**Chitin Uses in Agriculture**

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# Abstract

As sustainable agriculture becomes more urgent, biocontrol using natural compounds such as chitin, a carbohydrate chain polymer, and its derivatives, is a promising strategy. Chitin and its derivatives induce or enhance natural defensive mechanisms in plants. They are recognized as plant growth regulators, growth stimulants, and elicitors for the production of secondary metabolites. They have beneﬁcial effects as fertilizers, soil conditioning agents, plant disease control agents, antitranspirants, ripening retardants, and seed and fruit coatings.

# Keywords:

Agriculture • Sustainable Development • Chitin Fertilizer • Biocide • Elicitor • Plant Growth Regulator

# Abbreviations

|  |  |
| --- | --- |
| NH4+ | Ammonium |
| C:N | Carbon to Nitrogen Ratio |
| CAT | Catalase |
| CMV | Cauliflower Mosaic Virus |
| CERK1 | Chitin Elicitor Receptor Kinase 1 Enzyme |
| CEBiP | Chitin Elicitor-Binding Protein |
| DA | Degree of Acetylation |
| DHA | Dehydrogenase Activity |
| *F. oxysporum* | *Fusarium oxysporum* |
| GlcNAc | N-Acetylglucosamine |
| HMWC | High Molecular Weight Chitin |
| mRNA | Messenger Ribonucleic Acid |
| MAMP | Microbe-Associated Molecular Pattern |
| MAP | Modiﬁed Atmosphere Packaging |
| MDA | Microbial Dehydrogenase Activity |
| MW | Molecular Weight |
| N-P-K | Nitrogen to Phosphorus to Potassium ratio |
| NYDB | Nutrient Yeast Dextrose Broth |
| PGPR | Plant Growth Promoting Rhizobacteria |
| PPO | Polyphenol Oxidase |
| POD | Polyphenol Peroxidase |
| ROS | Reactive Oxygen Species |
| *S. marcescens* | *Serratia marcescens* |
| SOD | Superoxide Dismutase |
| sp. | species |
| EPA | The US Environmental Protection Agency |
| TMV | Tobacco Mosaic Virus |
| U.S. Pat. | United States Patent |
| w/w | Weight to Weight |
|  |  |

# Introduction

Due to strict federal, governmental and state regulations in the pesticide, fertilizer, and plant growth regulator markets (US EPA 2017), there is an increased demand for viable organic alternatives. Due to the constant unselective use of large amounts of synthetic agrochemicals to control the microorganisms that cause infection and plant diseases such as fungi and oomycetes, and to improve plantgrowth, this problem has become a widespread societal issue, of worldwide scale (Aktar et al. 2009). ‘Sustainable agriculture’ through the use of naturally friendly methods and products while replacing current plant protection products with lower environmental impact substances becomes more urgent every day. Among these, biocontrol using natural compounds is one of the most promising strategies.

In this regard chitin, the second most abundant biopolymer after cellulose, is one of the promising natural alternatives (Klemm 2004; Barikani et al. 2014). Chitin is a carbohydrate polymer composed of repeated 2-(acetylamino)-2-deoxy-D-glucose units (Fig. 4.1, left), with a chemical name poly-(1->4)-*β*-*N*-acetyl-D-glucosamine and molecular formula (C8H13NO5)n. Chitin is obtained from the exoskeleton of shellﬁsh, insects, or the cell wall of fungi, by treatment of the respective biomass with hydrochloric acid and sodium hydroxide (No et al. 1989; Cho et al. 1998; Barber et al. 2013; Younes and Rinaudo 2015).

While chitin is used in agriculture, a signiﬁcant drawback is that the polymer is insoluble in water and common solvents, and the ‘special’ solvent systems that are employed for chitin dissolution (Austin 1977; Yusof et al. 2001; Pillai et al. 2009; Xie et al. 2017) are not utilized in this industry. Because of insolubility of chitin, a water-soluble chitin derivative, chitosan, or oligosaccharides of chitin are often used instead in agricultural applications. Chitosan is the fully or partially deacetylated derivative of chitin (Fig. 4.1, center), which differs from chitin polymer by the presence of free amine groups on C-2 atom of D-glucose unit, in place of acetamide groups present in chitin. Due to presence of these free amines, chitosan is soluble in slightly acidic aqueous solutions, which are often employed in agricultural applications. Chitin can also be partially depolymerized into oligosaccharide derivatives of varied chain length (Fig. 4.1, right, top: dimer), or even fully depolymerized to *N*-acetylglucosamine GlcNAc (Fig. 4.1, right, bottom: monomer), which is also widely applied for the purpose (Ramírez et al. 2010).

Experimental trials on crop plants demonstrated the beneﬁcial use of chitin and its derivatives as fertilizers, soil conditioning agents, plant disease control agents such as fungicides, oomyceticides, bactericides, nematicides, antitranspirants, fruit retardants, and seed coatings. Chitin and its derivatives enhance or induce natural defensive mechanisms in the plant (Hassan and Chang 2017), and are recognized as plant growth regulators, growth stimulants, anti-stress agents, and elicitors for the production of secondary metabolites (Orzali et al. 2017).

Because the whole family of compounds, chitin, chitosan, and oligosaccharide derivatives, is safe for humans, farm animals and the environment, the overall production of these compounds for agriculture is enormous. The production constituted 17,600 metric tons in 2015, with expectations to double by 2021 and reach 37,800 metric tons (Chitin Market 2017). Revenues are also expected to increase from $75 million to $170 million during the same period (Chitin Market 2017).

