Chapter 2

Chitosan in Agriculture: A New Challenge for Managing Plant Disease

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Additional information is available at the end of the chapter

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Abstract

In recent years, environmental-friendly measures have been developed for managing crop diseases as alternative to chemical pesticides, including the use of natural compounds such as chitosan. In this chapter, the common uses of this natural product in agriculture and its potential uses in plant disease control are reviewed. The last advanced researches as seed coating, plant resistance elicitation and soil amendment applications are also described. Chitosan is a deacetylated derivative of chitin that is naturally present in the fungal cell wall and in crustacean shells from which it can be easily extracted. Chitosan has been reported to possess antifungal and antibacterial activity and it showed to be effective against seedborne pathogens when applied as seed treatment. It can form physical barriers (film) around the seed surface, and it can vehicular other antimicrobial compounds that could be added to the seed treatments. Chitosan behaves as a resistance elicitor inducing both local and systemic plant defence responses even when applied to the seeds. The chitosan used as soil amendment was shown to give many benefits to different plant species by reducing the pathogen attack and infection. Concluding, the chitosan is an active molecule that finds many possibilities for application in agriculture, including plant disease control.

Keywords: chitosan, seed treatment, soil amendment, plant resistance elicitation, induced defence, elicitor

1. Introduction

The control and management of crop plants diseases has always been considered a subject of great interest for the huge economic losses associated with them. For many years, the control of pathogens has been performed mainly through the application of chemical pesticides, due to their easy application, the relatively low cost and the broad spectrum of action. Pesticides application for crop defence has been widely used since post-war years and led to a large yield growth in agriculture, contributing to economical development, reducing endemic diseases and protecting/restoring plantations, forests, harvested wood products [1]. In fact, plant diseases represent a critical problem to successful production. Agricultural productivity has demonstrated to get benefits from the utilization of pesticides both at quantitative and healthy level, e.g. when pesticides are properly used, they contribute to the higher production and quality characteristics of crops. However, the advantages in their use comprise several drawbacks problems related to two main aspects: the human health and the environmental impact. In fact, the chemical plant protection products, including even copper that is allowed in organic agriculture, are mostly toxic, persistent, bio-accumulative and extremely harmful not only for human health, but also for many living organisms [2]. Pesticides can contaminate environmental matrices coming up the aquifer [3], causing direct and permanent damage to the ecosystem. In addition, there is the real possibility that their residues can get into the food chain of consumers [4]. The massive use of these chemical substances has also favoured the emergence of resistance phenomena in the major crop pests [5] and the contemporary disappearance of many pests' natural enemies, such as bumblebees, butterflies and bees [6].

The pesticides application and their related effects constitute a topic of major concern, so that, according to the new Europe directive in favour of a sustainable agriculture, many plant protection products currently in use will be replaced with lower environmental impact substances. For this reason, many scientific works and researches have been focused in developing alternative approaches to the use of pesticides for managing crop diseases, through experimentations that have followed different paths including physical methods [7] integrated pest management and biological control [8]. A promising approach consists in the use of natural compounds such as plant extracts and their active principles (alkaloids, phenols, monoterpenes and sesquiterpenes, isoprenoids), which have been studied for their various antifungal, antibacterial and antioxidant properties [9–11] and animal derived compounds like chitosan. It has proved to be very interesting for controlling plant diseases [12]. In fact, it has been shown both to possess a broad-spectrum antimicrobial activity against several phytopathogenic organisms and to induce numerous biological responses in plants [13].

2. Chitosan

Chitosan is a linear polysaccharide that can be obtained from the deacetylation of chitin, a long-chain polymer of N-acetyl-glucosamine present and easily extracted from fungal cell wall and crustacean shells (**Figure 1**). From a practical viewpoint, the shells of marine crustaceans such as crabs and shrimps are very affordable for a commercial production of chitin. They represent a practical challenge because they are available as waste from the seafood processing industry. Recent advances in fermentation technologies suggest that the cultivation of selected fungi can provide an alternative source of chitin and chitosan [14].

