chitosan is a polycationic polymer that is formed when chitin is deacetylated. Chitin is a structural polymer found in crustaceans, insects, mollusks, fungus, shrimps and other marine organism [28–30]. In terms of function and structure, chitin produced by crustaceans and arthropods in their shells is extremely similar to cellulose generated by plants in cells. Chitin is most abundant polysaccharide found next to cellulose. Chitosan is more functional than chitin because of its amino-based functional groups that stretch along the chain consisting of 2-deoxy-d-glucosamine (GlcN) and 2-deoxy-N-acetyl-d-glucosamine (Glc-NAc) units [31]. The source and extraction process have an impact on the molecular weight, biological and physicochemical properties, and purity of isolated chitosan [32]. In addition, the protonation intensity of amine groups affects chitosan's functioning. The major properties include nontoxicity, mucoadhesiveness [33], hemostatic, film-forming capability [34], excellent adsorption matrix, antiviral, antibacterial and antifungal [35], and antioxidative [36], making it a very appealing component for use in a variety of applications. Previous research has included a number of investigations that are related to variety of application in the field of biomedical industry, food technology, agricuture and other vital areas. In this context, chitosan has potential role in meeting agriculture sustainable goal through plethora of application in the field of nanoscince, encapsulation of agrochemicals and other potential formulations in mitigating issues of biotic and abiotic stress.

Previous research has shown that chitosan application induce tolerance and alleviates abiotic stress such as drought, salinity, heavy metal toxicity and low temperature on various crops. This has been reviewed comprehensively by Hidangmayum et al. [3].Compared to bulk chitosan, chitosan nanoparticle exhibit enhanced physicochemical properties such as smaller particle size, high surface area, increased efficiency of encapsulated drug, low toxicity, suitable for blending with other bioactive compounds. Chitosan nanoparticle synthesis is obtained through various methods which are explained in the subsequent section of this paper. However, the most simple and versatile method is the ionic gelation without the use of external chemicals which could produce nano sized chitosan polymer. Polycationic chitosan is made to mixwith polyanionic molecules such as tripolyphosphate (TPP) drop wise under constant stirring to produce nanosized chitosan colloidal particle (refer to Fig. 1). Nano chitosan showed improved organic carbon status with better aggregation stability, both macro and micro, relative to other natural products such as zeolite for organic carbon content and aggregation stability [35]. Combination of chitosan nanoparticle and plant growth promoting rhizobacteria (PGPR) have been reported to increase growth parameters and plant metabolites as compared to PGPR treated alone [37]. Application of chitosan nanoparticles on coffee seedlings results in higher pigment content, enhanced photosynthetic rate and uptake of nutrients [38]. Application of chitosan and chitosan nanoparticles at 50 and 5 µg/ml in wheat seed showed significant positive result in seedling parameters, and other physiological attributes, respectively. Chitosan nanoparticle (5 µg/ml) treated seeds have shown higher adsorption capacity as compared to chitosan (50 µg/ml) alone. This proves that chitosan nanoparticle at low concentration can stimulate plant growth and development. Also, the level of IAA content and related gene required for growth and development were shown to increase in both the cases [39]. Encapsulation with active agrochemicals or metal components inside the chitosan domain also proved to have significant ability in inducing a myriad of defense related metabolites, antioxidant activities, higher adsorbtion abilites and promoting growth and development. The slow release mechanism of drugs provide sustained protection without affecting environment and ecosystem. The application of chitosan nanoparticle and its nanoformulations on various crops are described in last section of this review. These findings sparked researchers to further explore the role of chitosan based nanoparticles in promoting crop protection.

Synthesis of Chitosan Nanoparticle

The preparation of chitosan nanoparticles was first used in 1994 as a drug carrier via the emulsification and crosslinking process [40]. Since then, other techniques such as ionic gelation [41, 42], reverse micellar method [43, 44], precipitation [45, 46], sieving [45], emulsion droplet coalescence [47]and spray drying [27, 48] have been developed. The production method depends mainly on the application mode, for example, the mode of operation; the efficiency of the active ingredient's encapsulation depends on the hydrodynamic size and shape of the prepared nanoparticles, their

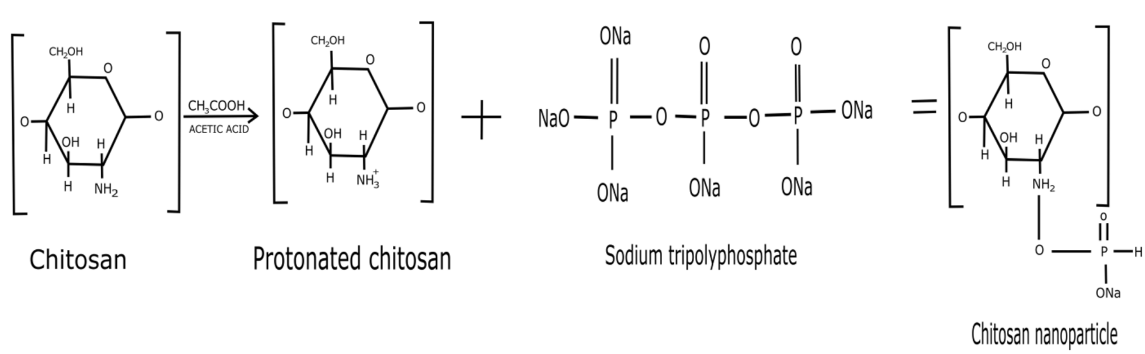

Fig. 1 Schematic diagram for synthesis of chitosan nanoparticle

thermo-mechanical activity and the degree of toxicity [27, 49].

Ionic Gelation

Ionic gelation is the most common and effecient method for synthesis of stable and non-toxic chitosan nanoparticles. This technique was first reported by Calvo et al. [41]. It is based on the electrostatic interaction between the polycationic amino groups (NH^{3+}) and polyanionic cross linkers. Firstly, chitosan is dissolved in aqueous weak acidic solution such as acetic acid, lactic acid, etc. to protonate amine group. After that the cross linker agent which is commonly used as TPP (tripolyphosphate) is added drop-wise under constant stiring. This undergoes ionic gelation due to formation of complex TPP and cationic chitosan by electrostatic forces and precipitates to form nano sized particles. Kleine-Brueggeney [50] designed and studied the formation of nanoparticles through inotropic gelation with different degrees of acetylation ranging from 0 to 47% and molecular weight of 2.5–282 kDa. Interesting results were obtained where different DA (Degree of acetylation) behaved differently and high molecular weight were found to have more preference in incorporating than low molecular weight ones as determined by SEC-HPLC.

