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Use of biostimulant compounds in agriculture: chitosan as a sustainable option for plant development

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Abstract

A novel and sustainable strategy to solve problems caused by stress in plants is the use of naturally prepared solutions called biostimulants. These products in the last decade have received attention by the scientific communities of greatest relevance in agricultural systems because they modify physiological processes to improve crop production and quality. Within this group, one of the biopolymers with the greatest number of beneficial properties is chitosan, a deacetylated form of chitin found in the exoskeletons of crustaceans, fungal cell walls and in the cuticle of insects. In many species of crops the application of chitosan is studied. Several studies have demonstrated its property as an antiperspirant, plant growth promoter and defense system booster in stressful situations. There is evidence that chitosan is one of the most suitable compounds to use together with macro and micronutrients, due to its wide range of characteristics that include biocompatibility, biodegradability, high permeability, cost-benefit ratio, low toxicity, and excellent film-forming capacity. that are used as covers, in addition to that their uses can be extended with pesticides, herbicides, genetic material and plant hormones. The general objective of this review is to describe the role of biostimulants in agriculture, emphasizing the use of chitosan and its effects on plants, in addition to the relationship and interaction it presents with key micronutrients in plant nutrition such as iron and zinc.

Keywords: chitosan; defense inducer; iron; nanoparticles; plants; zinc

Introduction

According to United Nations estimates, the world population will expand by approximately 2.2 billion by 2050. Therefore, the greatest problem worldwide is centered on how to ensure that the growing world population has enough food and that it is of a quality necessary for meet the nutritional needs of this population (FAO, 2019). In addition to the above, resources such as water and soil are increasingly scarce, in addition to

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the increasingly severe and frequent climatic changes that aggravate the situation (Singh and Prasad, 2014; Rouphael and Colla, 2018).

Unfortunately, the main objective of agricultural production systems is to increase profitability for farmers and agricultural industries, using for this the excessive and dependent application of inorganic fertilizers, leaving in the background what is related to the quality of the products and their effects in human health, making work difficult for agricultural scientists. However, the focus is now on the quality of the products and the sustainability of the production systems (Bulgari *et al.*, 2015; Reyes and Cortés, 2017; Méndez-Argüello and Lira-Saldívar, 2019).

Various types of stress caused by biotic factors (bacteria, fungi, or viruses) and abiotic factors (soil composition, salinity, pH, extreme temperatures, drought, pollution, humidity, wind or UV radiation) are the main responsible for quality losses. and production of agronomic crops. Despite the great advances in this area, the current understanding of the mechanisms involved and the strategies to mitigate these effects is limited (Yakhin *et al.*, 2017; Drobek *et al.*, 2019).

A novel and sustainable strategy to solve these types of problems caused by stress is the use of naturally prepared solutions called biostimulants. These products have begun to have a greater relevance in agricultural systems with the aim of modifying physiological processes and optimizing production and quality; receiving important attention in the last decade by and the scientific communities, significantly increasing the number of publications related to the mechanisms in which biostimulants help to face stress situations and increase the productivity of agricultural crops (Bulgari *et al.*, 2015; Yakhin *et al.*, 2017; Van Oosten *et al.*, 2017; Drobek *et al.*, 2019).

The most complicated part of the application of these new technologies is to obtain materials with properties equivalent to those of fully synthetic products, and which also retain their functionality. Within the group of biostimulants, the use of chitosan stands out, which is the deacetylated form of chitin, which in turn is the second most abundant waste material and comes mainly from the exoskeleton of crustaceans and insects. Different authors have reported positive effects on vegetative growth, concentration of photosynthetic pigments and performance in more than 20 plant species, and it has also shown antifungal effects and inducer of defense mechanisms in plants (Pichiyangkura and Chadchawan, 2015; Ibrahim and Ramadan, 2015; Choundary *et al.*, 2017).

Based on the above, the general objective of this review is to describe the role of biostimulants in agriculture, emphasizing the use of chitosan, its effects on plants and the relationship and interaction with key micronutrients in plant nutrition such as iron and zinc.

Biostimulants: concepts and definitions

The word biostimulant has been used, in horticulture, to describe substances that promote plant growth without being nutrients, soil improvers or pesticides. This concept was first used in 1997 by the Department of Crop and Soil Environmental Sciences of the Virginia Polytechnic Institute and Virginia State University, which defined biostimulants as "materials that, in minute amounts, promote plant growth", referring mainly to extracts of algae and humic acids (Du Jardin, 2015).

Over the years, the concept has undergone many modifications due to changes in the diversity of inputs that can be considered within this group, in addition, there are two industrial branches that have their own definitions about the term biostimulants (Calvo *et al.*, 2014). The European Council of the Biostimulant Industry (EBIC), defined biostimulants as follows: "Plant biostimulants contain substances and/or microorganisms whose function when applied to plants or the rhizosphere is to stimulate natural processes to improve and/or benefit nutrient uptake, nutrient efficiency, abiotic stress tolerance, and crop quality. Biostimulants do not have direct action against pests and, therefore, do not fall within the regulatory framework of pesticides" (Rouphael and Colla, 2018).

