# Polymer-Based Nanoparticles (NPs): A Promising Approach for Crop Productivity

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**Abstract** Recently, the production of nanoparticles (NPs) has become a new and pioneering approach that can be exploited in several areas such as medicine, agriculture, cosmetics, engineering, and environmental fields. The wide applicability is due to their particular physicochemical properties, like small size with the big superficial area, and high electronic properties. Regarding agricultural fields, different kinds of NPs were applied. Among the successful nanomaterials that were used to promote plant growth and productivity of crops, there are polymer-based NPs (i.e., polysaccharide NPs, protein NPs, and lipid NPs). These nanomaterials were used as a plant growth promoter and nanostructured carriers of antioxidants, phytohormones, fertilizers (NPK), volatile organic compounds, and agrochemicals (herbicides, fungicides, and so on). Although there has been remarkable progress in the development of polymer-based NPs to induce vegetative growth and the quality of fruits and vegetables as well as to cope with environmental stresses, their mechanisms remain unclear. Therefore, the present chapter will address three important

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points: (1) the effects of polymer-based NPs on plant physiology and fruits, (2) their mechanisms in protecting plants from biotic and abiotic stress, and (3) how they affect beneficial microorganisms (arbuscular mycorrhizal fungi and rhizobacteria). The application of NPs in sustainable agriculture is a promising solution instead of agrichemicals, which are unsafe to the ecosystem. The investigation of this novel approach could be an efficient application in plant pathology and crop productivity.

**Keywords** Sustainable agriculture · Nanoparticles (NPs) · Crop production · Plant protection

## 1 Introduction

Nanotechnology has been defined for the first time by the US Environmental Protection Agency [1] as the discipline of studying and managing either substances, atom arrangement, or procedures that function at a scale less than $10^{-7}$ m, where unique physical properties make novel applications possible [2]. This science is a pioneering field of interdisciplinary research that includes several fields such as physics, electronics, chemistry, biology, and medicine. In the nonagricultural field, this term is related to materials science and biomass conversion technologies, by applying colloidal particles between 10 and 1000 nm in size [3]. In addition, it has found several uses in the agricultural fields, such as nanofertilizers, nanopesticides, nanobiosensors, or as environmental remediation agents [4]. The application of nanomaterials (NMs) in agronomy wants to substitute the use of chemical substances by smart delivery of compounds, to reduce the depletion and the loss of fertilizers' nutrients, and to raise yields by providing an adequate amount of water and nutrients [5]. Recently, with the circumstances of climate change such as high temperature, drought, lack of arable land, salinity, water scarcity, frequent flash floods, urbanization, and high demand for food, the application of nanotechnology for the agricultural countries could be a good solution to increase the farm productivity, to control the nutritional needs of the plant, and to minimize the use of fertilizers with the costs of agricultural production and environmental pollution [6]. In the NMs, particle dimension and their form, pore volume, and raggedness are physical characteristics that precise the surface-to-volume ratio, which in turn influences other features especially the charge or the fee energy per unit area [7]. Recently, the manufacture and creation of nanoparticles (NPs) have grabbed considerable attention because of their faster impact with a lower dose, their faster penetration through membranes, and their great potential in the different application fields, especially agriculture [8]. Nowadays, enormous innovative NP products were produced. Some of them were synthesized naturally by plants or microbial species (i.e., *Limnothrix* sp. and *Lyngbya majuscula*), such as gold and silver NPs, while others were developed by a human for multiple uses [9–11]. Three kinds of NPs (i.e., metal, polymeric, and semiconductor NPs) have been engineered to ensure a slow release of fertilizers, phytohormones, and active compounds, to encapsulate microorganisms, to trigger signals, as well as to increase plant growth, its development, its resistance against phytopathogens, and the yield [12–15]. Polymeric NPs are particles made from a class of multitalented materials such as poly-sugars (i.e., alginate, starch), polypeptides (i.e., gelatin), or lipids (i.e., beeswax) [16]. These materials have been extensively used in agriculture because of their diverse features like biodegradability, not a hazard to the environment, biocompatibility, as well as adsorbing ions [17]. Moreover, polymer-based NPs have additional features, essentially large surface area, high reaction activity, and surface modifiability, which allow them to be used for several purposes than raw material or metallic and semiconductor NPs [18, 19]. Recently, different methods were developed for the production of polymeric NPs depending on the type of polymer used, its molecular weight, its dose, and the goal of their application [20]. Ultimately, they have been appraised as a crucial element for the development of sustainable agricultural practices due to their positive impact on the environment as well as their beneficial outcome on many fruits and vegetables; however, in some cases, their application shows neutral or negative effects depending on plant species, application mode, and soil composition. The purpose of this chapter is to present the latest advances in the production of polymer-based NPs as promising approaches of nanotechnology, as well as the major results regarding their application on plants' and fruits' production.

## 2 Polymer NPs: Types and Preparation Methods

The polymeric NPs could be nanocapsules or nanospheres in shape; this difference was explained in the study of Yoo and Park [21] where they mentioned that the nanocapsules are characterized with a polymeric cavity structure and inner hydrophobic phase (Fig. 11.1a), while nanospheres are described as an organized solid matrix with the polymeric chains (Fig. 11.1b). Several kinds of natural or synthetic

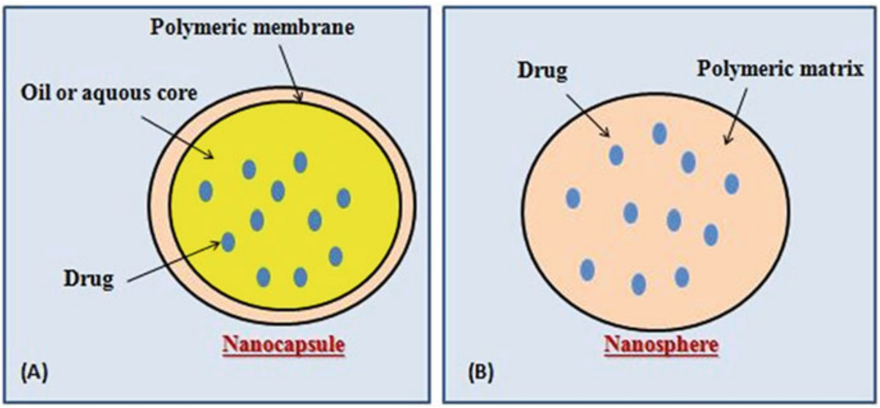


**Fig. 11.1** Schematic illustration of (**a**) nanospheres and (**b**) nanocarpsules applied in agriculture as polymeric NPs

polymers could be a source of polymer-based nanoparticles (NPs) by top-down or bottom-up methods.

The most functional natural nanopolymers that are widely used in agriculture are chitosan, alginate, pectin, cellulose, starch, lignin, polyaspartic acid, and beeswax [22–24]. Their use is related to their low cost, simple and mild preparation methods, stiffness, eco-friendliness, and low toxicity, and they can be applied by spray or as a soil drench.