Emulsion Cross-Linking

First, in this process, chitosan solution is emulsified to prepare the emulsion of water in oil and then aqueous droplets are further stabilized by a suitable surfactant, and then reacted with a suitable cross linking agent such as glutaraldehyde, formaldehyde, genipin, glyoxal, sulphuric acid, poloxamer, etc. resulting in formation of nanoparticle [51]. The amino groups of chitosan are cross linked with the aldehyde groups of glutaraldehyde and precipitate to form particles.

Spray Drying

This method is used for the synthesis of dry powder, pellets and granules from chitosan solution and suspensions [52]. This technique employed the use of a nanospray dryer where the acidic aqueous chitosan solution is passed along the cross linker and active ingredient via a hot air stream nozzle. Due to atomization, minor droplets are collected. The solvent is, therefore, evaporated at the end of the reaction and a nano-sized dry form is obtained. The compressed air induces chitosan to cross-link and decrease in size. However, there are some critical parameters to be optimized such as particle size of needle type, air flow rate, degree of crosslinking and temperature [53]. The process is very flexible and can be used for thermo sensitive drugs with high or low water solubility and hydrophilic or hydrophobic polymers [54].

Emulsion Droplet Coalescence Method

In this method, cross linking agents are absent and is based on the use of emulsion and precipitation principles. Firstly, a stable emulsion is prepared containing chitosan solution and drug to be loaded along with liquid paraffin oil. Secondly, another stable emulsion is prepared using chitosan solution and sodium hydroxide along with liquid paraffin oil and, lastly, the two stable emulsions prepared are mixed under high speed stirring to generate collision between different droplets randomly producing nanosized particles [55]. Since it does not use crosslinkers, chitosan amino groups are freely available to bind to active ingredients and can achieve greater encapsulation efficiency with lower nanosize as compared to the cross-link emulsion method. Using this method chitosan nanoparticle loaded gadopentetic acid (452 nm) was synthesized with encapsulation efficiency of 45% [47]. Similarly, synthesis of chitosan nanoparticle using this method was achieved by loading 5-fluorouracil which resulted in uniform content and finer droplets [56].

Reverse Micellar Method

This method utilized the basic principle of reverse micelle to produce thermodynamically stable and monodisperse unit, and produce small sized nanoparticle without uniform distribution compared with other methods. First, a surfactant and organic solvent are mixed to form fine, translucent droplet solution, and acidic aqueous solutions of chitosan are added to the isotropic reverse micelle with continuous vortexing along with the desired ingredients to be encapsulated. Then, in a constant stirring mode overnight, a cross-linking agent is added which leads to nanosize chitosan. Lastly, organic solvent is evaporated to obtain the dry matter and surfactant is removed via salt precipitation. Then, the mixture is centrifuged and a nanosize particle is obtained. Monodispersed, stable and narrow size are important attributes of this method. However, this method is tedious and laborious requiring a lengthy comprehensive process compared to other methods reported [39]. Chitosan produced through a reverse micellar method is beneficial to retain greater control over the size and distribution of particles.

Sieving Method

This method as its name suggest is only useful for obtaining monodispersed nanoparticles through filtering using the fixed sieve size. This was inventedby [45] owing to the challenging issue of stable and monodispersed chitosan nanoparticles by different methods.

Challenges in Synthesis of Chitosan Nanoparticles

Chitosan nanoparticles face some challenging issues first being the insolubility of bulk chitosan in neutral media, thus hampering the bioactivities of anti-microbial activities and phytoxicity to plants. Researchers have come up with different techniques to increase its solubility without compromising bioactivities. Thanks to the presence of flexible chemical structure which could tune it to our desired physiochemical properties through incorporation of different compounds. For example, modified chitosan such as tri-ethylene diamine dithiocarbamate chitosan and orthohydroxy-phenylaldehyde thiosemicarbazone chitosan possess enhanced solubility and antimicrobial activities than bulk chitosan [57]. Being a polycationic polymer, it is unstable in variable pH since it is only dissolved in acidic condition due to protonation of its amine group and precipitates in basic condition due to de-protonation. So, the chemical stability is a challenging issue for use in agriculture [58].

Chitosan nanoparticle synthesis for agricultural application needs a large amount which is quite a sheared task. In this context, many researchers have reported the synthesis of ionic gelation to be more reliable method for large scale up synthesis for use in agriculture mainly for seed priming and foliar application [59, 60]. Further, various factors such as degree of polymerization, chitosan and tripolyphopshate mass ratio, pH, temperature and rate of mixing influenced the physicochemical properties such as size, shape, stability and yield of synthesized nanoparticles [61, 62]. Other methods of synthesizing nanoparticles have been comprehensively reviewed including its pros and cons by Kashyap et al. [27].

Controlled Release Formulations

Issue of global population coupled with global warming has threatened our food production and food security due to use of synthetic agrochemicals which have deleterious effects on environment and ecosystem. Many international organizations such as Codex Alimentarius, the Joint United Nations World Health Organization (WHO) and the Food Agriculture Organization (FAO), the European Union, and other influential organizations have set the permissible maximum residual levels of pesticides (MRLs) to regulate appropriate food quality standards [63]. In this context, chitosan-based nanoparticles can benefit from synthetic agrochemicals because on the basis of their biodegradability, they can escape any strict regulation of nanomaterial applications [64]. Roy and co-worker [65] reported that agrochemicals and pesticides reach the target site with as low as 0.1% while remaining are lost through leaching and evaporation leading to environmental hazards.

In the late 1980 s, the idea of microencapsulation emerged as research for addressing the issue of stability of carrier molecules and as an option to liposomes since these are unstable in biological solution [66]. In the following years research was focused on exploring dissimilar matrices for encapsulating and control release of active ingredients. To this, chitosan stand out to be the most versatile polymer for encapsulation application.The presence of functional amine groups can bind with versatile compounds Also, its flexibility to control our desired formation due to the presence of deacetylated surfaces and ability to form hydrogels, scaffolds,films, fibres, micro and nanoparticles are benefits associated with chitosan [67, 68].