While there are multiple reviews available on chitosan in agriculture (El Hadrami et al. 2010; Sharp 2013; Malerba and Cerana 2018), we would like to narrow down this mini-review to the agricultural use of chitin polymer, and not the chitosan of much lower degree of acetylation (DA) and much higher solubility. The emphasis of this review is thus given from an industrial/applications perspective rather than a molecular biology viewpoint. Table 4.1 summarizes the main points that are covered in this review.



**Figure 1**. Structure of chitin (left), chitosan (center), *N*,*N*-diacetylchitobiose dimer (right: top) and GlcNAc monomer (right: bottom)

**Table 1.** Chitin use in agriculture and its mode of action

|  |  |
| --- | --- |
| ***Action*** | **Mode of action** |
| *Fertilizer* | 1. Utilization of nitrogen from chitin’s acetamide groups: chitin is enzymatically degraded to its oligomers under the inﬂuence of endochitinases and exochitinases, and then to monomers under the inﬂuence of *β*-*N*-acetylhexosaminidases bacteria. Monomers, in turn, decompose to ammonia and nitrates for direct nitrogen uptake by plants (Chernin and Chet 2002; Andronopoulou and Vorgias 2004; Manucharova et al. 2006); 2. Eliciting activity of chitin monomers/oligomers and resultant stimulation of innate plant defense mechanisms (Velásquez and Pirela 2016). |
| *Biocides: Fungicides, Oomyceticides, Bactericides, Antivirals, Nematicides* | 1. Chitin has no ‘direct’ antibacterial effect. Its biocidal action is brought about by induction of plant defense mechanisms where chitin and its oligomers act as elicitors to induce plant immunity (Shibuya and Minami 2001; Okada et al. 2002; Velásquez and Pirela 2016); 2. Degrading cell walls of pathogens using chitinases (Velásquez and Pirela 2016);*Note*: Activity depends on molecular weight of chitin polymer chain (Eguza et al. 2015);   *Note*: Activity depends on MW of chitin polymer chain (Egusa et al. 2015). |
| *Elicitor for the production of secondary metabolites* | 1. Plants are able to recognize chitin via speciﬁc receptors such as chitin elicitor-binding protein (CEBiP, Kaku et al. 2006) or the chitin elicitor receptor kinase 1 enzyme (CERK1, Miya et al. 2007). Such ‘recognition’ of elicitor by the plant serves as a signal for the plant to initiate a systemic acquired resistance in healthy uninfected plant tissues, which allows the entire plant to prepare to repel an attack (Shibuya and Minami 2001); 2. Enzymes which are responsible for the formation of phytoalexins normally exist in an inactive state in a healthy plant cell and are bound by certain inhibitors. Such inhibition gets removed under the action of an elicitor (Velásquez and Pirela 2016). |
| *Direct growth regulator/ bio-stimulant/ Anti-stress agent* | 1. Induction by PGPR through: (1) nitrogen ﬁxation, making nitrogen available nutrient to the plant, (2) degrading cell walls of pathogens using chitinases, and (3) growth regulation through activation of various signaling molecules (Maximov et al. 2011); 2. Eliciting activity discussed above.   *Note*: Bio-stimulants are specifically formulated multicomponent products; thus, classification should be based on efficacy testing, without elucidation of a specific mode of action (Yakhin et al. 2017). |
| *Antitranspirant, wood and leaf sealant* | 1. Formation of a barrier minimizing evaporation from tissues and preventing the pathogen from invading plant healthy tissues (Hirano et al. 1996).   *Note*: Activity depends on the length of chitin oligomers and longer oligomers demonstrate higher activity. Chitosan oligomers did not display any noticeable activity (Barber et al. 1989). |
| *Postharvest*  *Biocontrol* | 1. Unknown |
| |  | | --- | | *Seed Treatment* | | 1. Some seed treatments express antifungal, antiviral, antibacterial properties, others promote seed’s germination rate and plant growth (Yu et al. 2008). |

This article is an abridged version of the chapter by Crini (2019).

# Chitin as a Fertilizer

Fertilizer is any material that is applied to soil or a plant in order to supply the plant with needed nutrients (US EPA 2018a). There are several classiﬁcations of fertilizers, such as those based on the type of speciﬁc nutrient that fertilizer supplies: nitrogen, potassium, phosphorus and ‘compound’ fertilizers (Cole et al. 2016). Nitrogen fertilizers are typically used to promote leaf growth (Liu et al. 2014), while phosphorus fertilizers promote growth of the roots, ﬂowers, seeds, and fruits (Razaq et al. 2017). Potassium fertilizers promote strength of the stem, movement of water in the plant, and also improve ﬂowering and fruiting (Barber et al. 1963).

According to their origin, fertilizers can be divided into organic and inorganic. Inorganic fertilizers are man-made chemicals widely available on the market, while organic fertilizers are mostly plant or animal derivatives. Even though the growth effect on crops from organic and inorganic fertilizers (Anwar et al. 2005; Amujoyegbe et al. 2007) are reported to be comparable, the use of inorganic fertilizers has damaging effects on the soil (Šimek et al. 1999). Many inorganic fertilizers are harmful for humans and the environment, posing concerns about their safety (Viets and Lunin 1975; Damalas and Eleftherohorinos 2011). With growing public concern of possible harm from man-made fertilizers and growing interest in organic food, interest in chitin as a natural fertilizer has also increased (Harper and Makatouni 2002). In agriculture, chitin is used as a component for the preparation of fertilizers and is considered to be an ‘organic’ fertilizer of ‘nitrogen type’.