## *2.1 Chitosan NPs*

The *N*-deacetylation of chitin leads to obtaining chitosan, a natural and plenty polysaccharide worldwide. This biopolymer can be derived from different sources like the exoskeleton of crustaceans [25], fish scales [26], insects [27], and fungi [28]. The deacetylation of chitin could be chemically or biologically. The study of Ohya et al. [29] gave rise to chitosan NPs for the delivery of antineoplastic agents like 5-fluorouracil. Over the years, researchers have developed new methods based on varied parameters such as stability retention time, size, and drug-loading capacity [30]. Chitosan NPs could be obtained using various methods such as emulsification; precipitation, in addition to ionic or covalent crosslinking; or amalgamation between the two last methods. The primary method reported for the formulation of chitosan NPs is emulsification and crosslinking based on the interaction between an aldehyde group of the crosslinker and the amino group of chitosan. To carry out this method, it is essential to prepare an emulsion formed from a mixture between an aqueous solution and an oily phase. In the study of Jameela et al. [31], glutaraldehyde crosslinker agent is mixed with toluene, and Span 80 is used as a stabilizer. Briefly, after homogenization of the phases intensively, NP beads were formed after crosslinking. Centrifugation could be used to separate easily NPs from the emulsion with multiple washing steps. Finally, the obtained NPs undergo vacuum or freeze-drying (Fig. 11.2).

The physicochemical features of chitosan play an important role in the precipitation method, i.e., the non-solubility of this biopolymer in alkaline media will facilitate the formation of precipitates. In brief, the realization of this method requires the preparation of an alkaline solution from which the chitosan will be blown using a compressed air nozzle forming droplets, followed by filtration or centrifugation to separate, filter, and purify the particles. Finally, the obtained particles undergo intensive washing by altering between hot and cold water as shown in Fig. 11.3.

The principle of the ionic crosslinking method depended on the conglomeration of chitosan with negatively charged macromolecules or the presence of a crosslinking agent. The most common crosslinker used in this method is tripolyphosphate. This method is recognized as the ionic gelation process because there is a formation of gels due to ionic linkage as highlighted in Fig. 11.4.

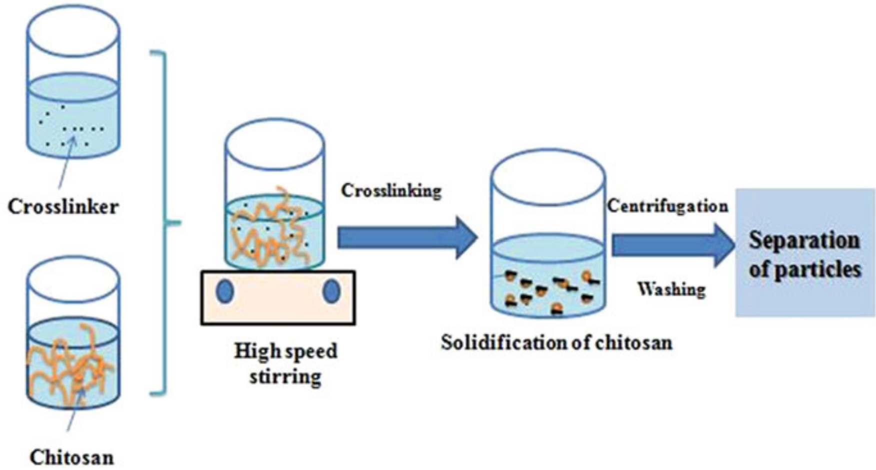


**Fig. 11.2** Preparation of chitosan NPs by emulsification and crosslinking method

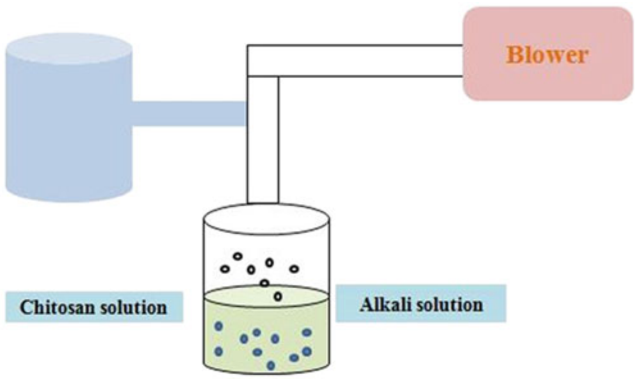


**Fig. 11.3** Chitosan NPs' preparation by precipitation method

Chitosan NPs play an important role as nanocarriers that ameliorate the stability and the delivery of active compounds [32]. The application of chitosan in agriculture as NPs is advantageous because a small dose has a great effect. In addition, its biological properties give them the possibility of being applied without any risk to humans or the environment [33].

## *2.2 Alginate NPs*

Alginate is a natural hydrophilic polysaccharide extracted from the rigid layer of various species of brown algae. Alginate is characterized by a linear block polymer

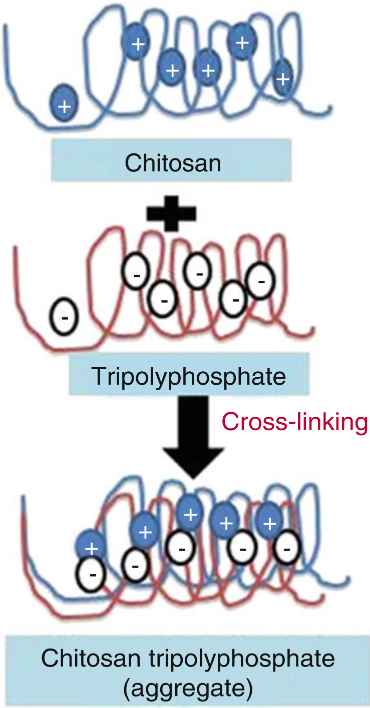


**Fig. 11.4** Chitosan NPs' preparation by crosslinking method

of β-D-mannuronate and α-L-guluronate units; their rate will influence its physical characteristics and orients its industrial application [34]. Alginate (NPs) could be found as alginate nano-aggregates or alginate nanocapsules. The complexation of alginate is easily done by adding a crosslinker agent like calcium from calcium chloride or by mixing it with chitosan as polyelectrolyte [35]. The other method for the preparation of nano-alginate is based on adding alginate to an oil emulsion solution [36].

## 2.3 *Pectin NPs*

Pectin is a biocompatible and negatively charged polysaccharide that consists of α-D-galactopyranosyluronate units [37]. Several studies have shown the possibility of the formation of NPs based on pectin. Nanospheres of pectin were formed by adding $Ca^{2+}$ and $CO_3^{2-}$ ions [38] or glutaraldehyde as crosslinking agents [39]. In the agricultural field, calcium pectinate NPs were formulated as water reservoir, and their application could resolve the problem of scarcity of water in drought-stricken countries [40].

## 2.4 Cellulose and Starch NPs

Cellulose is the largest ubiquitous polysaccharide in the world. This linear polysaccharide is a repetition of glucose units linked with β-D-(1 → 4) linkages. Starch is widely spread in plant tissues, as glucose units linked with α-D-(1 → 4) and/or α-D-(1 → 6) linkages [37]. According to the literature, these two famous polysaccharides have shown their feasibility of being in the form of NPs in the agricultural field. The study of Zhang et al. [41] highlighted a facile synthesis of cellulose NPs with high yield. In addition, many studies reported the preparation of cellulose NPs as nanowhiskers [42–44], i.e., the study of Gu et al. [45] mentioned a simple method of preparation of cellulose NPs via an ultrasound-assisted etherification and a subsequent sonication process. On the other side, the study of Hasanin [46] reported the possibility of the extraction of starch NPs from potato peel waste via simple alkali extraction followed by ultrasonic treatment.

## 2.5 Lignin NPs

After cellulose, lignin is the most spread and renewable organic material on the earth extracted from biomass [47]. This biopolymer is characterized by its irregularity and complexity, containing three phenylpropanoid compounds (sinapyl, coniferyl, and coumaryl) [48]. The insolubility property of lignin in water restricts its applications. However, recently some papers have been shown the possibility of preparing aqueous lignin NP dispersions [49, 50], and it has also been reported to have the ability to improve the water absorption of materials [22]. Spherical lignin NPs were prepared using a simple method by dissolving the non-hardwood in tetrahydrofuran [51]. In addition, Yearla and Padmasree [52] have fabricated dioxane lignin NPs by nanoprecipitation method from the solid wood dioxane lignin and nonsolid wood alkali lignin.