Chitosan Matrix for Controlled Release and its Mechanism of Action

Control release can be defined as "the permeation-regulated transfer of an active ingredient from a reservoir to a targeted surface to maintain a predetermined concentration level for a specified period of time" [69]. A control release matrix can produce active ingredients which predetermined its concentration and sustain its release for a period of time. The highly regulated release behaviour associated with continuous release of chitosan encapsulated bioactive compounds improves bioavailability, mobility with accumulate long time in plant tissue. Their control release mechanism depends on the morphology, size, density and physicochemical characteristics and other factors such as pH, solubility and the enzymes present in the drug encapsulated inside the chitosan domain [70].

Thus, the sustained release of active ingredients aims to address the issues of excessive usage of synthetic chemicals and leaching loss by reducing adversities on environment. Two types of stimuli can induce the release of encapsulated chemicals: (1) Biotic stress such as pathogens and infestation of insects (2) Abiotic stress such as change in pH, temperature, salinity, drought, flooding [51, 71]. Several mechanisms of drug release from chitosan nanoparticles exist, such as chitosan polymer swelling, polymer diffusion, polymer erosion or degradation, or a combination of both [27] (Fig. 2). The drug penetrates through the polymer matrix to the surrounding medium in the diffusion-controlled release process. The polymer chains form the membrane barrier, which makes it difficult for the drug to cross this barrier, acting as the drug release rate limiting membrane. Polymer swelling happens as water is absorbed before the polymer dissolves and the release mechanism of the polymer is characterized by the

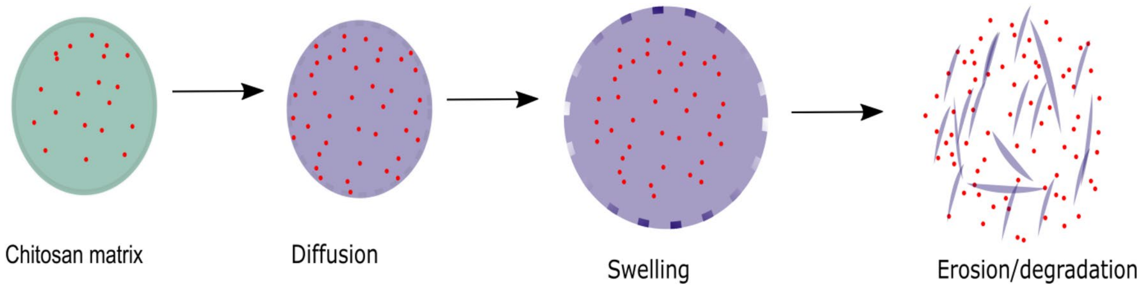

Fig. 2 Release mechanism of chitosan nanoparticles

solubility of the polymer in water or the surrounding biological medium. When the chitosan matrix meets the biological medium and swelling begins, the chitosan polymer chain begins to disengage, followed by the released drug. The hydrophilicity of the polymer, the rate of swelling and the density of the polymer chains usually affect the profile of drug release [72]. Simultaneously or separately, erosion and deterioration take place. Degradation of the polymer as a bond break can cause subsequent physical erosion. Swelling, diffusion and dissolution are involved. Polymer degradation depends on the pH of the surrounding medium, the form of drug encapsulated, and the shape and size of the nanoparticles [73]. Diffusion and degradation occur in chitosan. The initial burst of particles from the active ingredient is detected. This is due to the adsorption of active ingredients in the domain of chitosan. A gradual and steady release of ingredients until this initial burst is depleted. Due to the breaking of molecules that were bound to the surface of the chitosan domain, a rapid burst of agrochemicals (30 % spinosad and 75 % permethrin) was observed within 5 h [74]. The release kinetics of chitosan microspheres loaded with indomethacin were associated with the surrounding medium's chitosan concentration and pH 75]. Dynamic swelling of chitosan nanoparticle decreases with the increase in cross-linking [45]. Also, under different conditions such as pH, degree of cross-linking and polymeric compositions, Khan [76] studied the swelling mechanism of chitosan hydrogels. They found that with the rise in poly (vinyl alcohol) hydrogels at higher pH, increased swelling was seen. Also, crosslinking ratio was inversely proportional to the swelling of hydrogels. Martnex-Ruvalcaba and co-workers [77] described similar findings where regulated drug release increased with an increase in drug content, while drug release decreased as the crosslinking agent ratio increased in the structure of the hydrogel due to strong polymer interaction. Similar findings have been observed in particles of chitosan-polyvinyl alcohol (PVA) where drug release has been observed under various conditions [78].

Uptake and Translocation of Chitosan Based Nanoparticles

Nanoparticle's uptake takes place in two modes, namely foliar uptake and root uptake (Fig. 3). Many studies have reported that foliar uptake of nanoparticle is through cuticular and stomatal pathway. In cuticular mode of entry, it faces many challenges owing to the presence of waxy cuticle to prevent water loss and to prevent foreign solutes to pass through it. Uptake of solutes across the cuticle is generally limited due to its petite size ranging from 0.6–4.8 nm [16, 79]. The stomatal uptake of nanoparticles is generally the only evident pathway from leaf to the internal tissues. Many studies have observed the movement of nanoparticles through stomatal pathway including in *Allium porrum*, *Arabidopsis thaliana*, *Cucurbita pepo*, *Lactuca sativa* and *Citrullus lanatus* using CLSM or Micro-XRF and TEM [16, 80]. Stomatal aperture ranges from 3 to 10 micrometre in width and 25 micrometre in length [16], however, the actual size exclusion limit (SEL) of nanoparticle entry is not known in totality and is dependent on the species of plant, their leaf morphology, stomatal size and density. Nanoparticles may be transported through xylem or phloem. However, the exact mechanism of translocation of nanoparticles is not known in totality. Research done on watermelon where foliar application of nanoparticle, size ranging from 24–47nm was found to occur through stomatal pathway which was observed through TEM and translocated from shoot to roots via phloem sieve tubes [81]. Chitosan loaded with NPK nanoparticles of different sizes applied through foliar mode in wheat plants showed that nanoparticles were localized inside xylem and phloem as observed through HRTEM image [23]. Interestingly, another set of experiment applied with the same nanoformulation in bean plant after 30 days of its application reported that nanoparticles were localized in phloem and not in xylem tissue, using HRTEM image [82]. Foliar application of chitosan Zn nanoparticle in wheat plants has shown that nanoparticles enter through stomatal pore which is evident from the localization of Zn in the stomata region observed using FESEM and fluorescence