An important quality of nitrogen fertilizer is the carbon to nitrogen (C:N) ratio, the ratio of mass of carbon to mass of nitrogen in fertilizer (USDA 2011). This C:N ratio (w/w), among other factors, determines how fast the fertilizer decomposes and hence becomes available for the plant (USDA NRCS 2011). Efﬁcient fertilizers maintain a C:N ratio of some 25–30. Too high carbon to nitrogen ratio, more than 25:1 with excess of carbon usually means that fertilizer will decompose slowly. If the C:N ratio is, contrarily, too low with excess of nitrogen, it may lead to immobilization of plant nutrients in the soil (Jat et al. 2012). Chitin and chitosan have C:N ratio of ca. 6 for fully deacetylated chitosan, to 7 for fully acetylated chitin. Another important ratio is the percentage of nitrogen (N), phosphorus (P), and potassium (K) that the product contains; this is usually indicated as ‘N-P-K ratio’ on the fertilizer label; chitin has no phosphorus or potassium (Kaplan et al. 2016).

In respect to the mechanism of action, the fertilizer effect of chitin or chitosan is caused by either biodegradation of the polymer in the soil into ammonia-derived compounds which have fertilizer effect on their own due to presence of aminogroups, promoting of growth of selected microorganisms (Dahiya et al. 2006). Said decomposition of chitin in the soil is achieved by bacterial chitinases, enzymes that degrade chitin. There are many different chitinases with optimal temperature of action from 30 to 60 oC and optimal pH of 4.0–9.0. Chitinolytic enzymes are divided in three types: endochitinases, exochitinases, and *N-*acetyl-*β*-1,4-Dglucosaminidases, also called **β**-*N*-acetylhexosaminidases (Andronopoulou and Vorgias 2004). Endochitinases catalyze the hydrolysis of random bonds over the whole length of the chitin polymer chain, producing soluble oligomers that are further degraded. Exochitinase catalyzes the process of releasing diacetylchitobiose units at the polymer ends. Finally, *β*-*N*-acetylhexosaminidases produce GlcNAc monomers from oligomers (Velásquez and Pirela 2016). At the end of the cycle, chitin decomposes to ammonia and nitrates and can be consumed by plants.

Such decomposition of chitin under the inﬂuence of various bacteria such as *Flavobacterium sp*. and actinomycetes *5A* and *8A*, and F. oxysporum fungus has been studied, and while there was no difference in CO2 production between the chitin-amended and -unamended microcosms, production of NH4+ was signiﬁcantly higher in all of the chitin-amended microcosms (Gould et al. 1981).

Even before a puriﬁed chitin polymer became available as a fertilizer, people started to use shrimp and crab meal in the form of a ground biomass as a good source of nitrogen for soil. It was demonstrated that crab and shrimp meal is high in nitrogen, phosphorus, several micronutrients, and that using crab and shrimp meal as a fertilizer improves crop and plant growth (Costa 1977). Shrimp meal fertilizer, for example, contains 8.5% of nitrogen, 2.6% of phosphorus, and 1% of potassium with N-P-K ratio of 8.5–2.6-1 (Gravel et al. 2012).

In these crab and shrimp meal fertilizers, chitin is the main component containing nitrogen. There are many commercial versions of shrimp/crab shell fertilizers on the market: Down to Earth Crab Meal Fertilizer, Neptune’s Harvest CS604 Crab Shell Multi-Purpose Plant Food, OptiVeg Chitin Based Soil Amendment, Tidal Vision All-Natural Plant Size & Immune Booster, Plant Magic Plant Food 100% Organic Fertilizer, *etc*.

Shrimp and crab meals have also been compared to man-made fertilizers. Aklog et al. (2016) demonstrated an increase in the growth of tomato plants fertilized hydroponically with a mixture of proteins, chitin, and calcium carbonate prepared by mechanical milling of the crab shells to the level of nanoﬁbers (no chemical treatment was applied). Tomato plants were treated with this mixture once per week. Results were compared with plants treated with distilled water (control) and with plants treated with commercial fertilizer HYPONeX, with N-P-K ratio of 10–10-10. It was demonstrated that protein/chitin/calcium carbonate mixture while was effective as a fertilizer, was not as efﬁcient as commercially available HYPONeX fertilizer. It was hypothesized that the presence of calcium carbonate in shrimp meal results in lowering the fertilizer’s decomposition rate and, thus, results in a slow release of fertilizer (Helyar and Anderson 1974; Fenn and Kissel 1975).

To offset this undesired outcome, the use of puriﬁed chitin polymer as a plant fertilizer was ﬁrst shown by Peniston and Johnson (1980) who reported that “chitin can be used as a fertilizer to release nitrogen, slowly, into the soil and thereby over a relatively long period of time increase the nitrogen content of the soil.” Hence, in order to demonstrate fertilizing ability, chitin must degrade and release nitrogen; this process of chitin degradation is caused by chitinoclastic bacteria (Zobell and Rittenberg 1938; Manucharova et al. 2006). This process deﬁnes how chitin degrades and therefore, releases nitrogen in the form consumable for plants; the type and the time of chitin degradation deﬁnes the chitin’s fertilizing ability.