## 2.6 Polyaspartate NPs

Polyaspartate (PASP) is a biodegradable functional and hydrophilic material. This biopolymer is often used in wastewater treatment due to its high potential of metal chelating via the carboxyl and amino groups [53]. PASP is developed as a nanoprobe for the detection of iron ions [54]. In addition, another study highlighted a new nonstoichiometric polyelectrolyte complex NPs based on chitosan and PASP sodium salt [55].

### 2.7 Beeswax NPs

In 1960, gas-liquid chromatography determined the components of beeswax [56]; this material as other lipids formed from a mixture of several classes of components composed from a series of substances with a long chain varying by two carbon atoms. Beeswax NPs are considered solid lipid NPs (SLNs); these kinds of NPs were introduced in 1991. Their good tolerability, biodegradability, physical stability, macroscale production efficiency, and feasibility to include in their lipid core the lipophilic drugs have attracted big attention in different fields. In the same sense, a study focused on the feasibility of preparing SLN by employing beeswax and carnauba wax to incorporate ketoprofen [57].

## 3 Effects of Polymer-Based NPs on Plants

### 3.1 Application of NPs as a Growth Promoter

Plant growth promoters are all substances produced by plants to regulate their biological processes and to ensure their safety. Even though a little amount of these substances trigger major plant physiological and biochemical changes, in some cases, they can have a neutral or an inhibitory effect, depending on the target part of the plant and the moment of their biosynthesis. Therefore, an exogenous application of natural or synthetic plant growth promoters at the most suitable growth stages will ensure rapid growth [58].

Few reports showed that polymer-based NPs can act as a plant growth promoter and induce seed germination and vegetative growth [59]. For instance, chitosan NPs promote the growth of biophysical properties of coffee plants in greenhouse conditions by increasing the pigment content, mineral and water assimilation, and respiration rate [60]. Also, a low dose of chitosan NPs increases the germination and the growth of wheat compared to bulk chitosan [61]. Due to the different properties of NPs, their interaction with the plant allows morphological, genetic, and physiological changes. Several factors can affect the NPs' effect on the plant such as surface covering, chemical composition, and reactivity. Regarding alginate NPs, the study of Sharma et al. [62] showed that these NPs play an important role as water reservoirs, which mixing them with plant's soil exhibits better growth compared with the control. This study proved that alginate NPs could be a good material to ensure agricultural sustainability due to their high water retention capacity. For pectin NPs, the study of Abdelrahman et al. [63] depicted large information about the stimuli-responsive formulation made from mesoporous silica NPs-$NH_2$-Pectin, a delivery system for agrochemicals into plant cells. In addition, nanocellulose is known also to be applied in agriculture due to its surface dimension, high width-to-length rate, great transparency, and high toughness [64]. In the same sense, cellulose NPs can also be used as a nanocarrier system of macronutrients (i.e., nitrogen) and to release slowly for 2 months [65]. Moreover, lignin NPs (LNPs) have been successfully applied in the agricultural field as biostimulants of maize [66]. It has been found that the maize seeds treated with LNP revealed important physiological and biochemical responses such as an increase in seed germination rate, radical length, and the content of photosynthetic pigments and flavonoids. The benefic effect of nano-biopolymers in the agricultural field is related to the high potential of those biomaterial properties including mechanical, thermal, barrier, and physicochemical properties [67]. As reported in the literature, few experiments have studied polymeric NPs' effects on plants grown in normal conditions (without stress or biostimulants presence). Therefore, further studies should shed light on the beneficial and adverse effects of NPs on cell growth, metabolic responses, and gene expression.

## *3.2 Application of NPs for Controlling Environmental Stresses*

Plants are sessile living organisms, which makes them very sensitive to sudden and slow changes in their living environment. These adverse environmental conditions are coming from living and nonliving factors of an ecosystem such as fungi, herbivores, bacteria, insects, salinity, drought, heavy metal, as well as low and high temperatures. These stresses could affect the different aspects of the plant through disruption of metabolic pathways and physiological and biochemical activities as well as the induction of cell dysfunction [68]. These changes vary depending not only on the type and period of stress but also on the stage of the plant [69]. To sustain life and to overcome the adverse effects of stress, plants must evolve a wide range of defense mechanisms such as reducing the stomatal aperture, producing phytohormones, changing gene expression, accumulating osmolytes, and scavenging the ROS system [70–73]. These mechanisms might improve plant tolerance against environmental constraints and protect cells from oxidative damages [74]. Nevertheless, when stress is severe and unmanageable by plants, an effective approach must be taken into consideration to control stress. The application of nanotechnology techniques has already been considered, in regard to improving the growth and protection of plants. It has been proven that NPs acting as biofertilizers, biopesticides, or microbicides could help plants deal with different environmental stresses and improve their productivity [75–77]. Moreover, the application of NPs under environmental stresses has a regulatory effect on plants [78–80]. The positive effect of NPs has been observed on several plants such as broad bean [81], rice [82], tomato [83], wheat [84], cowpea [85], and cumin [86]. Numerous investigations exhibited that the impact of NPs on plants could be phytotoxic [87, 88] depending on the nature and dose of NPs [89]. Nevertheless, polymer-based NPs which are known for their low toxicity would be the promising approach that could maintain green agriculture [90]. Chitosan-based NPs are bio-elicitors and nontoxic with high permeability and high film-forming capacity [91], which possess various physicochemical characteristics and biological activities. To date, there are only a few reports that showed the effect of polysaccharide NPs on plant defense and protection. Thus, in this section, we will review the effects of chitosan NPs as an eco-friendly mechanism which enhance plant tolerance to some environmental stresses such as salt, drought, heavy metal, and biotic stress.

### 3.2.1 Role of Chitosan-Based NPs in Plants Exposed to Salt Stress

Salinity is regarded as one of the major nonliving factors hindering plant growth and productivity [92]. The excess of $Na^+$ and $Cl^-$ ions in irrigation water or soil could cause many metabolic disorders such as oxidative stress, nutrient imbalance, and ionic toxicity [93]. Thus, chitosan NPs' application has shown great potential in mitigating adverse effects of salt stress (Table 11.1).

For example, Sheikhalipour et al. [95] suggested that the treatment with 20 mg $L^{-1}$ of selenium functionalized using chitosan NPs enhances the shoot height (19%), root length (13%), leaves' fresh weight (16%), root fresh weight (12%), leaves' dry weight (24%), and root dry weight (13%) under 100 mM of salt stress, compared to control. The growth-promoting effect of chitosan-based NPs might be due to the stimulation of the biosynthesis of some phytohormones such as auxin and gibberellins which are involved in plant growth [110]. Also, Sen et al. [96] showed that proline, sugar, and protein content were substantially increased by the application of nano-chitosan under salt stress. The accumulation of osmoprotectant compounds in the cytoplasm plays a critical role in osmotic adjustment in plants (Fig. 11.5). This accumulation is maybe due to the increase in the synthesis of these compounds or reduction of their degradation in response to saline conditions. Analogous responses have been denoted by Hassan et al. [98], which added that the increase in the number of osmoprotectant compounds is mainly due to the increase in the level of both enzymatic antioxidants, such as glutathione S-transferase (GST), and nonenzymatic antioxidants (phenolic compounds, ascorbate (ASA), carotenoids, flavonoids, and glutathione (GSH)) (Fig. 11.5). Furthermore, the use of chitosan NPs induced the expression of superoxide dismutase (SOD) and jasmonic acid genes, which activate the antioxidant system [111]. In this way, the unpleasant effects of saline stress could be decreased or avoided.