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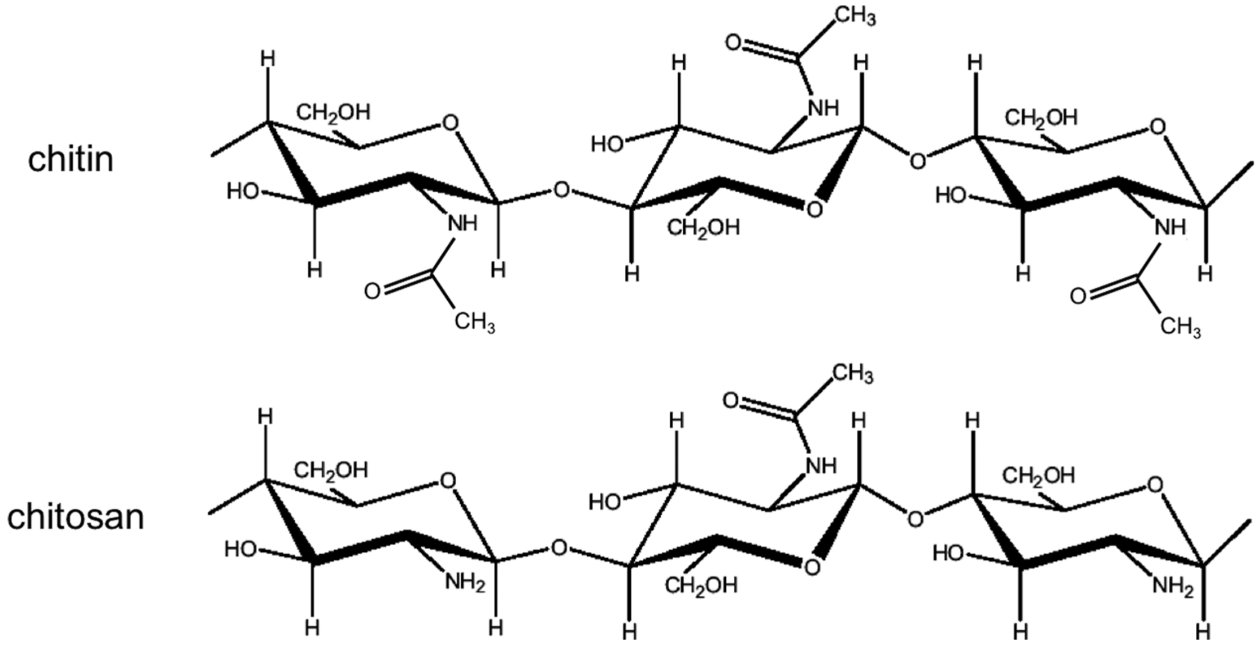

Figure 1. The structure of the molecules of chitin (β-(1-4)-N-acetyl-D-glucosamine) and chitosan (β-(1-4)-D-glucosamine) that results from the partial deacetylation of chitin.

In addition to its low cost production, chitosan also possesses other biological properties such as non-toxicity, biocompatibility and biodegradability, which make chitosan a sustainable and eco-friendly molecule.

Chitin is the second renewable carbon source after lignocelluloses biomass and, in fact, about 1600 tons of chitin are annually produced [15]. For industrial production, solid chitin is soaked in 40–50% (w/v) NaOH. This process removes more than 80% of the acetyl residues and converts N-acetyl-D-glucosamine into β-1,4-D-glucosamine. Complete deacetylation is possible by repeating the alkaline treatment. Therefore, the term "chitosan" is not uniquely related to a defined compound, but to a group of commercially available copolymers that are heterogeneous for deacetylation degree, molecular mass, polymerization degree and acid dissociation constant (pKa value) [13]. These different characteristics, in particular the degree of deacetylation and the molecular weight, influence the physic-chemical properties (including viscosity and solubility), and they have a direct influence on the biological properties of the substance and the effects in plants and pathogens.

All these characteristics make chitosan very useful for several industrial applications, namely cosmetology, food, biotechnology, pharmacology, medicine and, more recently, agriculture [16].

Based on the current state of research and progress in agriculture, this chapter will consider the potential uses of this natural compound in plant disease control, based mainly on a dual mode of action involving a direct antimicrobial activity and an indirect resistance induction that elicit several defence responses in the plants. The upgrading of the plant defence mechanisms against pathogen represents a very innovative approach for crop protection [15], and it will be described separately. Moreover, the last advanced researches such as seed treatment and soil amendment applications will be described. Postharvest fruit application will not be included because extensively discussed in the literature [17].

2.1. Antimicrobial activity

One of the most studied proprieties of chitosan is its high antimicrobial activity against a wide variety of microorganisms such as fungi, bacteria and viruses (**Table 1**). An antimicrobial substance is defined as a substance that kills or inhibits the growth of microorganisms [18].