Xu et al. (2005) reported use of chitin and chitosan as slow release fertilizers, and compared them with a commercially available slow release fertilizer Osmocote 18–11–10, and control with no actives, in oil-contaminated beach sediments soil type. In addition to use of chitin, chitosan and Osmocote, two mixtures, namely chitin/Osmocote and chitosan/Osmocote were also studied (Xu et al. 2005). As a measure of the activity, microbial dehydrogenase activity (DHA) and respiration rates were determined on the days 0, 3, 7, 14, 21, 28, 36, 42, 49, and 56. It was found that chitosan had a high fertilizing activity, although not as high as Osmocote. Chitin was found to be effective albeit less than Osmocote, as a nutrient source, and released considerably higher amounts of nitrogen than chitosan (Xu et al. 2005). Chitin also resulted in much higher dehydrogenase activity of microbes relative to the control, acting as both a carbon and nitrogen source (Xu et al. 2005). In contrast, the presence of the chitosan had no noticeable beneﬁcial effect on dehydrogenase activity. However, due to the presence of free amino- groups on chitosan, it was able to chelate polycyclic aromatic hydrocarbons in oily sediments, improving the oil conditions (Xu et al. 2005).

Xue et al. (2018) reported effect of nanochitin in the form of nanowhiskers on the enhancement of the growth yield in wheat. The measurement of chitin activity was conducted through determination of photosynthetic rate, and yield/quality of the grains. It was found that the crop yield was proportionally dependent on amount of chitin, although chitin’s effect on quality of grains was not that straightforward and certain amounts chitin seemed to be excessive.

# Chitin as a Biocide (Fungicide, Oomyceticide, Bactericide, Antiviral, Nematicide, Insecticide)

The US EPA deﬁnes biocides as ‘any poison that kills a living organism’ (US EPA 2018b). Since such deﬁnition is a relatively broad one, biocides have been divided in smaller groups according to the type of living organism that they poison. Biocides, hence, include fungicides which are active toward fungus, oomyceticides which are active toward oomycetes, bactericides which are active toward bacteria, antivirals which are active toward viruses, nematicides which are active toward nematodes, and insecticides which are active toward insects (Milholland 1973).

Fungicides are a very important type of biocide. Fungi are dangerous for plants causing numerous plant diseases such as gummy stem blight and Alternaria leaf spot on berries, leaf blight and powdery mildew on carrots (Vulsteke et al. 1996), white mold on beans (Steadman 1983), *etc*. Due to oomycetes being fungi-like organisms, oomyceticides’ mechanism of action is the same as that for fungi; often oomyceticides are referred to as to fungicides (Beakes et al. 2012). While chitosan derivatives demonstrated pronounced ‘direct’ fungicide activity (Parra and Ramírez 2002) arising from chitosan being an antibacterial agent on its own, chitin does not exhibit signiﬁcant ‘direct’ antibacterial properties due to the absence of free amino groups in the structure. It was reported that ‘direct’ antimicrobial activity of chitosan is linearly proportional to DA, *i.e*., to the number of free amino groups (Goy et al. 2009). Higher number of protonated amino groups in chitosan correlated with an increase in its antibacterial properties, while a low DA correlated with lack of such properties.

The suggested antibacterial action of chitosan is explained by the interaction of positivelychargedchitosanmoleculeswiththenegativelychargedpathogensurface, leading to damage of the pathogen cell due to an increase in the cell membrane permeability, i.e., the ionic surface interaction resulting in cell wall leakage (Rabea et al. 2003). Also important is the formation of an impermeable layer of chitosan on the cell resulting in the inability of a cell to receive needed nutrients, i.e., the formation of an external metal-chelating barrier that results in the suppression of essential nutrients to microbial growth (Xing et al. 2015). One more mechanistic hypothesis suggests the binding of chitosan with microbial DNA inhibiting mRNA and protein synthesis (Goy et al. 2009), although the exact mechanism is not fully understood.

Even though chitin lacks ‘direct’ antimicrobial activity, it is included in multiple fungicidal formulations such as a patented immune-prophylactic fungicidal mixture (IN 2009CH01198 A 20111202), made of acetylsalicylic acid, monosodium of 1-hydroxy ethylidene-1,1-diphosphonic, CM-cellulose, potassium sulphate, and chitin (Sundaresan 2011). The reason for including chitin in these herbicidal formulations is indirect inhibition of the pathogens caused by chitin decomposition by-products. Chitin also supports growth of microorganisms, and exhibits eliciting activity; this phenomenon is covered in the next section of this review. When chitin acts as an elicitor, the protective action is brought about not by ‘direct’ antibacterial action of the compound but by activating the plant’s immune defense mechanisms instead (Velásquez and Pirela 2016).

Such eliciting activity takes place through the activation of chitin-degrading enzymes, chitinases, that decompose chitin to oligomeric fragments. When plants undergo fungal attacks, chitinolytic enzymes are released by plants. These fragments act as elicitors to induce plant-innate immunity against the invading pathogen (Pusztahelyi 2018). Once chitinolytic enzymes degrade chitin to respective oligomers, they are recognized as one of the microbe-associated molecular patterns (MAMPs) which in turn triggers various defense responses in plants (Okada et al. 2002; Shibuya and Minami 2001).