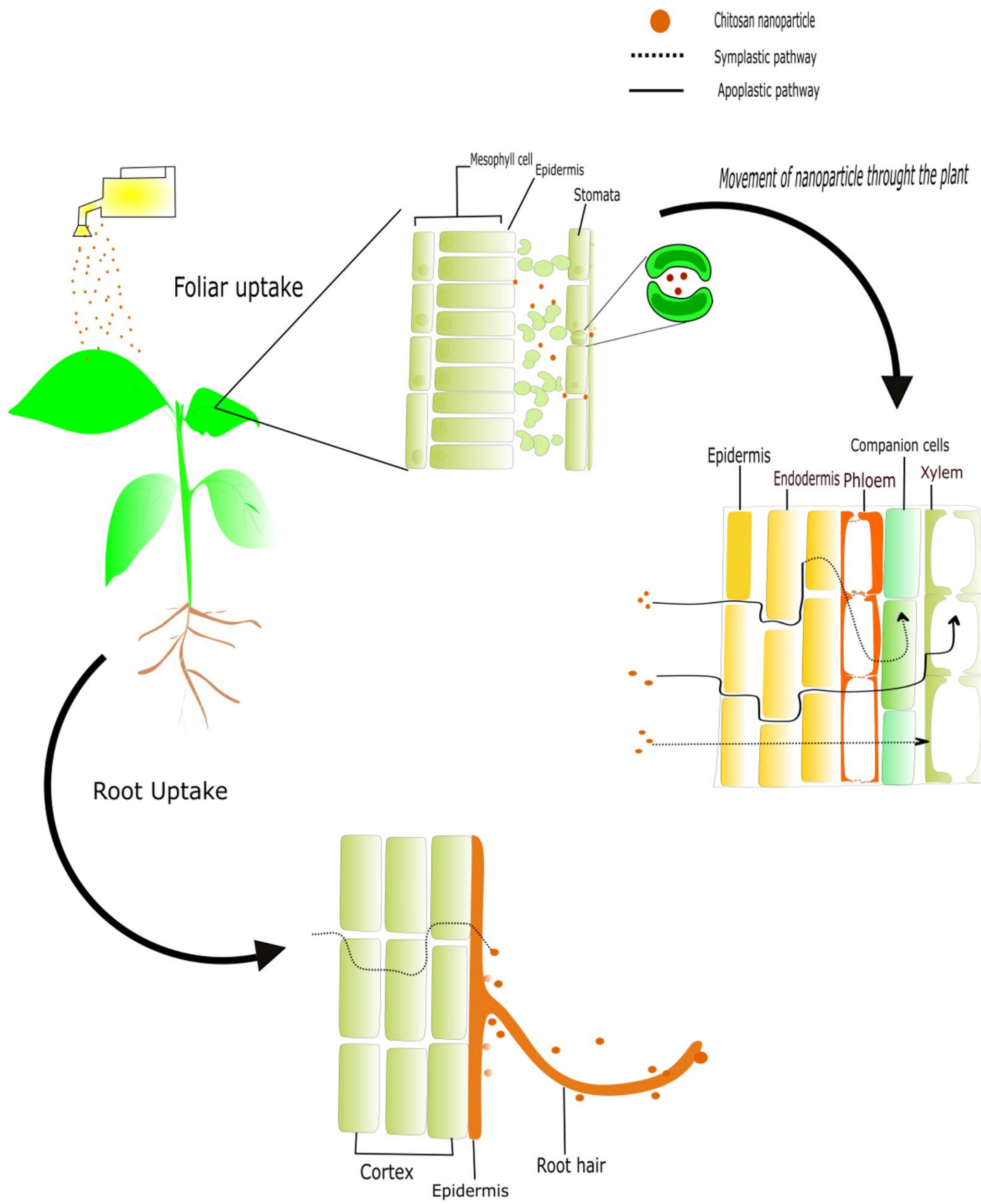

Fig. 3 Schematic diagram of chitosan nanoparticles uptake and translocation

microscopy. Also, high Zn content was observed in the embryo, endosperm and aleurone layer as detected through confocal laser scanning microscopy [83]. In root uptake, nanoparticle exhibiting net positive charge has potential to adsorb because root hairs exhibit highly negative charge due to presence of mucilage, organic acids and small molecules. Here, root cuticlar pathway is generally restricted as that of foliar pathway. Root epidermis provides nanoparticles to enter through apoplastic pathway as many studies have supported this claim inside apoplastic region by TEM or CLSM. e.g., ZnO nanoparticle size of 20 nm in rye grass [84] and 20–80 nm of Ag nanoparticle in *A. thaliana* [85].

Applications of Chitosan/Oligo-Chitosan/Nano-Chitosan in Regulating Abiotic Stress in Plants

Salinity stress

Salinity has a significant impact on the worldwide growth of plants. More than 20% of all agricultural land in the world is projected to have high salinity [86]. About 800 million hectares of arable land that is around 6% of the total land area is affected by salinity which results in negative effect on crop growth and development [87]. Salinity affects whole plant system both physiologically and biochemically inhibiting nutrient uptake and water upatke. Salt stress modulates biochemical reaction and accumulate reactive oxygen species (ROS) which disrupt cellular machinery and causes oxidative stress. Many studies have reported that salinity induced accumulation of MDA caused lipid peroxidation of cellular membrane. However, there are significant reports where chitosan or chitosan derivatives regulate and alleviate salt induced stress [3]. Chitosan treatments at low concentration were able to mitigate osmotic stress caused by salt stress in safflower (*Carthamus tinctorius*) and sunflower (*Helianthus annus* L.) [88]. In addition, there are reports of chitosan and oligo-chitosan treatment mitigating salt stress in wheat [89], chickpea [90], lentils [91], isagbol [92], ajowan [90], sunflower [88], fenugreek [93] and maize [94]. Previously, chitosan confering abiotic stress in different crops have concisely been reviewed [3]. However, recent reports have been updated in Table 1. Nanochitosan may be more successful in these crops because they have a higher surface area due to small particle size, greater adsorption ability, nontoxic and ability to encapsulate with other molecules with good encapsulation efficiency. Research done in bean plant (salt sensitive) shows improved seed germination when treated with chitosan nanoparticle at 0.1%, 0.2% and 0.3% under 100 mM salt concentration [86]. Nitric oxide encapsulated inside the chitosan domain has been shown to be more effective in combating salt stress in maize than free donor NO [95]; they reported increased bioavailability of NO in the plant, the encapsulated NO donor improved S-nitrosothiols content in leaf, enhanced level of photosynthetic rate (PSII) and chlorophyll content in all treated plants. Solid matrix priming with nanochitosan in mungbean seedlings alleviated the negative effect of salinity and improved growth,chlorophyll content and protein level of the plants [96]. Tomato plants treated with chitosan-polyvinyl alcohol hydrogels with or without copper nanoparticles subjected to salt stress were found to increase the expression of genes responsible for jasmonic acid (JA) and superoxide dismutase (SOD), which are essential for detoxification [97].