### 3.2.2 Effect of Chitosan-Based NPs in Plants Exposed to Drought Stress

Drought stress is the major environmental problem that humanity is facing today. During water deficiency, closing stomata is the first reaction done by the plant to limit water loss. But, if it lasts for a long time, it could drastically reduce agricultural yield by more than 50% [112]. Overall, drought stress could affect the morphological, physiological, and nutritional characteristics of plants [113]. The use of chitosan-based NPs can mitigate its adverse effects (Table 11.1 and Fig. 11.5). Leung and Giraudat [114] reported that chitosan application could induce hydrogen

**Table 11.1** Different results regarding the nano-chitosan impact on plant growth and its metabolism under environmental stresses

| Plant species | Polymer-based NP treatments | Type of stress | Impacts | References |
|---|---|---|---|---|
| Maize (*Zea mays* L.) | Nitrogen monoxide delivering chitosan NPs | Salt stress | –Amelioration of Photosystem II activity, chlorophyll content, and plant growth | [94] |
| Stevia (*Stevia rebaudiana* Bertoni) | Selenium-chitosan NPs | Salt stress | –Increase of plant growth, photosynthetic performance, and antioxidant enzyme activity<br>–Reduction in electrolyte leakage and the content of $H_2O_2$ and MDA | [95] |
| Mung bean (*Vigna radiate* L.) | Nano-sized chitosan | Salt stress | –Improvement of plant development<br>–Increase in photosynthetic pigment<br>–Reduction in $H_2O_2$ and MDA content | [96] |
| *Bean (Phaseolus vulgaris L.)* | Chitosan NPs | Salt stress | –Promotion of seed germination<br>–Increase in the level of chlorophyll a and b, CAT, proline, RWC, carotenoids, and antioxidant enzymes | [97] |
| *Periwinkle (Catharanthus roseus L.)* | Chitosan NPs | Salt stress | –Accumulation of CAT, APX, and glutathione reductase | [98] |
| *Periwinkle (Catharanthus roseus L.)* | Chitosan NPs | Drought stress | –Enhancement of plant growth<br>–Induction of CAT and APX enzyme activity<br>–Raise in the content of alkaloid and the expression of *deacetylvindoline-4-O-acetyltransferase*, *geissoschizine synthase*, *peroxidase 1*, and *strictosidine synthase* genes | [99] |
| Sugarcane (*Saccharum* spp.) | *S*-Nitroso-glutathione chitosan NPs | Drought stress | –Increase in photosynthetic rates and root/shoot ratio | [100] |
| Cotton (*Gossypium* L.) | Nano-chitosan-NPK | Drought stress | –Increase in stem length and number of fruiting nodes per seedling<br>–Improvement in the number and weight of open bolls, seed ratio, and lint percentage | [101] |

(continued)

**Table 11.1** (continued)

| Plant species | Polymer-based NP treatments | Type of stress | Impacts | References |
|---|---|---|---|---|
| Wheat (*Triticum aestivum* cv. Pishtaz) | Chitosan NPs | Drought Stress | –Increase in leaf area, the content of relative water and photosynthetic pigments, activity of catalase and superoxide dismutase, and rate of photosynthesis<br>–Improvement of yield and shoot and root biomass | [84] |
| Tomato (*Solanum lycopersicum* L.) | Chitosan NPs | Cadmium stress | –Reduction in MDA content<br>–Improvement in the content of enzymatic and nonenzymatic antioxidants and osmoprotectants | [102] |
| Moldavian balm (*Dracocephalum moldavica* L.) | Chitosan-selenium NPs | Cadmium stress | –Reduction in MDA and $H_2O_2$ levels<br>–Improvement of agronomic traits, photosynthetic pigments, and chlorophyll fluorescence parameters<br>–Increase in the level of proline, phenols, essential oils, and antioxidant enzyme activities | [103] |
| Thorn apple (*Datura stramonium* L.) | Chitosan NPs | Cadmium stress | –Increase in peroxidase and polyphenol oxidase activities | [104] |
| Tomato (*Solanum lycopersicum*) | Copper-chitosan NPs | Pathogenic fungi | –Inhibition of mycelial growth<br>–Delay in spore germination | [105] |
| Tomato (*Solanum lycopersicum* L.) | Chitosan NPs | Wilt disease (*Fusarium andiyazi*) | –High antifungal activity<br>–Upregulation of PR proteins and antioxidant genes | [106] |
| Chick pea (*Cicer arietinum* L.) | Chitosan NPs | –*Fusarium oxysporum*<br>–*Pyricularia grisea*<br>–*Alternaria solani* | –Promotion of the growth of chickpea seedlings<br>–Inhibition of the growth of phytopathogens | [107] |
| Maize (*Zea mays* L.) | Copper-chitosan NPs | Curvularia leaf spot | –High activity of plant defense enzymes such as phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (PPO) | [108] |
| Wheat (*Triticum turgidum* L.) | Chitosan NPs | Fusarium head blight disease | –Reduction in the density of hyphal branches and number of colonies formed | [109] |