The antifungal effect of chitin has been validated in a ﬁeld study on beans and radishes, in which root-rot and vascular wilt diseases, respectively, were caused by *Fusarium sp*. fungi (Leuba and Stossel 1986). A signiﬁcant reduction of plant disease severity was observed after soil application of chitin which acted as ‘indirect’ fungicide through acceleration of action of antibiotic-producing actinomycetes bacteria (Leuba and Stossel 1986). The aforementioned indirect fungicidal activity of chitin also depends on the molecular weight of the polymer. It has been shown that high molecular weight chitin in nanoﬁbrillated form when used as pre-treatment of Arabidopsis leaves effectively reduced pathogen infection by both the fungus Alternaria brassicicola and the bacterium *Pseudomonas syringae pv*. *tomato DC3000* (Egusa et al. 2015).

Nematicide is a substance that can kill plant-parasitic nematode worms (Chitwood 2003). There are many species of nematodes and more than half of them are parasitic. Nematodes live in both salt and fresh water, in the soil from arctic to tropical regions, and from low to high elevation. Depending on the species, nematodes can be useful or harmful to the plants. The most popular way to apply plant nematicide is through soil fumigation/ irrigation (Taylor 1963). Chitin, as a mixture with urea, has been shown to be effective in decreasing the number of plant parasite nematodes. Thus, experiments were conducted on several plants (Westerdahl et al. 1992): tomato (root-knot nematode species: *Meloidogyne incognita*), potato (root-knot nematode species: *Meloidogyne chitwoodi*), walnut (rootlesion nematode species: *Pratylenchus vulnus*), and brussels sprouts (beet cyst eelworm species: *Heterodera schachtii*) through irrigation using a subsurface drip irrigation system. These experiments demonstrated a signiﬁcant nematode control. Addition of chitin-containing mixtures to the soil rather than irrigation has also demonstrated a similar effect (Radwan et al. 2011). It was reported that chitosan was more effective as a nematicide than chitin (Radwan et al. 2011).

It is not expected for chitin to demonstrate insecticide properties because chitin naturally functions as scaffold material in insects. However, chitin derivatives act as antivirals, i.e., substances able to kill a virus. Plant viruses are intercellular parasites without any molecular structure that replicate themselves without a host (Lodish et al. 2000). Tobacco mosaic virus (TMV) and cauliﬂower mosaic virus (CMV) are typical examples of plant viruses, although it is important to note that plant viruses are studied signiﬁcantly less than animal and human viruses. Commercially available Cytovirin and Trichotherin are typical antiviral substances used on plants (Shanks and Chapman 1965). It was reported that chitosan is able to inhibit development of viruses and increase the immune response in plants (Pospieszny et al. 1991). There is no proposed explanation for viruses being inactivated by chitosan; neither have studies of use underivatized chitin on viruses been conducted. There is a hypothesis that the effectiveness of chitosan against plant viruses is brought about by modifying the plants response to infection and disrupting the viral particle transfer (Sharp 2013). It was also shown that chitosan derivatives, such as chitosan sulphate, act through interaction with a positive charge on the cell surface glycoprotein. As a result, this part of the cell becomes shielded and the virus cannot bind to the cell (Okazaki et al. 1991; Neyts et al. 1992; Kari and Gehrz 1992).

# Chitin as Elicitor of Plant Response

Partially covered in the sections above, chitin acts as a substance that enhances plant resistance through the activation of defense genes and altering the metabolism of plant tissues, eliciting plant response. Elicitors are artiﬁcial ‘parasite’ compounds recognized by the plants (Mishra et al. 2012). Recognition of the elicitor by the plants serves as a signal for these plants to initiate a plant-innate immune response, usually when the elicitor compound comes into contact with the surface of the plant. Both chitin and chitosan are considered to be powerful elicitors, which is typical for carbohydrates (Shibuya and Minami 2001).

Not much is known about the mechanism of action of elicitors. Typically, plant tissues produce special substances in response to contact with an elicitor called phytoalexins (Keen and Bruegger 1977), and each plant family produces phytoalexins of different types and chemical structures, synthesized by different enzyme systems (Hammerschmidt 1999). Due to such variability, it is very difﬁcult to study elicitors’ mechanism of action. It has been proposed that a group of genes responsible for the formation of enzymes that synthesize phytoalexins is suppressed in a healthy plant (Shimizuetal.2008). Induction of plant response is associated with the expression of these parts of the cell’s genome, resulting in the synthesis of enzymes. Thus, it has been shown that two phytoalexins in rice (Oryza sativa), namely phytocassanes and momilactones, were induced by chitin elicitor (Shimizu et al. 2008).

The same authors showed that plants are able to recognize high-molecular-weight chitin (HMWC) via speciﬁc receptors (Shimizu et al. 2010). Kaku et al. (2006) identiﬁed this plasma membrane glycoprotein receptor, chitin elicitor-binding protein (CEBiP). Another hypothesis suggests that enzymes which are responsible for the formation of phytoalexins normally exist in an inactive state in a healthy plant cell and are bound by certain inhibitors. Such inhibition gets removed under the action of an elicitor. Thus, in the Brassicaceae family (Arabidopsis genus) and the Fabaceae family (Medicago genus), the chitin elicitor receptor kinase 1 enzyme (CERK1) functions as a component for the perception of the chitin oligosaccharide (Miya et al. 2007).