Drought Stress

Drought affects many aspects of plant development at morpho-physiological, biochemical and molecular level resulting in reduced growth and yields. Drought stress causes impaired chloroplasts, reduced chlorophyll content and enzyme activity involved in the calvin cycle of photosynthesis. It causes disruption in CO_2 intake due to closure of stomata thus leading to reduction of photosynthesis and plant growth [98]. However, chitosan induces stomatal closure and follows ABA-dependent pathway. ABA activity was known to induce stomatal closure and reduce transpiration [99] and mechanism behind chitosan induced stomatal closure is not known in totality. Chitosan treated bean leaves have shown to increase ABA activity leading to stomatal closure [100]. Similarly, foliar applied chitosan in pepper has antitranspirant activity and reduces water use by 26–43% via stomatal closure [99]. Similarly, antitranspirant activities were found in Bean (*Phaseolus vulgaris* L.) and barley (*Hordeum vulgare*) [101, 102]. Chitosan pretreatment enhanced the production of stress protective metabolites in white clover which resulted in alleviation of drought stress [103]. Foliar treatment with chitosan on *Thymus daenensis* alleviated drought stress without compromising on essential oil production and dry matter content [104]. Higher proline accumulation in plants indicate positive response which is responsible for stress adaptive mechanism and reduced water loss by lowering the leaf water potential. Many researches have reported chitosan induced higher level of proline content.e.g. in thyme plant [104] and safflower [105]. However, in castor bean (*Ricinus communis)*, it is reported that proline level has no affect [106]. Similar results were found in blackberry treated with oligo-chitosan where no significant level of proline was observed in treated and control plant [107]. Increase proline accumulation indicated plant stress but maintaining a constant level may also indicate plant's adaptation to stress. Thus, it is considered that chitosan treatment in different plant species follow different mechanisms. Chitosan induces several antioxidative enzymes and promotes plant growth. This is shown in apple seedlings where treatment with chitosan increased SOD, CAT and MDA activity thereby reducing lipid peroxidation and alleviating drought stress [108]. Chitosan also contributes to increased soluble sugar content in peas, sugar beets and black poplars [109]. These sugars such as glucose and fructose have potential to alleviate drought mitigation strategies through signal transduction, modulate stress response and increase growth and development. Also, chitosan treatment in white clover upregulated various stress related genes involved in carbohydrate transport and metabolism which have potential in mitigating drought stress [103]. Also, chlorophyll content and photosynthetic activity was reported to increase after chitosan treatment [110]. Additionally, chitosan application in apple explants grown on agar medium at 40 mg/L concentration was found to lower the deleterious effect of salt stress [111]. Chitin oligosaccharides treatment in maize, soybean and beans also found to increase photosynthesis level [112, 113].

Chitosan nanoparticles application in barley plant at 60 and 90 ppm concentration through soil and foliar modes of application resulted in reducing harmful effects of late season drought stress which is evident with the improvement in relative water content (RWC), plant growth and yield [114]. Application of nanochitosan through foliar application improved water status of plants in pearl millet subjected to salt stress by reducing stomatal conductance

Table 1 Recent reports of chitosan/oligo-chitosan regulating abiotic stress in some crops

Plant	Stress	Characteristic/method applied	Effect	References
Tomato (*Solanum lycopersicum* L.)	Salinity	Foliar	Increased morphological traits, photosynthetic pigments, osmolytes, total phenol and antioxidative activity	[145]
Wheat (*Triticum aestivum* L.)	Heavy metal	Low molecular weight, seed priming	Low concentration (0.1 & 0.25 mg/L) alleviated the damage caused by Al toxicity	[146]
Wheat (*Triticum aestivum* L.)	Heavy metal	DA: 80%, Seed treatment	1 kDa effectively mitigated the Cd toxicity through increased antioxidant activity, soluble protein and soluble sugar	[147]
Soybean (*Glycine max*)	Heavy metal	Seed, soil, foliar application	Increase antioxidative enzymes, proline, and physiological triats, lower nickel uptake and alleviate nickel toxicity	[148]
Maize*(Zea mays)*	Salinity	Soil application	Increased antioxidant activities and growth parameters, alleviated salt induced stress	[149]
Barley (*Hordeum vulgare* L.)	Drought	Soil application	In combination with biochar it increased morpho-physiological parameters, and antioxidant activities thus alleviating drought stress	[150]
Tea (*Camellia sinensis*)	Cold	Oligo-chitosan Foliar	Enhanced antioxidant activities, photosynthesis and carbon processes, activated genes related to stress signalling thus alleviating cold stress	[122]
Chrysanthemum (*Dendranthema grandiflorum*)	Drought	Oligo-chitosan: deacetylation > 95%, Foliar	Increased morphophysiological traits, antioxidant activities and higher expression of stress related genes leading to enhanced tolerance to drought stress	[151]
Potato (*Solanum tuberosum* L.)	Drought	Oligo-chitosan: 82.20 Chitosan: 337.73, Foliar	Chitosan (75 mg/L) and oligo-chitosan (50 mg/L) increased plant growth trait and defence mechanism thus mitigating drought stress	[152]
Safflower (*Carthamustinctorius* L.)	Salinity	Molecular weight: 50 kDa, media supplement	Enhanced production of secondary metabolites and alleviated salt induced stress	[153]
Marjoram (*Origanum majorana* L.)	Drought	Foliar	Increased morpho-physiological traits and antioxidant activities, higher expression of genes related to stress and oil composition	[154]
Milk thistle (*Silybum marianum* L.)	Drought	MW: 1526.5 g/mol, DA: 75–85%, viscosity: 20–300cP Foliar	Increased growth of morpho-physiological, yield and phytochemicals traits, increase in flavonoids (Silybin)	[155]

MW molecular weight; *DA* degree of acetylation

and transpiration [115]. Foliar application of chitosan nanoparticle in periwinkle (*Cartharanthus roseus*) resulted in mitigation of drought stress through increased proline accumulation and antioxidative activity. Also, it is reported to increase alkaloid content and activate gene responsible for defense enzyme production [116]. S-nitrosoglutathione, a NO donor, encapsulating chitosan nanoparticle has been shown to mitigate the negative impact of drought stress in sugarcane plants, which is obvious with a higher root biomass and higher photosynthetic rate as compared to those with free S-nitrosoglutathione treatment alone [117]. Also, study in wheat plant treated with chitosan nanoparticle through soil and foliar application at 90 ppm subjected to water deficit condition improved physiological and biochemical attributes of the plant [118]. Some of the recent reports utilizing chitosan nanoparticles in confering abiotic stress are listed in Table 2.