A broad spectrum fungicidal activity of chitosan has been described: it inhibits *in vitro* fungal growth of many pathogenic fungi, for example, *Botrytis cinerea, Alternaria alternata, Colletotrichum gleosporoides* and *Rhizopus stolonifer*. The inhibition was observed at different pathogen development stages such as mycelial growth, sporulation, spore viability and germination, and on the production of fungal virulence factors [14]. Moreover, the antifungal activity was also demonstrated *in vivo* in many different plant-pathogen systems, such as in pear against *A. kikuchiana* and *Physalophora piricola* [19], in grapevine and in strawberry against *B. cinerea* [20, 21], and in dragon fruit against *C. gleosporoides* [22]. In rice, the antifungal activity against *R. solani* was further demonstrated by transmission electron microscope observations and pathogenicity testing [23].

Chitosan also prevents the growth of several pathogenic bacteria including *Xanthomonas* [24], *Pseudomonas syringae* [25] *Agrobacterium tumefaciens* and *Erwinia carotovora* [26]. However, the antimicrobial effectiveness of chitosan seems to be higher against fungi than bacteria [27], and among bacteria often been higher against Gram-positive than Gram-negative ones, even if this efficiency is somewhat controversial. This could be explained by the different structure of the bacteria surface and cell wall composition [28].

Besides these activities, chitosan is able to inactivate the replication of viruses and viroids thus limiting their spread [29], even though relatively few research studies on its antiviral activity have been reported [30].

The exact mechanism of the direct antimicrobial action of chitosan is still ambiguous, and different mechanisms have been proposed and described [14, 28], but none of them are mutually exclusive. The main mode of action proposed is related to its cationic properties [31] hypothesis supported by the lack of antifungal activity of uncharged chitin oligomers [32]. In fact, unlike chitosan, the polymeric form of chitin is naturally uncharged, and it does not show substantial antimicrobial activity. Basing on this model, the positive charges on the chitosan molecules interact with negatively charged pathogen surfaces (electrostatic interactions); this leads to the cell structure destruction, causing an extensive cell surface alterations and increasing membrane permeability [33–35]. Another proposed mechanism involved the alteration of cell permeability by chitosan that includes its deposition onto the pathogen cell surface, and consequent creation of an impermeable polymeric layer that prevents the uptake of nutrients in the cell, and in the meantime changes of the metabolite excretion in the extracellular matrix [28].

Chitosan is also able to chelate some essential nutrients, metal ions and trace elements necessary for bacterial and fungal growth [12, 28], inhibiting thereby toxin production and microbial growth [36].

2.2. Plant resistance elicitation

All plants, whether they are resistant or susceptible, respond to pathogen attack by the induction of a coordinate signalling system, which results in the accumulation of different gene products. The responses to pathogen attack are effective at different levels: at first, the pathogen recognition leads to the development in the plant of a rapid localized cell death, also known as the hypersensitive response (HR), that causes necrosis at the site of infection (local response). Then, even in uninfected parts of the plants, a systemic expression of a broad spectrum and long-lasting resistance against further pathogen infection is triggered. This leads to the production of reactive oxygen species (ROS), the activation of defence-related genes as well as an enhanced expression of genes related to the production of molecules, such as phytoalexins, terpens, pathogenesis-related (PR) proteins and many enzymes involved in defence mechanisms (phenylalanine ammonia-lyase [PAL], polyphenol-oxidases [PPOs] and peroxidases such as guaiacol-peroxidase (G-POD) and ascorbate peroxidase [APX]) [37–39].

The signals able to trigger the defence mechanisms in plants are called elicitors, and they can be produced in the site of infection both by infected plant cells (endogenous elicitors, released by the plants upon contact with the pathogen) and by the pathogen itself (exogenous pathogenic elicitors). They consist of several compounds including oligosaccharides, lipids, peptides and proteins, and they are capable, even at low concentrations, to act as signal molecules inducing the plant to trigger the defensive responses [40]. The elicitors, once recognized by transmembrane receptors of plant cells, induce an immune response, both locally (around the infection site/application) and systemic, through the translocation of signalling molecules in distal tissues [41]. The increasing knowledge of the mechanisms underlying the plant response to pathogen attacks has supported the idea that it is possible to achieve a broad-spectrum disease control and an increased protection against virulent pathogens by artificially inducing the plant's own resistance mechanisms using substances with elicitor function. It is now well-documented that treatment of plants with various eliciting agents leads to an induced resistance against subsequent pathogen attacks, both locally and systemically. Therefore in order to enhance plant resistance in agriculture the use of elicitors is becoming a very attractive and eco-friendly tool that could provide efficient alternatives to the usage of chemical pesticides for managing plant diseases, thus reducing their environmental negative impacts.

