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Review Article

Chitosan: A promising plant stimulant

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Abstract

Chitosan is a natural polymer produced from the cell walls of fungi and the exoskeleton of crustaceans. Chitosan has exceptional qualities, such as non-toxicity, biodegradability, biocompatibility, affordability, and its capacity to function as a proteinase inhibitor by way of the formation of phytoalexin. Furthermore, positive ionic charges on the chitosan molecule increase plant immunity and defense systems against pathogens, promote plant growth, and increase yield.

Chitosan plays a significant role, particularly in reducing adverse effects of abiotic stress like salinity and drought, by affecting the formation of Reactive Oxygen Species (ROS) such as proline, antioxidant enzyme activities, and total soluble sugars, which reverse the adverse effects of stress, improve the initiation of the antioxidant system, and increase photosynthesis, consequently improving plant growth and yield. Furthermore, chitosan treatment could suppress virus infections regardless of virus types as well as plant species.

There are numerous physiological responses to chitosan application to improve plant immunity and defense systems, such as the synthesis of antioxidant enzymes to enhance plant resistance against pathogens, particularly fungi and bacterial infections. There are numerous benefits to chitosan, including inducing defense genes in numerous species, improving salt tolerance, enhancing plant growth under abiotic stress, improving germination parameters, increasing seedlings' survival, and consequently, improving plant resistance to pathogen infection, increasing tolerance to abiotic stress, and improving plant growth and productivity. Furthermore, it extends the shelf life and improves the fruit quality of numerous fruits when used as a coating film.

Abbreviations

AMV: Alfalfa Mosaic Virus; COD: Chemical Oxygen Demand; DPPH: Diphenyl-1-Picrylhydrazyl; PPO: Polyphenol Oxidase; TMV: Tobacco Mosaic Virus; TNV: Tobacco Necrosis Virus

Background

Due to the severe climate changes that face the agricultural sector, there is an urgent requirement to use new technologies in agriculture to increase crop production.

Therefore, there is more interest in using advanced techniques and products that are eco-friendly to stimulate plant defenses, continue progress in the agricultural sector, and produce adequate food for humanity (Trivedi, et al. 2017).

Chitosan is a biopolymer produced from the cell walls of fungi and the exoskeleton of crustaceans; it is also obtained from shellfish waste [1]. Chitosan is obtained by partially deacetylating chitin. The unique structure of chitosan is due to its molecule components, which contain three functional groups, namely the amino group and the primary and secondary hydroxyl groups responsible for improving their affinity [2].

Chitosan has been used in numerous industries, including agriculture since it was used as a biostimulant in the 1980s. Since then, chitosan has been showing significant improvement in the germination and survival rate of seedlings and stimulating the growth and flowering of different crop species, such as cereals, fruits, and medicinal crops. In addition to its exceptional qualities, which include non-toxicity,

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biodegradability, biocompatibility, and affordability, and its capacity to function as a proteinase inhibitor by way of the formation of phytoalexin.

There is more interest in using chitosan as a biopolymer on a large scale in the agricultural sector due to its unique physiological characteristics, bioactivity, and great potential, particularly as stimulating plant growth under environmental stress and as an antimicrobial agent. In addition to its use as a safe coating material for vegetables and fruits to extend shelf life, particularly for perishable commodities, it is also degradable, which protects the environment.

Chitosan ranks among the most significant plant elicitors, earlier studies have demonstrated it triggers a plant's defense mechanism against a variety of pathogens, such as viruses and fungi [3].

There is a wide range of applications for chitosan in agriculture, including food packaging, stimulating plant growth, feed additives, antimicrobial agents, etc. Since then, the use of chitosan has led to significant improvements in the growth and productivity of various crops. In addition, chitosan-coated films are used to preserve foods and extend the shelf life of numerous fruits.

This review describes the physiological reactions of chitosan and the current beneficial responses to plant immunity, defense mechanisms, germination, plant growth, photosynthesis, etc., in addition to investigating the beneficial effect of chitosan on the growth of aerial parts and roots as well as the productivity of various plants.

What is the importance of chitosan?

Chitosan has received great attention in recent years due to its distinct properties, such as being immune-stimulating, antibacterial, and antifungal [4].