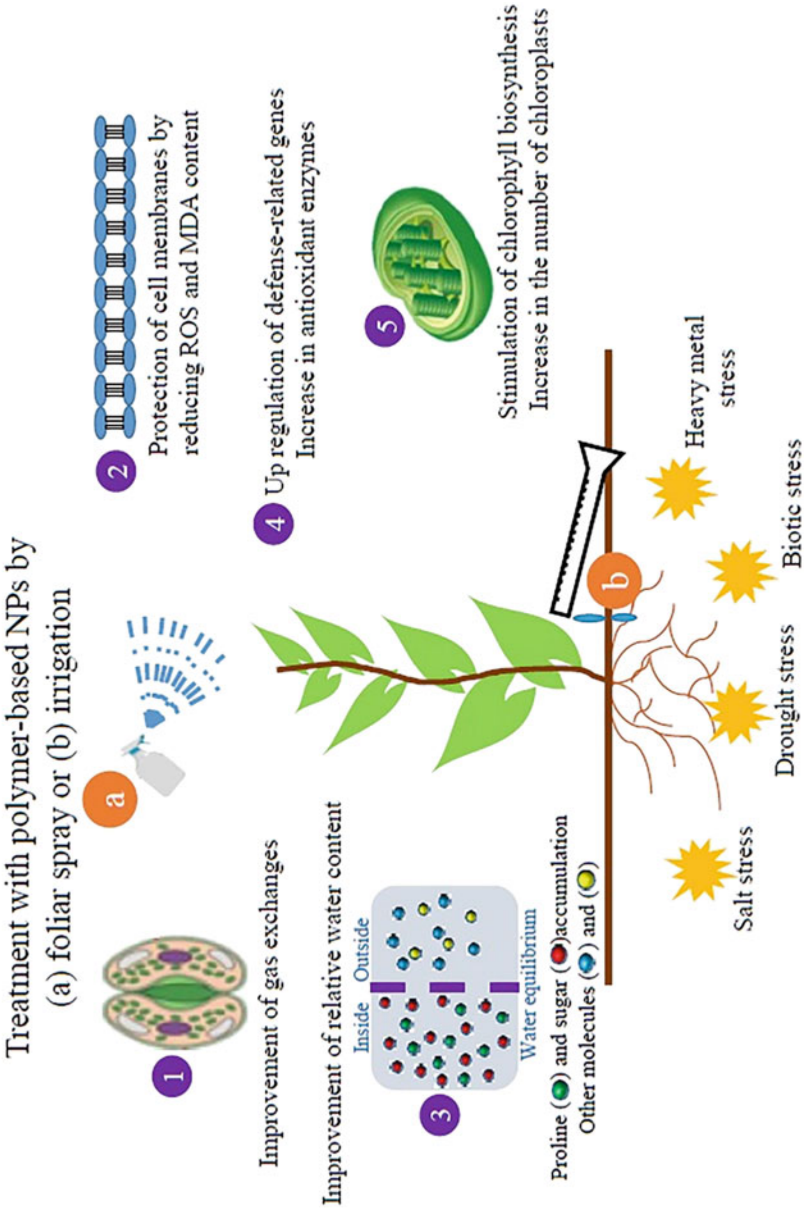


**Fig. 11.5** Schematic diagram shows the effect of chitosan NPs on plants exposed to abiotic/biotic stresses

peroxide ($H_2O_2$) production in the guard cells which therefore increased the levels of abscisic acid leading to inhibiting the opening of stomata. Furthermore, a recent study has shown that the application of chitosan NPs at 90 ppm on wheat significantly increased leaf area, SOD and catalase (CAT) activities, and chlorophyll content [84]. The increase in chlorophyll content under water stress conditions could be linked to the induction in the endogenous level of cytokinins, a stimulator of chlorophyll synthesis, or to the release of mineral elements from chitosan such as nitrogen which is an essential component in the tetrapyrrole ring of chlorophyll. Furthermore, foliar spray of 0.1% chitosan nanoemulsion has induced changes in the photosynthetic apparatus of pearl millet [115]. These changes might be referred to the increase in the number of chloroplasts; the improvement of the activity of RuBisCo, which is a key enzyme of photosynthesis; and the limitation of $CO_2$ diffusion into the leaf, which therefore caused stomatal closure. In addition, Silveira et al. [100] reported that S-nitrosoglutathione filled into chitosan NPs effectively alleviated the detrimental effect of water stress on sugarcane plants. Also, the potential role of chitosan NPs in protecting plants under drought stress could be due to their ability to delay the release of nitric oxide.

### 3.2.3 Impact of Chitosan-Based NPs on Plant Exposed to Heavy Metal Stress

The pollution of water or soil by heavy metals causes a crucial environmental concern worldwide. The increased level of heavy metal negatively affects soil quality, plant growth, as well as human living [116]. Cadmium is one of the most toxic heavy metals because it is characterized by its high mobility in plant cells [117]. It could disrupt plant metabolism and cell membrane structure and function, dysregulate phytohormones' biosynthesis and signaling, reduce photosynthetic efficiency, and lead to membrane lipid peroxidation [118, 119]. Different studies have demonstrated that chitosan NPs could alleviate cadmium toxicity effects (Table 11.1). The translocation of cadmium ions from roots to leaves could be reduced by chitosan application; thereby plant growth is enhanced. The effect of chitosan could be due to its large number of active amino and hydroxyl groups, which chelate $Cd^{2+}$ ions and store them in vacuoles and other organelles to maintain heavy metal homeostasis [120]. Also, the application of chitosan-selenium NPs could be an effective tool against the adverse effects of cadmium because they protect cell membranes and decrease $H_2O_2$ and malondialdehyde (MDA) content [103] (Fig. 11.5).

### 3.2.4 Impact of Chitosan-Based NPs on Plants Exposed to Biotic Stress

Regarding biotic stresses, plants can defend themselves and minimize damages occurred from herbivores' attacks and pathogen disease through multiple defense mechanisms. Among them, there is the building of a rigid cell wall structure to limit the invasion of microorganisms inside the plant cells and the production of lignin, toxic chemicals such as α-tomatine, and pathogen-degrading enzymes or making symbiotic relationship [121–125].

To prevent the loss of agricultural products, farmers are focusing now on the cultivation of plants with genetic disease resistance or applying the plant resistance inducer like NPs (Table 11.1 and Fig. 11.5). In tomato, Chun and Chandrasekaran [106] depicted that chitosan NPs have antifungal activity against phytopathogenic fungi, and this is probably due to the activation of plant defense genes such as chitinases and glucanases. Likewise, Cu-chitosan NPs were successfully controlled by *Alternaria alternate*, *Macrophomina phaseolina*, and *Rhizoctonia solani* which their growth was reduced by about 89.5, 63.0, and 60.1%, respectively, in comparison to the control [106]. Also, another study showed that the application of a very low dose of chitosan NPs (0.1%) could inhibit spore germination (up to 87.6%) [106]. Furthermore, their application on finger millet leaves has delayed the blast symptom expression by about 64% [107]. This protection could be due to either the activation of the defense response in plants or the inhibition of fungal RNA synthesis. Although chitosan is effective against bacteria [126]; however, to our knowledge, there are no data about the antibacterial potential of nano-chitosan. Thus, it might be interesting to study their ability to protect plants from harmful bacteria (i.e., *Pseudomonas syringae* pv) and their mechanisms on defense phytohormones, plant metabolome, and defense enzymes.

### *3.3 Application of NPs with Biostimulants*

Over the past three decades, several innovative agricultural methods and techniques have been proposed to improve agricultural production and to reduce the use of agrochemicals which have adverse effects on the ecosystem. Among the promising innovation approach that respects the environment, there is the use of biostimulants [127]. They are generally substances and microorganisms which when either primed seedlings and seeds or applied to a growing substrate could modify all plant processes (physiology, biochemistry, and molecular responses) [127]. Indeed, the application of biostimulants offers potential benefits, such as the improvement of seedlings' development, yield, and nutrient assimilation, the acceleration of flowering and fruit set, as well as the increase of plant tolerance to environmental stresses [128, 129]. Biostimulants could be divided into two main groups: i) beneficial microorganisms, such as plant growth-promoting (PGP) fungi, endomycorrhizal fungi (AMF), and PGP rhizobacteria (PGPR), and ii) substances from plants and animal materials, such as chitosan, alginate, and humic acid or organic materials like vermicompost, compost, and seaweed extracts that are rich in organic and amino acids as well as phytohormones [130]. Recently, several scientific researchers have tested whether the effect of biostimulants on plants could be promoted by adding either chitosan NPs, alginate NPs, or fullerene NPs.

### 3.3.1 Beneficial Microorganisms

In the earth, various fungal and bacterial strains could colonize the rhizosphere and plant tissues for a mutually beneficial exchange. For example, some of them could suppress pathogenic microorganisms through competition for food, while others could fix the atmospheric nitrogen via root nodules; increase the uptake of nutrients that are limited in the soil and those that are inaccessible by plants, such as polyphosphates, via mycelium; as well as produce secondary metabolites (volatile compounds, phytohormones, polymers) that could either control cell functions and metabolisms or prime plants for defense responses [131–133].

Recently, there has been a major increase in the synthesis of metal NPs [134]. Most of these synthesized NPs were used to fertilize soil without altering the growth of the microbial community. Additionally, some antagonistic microorganisms that belong to the PGP fungi class, such as *Trichoderma viride and T. harzianum*, can naturally synthesize chitosan NPs and silver NPs, respectively, with a very low average size, around 25–89 nm [135, 136]. However, the mechanisms leading to the biosynthesis of polymer-based NPs by *T. viride* remain unknown. Some studies have demonstrated that the biosynthesis of these NPs would require the presence of pathogens, such as *Sclerotinia sclerotiorum* [137]. Upon sensing the cell wall of pathogens, *Trichoderma* strains could secrete NADA co-enzyme and NADA-dependent enzymes (nitrate reductase) which are important for NP biosynthesis [137, 138].