Moreover, it is known that chitosan at low molecular weight acts as a potent biotic elicitor, able to induce plant defence responses and to activate different pathways that increase the crop resistance to diseases [13, 15, 28, 42, 43]. The most studied plant responses to chitosan treatment are the formation of chemical and mechanical barriers and the synthesis of new molecules and enzymes involved in the defence response [15, 37]. In some cases, chitosan causes the induction of the hypersensitive response, mainly around the infection site, that leads to the programmed cell death [44]. This hypersensitive response can be followed by systemic response of the plant defence mechanisms. These latter mainly include the synthesis and accumulation of secondary metabolites with active roles in defence: phenolic compounds such as lignin, callose, phytoalexins, PR proteins (pathogenesis-related proteins) and the modulation of the activity of key enzymes of metabolic pathways involved in the defensive response, such as the PAL, peroxidases and chitinase (**Figure 2**) [45–48].

The mechanism of action of chitosan and the responses induced by the latter in plant-pathogen interaction is not yet entirely clear. As written above, the plant through transmembrane cell receptors recognizes the elicitors, but the specific receptor for the chitosan has not yet been identified [37]. Protein kinase cascades that may relay the signal to transcription factors (TFs) have not been identified as well. Various models have been proposed to explain the role of chitosan in the activation of plant defence genes. A proposed direct elicitation of gene activity in plant defence implicates chitosan/DNA interactions (**Figure 2**). The proposed predictive models assume that chitosan induces the activation of defence genes by modifying the structure of DNA (chromatin remodelling) along with reductions in the architectural transcription factor high mobility group HMG A [43, 49] or by the interaction with the DNA polymerase complex [50].

The defensive responses that are induced by chitosan treatment may depend on the pathosystem and, even in the same crop, on numerous factors, including the type of treatment application (**Table 1**).

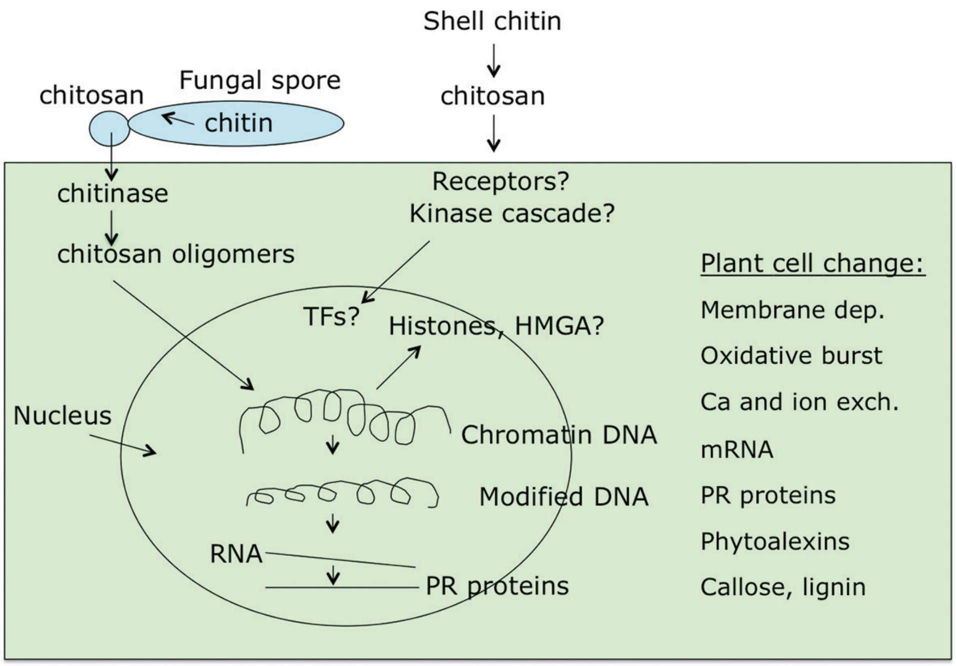

Figure 2. Some proposed effects of chitosan as elicitor of plant defence responses [43]. The cellular and molecular changes elicitated by chitosan can be summarized in: membrane depolarization, oxidative burst, influx and exit of ions such as Ca^{2+}, activation of MAP-kinases, chromatin and DNA alteration, increase in PR gene mRNA, PR proteins synthesis, phytoalexins accumulation, lignification and callose deposition. TFs: transcriptional factors. HMG A: architectural transcription factor high mobility group. PR proteins: pathogenesis-related proteins.