Chitosan has distinct properties that improve plant growth under different conditions, including non-toxicity, biodegradation, and biocompatibility with various plants, in addition to antifungal activity and reduced oxidative stress, particularly inhibiting proteinase through the formation of phytoalexins, in addition to its cheap cost, which increases farmers' profitability.

Mode of action of chitosan

Positive ionic charges on the chitosan molecule enhance the plant immunity and defense system against pathogens by forming chemical and physical barriers, which in turn promote plant growth and increase production [5]. Furthermore, chitosan acts as a proteinase inhibitor through the formation of phytoalexin, which improves water use efficiency, boosts mineral nutrient absorption, enhances photosynthesis, and lowers oxidative stress to increase plants' tolerance to salinity [6].

The efficiency of chitosan as post-harvest prevention against fungal diseases could be due to the high penetration of cell walls and induced activation of defense response [7].

Role of chitosan under abiotic stress

Under abiotic stress, particularly salinity and drought, chitosan application induces the formation of reactive oxygen species, such as proline, antioxidant enzyme activities, and total soluble sugars, which effectively reverse the adverse effects of salinity, minimize oxidative damage, improve the initiation of the antioxidant system, and increase photosynthesis, consequently improving plant growth and yield [8].

Chitosan plays an important role in improving plant growth under salinity conditions (Figure 1), such as reversing the adverse impact of salinity on plant growth, improving vegetative growth, inducing the accumulation of K+ in leaves, and decreasing oxidative stress markers.

Chitosan and biotic stress

The antiviral activity of chitosan depends on its structure and molecular weight, the efficiency of chitosan is due to its stimulant innate plant immunity effect that elicits defensive responses and its antimicrobial properties.

There are many factors that affect the antimicrobial activity of chitosan, including the type of pathogens, positive charge density, pH, molecular weight, solubility, chemical modification, chelating capacity, and concentration [9].

Previous studies have shown that chitosan treatment could suppress virus infections regardless of virus types as well as plant species. It was found that the antiviral activity of chitosan increased as its molecular weight decreased [10], this may be due to the small molecules having more penetrability across the cell wall and integuments of the seed. For instance, (oligochitosan) one of the derivatives of low-molecular-weight chitosan, is more effective in suppressing infection with the Tobacco Mosaic Virus (TMV) [11].

Treating seeds before cultivation with chitosan reduces the incidence of viral diseases. For example, treating bean seeds

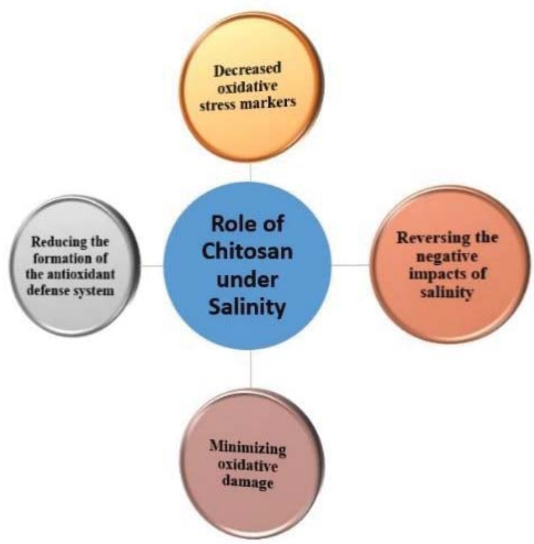

Figure 1: The main role of chitosan in plants under sanity conditions.

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with chitosan reduced infection with Alfalfa Mosaic Virus (AMV). Furthermore, chitosan inhibits the infection caused by bacteriophages, by inhibiting their reproduction at the cellular level [12]. It is well documented that chitosan induces phago resistance in cultures of industrial microorganisms to prevent unwanted phagocytosis contamination by virulent phages or bacteriophages or by induction in lysogenic cultures. Moreover, chitosan-activated systemic acquired resistance against Tobacco Necrosis Virus (TNV) ensued from programmed cell death Katiyar, et al. [9].

Role of chitosan in defense mechanisms

Chitosan induces plant immunity by activating other systems, including transduction, cascades, and involved elicitor-responsive genes, which boosts plant immunity and strengthens the plant's resistance to infection.