Temperature and Heavy Metal Stress

Extreme temperature and metal contaminated soil are affecting global agricultural scenario with the rapid change in global climate and rise in synthetic chemicals use thus depleting soil quality and health affecting global food production. Although research on the application of chitosan nanoparticles conferring tolerance against temperature and heavy metal stresses are few, there are various reports on the use of bulk chitosan. Priming with chitosan nanoparticle in maize seeds subjected to low temperature at 15 °C has shown to improve seedling parameters with lower mean germination time [119]. Recently, similar results were obtained where chitosan treated ball pepper (*Capsicum annum* L.) showed increased germination attributes at low temperature along with the increased activities of glucanase and chitinase enzymes which are stress defensive enzymes [120]. Oligo-chitosan treatment with different DP (Degree of polymerization) induced protection against chilling stress in wheat [121]. Similarly, in tea plant oligo-chitosan induced protection from cold stress through activation of genes related to antioxidant, photosynthesis and carbon metabolism [122]. Application of chitosan with varying molecular weight (5 kDa and 1 kDa) induced protection from cadmium toxicity in hydroponically grown edible rapeseed [123]. Also, bulk chitosan and zinc application in late sown dry bean plant (*Phaseolus vulgaris* L.) reduced the negative impact of heat stress [124]. Chitosan has proven to effectively form complexes with metal ions Pb(II), Cu(II) and Ag(I) in soil along with other mineral ions like Cl^-, K^+ and NO_3 (Kamari et al. 2011), due to the presence of amino and hydroxyl groups which are beneficial for phytoremediation and biofortification programmes [125, 126]. In this context, bulk chitosan or chitosan nanoparticle alone or encapsulated with other effective molecules known to induce defense enzymes or mitigate heavy metal stress/toxicity could be beneficial. Furthermore, chitosan nanoparticle could be more prominent than bulk form in relation to adsorption, solubility, translocation and bioactivity of encapsulated active ingredients with controlled release mechanism.

Mechanism of Action to Combat Abiotic Stresses

Plant abiotic stress offers different primary stimuli depending on the stresses induced. During signaling events, the primary signal induced by the specific receptor molecules activates secondary signalling cascade such as reactive oxygen species (ROS) and ionositol phosphate which are then transduced to release Ca^{2+} in the cells. This event causes phosphorylation driven alteration of specific protein or transcription factor regulating the expression of specific genes involved in stress response [127]. Multiple receptors that are then transduced to secondary events and downstream signaling cascades interpret the primary stress signal at various times and locations from the primary signaling site [127]. These secondary signalling events are exchanged between different pathways of stress response that provide plants with cross protection [127].

There are numerous reports citing chitosan exhibiting defense response to stress, however, the exact mechanism of action is not known fully. Plant responds to stress, either biotic or abiotic, through activation of defense related metabolites and activation of genes related to stress protection. When treated with chitosan plant sense stress signal since they perceived chitin-containing organism [128]. Receptor molecules for chitin binding have been identified in various crops [129], a glycoprotein (family of lectins) from mustard (*Brassica campestris*) [130], in *Arabidopsis*, using mutant shows chitosan can induce a receptor like kinase gene which can bind with chitosan, however, on contrary, in another research done by Povero [131], chitosan signalling is perceived independent of chitin elicitor receptor kinase. Thus, chitosan binding receptor is not elucidated clearly. Therefore, in these various pathways that communicate with each other, the involvement of a chitosan as an elicitor that evoked stress response for distinct abiotic stress mitigation could be more pronounced in nanosized chitosan.

The essential metabolic process within the plant, such as photosynthesis and protein synthesis are disrupted by extreme temperatures, drought and high salinity in the soil, resulting in reduced growth rate, crop growth and quality [86, 132]. Photosynthesis carbon fixation and redistribution are negatively affected by extreme temperature such as heat; also it disrupts the chloroplast functioning resulting in impaired electron transport chain [132]. In drought stress, plant responds by activating stomatal closure and decreased activity of photosynthetic enzymes such as ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) [132]. Also, to tolerate drought stress, plant responds by increasing drought responsive metabolites such as amino acids, sugars and polyols to regulate the turgor pressure [103]. Higher level of osmoprotective amino acid proline was found in barley plant treated with chitosan grown under water deficit conditon [114].

Salt stress affects the plant developmental process such as disruption in cell membrane, creating osmotic stress and accumulation of sodium (Na^+) and chloride (Cl^-) ions thus causing ionic imbalance. Plant's response through accumulation of stress protective metabolite like proline is an adaptive feature of salt stress [89]. Application of nanochitosan in maize has higher levels of organic compounds such as aldehydes, ketones, phenols, etc. that are defensive metabolites against stresses [37]. Additionally, plant response includes increased levels of antioxidants and antioxidative enzymes to confer protection against elevated level of ROS which are product of a broken electron transport chain, and expresses unique proteins known to have a defensive role against these stresses, such as late embryogenesis abundant protein (LEA) [100, 118]. Enhanced activities of antioxidants like catalase (CAT) and superoxide dismutase (SOD) enzymes are recorded when treated with nanochitosan in barley subjected to drought stress [114].

In addition, the application of chitosan has increased proline levels, which are stated to be correlated with enhanced proteinase enzyme activity [98]. Furthermore, application of chitosan was reported to induce abscisic acid (ABA)-dependent antitranspirant activity leading to stomatal closure [100]. ABA is known to induce stomatal closure upon stress stimuli and activates the defense related genes [132]. Similarly, oligo-chitosan induces gene expression related to auxin and gibberellin synthesis and activation of JA (jasmonate) and ET (ethylene) signalling pathway in rapeseed [133] which are defence response against biotic and abiotic stresses.