Even though it is known that chitin elicits plant defense responses, polymeric chitin is insoluble in common solvent systems, thus many studies on chitin-induced immune responses have been conducted using chitin oligomers of low molecular weight. To determine whether high molecular weight chitin can result in the same plant reaction and provoke plant resistance, chitin nanoﬁbers of submicron thickness have been prepared from high molecular weight polymer and then evaluated (Mayumi et al. 2015). Chitin nanoﬁbers have been dispersed in water and Brassicaceae family sprouts (Arabidopsis seedlings), 10-days old, were treated with aqueous dispersion of chitin nanoﬁbers. Chitin triggered production of reactive oxygen species (ROS, important for signaling role in growth, development) and induced defense-related gene expression in Arabidopsis seedlings. Furthermore, pre-treatment of Arabidopsis leaves with chitin nanoﬁbers effectively reduced pathogen infection by both the fungus Alternaria brassicicola and the bacterium Pseudomonas syringae pv. tomato DC3000. These results demonstrated that chitin nanoﬁbers exhibited elicitor activity.

Similarly, the chitin nanoﬁbers of various nanoﬁbrillation degree were evaluated for elicitor activity in cabbage and strawberry plants. Here, cabbage and strawberry plants challenged with fungal pathogens, Alternaria brassicicola and Colletotrichum fructicola, were grown in a soil/chitin nanoﬁbers mixture. Plants demonstrated a reduction in the number of spots caused by pathogens. Gene expression analysis revealed that the defense-related genes in cabbage plant grown in chitin nanoﬁbers-containing soil were signiﬁcantly upregulated, indicating that chitin nanoﬁbers are suitable for systemic stimulation of disease resistance, in both cabbage and strawberry plants (Parada et al. 2018).

# Chitin as a Bio-Stimulant

Agricultural bio-stimulants are formulations of compounds applied to plant foliar or soils, to promote ‘productivity’ either as plant growth and yield or quality and stress tolerance, through stimulation of natural processes and enhancing nutrient uptake and efﬁciency. Given that the particular composition typically includes various compounds and differs from crop protection products in that there are no direct actions against pests or disease, there is an evident difﬁculty in determining their modes of action.

Yakhin et al. (2017) in their review on bio-stimulants in plant science, proposed the deﬁnition of a bio-stimulant as ‘a formulated product of biological origin that improves plant productivityasa consequenceof thenovel, or emergent properties of the complex of constituents, and not as a sole consequence of the presence of known essentialplantnutrients,plantgrowthregulators,orplantprotectivecompounds,and suggested that bio-stimulant testing should be conducted based solely on efﬁcacy testing, without a requirement for the elucidation of a speciﬁc mode of action (Yakhin et al. 2017). Yet one well-established mode of action has been through bio-stimulation induced by rhizobacteria. PGPRs are the soil bacteria living on plant roots and beneﬁcially involved in promoting plant growth (Kevin 2003). PGPRs are classiﬁed into two classes by the types of relationships they form with the plant, namely rhizospheric, those that colonize the surface of the root and endophytic, those growing within the host plant in the apoplastic space).

The main processes PGPRs are involved into are: (1) nitrogen ﬁxation, the process of conversion of gaseous nitrogen (N2) into ammonia (NH4+) making nitrogen available as a nutrient to the plant, (2) degrading cell walls of pathogens using chitinases, and (3) growth regulation through activation of various signaling molecules (Maximov et al. 2011). Thus, PGPR Bacillus bacteria is able to emit chitinases into culture medium and PGPR S. marcescens GPS 5 is able to hydrolyze high molecular weight chitin into chitin oligomers. In the case of pathogen containing chitin as an essential part of the carbohydrate skeleton of the fungal cell wall, Bacillus bacteria is able to emit chitinases resulting in fungicidal action. Contrarily, when chitin is used as a fertilizer, PGPR S. marcescens GPS 5 results in direct enhancement of nitrogen uptake by plant and eliciting activity of resultant chitin oligomers that promote plant immune response.

# Chitin as Antitranspirant, Wood Sealant and Leaf Sealant

Antitranspirants are compounds applied to the leaves of plants to reduce transpiration through the formation of a physical barrier around pathogen penetration sites. Chitin has been shown to form a physical barrier preventing the pathogen from invading plant healthy tissues. Hirano et al. (1996) reported that application of various chitin ﬁlms onto the tree bark tissues (Dendropanax, Camellia, Maple, Cherry, and Spear-ﬂower) resulted in enhanced tissue healing. The composition of ﬁlms included rubber for easier adherence, and the ﬁlms of the following compositions have been applied to the wounded tree bark: 1/1/1.2 chitin/chitosan/rubber, 1/1.1/1.1 chitin/starch/rubber, 1/1.1/1.1 chitin/cellulose/rubber, and 1/2.3 chitin/rubber. In addition, a composition with no rubber has also been attempted, such as a nonwoven chitin-cellulose 1/1.2 sheet.

Data indicated that only chitin was active for stimulation of chitinase in the wounded tree bark tissues, while no activity was found for cellulose, rubber, and starch. The chitin ﬁlm’s activity was attributed to the degradation of chitin into oligosaccharides and their eliciting action, however no in-depth study of this mechanism with elucidation of speciﬁc proteins or enzymes participating in this mechanism has been conducted. Depending on the composition, biodegradation, and thus healing, took 4–24 weeks after implantation.

Barber et al. (1989) tested chitin and chitosan oligomers for ligniﬁcation of wounded wheat leaves and a signiﬁcant portion of this study was directed to comparison of chitin and chitosan activity as leaf sealants. The monomer of chitin, N-acetylglucosamine, and dimer did not show any measurable activity in ligniﬁcation while higher molecular weight chitin oligomers (trimer, tetramer, pentamer and hexamer) exhibited signiﬁcant dose-dependent activity, proportional to molecular weight of oligomers with highest activity being found for hexamer (highest MW oligomer tested in this study). It was also shown that chitosan oligomers, in contrast, did not demonstrate any noticeable activity, conﬁrming the difference between chitin and chitosan and a need for acetamide group.