Furthermore, it induces the accumulation of phytoalexins, which leads to enhanced protection from fungal infection. Foliar application of chitosan on orchid (*Dendrobium Missteen*) plants reduced the severity of leaf spot disease and increased the length of inflorescences [13], also, chitosan improved the growth of freesia plants (*Freesia Eckl*. ex Klatt) and increased plant height and chlorophyll content [14].

Application of chitosan induces the activity of Polyphenol Oxidase (PPO) which is associated with forming reactive oxygen species, consequently improving the plant's resistance to pathogens. Chitosan may be involved in the signaling pathway for the biosynthesis of phenolics, moreover, chitosan stimulates the formation of chitinase and chitosanase that can degrade the cell walls of some phytopathogens, then enhancing host plant defense systems [15].

Chitosan induces the expression of various genes involved in plant defense responses such as genes encoding and protease inhibitors, Furthermore, probably alleviates the membrane lipid peroxidization and reduces phytotoxicities in plant cells, which can reduce plant cell stress caused by high Chemical Oxygen Demand (COD) in polluted water [16].

The antifungal action of Chitosan involves more pathways to increase plant resistance against fungal diseases, as it affects the biosynthesis of fungal cell membranes, or reduces the ability of pathogens to infect the plant [17].

Benefits of Chitosan

Chitosan enhances the effectiveness of plants to tolerate the negative effects of abiotic stresses (salinity, drought, heavy metals, and cold). It also stimulates defense mechanisms in plants to withstand biotic stress (fungi, bacteria, and insects), which leads to improved plant growth [9]. Chitosan affects many physiological responses such as plant immunity, and defense mechanisms that include the production of antioxidant enzymes to increase the plant's tolerance to pathogens [18].

The application of chitosan elicits defense responses at the cellular, biochemical, and gene expression in plants against bacterial disease, thereby improving growth and yield.

Chitosan stimulates the production of phenylalanine Ammonia Lyase, polyphenol oxidase, and tyrosine ammonia lyase, i.e. superoxide dismutase, catalase, and peroxide activities against the conditions.

Moreover, the stimulating effect of Chitosan on plant growth could be due to its high fertilizing ability and supply of essential elements for plants, consequently, improving growth and promoting the yield of various crops.

Furthermore, oligochitosan is effective in stimulating plant innate immunity against plant diseases in various plants [19].

1. Enhance salt tolerance for numerous plants.
2. Improves plant growth under abiotic stress.
3. Accelerated germination parameters.
4. Promoted ornamental plants' early flowering.
5. Increased dry matter (fresh and dry weights).
6. Enhancing seed production.
7. Increase the production of phytoalexin in germinating seeds of legumes and solanaceous plants.
8. Using chitin in a growth medium increases extracellular peroxidase activity.
9. Increased antioxidant activity of 2, 2-diphenyl-1-picrylhydrazyl (DPPH).
10. Induces defense genes in numerous species.
11. Increased antioxidant activity in sweet basil (*Ocimum basilicum*).

Application of chitosan in agriculture

Chitosan has unique physiological and biological properties that contribute to its uses in numerous fields, including agriculture, as a coating material for fruits, vegetables, and seeds. Chitosan has positive ionic charges, which increase its ability to bind to negatively charged molecules, whether fats, metal ions, proteins, or large molecules [9]. Therefore, chitosan stimulates plant immunity and protects them from pathogenic organisms, improves growth, and increases plant productivity [20].

Recently, chitosan has been used to enhance plant growth and increase productivity, as it works to stimulate plant immunity, protect them from pathogens, and protect fruits from microorganisms (bacteria and fungi).

Chitosan improves the germination of cereal crops: Pre-cultivation treatment of seeds of cereal crops with chitosan improves the germination rate, by forming a layer of the seeds that reserves moisture and increases the uptake of water, consequently improving germination rate. Moreover, chitosan improves seedling survival, and growth parameters of numerous plants, including Wheat (*Triticum aestivum*), [21]

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Carum copticum [22], common bean (*Phaseolus vulgaris* L) [20], Maiz (*Zea mays*) [23].