Moreover, the application of chitosan NPs, with a diameter size between 50.748 and 141.772 nm, in combination with *T. viride* has increased plant disease resistance and reduced maize late wilt caused by *Cephalosporium maydis* [139]. This reduction was related to the increase in the activity of acid phosphatase enzyme.

As far as we know, no study had studied yet the impact of chitosan NPs produced by *Trichoderma* spp. on their growth as well as whether the exogenous application of these NPs affects the action mechanisms of these fungi. Moreover, some studies reported that chitosan could interact with *Trichoderma* spp. in different ways, depending on the dose applied. Zavala-González et al. [140] have reported that the growth rate of *T. pseudokoningii*, *T. harzianum*, and *T. koningiopsis* was around 22–53%, 68–81%, and 100%, respectively, under the application of 0.5 mg mL$^{-1}$ of chitosan to different growing media. Additionally, when a high dose of chitosan (1 to 2 mg mL$^{-1}$) was applied, the growth of *T. koningiopsis* strain was not affected because its cell wall had a lower level of linolenate (C18:3) and a high level of stearate (C18:0), in contrast to those chitosan sensitive, like *T. harzianum*, which had a high level of polyunsaturated free fatty acids [140–142].

Chitosan NPs may have positive or negative effects on AMF growth as well as their impacts on plants. The effect of chitosan NPs on mycorrhizal symbiosis could be related to various factors including size, concentration, and crosslinker used for the preparation of NPs, physicochemical properties of soil, and fungal species [143]. El-Gazzar et al. [139] reported that chitosan NPs (0.1 g mL$^{-1}$) applied to seeds increased the frequency and intensity of *Glomus mosseae* colonization as well as the number of arbuscules, therefore controlling late wilt. However, El Amerany et al. [144] reported that foliar spraying of leaves with chitosan NPs (250 mg $L^{-1}$ to 1000 mg $L^{-1}$) reduced mycorrhization rate as well as the transcript level of fungal biomass (*RiBtub*) and AM-specific phosphate transporter (*SlPT4*) genes, and therefore, tomato plant biomass and flowering of mycorrhizal plants were as well affected. The failure of the mycorrhizal network was supposed to be related to the down expression of an acidic isoform of the chitinase (*Chi3*) gene that is important for fungal growth.

Regarding PGPR, some studies demonstrated that chitosan NP application has strengthened the activity of *Bacillus* spp. and *Pseudomonas aeruginosa* due to the increase in the level of enzymatic indicators of soil health (dehydrogenase, fluorescein diacetate, alkaline phosphatase, nitrogenase reductase, nitrous oxide reductase, nitrite reductase, nitrate reductase), the growth (seed germination, leaf area, stem height, root length) of *Zea mays* and *Bidens pilosa*, as well as the accumulation of plant metabolism (alcohol, add ester, hydrocarbons) [145, 146]. Additionally, Atalla et al. [147] reported that the use of Cu-chitosan NPs, with a diameter size of 220 nm, in combination with fermented maize wastes by *Pseudomonas fluorescens* and *T. harzianum* reduced maize diseases. The production of antimicrobial protein, such as α-amylase and β-amylase, by beneficial microorganisms under Cu-chitosan NPs application, was demonstrated to be the most important metabolites involved in reducing disease severity. Moreover, chitosan NPs could be used as herbicide (imazapic and imazapyr) nanocarrier to reduce their toxicity on plant development; however, the encapsulated herbicides could alter the growth of beneficial bacteria [146].

Polymer-based NPs are used not only for the encapsulation of substances but also nano-enclosing of beneficial microorganisms. For example, alginate-silica NPs were used for enclosing either *Pseudomonas fluorescens* VUPF5, *Bacillus subtilis* VRU1, or their metabolites (i.e., auxin), and these nanoformulations had a positive impact on shoot number and length, root architecture, and fresh weight of pistachio [148, 149].

A summary that shows polymer-based NP effect on different beneficial microorganisms was illustrated in Fig. 11.6.

### 3.3.2 Substances and Organic Materials

Despite the outstanding results that have been achieved through the application of beneficial microorganisms, in terms of improving yield and soil fertility, however, in some cases, their application is not feasible in the presence of harmful or competing microorganisms and because of the inappropriate soil conditions, such as high level of phosphate, as well as a slow population development [150, 151]. Thus, emerging interest has been focused recently on providing an organic amendment like compost which could be produced in a huge quantity through the controlled fermentation (or composting) of different kinds of organic wastes (plant residues, animal manure, and urban, municipal, and industrial waste) and for the shortest period [152].