# Chitin as an Agent for Seed Treatment

Seed treatment is a process of coating seeds with chemical or biological substance that has properties beneﬁcial for the plant. There are many reasons to use seed treatment. Some seed treatments have antifungal, antiviral, antibacterial, or insecticide properties while others promote seed’s germination rate and plant growth.

Among the advantages of seed treatment is the lowering of the amount of chemicals needed for plant growth, ease in controlling herbicidal spills, uniform distribution of chemicals, perfect timing and targeting. The main disadvantages of themethodarethesameasthoseassociatedwiththeuseofpesticidessuchasriskfor workers, and potential harm for the environment (ODA 2001). Typical seed treatment agents can be contact or systematic. Systematic agents penetrate the seed shell and affect seed movement inside of the plant. Contact agents remain on the surface of the seed. In general, chemicals that are chosen for seed treatment are the same as those chosen for an adult plant. For example, streptomycin is often used as a bactericidal seed treatment (Taylor and Dye 1975) and difenoconazole (Brancato et al. 2018) is applied as a fungicidal seed treatment, etc.

Both chitin and chitosan can be used as standalone seed treatments, or be supplemented to existing formulations. It has been shown that chitin has a positive effect on seed germination, increasing grass seed germination rates. Chitin also increases the production of carbohydrates in plants, and improves their frost tolerance. For example, the addition of chitin to chitinolytic PGPR B. subtilis AF 1 has been tested (Manjula and Podile 2005). Here, chitin was supplemented to AF 1 formulation, and this formulation evaluated as a seed treatment. Addition of chitin increased the emergence and dry weight of pigeon pea seedlings by 29 and 33%.

# Chitin as an Agent for the Biological Control of Postharvest Diseases

The United Nation Environment Program has estimated that about one third of all food produced in the world in 2009 was lost or wasted (Lipinski et al. 2013). About 24% of all food lost was due to storage and handling. There are several approaches to this problem and the postharvest treatment of fresh fruits and vegetables is one of them.

When fruits are collected from the plants, the concentration of the biologically active molecular gases, such as O2 and CO2, changes. As a result, the cell renovation process stops, metabolic loss increases, fruits ripen, and then degrade. The gases’ concentration and respective exchange rate depends on several factors that can be taken into account in order to increase postharvest life span of fruits and vegetables (Dhall 2013). There are many different types of postharvest treatments, such as heat treatment, irradiation, coatings, etc. (Mahajan et al. 2014). Different treatments have different beneﬁts, for example, heat treatment can postpone ripening, edible coating helps to minimize loss of moisture, antimicrobial agents provide protection against microbial growth, etc.

Depending on the produce, the relevant procedure has to be chosen. For instance, it is common for bananas and mango to be treated with nitrogen oxide, while carrot and strawberry in general are getting coated with edible coatings (Mahajan et al. 2014). Several parameters are usually evaluated: browning index, leakage rate, assay of enzymes such as (1) superoxidedismutase or SOD, an enzyme that protects plants from decomposition, (2) Catalase or CAT, enzymes that participate in the breathing process, (3) polyphenol oxidase and peroxidase or PPO and POD, respectively, enzymes that participate in browning mechanism.

While chitosan is an effective edible coating material for postharvest fruits, and coatings prepared from it have been shown to be effective on cherry tomatoes (Won et al. 2018) and mushroom (Liu et al. 2016), etc., there is not much literature available related to coatings made of chitin, probably due to the insolubility of chitin polymer.Ghoshetal.,reportedanincreaseintheshelflifeoflitchiafteritwascoated with chitin-containing wax (Ghosh et al. 1998). Here, modiﬁed atmosphere packaging (MAP) was used together with coating and consisted of wax emulsion, chitin in tartaric acid and shellac. Samples without coatings were considered as a control. Weight loss after 15 days of storage was 1.16% in samples coated withchitin,3.19% for samples coated with shellac, and 3.23% for samples coated with pure wax. The control group demonstrated a 3.47% weight loss. Titrable acidity and amount of ascorbic acid were measured. Both of them decreased with time, but samples coated with chitin demonstrated more acidity and more ascorbic acid after 15 days of storage. Total amount of sugar increased with storage time, but it was lower in samples covered with chitin compared with all other tested coating and control.

Sun et al. (2018) demonstrated the effect of chitin coating on tomatoes. Chitin suspension was used for coating. Tomatoes were immersed in chitin suspensions with 0.1%, 0.5% and 1% of chitin. The control group was immersed in water. After that tomatoes were wounded and Botrytis cinereal were injected in tomatoes on the wound side. Effectiveness of chitin in reducing bacterial activity was measured. It was demonstrated that chitin increases the ability of tomato to resist to Botrytis cinereal.

One typical problem is the attack of harvest by mold. Chitin has been used for enhancing the antagonistic activity of yeasts employed in postharvest biocontrol. Thus, the recent study conducted to evaluate the effect of chitin on the antagonistic activity of yeast species Cryptococcus laurentii used against the postharvest blue mold rot caused by Penicillium expansum in pear fruit showed an increase in the activity of the yeast with addition of 0.5–1.0 wt% chitin (Yu et al. 2008). While the addition of chitin did not inﬂuence the growth of C. laurentii yeast in nutrient yeast dextrose broth (NYDB) media, its population was found to increase rapidly in pear fruit wounds. Moreover, the biological activity of C. laurentii against blue mold rot on pear fruit was greatly enhanced. It was suggested that enhanced population of yeast was likely a major reason for increased biocontrol, although the mechanism for the enhancement of the yeast growth in fruit after chitin treatment remained unclear.