3. Chitosan seed treatments

In agriculture, "Seed treatments are the biological, physical and chemical agents and techniques applied to seed to provide protection and improve the establishment of healthy crops" [International Seed Federation (ISF)]. It represents the first line of defence for seeds and seedlings against pests infecting the seed teguments or living in the soil (seedborne and soilborne pathogens). It provides protection during the critical stage of germination and the very first seedling development, when seeds and seedlings are unable to protect themselves from invasive pathogens [51]. The substance applied to the seeds can be of various nature such as chemical pesticides, biochemical substance, natural compounds [8, 52], and there are many different techniques that can be used for this purpose. Among them, seed coating and dressing represent a common procedure of seed treatment applied for preventing diseases and pests [53, 54] other than improving the seedling performances, i.e. the seedling emergence time, synchronized emergence, improve germination percentage, emergence rate and yield in many field crops [52, 55–57].

The technique of seed dressing involves the application on the seed surface of thin layer of the active product, such as pesticides, fertilizers or growth promoters [57], often in combination with other additives. These other components may include colour (effective pigment), filming agents, surfactants or tackifiers. These products come in dry formulations as powders or liquid formulations are also available for sprays, dips, fluid drilling gels and solid matrix priming.

Chitosan represents an interesting prospective in this field because it could cover different aspects thanks to its variety of properties mainly related to the molecular weight [15]. The high molecular weight confers to chitosan biopolymer characteristics, so that it can be used as film, forming physical barriers (film) around the seeds preventing the pathogen infection [58, 59].

The low molecular weight chitosan posses high antimicrobial activity, which increases with weight decreasing, demonstrated against a wide variety of microorganisms such as bacteria, yeast and fungi [60, 61] even if some controversial evidences for a correlation between bactericidal activity and chitosan molecular weight have been found [62]. Thanks also to its ability to induce plant resistance, low molecular weigh chitosan has great potential as protector against diseases.

An interesting application on seed is the use of chitosan as film coating as a delivery system for fertilizers, plant protection products and micronutrients for crop growth promotion [13]. Chitosan in fact can vehicular and protect other antimicrobial compounds such as essential oils. Essential oils have demonstrated antimicrobic activity [10, 11, 63] but are very volatile and their incorporation into coating can ensure a better persistence of the active ingredient on the surface and maintain high concentration of active molecules [64].

For example, different kinds of chitosan seed coatings with or without essential oils like thyme (*Thymus vulgaris*) and tea tree (*Melaleuca alternifolia*) essential oils, incorporated at different concentrations and applied with different thickness, have been studied for controlling disease and reduce the risk of pathogen attack [65]. The treatment effectiveness in reducing fungal development was evaluated both on *Fusarium* spp. naturally infected wheat seeds and on seeds artificially infected with *F. graminearum*, one of the causal agents of root and foot rot in cereals (**Figure 3**). Results showed that coating treatment with essential oils reduced fungal growth on seeds without affecting germinability and lowered severity on seedlings at the first developing stages. Scanning electron microscope studies allowed to monitor the superficial structure stability of the coating treatment on seeds after the imbibition processes [65].