Interestingly, oligo-chitosan treatment in gravevine is reported to increase phenolic compounds such as anthocyanins which are responsible for protection against biotic

Table 2 Chitosan nanoparticles and its encapsulated molecules under abiotic stress

Nano-matrix	Stress	Crop	Characteristics	Effect	Reference
Chitosan + Nitric oxide	Salinity	Maize (*Zea mays*)	MD: 38.81 nm ZP: +17.7 mV PDI: 0.30 EE: 91.07%	Alleviated salt stress and increased plant growth	[95]
Chitosan/TPP + Nitric oxide	Drought	Sugarcane (*Saccharum officinarum* L.)	MD: 104.8nm PDI: 0.345 ZP: 17.5	Higher photosynthetic rate, high root/shoot ratio in water deficit condition and slow release of nitric oxide improved tolerance to water stress	[118]
Chitosan/TPP	Salinity	Mungbean (*Vigna radiata* L.)	MD: 254nm ZP: 103 mV PDI: 0.501	Overcome the adverse effect of salinity stress through increased antioxidant, increased content of chlorophyll and growth	[96]
Chitosan/TPP	Drought	Periwinkle (*Catharanthus roseus* L.)	–	Improved drought stress tolerance, Induced antioxidants activities, Increased alkaloid content and induced genes expression related to drought stress	[116]
Chitosan	Drought	Barley (*Hordeumvulgare* L.)	MD: >100nm	Significant increase in leaf area, chlorophyll, and number of grain spike and increased antioxidant activity, mitigated harmful effect of drought stress	[114]
Chitosan/TPP + Salicylic acid	Heavy metal	*Isatis cappadoci-ca* Desv	–	Increased Root length, shoot height and biomass under arsenic toxicity	[156]
Chitosan/TPP + NAC(N-acetyl cysteine)	Ozone	Durum wheat (*Triticum durum* L.)	MD: 178 PDI:0.397 ZP: 47.2	Increased level of antioxidant activity, Ascorbic content, triggered plant defence against ozone stress	[157]
Chitosan Selenium nanoparticle (Cs-Se-Np)	Salinity	Bitter melon (*Momordica charantia*)	DA: 75–85% MW: 310–375	Increased antioxidative activity, proline, relative water content, K^+, decreasing MDA, H_2O_2 and Na aggregation, increased yield and essential oil	[158]

MD mean diameter; *DA* degree of acetylation; *MW* molecular weight; *ZP* zeta potential; *PDI* polydisperity index; *EE* encapsulation effeciency

and abiotic stress [134]. Similarly, oligo-chitosan treatment in blackberry (*Robbus* spp.) increase total soluble phenolic content. Also, treatment with chitosan in ball pepper (*Capsicum annuum* L.) is reported to increase chitinase and glucanase activities in both seed and seedling [120]. Upregulation of genes for phenolic biosynthesis after chitosan treatment has been reported previously [135, 136]. In addition, enhanced level of carbon and nitrogen metabolism was found in wheat treated with chitosan [137]. Such involvement of protective secondary metabolites biosynthesis has potential role in resilience to biotic and abiotic stress [107]. Chitosan and its oligosacharides also induce growth and development in gravevine [138], orchid [139], bean [126, 140], potato [141] and wheat [142]. Additionally, chitosan nanoparticle stimulates plant growth and yield of plants such as wheat [143], coffee [38], chilli [144] and maize [37]. Some of the recent reports on chitosan nanoparticle with or without encapsulation regulating growth and development are listed in Table 3.

Chitosan Based Biogenic Nanoparticles

Biogeneic synthesis of nanoparticle is a new approach which is gaining attention due to its low toxicity as compared to chemical synthesis. Biological agents such as plant extract and microbes have been used for synthesis of nanoparticles. Also, plant extracts provide an excellent stabilizing ability and both reducing and capping agents for synthesis of nanoparticles [168]. Also, chitosan acts as an excellent carrier molecules and fabrication flexibility for incorporation of biogenic nanoparticles. For example Chitosan based nano-composite films using biogenic silver (Ag) nanoparticles obtained from *Nigellea sativa* extract exhibit controlled released of Ag^+ ions and improved antimicrobial and antioxidative potential of the film [169]. Also, chitosan based iron nanoparticle incorporating *Moringa oleifera* leaves exhibits improved seed germination and growth parameters in corn [170]. Moreover, chitosan nanoparticle incorporating green tomato fruit extracts exhibits strong antibacterial

Table 3 Chitosan nanoparticles and their encapsulated molecules regulating plant growth and development

Nano-matrix	Crop	Characteristics	Benefits	Reference
Chitosan/TPP + Salicylic acid	Wheat (*Triticum aestivum* L.)	MD: 368.7 ZP: +34.1mV PDI: 0.01	Enhanced antioxidant, photosynthetic activities, and promoted grain weight	[22]
Chitosan/TPP + Zinc	Wheat (*Triticum aestivum* L.)	MD: 250–300 nm ZP: +42.34 mV PDI: 0.28 EE: 10–40%	Enriched zinc content in grain as compared to conventional micronutrient fertilizer	[83]
Chitosan/TPP+ Salicylic acid	Lettuce (*Lactuca sativa)*	MD: 1.57–2.45 µm PDI: 0.08–0.22 EE: 59–99%	Induced defense related protein and improved growth	[159]
Chitosan/TPP + Thiamine	Chickpea (*Cicer arietinum*L.)	MD: 596 nm ZP: +37.7 mV PDI b1 EE: 90%	Improved germination and seedling vigor, higher IAA level and defense related enzymes	[160]
Chitosan/alginate + Gibberellic acid	Tomato (*Solanum lycopersicum L.*)	MD: 450 nm ZP: −29 PDI: 0.3 EE: 100%	Significant observation on growth and development	[161, 162]
Chitosan/y-polyglutamic acid + Gibberellic acid	Common bean (*Phaseolus vulgaris* L.)	MS: 134 nm ZP − 27.9 mV PDI 0.35 EE: 61%	Improved germination of seeds, root growth and leaf area and avoidance of degradation of GA3	[162]
Chitosan/poly-methacrylic acid + NPK	Wheat (*Triticum aestivum* L.)	–	Increased in harvest index, crop index, and mobilization index subjected to sandy soil	[24]
Chitosan/TPP + NPK	Coffee (*Coffea canephora* var. Robusta)	MD: 500 nm ZP: 50 mV	The nanoparticles showed 240 h of sustainable release of NPK, enhanced photosynthetic capacity and growth parameters.	[163]
Chitosan/TPP	Robusta coffee (*Coffea canephoraPiere* var. Robusta)	MW:600 kDa, DA: 85%, pH 6.0 MD: 420, 750 and 970 nm	Increased chlorophyll content by 30–50 %,Improved nutrient uptake by 10–27 % N, 17–30 % P, 30–45 % K and photosynthesis rate (30 %).	[38]
Chitosan/TPP inonic	Chilli (*Capsicum annum* L.)	MW: 110 kDa, DA: 85–90%, PDI: 0.416 MD: 163 nm a, ZP: +60.4 mV	Increase of 77 % and 28 % in total fresh root and leaf mass, boosts catalase and peroxidase activities by 33 % and 23 %, respectively	[144]
Chitosan/TPP	Bean (*Phaseolus vulgaris* L.)	MW:100–399 kDa MD: 46 nm PDI: <1	Enhanced seed germination (123 % after 72 h) and radical length (231 % after 72 h) under salinity stress.	[86]
Chitosan/TPP	Maize (*Zea mays*)	MD: 80–100 nm d	Improved seed germination, plant height, and leaf area by 2 folds	[37]