# Outlook

Synthetic plant protection products are mostly toxic, bio-accumulative, persistent in the environment and harmful for both humans and animals. Use of chitin polymer as a promising environmentally-friendly natural alternative to synthetic herbicides, in order to control plant diseases and to regulate plant growth, has recently attracted signiﬁcant attention. According to EPA’s ﬁnal registration review decision, chitin ‘satisﬁes the statutory standard and can perform its intended function without unreasonable adverse effects on human health or the environment’ (US EPA 2018a, b, case #6063). The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) FIFRA Section 3(g) and 40 CFR 155.58(c) provides authority for this action (UA EPA 2008).

In agriculture, the most common use of chitin is a fertilizer, due to the polymer’s ability to slowly degrade, releasing needed nutrients to plants; chitin provides no risk of overfertilizing. Likewise, chitin is utilized as a soil conditioning agent which improves the soil structure and increases its ability to hold water. It also has been shown that chitin acts as a PGPR stimulator, and participates in direct enhancement of nitrogen uptake by plants. In addition, chitin has been shown to enhance or induce natural defensive mechanisms in plants being recognized as a plant growth regulator. Both chitin and its derivatives are considered to be powerful elicitors, inducing plants’ phytoalexins activity and promoting plant immune response.

Even though chitin lacks ‘direct’ antimicrobial activity, it is often utilized as a fungicide due to indirect inhibition of the pathogens caused by chitin decomposition by-products, and the polymer’s eliciting activity. The fungicidal activity of chitin depends on its molecular weight. Furthermore, chitin is also applied as a plant nematicide through soil fumigation/irrigation or addition of chitin-containing mixtures to the soil. Chitin has been shown to form a physical barrier preventing the pathogen from invading a plants healthy tissues and improves ligniﬁcation of wounded leaves. It can be used as standalone seed treatment. In food postharvest applications, chitin increases the ability of fruits to resist diseases and is used as a postharvest biocontrol treatment. Chitin also increases carbohydrate production in the plant, and improves plant’s frost tolerance. Table 4.2 below summarizes the products currently used in agriculture where chitin can make a difference.

We would also like to note here that while chitosan is extensively used in agricultural applications, chitin is not used extensively – partially due to its low solubility but also because of no commercial production in North America. Mari Signum Mid-Atlantic, LLC founded in 2016 is integrating its unique chitin extraction process (Shamshina and Rogers 2018) into an industrial-scale facility in Richmond, VA with estimated initial annual production of 210,000 pounds of chitin biopolymer, extracted from an approximate volume of 1 million pounds of raw shell biomass. One of the markets Mari Signum Mid-Atlantic, LLC is planning to tackle is the use of chitin in agriculture.

**Table 2.** Current agricultural products used

|  |  |  |
| --- | --- | --- |
| Type | Action | Representative Examples of Current Products |
| *Biocide* | Fungicide, Oomyceticide, Bactericide, Viricide (antiviral), Nematicide, Oomyceticide | Various synthetic and organic pesticides, fungicides, viricides (depend on type of disease) |
| *Fertilizer* | Fertilizer | Various granulated fertilizer product (soil-dependent) |
| *Growth Regulator (yield improvement)* | Direct growth regulator / bio-stimulant/ Stress alleviator | Various synthetic growth regulators and hormone inhibitors, humic and fulvic acid, seaweed extracts. Commercial names, e.g., NPK Industries Raw Humic Acid Fertilizer, Pac Low Plant Growth Regulator, Botanicare Seaplex. |
|  | Elicitor for the production of secondary metabolites | Jasmonates, salicylates, benzoic acid. Commercial products, e.g., Jasmonic Acid 95% – Plant Growth Regulator Kits |
|  | Antitranspirant | Synthetic polymers: e.g., di-1-p-Menthene. Commercial names, *e.g*., Wilt Pruf 07011 Antitranspirant Concentrate, Haven® antitranspirant |
|  | PGPR stimulator (plant growth promoting rhizobacteria, PGPR). | Commercial names, *e.g*., Asthra (liquid PGPR), Polyglycerol Polyricinoleate by A.B. Enterprises, PGPR 4150 by company called Palsgaard |
|  | Mycorrhizal stimulators (work by attaching themselves to the root of the plant extending the root system in order to get more water and nutrients) | Commercial names, e.g., Humboldt Nutrients Myco-Madness, Microbe Life Hydroponics Plus-C, Plant Success - PRPSGW25 by Neobits |
| *Ripening Retardant* | Ripening retardant | Controlled-environment storage, 1-methylcyclopropene |
| *Soil Conditioning* | Soil conditioning | Polyacrylamide and cellulose-based products. Commercial products, e.g., Soil binder granular polyacrylamides, liquid polyacrylamides; soil polyacrylamides power blocks |

**Notes**

Dr. Robin D. Rogers is a named inventor on related patents and applications and has partial ownership of 525 Solutions, Inc., and Mari Signum Mid-Atlantic, LLC. J. L. Shamshina is an inventor on related patents and applications, former employee of 525 Solutions, Inc., and CTO of Mari Signum Mid-Atlantic, LLC.REFERENCES

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