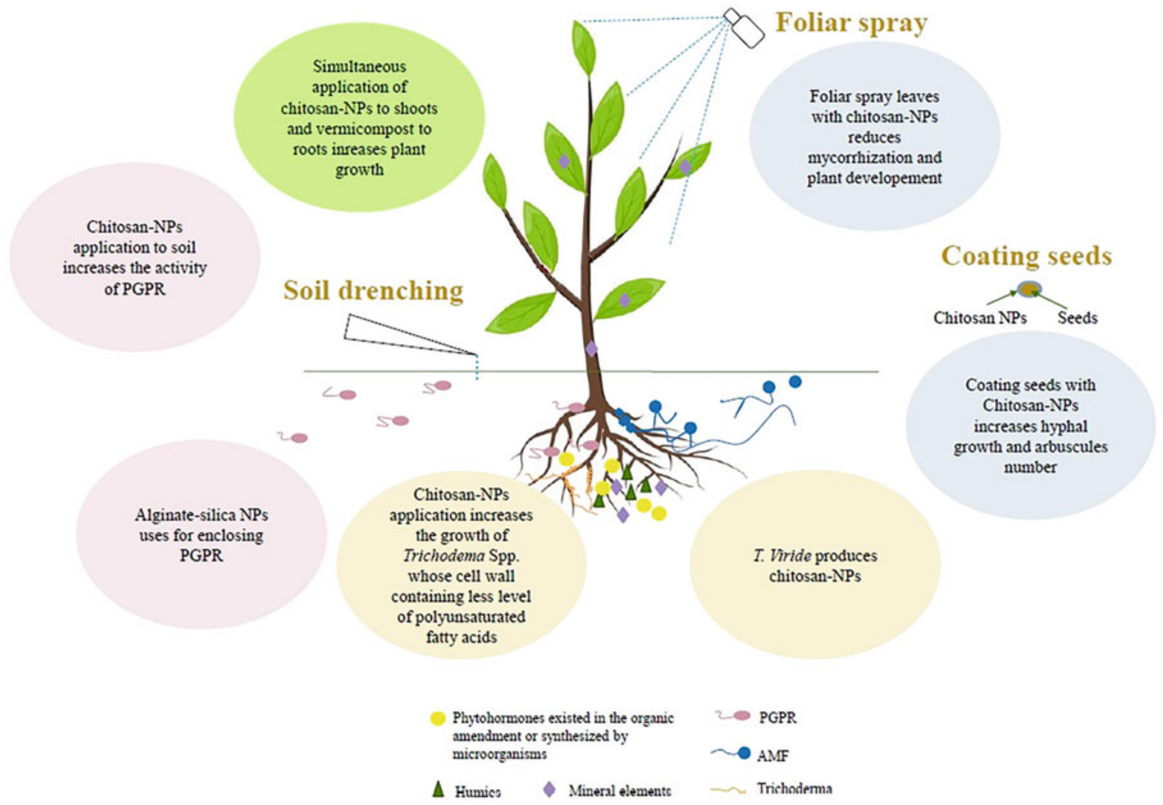


**Fig. 11.6** Illustrations of chitosan and alginate NPs' impacts on soil beneficial microorganisms

The production of compost has dual benefits. It could solve the problem of the deposition of a large number of unwanted residues as well as be an amendment for improving crops' traits. The agronomic effects of compost are due to its richness in nutrients; phytohormones (i.e., indole acetic acid); simple compounds of low structural complexity, such as phenols, quinones, carboxylic acids, and ethers; and the presence of bacterial and fungal groups [153, 154]. The richness of compost makes them able to improve water and nutrient retention capacity, plant growth and its development, and disease suppression [155]. Also, its components can interact with other biostimulants, therefore affecting their impacts on plants. For instance, some studies showed that fullerene (C60) NPs could absorb humic acids in the presence of $Ca^{2+}$ [156], while El Amerany et al. [151] showed that compost components could bind the amino groups of chitosan and this combination has increased shoot and root biomass, leaf area, stem development, chlorophyll fluorescence, and nutrient uptake (i.e., N, P, Mn, Mg, Na, Ca, Si, Fe, and Zn) of tomato plants. In regard to chitosan nanoscopic morphology, Ibrahim et al. [157] showed that chitosan NP (40 ppm) application in combination with vermicompost (6 tons/feddan) has increased plant height, branch and capsule number, herb fresh and dry weights, volatile oil level, and mineral nutrient (N, P, and K) content of black cumin (*Nigella sativa* L).

## 3.4 Effects of Polymer-Based NPs on Fruit Development and Quality

Fruit production is a critical phase for any fruiting crop species. It depends on several stages including flower formation, anthesis, fertilization, fruit set initiation, growth, maturation, and ripening. To get high fruit quality with freshness and long shelf life, scientists are trying to find methods or chemical additives that could trigger positive effects on the plant system [158–160]. Among these methods, they are the use of polymer-based NPs that have proven their effectiveness on plant growth performance. Chandra et al. [161] showed that chitosan NPs' application to shoots has induced the transcript level of β-1,3-glucanase gene that involves in increasing plant protection against harmful microorganisms and ameliorates plant development and physiology which include cellular division, flower production, seed maturation, and pedicel abscission. This is in concurrence with the acquired results by Behboudi et al. [84]. Recently, new formulations based on chitosan NPs containing phytohormones or inorganic elements were tested on shoots, roots, and seeds, to see whether they could impact fruit growth and its quality. For example, Kumaraswamy et al. [162] reported that applying SA-chitosan NPs to either seeds or leaves of maize was an effective approach that reduced flower alteration and yield loss caused by *Fusarium verticillioides*, while other studies showed that priming seeds and leaves of maize with either Cu-chitosan NPs, Zn-chitosan NPs, or chitosan-silicon NPs has increased grain yield by about 29%, 51%, and 187%, respectively, in comparison to non-treated plants [108, 163, 164]. Promotion in the number of spikelets and grain yield was also observed after foliar spray of chitosan NPK NPs and chitosan NPs containing N-acetyl cysteine on wheat (Table 11.2).

Also, the study carried out on tomatoes showed that priming seeds with chitosan (or chitosan-alginate) NPs containing $GA_3$ has a positive effect on fruit productivity [171]. Fruit number was induced by about 60% under the application of chitosan NPs containing $GA_3$, and fruits' fresh weight was increased by about 28% after the application of chitosan-alginate NPs containing $GA_3$, in comparison to control. The difference in effect could depend on the divergence in physicochemical features of polymers, chitosan, and alginate, which influence the duration of $GA_3$ release. Santo Pereira et al. [171] demonstrated that the release of $GA_3$ from chitosan NPs was faster than from the chitosan-alginate NP system. During the bud formation phase, the exogenous application of $GA_3$ could stimulate flower bud development and flowering-associated gene (squamosa promoter-binding protein-like (*SPL*) and suppressor of overexpression of CO1 (*SOC*)) expression as well as increase flowering quality and the level of endogenous hormones such as GA and IAA [180, 181]. Also, its application during flower opening and fruit production could increase the development of fruit (size, growth, and setting) and its nutritional composition (sugars, flavonoids, anthocyanins, phenolic compounds, and antioxidants) [182]. Thus, the initial release of $GA_3$ from chitosan stimulated flower formation, and therefore fruit number is induced; however, the induction in fruit biomass under the application of chitosan-alginate NPs could be explained by their release of $GA_3$ during fruit growth and ripening. Even many reports showed to what extent can the application of polymer-based NPs, during seed germination to fruit maturation, be effective on agricultural productivity, but there is no study regarding their effect on fruit quality.

The totality of agricultural products intended for human consumption does not reach its recipients due to losses during the so-called "postharvest" which is a phase that includes stages between harvest and processing of products for food. This phase can affect the quality of climacteric fruits as well as the ripening of those non-climacteric fruits. To decrease the unfavorable effects of chemicals and environmental stresses on the quality of fruits during storage, the coating was applied. It is used as a passive or as an inactivate barrier to regulate transpiration and volatile compound loss [183]. During the twelfth and thirteenth centuries, a monomer such as wax was the first film used on fruits [184]. Then, new and transparent materials have been developed to be used for short-term storage. Nowadays, different kinds of polymer-based NPs are used to maintain the quality of bananas, grapes, raspberries, persimmon, mango, sweet cherries, strawberry, and guava (Table 11.2). Coating fruits with chitosan NPs could maintain the color and the firmness due to delaying the maturation process and the microbial growth (Fig. 11.7 and Table 11.2). Also, their incorporation in hydroxypropyl methylcellulose films could prevent water and aroma loss and protect fruits from oxidative reactions and mechanical damage [185].

Moreover, the incorporation of various compounds such as amino acids (i.e., phenylalanine), unsaturated fatty acids, polysaccharides (i.e., xanthan gum), synthetic additive (i.e., propylene glycol and polyethylene glycol), and mineral ions (i.e., $Ag^+$) into polymer-based NPs improves their efficiency and functionality (Table 11.2). Releasing these compounds (or ions) from the coating materials onto

**Table 11.2** Various impacts of polymer-based NPs on fruit attributes and composition

| Polymer-based NPs | Doses | Application modes | Species | Effects | References |
|---|---|---|---|---|---|
| **Polysaccharide NPs:**<br>–Chitosan NPs | 0.2% | Coating fruit | Cavendish bananas (*Musa acuminate* AAA group) | –Increased smoothness of the skin and slower discoloration | [165] |
| | Stock solution | | | –Inhibited the activities of hydrolytic enzymes that involved in starch degradation<br>–Reduced water loss and the pulp to peel ratio and, therefore, prolong the shelf life of fruits | [166] |
| | 1–12 g $L^{-1}$ | Coating fruit | Grapes (*Vitis labrusca* L.) | –Delayed the ripening process of fruits with no alteration of sensory characteristics | [167] |
| | 90 ppm | Foliar/soil application | Wheat (*Triticumaestivum* cv. pishtaz) | –Increased spike weight and length, number of grain per spike, and the level of proteins | [84] |
| | 5 g $L^{-1}$ | Coating fruit | Raspberry (*Rubus sanctus Schreber*) | –Decreased decay extension rate<br>–Increased the amounts of flavonoids, anthocyanins, and phenolic compounds and the activity of α-diphenyl-β-picrylhydrazyl (DPPH), phenylalanine ammonia-lyase, and guaiacol peroxidase | [168] |
| –Chitosan NPK NPs | 10% | Foliar spray | Wheat (*Triticum Aestivum*) | –Increased spikelet number, kernel weight, grain number, and weight | [169, 170] |
| –Zn-chitosan NPs | 0.04–0.08% | Foliar spray and coating seeds | Maize (*Zea mays* L.) | –Increased grain zinc content and yield | [163] |