Another main application of chitosan as seed treatment concerns the elicitation of the systemic resistance in plants. Basing on recent evidences, chitosan, when applied as a seed treatment, behaves as a resistance elicitor, inducing a physiologically enhanced defensive ability in seedlings and plants, whereby the plant's innate defences are potentiated [45, 66]. Chitosan is able to cross the seed teguments, probably by diffusing through microscopic ruptures caused by the imbibition [67] and to interact with embryo cells, influencing the plant cellular metabolism in the following stages of development. Using radio labelled chitosan, it has been showed that the chitosan applied to seed is transferred to the emerging seedling during their development [68]. The major effects produced by the seed/chitosan interaction can be summarize as follows: (a) the seeds germination index is enhanced, (b) the mean germination time and flowering time are reduced; (c) plant growth (e.g. shoot height, root length, and seedling, vegetative growth vigour) and biomass are increased [52]. In maize [69], rice [70] and wheat [71], the chitosan seed treatment increased the germination percentage and stress tolerance, and improved the vigour of the seedlings. In artichoke [53], chitosan seeds treatment resulted in a better growth of the seedlings (e.g. longer and better developed radicle and greener hypocotyls) and lower chance of being infected by fungi in comparison with the untreated seeds. The observed growth improvement by chitosan could be also related to the incorporation of nutrients (nitrogen) from chitosan. Chitosan seed treatment is also able to increase the content of important resistance markers, like phenols and the activities of defence-related enzyme, thus improving the plant resistance. Biochemical analyses on durum wheat and sunflower confirmed the ability of chitosan to induce plant defence increasing PAL, PPO, peroxidases, and chitinase activities and phenolic content in seedling. The enhancement of these plant defence mechanisms suggests the activation of systemic resistance processes. Laboratory results on the chitosan-induced resistance were also confirmed under field and greenhouse condition, where an enhancement of the number of emerged plants (**Figure 4**) and a reduc-

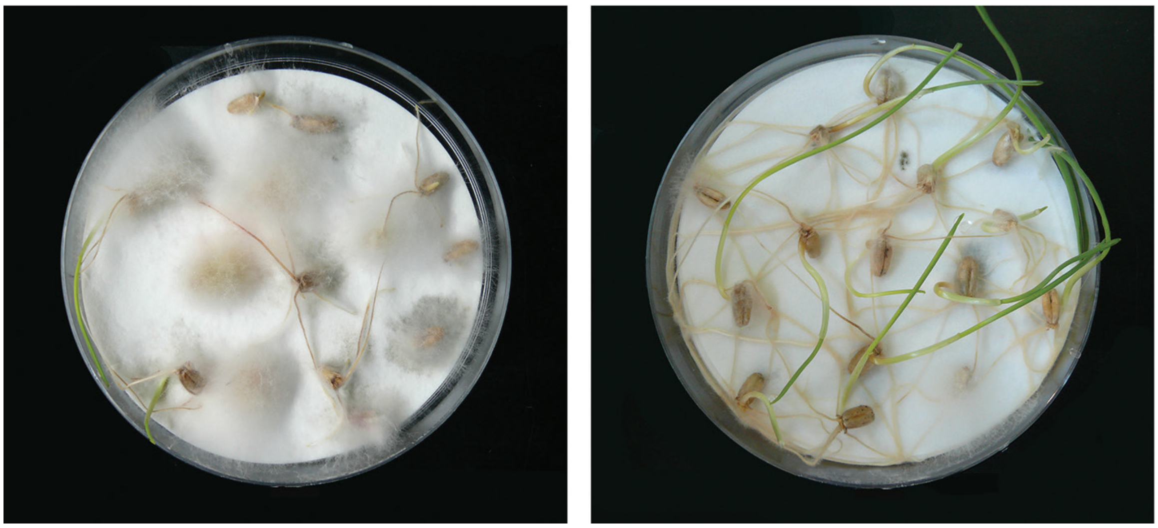

Figure 3. Blotter test for seed health analysis after 7 days of incubation at 25°C of durum wheat seeds artificially infected with *F. graminearum*, one of the causal agents of root and foot rot in cereals. Seeds were then coated with a solution of chitosan and tea tree oil. The chitosan/tea tree oil treatment reduced significantly the fungal infection on seeds (right) compared with the inoculated and not treated seeds (left).