Table 3 (continued)

Nano-matrix	Crop	Characteristics	Benefits	Reference
Chitosan + *Penicillium oxalicum* (Extra cellular protein)	Chickpea (*Cicer arietinum*)	MD: 10–30 nm , ZP: – 37 mV pH 4.8	Increased germination, seedling vigor index and vegetative biomass of seedlings by 3-folds.	[164]
NPK	Wheat (*Triticum aestivum*)	Observed inside the sieve tubes shows mean diameter of 26 and 31 nm b 20 nm b	Improved harvest index (24 %), crop yield (59 %), and mobilization index (42 %). Boost polysaccharides (10 %) and total saccharides (11 %).	[23]
Chitosan-polymethacrylic acid (CS-PMAA)	Bean (*Phaseolus vulgaris* L.)	MD: 20 nm	Increased plant growth, nutrient uptake, and biomass accumulation.	[82]
Chitosan-polymethacrylic acid (CS-PMAA) + NPK	Pea (*Pisum sativum*)	MD: 20 nm	Induced mitotic cell division (1.5 fold) and enhance of total soluble protein	[165]
Chitosan/TPP + Copper	Maize (*Zea mays*)	MD: 150 nm ZP: +22.6 mV PDI: 0.33 MW: low MW DA: 80%	Enhanced activity of alpha amylase and protease and encouraged the growth of seedlings.	[143]
Chitosan/TPP + Copper	Maize (*Zea mays*)	MW: 50–190 kDa, DA: 80% MD: 361.3 nm a, ZP: +22.1 mV PDI: 0.20	Increased seedling parameter, plant height, stem diameter, and root length	[6]
Chitosan/TPP + Zinc	Wheat (*Triticum durum* L.)	MD: 325 nm a, PDI: +42.3 mV MW: 60 kDa DA: 85%	Observed stomatal localization of nanoparticles. Up to 42 % increased zinc content in grain	[83]
Chitosan-polyglutamic acid + Gibberellic acid	Common bean (*Phaseolus vulgaris*L.)	MW: 290 kDa DA: 75–85% MD:134 nm a PDI: 0.35 ZP: 27.9 mV EE: 61%	Enhanced seed germination, leaf area and root development	[162]
Chitosan/TPP + Gibberellic acid	Bean (*Phaseolus vulgaris* L.)	MW: 27 kDa DA:75–85% pH 4.5 MD: 450nm ZP: -29.0 mV EE: 90 and 100%	Increased leaf area, chlorophyll and carotenoids amount Nanoformulation provided stability up to 60 days	[166]
Chitosan/TPP + Thiamine	Chickpea (*Cicer arietinum*)	MW: 27 kDa DA: 85% MD: 596 nm a ZP: +37.7 mV EE: 90%	Increased seed germination and induced defense related enzymes; Alsoa 10-fold increase in auxin level compared to the untreated seeds.	[160]

Table 3 (continued)

Nano-matrix	Crop	Characteristics	Benefits	Reference
Chitosan-polyvinyl alcohol hydrogels (Cs-PVA) + Copper	Tomato (*Solanum tuberosum* L.)	MD: 20nm	Enhanced expression of genes related to JA and SA, alleviated salt induced stress through regulation of oxidative and ionic stress	[167]

MD mean diameter; *DA* degree of acetylation; *MW* molecular weight; *ZP* zeta potential; *PDI* polydispersity index; *EE* encapsulation effeciency

activity against *Xanthomonas oryzae* pv. *Oryzae* [171]. Most of the applications of chitosan based biogenic nanoparticles in agriculture have focused on conferring tolerance biotic stress due to their immense antimicrobial potential and sustainability. However, application on conferring tolerance against different abiotic stresses would provide an overall mechanistic outlook of its potential for combined delivery in agriculture.

Conclusion and Future Perspectives

The preparation of chitosan based nanoformulations and their various applications related to plant growth and nutrition, their uptake mechanism and protection from abiotic stresses were outlined in this review. It can be inferred that nanostructured chitosans can be used as carriers of various bioactive ingredients owing to their ability to be carriers of encapsulation or immobilization.These are also interesting choices as drug delivery carriers and as growth and defense inducers in plants due to their favorable biological properties such as nontoxicity, biocompatibility, biodegradability and broad antimicrobial capability. Although the potential of chitosan-based nanoformulation and nano chitosan alone are well documented in many published literature, there are dearth of information related to abiotic stress management. It is still early to draw conclusions about chitosan based agronanochemicals and bioactive agents in plant's response to biotic and abiotic stress since data from real field condition is not known in totality. In fact, it was evident from laboratory study that chitosan based nanoformulations have good controlled release behaviour and long stability of bioactive compounds encapsulated inside chitosan nanoparticle, and have prosperous future for plant application. In order to allow the modification of chitosan-based nanoformulation with the desired properties, it is important to consider the physicochemical properties of chitosan nanoformulation such as molecular weight, degree of acetylation, compound to be encapsulated and other variables depending on the method of preparation, since these can greatly affect the bioactivity of plant. Chitosan is known to be nontoxic; however, nanosized chitosan and its encapsulated compounds needs to be carefully considered since its enhanced ability could accumulate in non-targeted site posing threat to environment and health issues. Further, holistic evaluations of the effects of these factors on morpho-physiological and at molecular level are, therefore, needed. In addition, more information is required to establish the mechanism of chitosan-based nanoformulation in a plant, where size of the particle, its morphology, solubility, concentration and other physico-chemical characteristics will determine the uptake and translocation. Also, to evaluate the actual movement of nanoparticle inside plant's system should be area of study of research in future.

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Conflict of interest The authors declare no conflict of interest.

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