(continued)

**Table 11.2** (continued)

| Polymer-based NPs | Doses | Application modes | Species | Effects | References |
|---|---|---|---|---|---|
| –Chitosan-silicon NPs | 0.8% | Foliar spray and coating seeds | Maize (*Zea mays* L.) | –Increased yield/plot | [164] |
| –Cu-chitosan NPs | 0.16% | Foliar spray and coating seeds | Maize (*Zea mays* L.) | –Increased ear length and weight as well as grain yield | [108] |
| –Chitosan/TPP NPs containing gibberellic acid ($GA_3$)<br>–Chitosan/alginate NPs containing $GA_3$ | 0.5 mg $mL^{-1}$ | Seed priming | Tomato (*Solanum lycopersicum*) | –Increased the number of fruits and productivity | [171] |
| –Chitosan NPs containing *N*-acetyl cysteine (0.2 mg $mL^{-1}$) | Stock solution | Foliar spray | Durum wheat (*Triticum durum* cv. Fabulis) | –Increased grain yield | [172] |
| –Chitosan NPs loaded with phenylalanine | 5 mM | Coating fruit | Persimmon (*Diospyros kaki*) | –Reduced $H_2O_2$<br>–Increased the level of MDA, soluble tannin, and total carotenoid as well as SOD, CAT, and APX activities and therefore chilling injury decreased | [173] |
| –Guar gum/AgNPs | – | Coating fruit | Mango fruit (cv. Seddik) | –Maintained the texture, titratable acidity, and the level of carotenoids<br>–Delayed ripening process | [174] |
| –Alginate NPs containing soybean oil with or without a $CaCl_2$ crosslinker | – | Coating fruit | Sweet cherries (cv. Bing) | –Increased titratable acidity and the level of soluble solid content, total soluble phenolics, anthocyanins, and antioxidant Capacity (DPPH) | [175] |
| **Protein NPs:**<br>Proteinoid polymer NPs containing auxin | – | Dispersion in the soil media | Lettuce (*Lactuca sativa*) | –Increased root length | [176] |

| | | | | | |
|---|---|---|---|---|---|
| **Lipid NPs:**<br>–Beeswax containing polysaccharides and propylene glycol | 10 g $L^{-1}$ | Coating fruit | Strawberry (*Fragaria ananassa*) | –Reduced weight and firmness loss, decay index, and color changes | [177] |
| –Candeuba® wax containing polysaccharide and polyethylene glycol | 65 g $L^{-1}$ | Coating fruit | Guava (*Psidium guajava* L.) | –Reduced weight loss<br>–Increased total solid soluble<br>–Maintained acidity value | [178] |
| –Candeuba® wax containing xanthan gum | 65 g $L^{-1}$ | Coating fruit | Guava (*Psidium guajava* L.) | –Reduced total color difference, respiration rates, and pectin methylesterase activity<br>–Increased ascorbic acid and phenolic compounds levels | [179] |

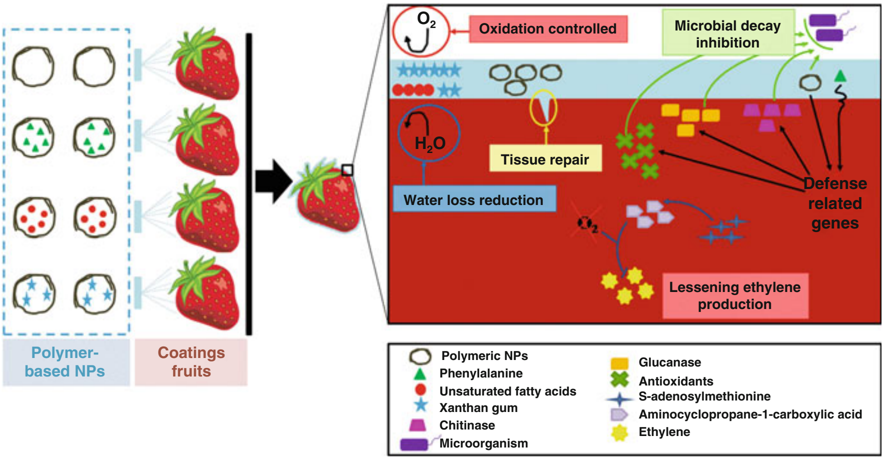


**Fig. 11.7** Proposed model illustrating the impact of coating fruits with polymer-based NPs

the surface of fruits increases the level of antioxidant (i.e., ascorbic acid, sugars, phenolic compounds) and antioxidant enzymes (SOD, CAT, and peroxidase ascorbate (APX)) and stops unwanted reactions, especially discoloration and enzymatic browning (Table 11.2 and Fig. 11.7).

## 3.5 *The Fate of Polymeric NPs*

Polymer-based NPs are complex molecules that are composed of three layers: (1) the outer layer or crust could interact and make complexes with materials, molecules, and ions; (2) the mantle is made of a chemically different material from the core, and this characteristic allows them to protect the encapsulated stuff; (3) the core is the inner space responsible for establishing the encapsulation property of NPs [186].

Polymer-based NPs are unstable particles due to their ability to change rapidly or to react with the active agent to reach a state considered relatively stable [187]. However, this interaction could lead to oxidation, ion exchange, deformation, assembly, and aggregation of polymer-based NPs and affect their sizes, structures, chemical composition, and their impacts on plant tissues as well soil structure and microbiota [187]. The literature shows that the nanometer size of NPs allows them to go through cells and react with molecules; but, as far as we know, no report has shown yet the fate of NPs after their application either on plants or soil microbia.

The cell wall of plants is a selective and protective barrier that protects cells from environmental factors and restricts the entry and the egress of substances. But, it is well known that only substances of small diameter size can enter plant cells through leaf's stomata which makes a hypothesis that leaf guard cells could be the target of polymer-based NPs [102]. Avellan et al. [188] hypothesized that NP uptake could be done via a plant cuticle that contains small hydrophilic pores or a stomatal pathway. Aziz et al. [189] showed that chitosan application could enter the stomata and translocate inside wheat plants through phloem cells. Also, Abdel-Aziz et al. [170] proved that chitosan NPs could be taken up by plants and move between leaves and roots through the phloem route.

Moreover, applying NPs, with a diameter size less than 50 nm, to shoots has been shown to accumulate in younger leaves, roots, and the rhizosphere soil; but their uptake and translocation are depended on their size and structure [188]. The accumulation of NPs in leaf surface increases for smaller sizes NPs. Regarding larger NPs, the particles efficiently cross the plant cuticle layer and then accumulate in the mesophyll cells or plant vasculature.

Even though various studies examined the foliar uptake of NPs as well as their mobility outside or inside plant cells, it remains unclear whether they were degraded when they passed through cells and what form of NPs was taken up by the plant.

## *3.6 Conclusion and Future Perspective*

It was concluded that the nature and the chemical properties of polymer NPs play an important role regarding their impacts on the plant system. They could increase plant growth and development, ameliorate fruit attributes and quality, stimulate fungal and bacterial growth, and enhance plant tolerance to water, salt, toxic elements, and pathogens. Despite many reports demonstrating their benefits on crops, there is no certain information regarding their action mechanisms. This could be attributed to the structure of polymer used, size of particles, or coated substances. But a clear insight to understand their impact on plant physiology, metabolic, and gene machinery are still missing. Also, given the importance of chitosan NPs on plants and microbiota, it remains unknown how another type of polysaccharide, such as lignin, starch, and cellulose as well as those from proteins and lipids, could affect the agricultural products.

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