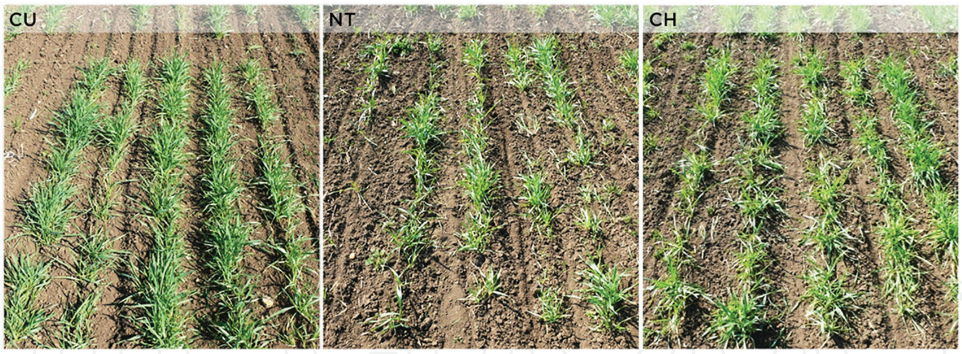

Figure 4. Parcels of field trial (Italy) sowed with durum wheat seeds cv. Simeto artificially infected with *F. graminearum* and then treated with chitosan (CH) or with a copper sulphate-based treatment (CU), commonly used as chemical treatment in organic farming. Chitosan treatment enhanced the number of emerged plants, if compared with no treatment (NT), as well as copper treatment.

tion in the disease severity (root and foot rot and downy mildew, respectively) were observed [45, 66]. Chitosan seed treatment also induced lignifications process, considered one of the first line of defences in plant-pathogen interaction: in chilli, the lignin content of seedlings obtained from chitosan-treated seeds was higher than that of untreated ones [72], thus giving to the plant a major protection against potentially penetration of invasive pathogen.

Chitosan seed treatment can also be effective in insect control because it stimulates plants to produce systemic antibodies with repellent effects on insect pests, as reported in soybean against *Agrotis ypsilon*, soybean pod borer and soybean aphid. The chitosan treatment developed an antifeedant rate of more than 80% against all these insects, together with increases in seed germination, plant growth and soybean yield [73].

4. Chitosan as soil amendment

As previously described, chitosan can be used in several ways to reduce plant disease levels and prevent the development and spread of diseases, thus preserving crop yield and quality. Chitosan as soil amendment was found to successfully decrease *Fusarium* wilt in several plant species [33, 74, 75]. Similar results were reported against *Cylindrocladium floridanum* [76], *Alternaria solani* [77] and *Aspergillus flavus* infections [78] after soil treatment with chitosan. Part of the observed effect of chitosan on the reduction in these pathogens comes from the fact that it enhances plant defence responses. It has been demonstrated that chitosan acts as an elicitor of plant systemic immunity by the accumulation of defence-related antimicrobial compounds, and it plays an important role in the activation of induced resistance [43]. For example, the innate immunity induction was observed in micropropagated kiwi plants after the addition of chitosan to the growth medium [46]. This includes the modulation of several enzyme activities, involved in detoxification processes as well as the increasing of the activity of enzymes involved in plant defence barriers (G-POD, APX, PAL and PPO) [46]. In addition, chitosan amendments were reported to induce callose formation, proteinase inhibitors and phytoalexin biosynthesis in many dicot species [79–81].

The amendment of soil with chitosan is eco-friendly, since in the soil chitosan can be degraded at a substantial rate, due to the enormous abundance and diversity of bacteria in most soils and the presumed presence of chitinases in a considerable fraction of the bacterial populations. Chitin degradation is mainly a bacterial process [82–85]. However, it still remains unknown the wideness of chitinolytic process due to soil bacteria population with different chitin degradation and whether fungi can also play a major role in this process. Works on microbial community members hypothesized a role of chitin in stimulating bacterial communities to a greater extent than the fungal ones [85, 86]. Among the bacteria genera isolated from chitin-treated soils, there were *Streptomyces* [86], *Stenotrophomonas* and *Bacillus* [87, 88]. However, due to the complexity of bacterial response in soil treated with chitin, the mechanisms behind the observed effects, in particular what are the active bacterial community, the timing of the chitinolytic activity and bacterial succession is still poorly understood. Probably, members of the actinobacteria have a key role in the degradation of complex organic molecules like chitinosan in field [88, 89].

In field conditions, chitosan alters the equilibrium of the rhizosphere, disadvantaging microbial pathogens and promoting the activity of beneficial microorganisms, such as *Bacillus, Pseudomonas fluorescens*, actinomycetes, mycorrhiza and rhizobacteria [90–93]. For instance, soil treatment with chitin and/or chitosan from shrimp waste has been shown to decrease the rate of infection of plant roots by nematodes [82, 84] and to enhance the suppressiveness against soilborne diseases [91]. Although not definitely proven in all cases, the mechanisms behind chitosan effectiveness are most often related to a change in the structure and/or activity of soil microbiota [94, 95]. Two hypotheses have been formulated regarding the response of the soil microbial communities to chitosan addition: (a) chitinolytic microorganisms, which are capable of hydrolyzing the chitinous hyphae of pathogenic fungi, increased their numbers and/or activities (b) secondary responders to the added chitosan have a detrimental activity against pathogens [96].