Chitosan stimulates defense mechanisms in plants and stimulates certain enzymes such as chitinase, pectinase, and glucanase. Chitosan improves plant growth by adjusting the osmotic cell pressure, thus increasing the availability and assimilation of water and nutrients [24].

Increase drought tolerance: Chitosan has contributed to improving the water status of the plant and increases the efficiency of photosynthesis. Therefore, coating seeds with chitosan before planting improves the water content in the seedling leaves and increases the formation of dry matter in the plant, which promotes plant growth under drought conditions according to Moolphuerk, et al. [25] on rice (Oryza sativa L.), and Ávila, et al. [26] on sorghum (Sorghum bicolor).

Chitosan reduces plant transpiration without affecting biomass production and yield of Lemongrass, therefore, chitosan could be used partially as an anti-transpiration [27].

Improving plant growth: Using low molecular weight substances like Oligochitosan increases the photosynthesis average and reduces negative impacts of salinity in wheat plants (Ma, et al. 2012).

Chitosan derivatives such as Chitogel improve net photosynthesis and stimulate vegetative growth of grapevine (*Vitis vinifera*) and fruit quality [28].

Moreover, chitosan lactate increased the shoot biomass of basil (*Ocimum basilicum* L.) and lemon balm (*Melissa officinalis* L.), through increased accumulation of valuable phytochemicals in Lamiaceae species [29]. Mohamed & Ahmed [30] have shown that chitosan promotes vegetative growth and enhances various processes in Navel orange (*Citrus sinensis* L.).

The inhibitory effect of chitosan on pathogens: Foliar spray of chitosan increases accumulation raises levels of hydrogen peroxide (H2O2) and induces a defense system through activation of defense-related enzymes. The application of exogenous chitosan induced the immune response in tomatoes against bacterial pathogens such as wilt disease and increased antioxidant enzymes [31].

Chitosan has been proposed as a potential substitute for treating postharvest illnesses in fruits and vegetables because of its antibacterial and antioxidant qualities as well as its low toxicity [32].

Furthermore, foliar application of chitosan during fruit growth enhances yield and improves fruit quality, which is reflected in the increasing marketable fruits of kiwi fruits [33,34].

Hua, et al. [35] claimed that chitosan derivatives particularly low molecular weight compounds improve the fruit of kiwifruit and reduce the activation of gray mold, inhibiting spore germination, and reducing the growth of Botrytis. cinerea mycelial on fruits.

Enhanced storage life and quality of fruits: Chitosan coating film is effective in food preservation and prolongs the shelf life of fruits by reducing the oxygen partial pressure in the package, balancing temperature and moisture between fruits and the package environment, reducing dehydration, delaying enzymatic browning in fruits, and reducing respiration.

Chitosan is used as a coating material for preserving fruits to extend their shelf life, reduce the infection of fungal diseases and increase the economic efficiency of packaging materials for many fruits such as Mango (*Mangifera indica* L. cv. Kent) Khalil, et al. [36], blueberry [37], Ziziphus mauritiana [38], papaya [39], strawberries [40], plums (*Prunus salicina* Lindl) Liu, et al. [41], citrus fruit [42], and fresh-cut oranges [43].

Conclusion

Chitosan, one of the biopolymers, is uniquely characterized due to the presence of positive ionic charges on the molecule, furthermore, it has exceptional qualities, such as non-toxicity, biodegradability, biocompatibility, affordability, and its capacity to function as a proteinase inhibitor, which improve plant immunity and stimulate defense systems against pathogens, consequently leading to improved plant growth and yield. Chitosan has an important role in reducing the negative effects of adverse environmental conditions such as salinity and drought by stimulating many physiological responses that increase plant immunity, activate defense systems, and enhance plant resistance to pathogens. It also stimulates reactive oxygen species such as proline, antioxidant enzyme activities, and total soluble sugars, which improve the initiation of the antioxidant system and increase photosynthesis, consequently improving plant growth and yield.

There are numerous benefits to chitosan applications that include improving seed germination, increasing seedling survival rates, and enhancing plant growth and productivity. Besides, treating fruits and vegetables with chitosan prolongs shelf life and improves fruit quality.

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