The beneficial effect of the chitosan seems to be linked not only to its impact on soil microbiota, but also on plant itself. Recently, an innovative bioremediation strategy uses the ability of chitosan to chelate minerals and other nutrients, making them more available for the uptake by the plant [97, 98]. This is important, since crop production is many times limited by low availability of essential mineral elements [99]. In agreement with this strategy, in Ref. [100], the effect of using chitosan oligosaccharides as a soil conditioner was demonstrated on the flowering and fruit growth of purple passion fruit. It was found that this soil conditioner increased significantly the numbers of flowers, fruit weight and juice production. The inclusion of soluble chitosan to hydroponic fertigation streams also promoted the growth and final yield of hydroponically cultivated potato microtubers [101].

Thus, if chitosan can increase absorption of essential minerals, enhancing the plant's nutritional value (biofortification), it is possible that it can also help plants to take up higher concentrations of toxic elements. In fact, the ability of chitosan to chelate certain ions also makes it an interesting compound to be used in phytoremediation. In Refs. [102, 103], the remediation of metal contaminated soil using chitosan as soil amendment was shown to be possible (**Table 1**).

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Plant	Disease/pathogen	Activity/defence response	Application	References
Pear	*A. kikuchiana* and *P. piricola*	Antifungal activity	Growth medium addition and postharvest treatment on fruits	[19]
Grapevine Strawberry	*B. cinerea*	Antifungal activity	Growth medium addition and preharvest spray treatment	[20–21]
Dragon fruit	*C. gleosporoides*	Antifungal activity	Growth medium addition and spray treatment on plants	[22]
Rise	*R. solani*	Antifungal activity	Growth medium addition, seed treatment and treatment on plants	[23]
	Xanthomonas, P. syringae, A. tumefaciens and *E. carotovora*	Bacterial growth inhibition	Growth medium addition and treatment on plants	[24–26]
Wheat	Root and foot rot/*F. graminearum*	Enhancing of phenol content and G-POD, APX, PAL and PPO activities; decreased disease incidence	Seed treatment	[45]
Kiwi	Healthy plants	Modulation of G-POD, APX, PAL and PPO activities	Growth medium addition	[46]
Maize	Healthy plants	Increased plant growth and biomass	Seed coating	[52]
Artichoke	Healthy plants	Enhanced seedlings growth	Seed coating	[53]
Soybean	*Agrotis ypsilon*, soybean pod borer and soybean aphid	Stimulation of systemic antibodies production with repellent effects	Seed treatment	[73]
Sunflower	Downy mildew/*P. halstedii*	PAL, PPO, peroxidases, and chitinase activities and phenolic content in seedling	Seed treatment	[66]
Chilli	*Colletotrichum* sp.	Increased lignin content	Seed treatment	[72]
Tomato	*Fusarium oxysporum* f. sp. *radicis-lycopersici*	Decreased disease severity	Soil addition	[74]
Celery	*Fusarium* wilt	Decreased disease severity	Soil addition	[75]
Purple passion fruit	Healthy plants	Increased flowers number, fruit weight and juice production	Soil addition	[100]
Potato	Healthy plants	Improved growth and final yield	Hydroponic fertigation	[101]

Table 1. Listing of some possible applications of chitosan in agriculture and the related effects (activity and plant defence responces).

5. Conclusion

The chitosan is an active molecule that finds many possible applications in agriculture with the aim of reducing or replacing more environmentally damaging chemical pesticides. Although it is a good alternative even in conventional farming, chitosan applications would find interesting opportunities particularly in organic farming, disadvantaged by the lack of effective tools for managing biotic diseases. The plant disease control in organic farming, especially those caused by fungal and bacterial pathogen, is currently based on copper treatments. However, the research of an ecological alternative is mandatory because of the environmental impact problems related to the use of this heavy metal. Thus, chitosan could represent an innovative eco-friendly strategy for managing plant diseases and replacing copper or reducing its use, thanks to its several properties such as those previously described. In fact, several studies have demonstrated the effectiveness of chitosan in protecting plants from biotic stresses by direct and/or indirect actions, but its interaction with pathogens and plants are still not fully understood. Chitosan application in the field, including formulation aspects, is one of the least studied issues and it needs further testing and validation.

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