For its part, the Coalition of biostimulants in the United States defines them as "substances, including microorganisms, that are applied to the plant, seed, soil or other growing media that can improve the ability of the plant to assimilate the nutrients applied or provide benefits for the development of the plant. Biostimulants are not nutrients for plants and, therefore, cannot make nutritional claims or guarantees" (Biostimulant Coalition, 2021). In more concise words, plant biostimulants are a diverse group of substances that can be added to crops and have positive effects on plant growth and nutrition (Figure 1), but also on abiotic and biotic stress tolerance, however, biostimulants are not considered as nutrients even though they facilitate their absorption (Van Oosten *et al.*, 2017).

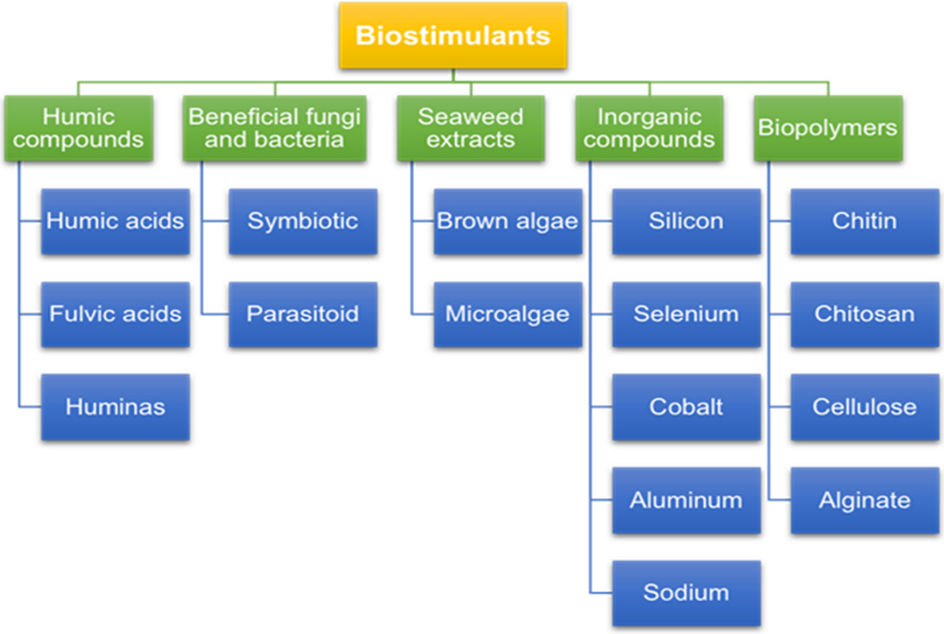

Figure 1. Types of biostimulants commonly used in agriculture

Use of biostimulants in agriculture and classification

The use and production of biostimulants is spreading from more developed countries to a larger number of countries. In Europe, the biostimulant market is expected to range between 1,500 and 2,000 million dollars in 2022, representing approximately half of the world market, with an annual growth rate of 10 to 12%. This is mainly due to the global trend to reduce the amount of fertilizer applied and the search for products that generate a lower environmental impact and are healthy to consume (Economic Overview of the European Biostimulants Market [EBIC], 2021).

Despite recent efforts to produce an international regulation on biostimulants, there is no legal definition of plant biostimulants anywhere in the world. This situation allows several substances and categories that can fall into this group. Despite this, some important categories are always included in various scientific works (Calvo *et al.*, 2014; Du Jardin, 2015; Yakhin *et al.*, 2017; Van Oosten *et al.*, 2017). The following section briefly details the categories that in our opinion represent the biostimulant market worldwide, with an emphasis later the compound of interest for this work.

Humic compounds

Humic compounds (HC) are found within the organic matter of the soil and are formed by the decompositions of plant and animal matter thanks to the action of microbial metabolism and represent the main reserve of organic carbon on the surface of the earth. The CH were classified into three groups: humins, humic acids and fulvic acids, originally categorized according to their molecular weights and solubility, being those with the lowest molecular weight the ones that tend to have the greatest positive biological effects on plants (Canellas *et al.*, 2015; Du Jardin, 2015; Halpern *et al.*, 2015).

HC can be extracted from many different sources, including soils, municipal waste, earthworms, coal deposits, peat moss, and leonhardite. HC have been shown to have a great variety of beneficial functions in the soil, such as controlling the availability of nutrients, the exchange of carbon and oxygen between the soil and the atmosphere; in turn, they are responsible for the transformation and transport of chemical substances that may become toxic to the microecosystems of the rhizosphere. These properties contribute to the regulation of many ecological and environmental processes that are crucial for the growth of plants and terrestrial life, they have also been reported to regulate the carbon and nitrogen cycle of the soil, in addition to improving the stabilization of the soil. soil structure (Calvo *et al.*, 2014; Canellas *et al.*, 2015; Halpern *et al.*, 2015).

More specifically Rose *et al.* (2014), concluded in an extensive analysis that in works where HC was applied, a total increase in dry weight of approximately 22% for the aerial part and 21% for the root part was achieved. For their part, Halpern *et al.* (2015) in their review emphasize various studies that mention the positive effect of HC, increasing the number of fruits and flowers in various crops. It also mentions the positive effects on the absorption of nutrients such as N, P, Zn and Fe, this is partly due to the positive effects it generates on the structure of the soil that was mentioned above but they also mention that CH can affect the morphology of the roots allowing greater absorption and greater activity of the H + -ATPase enzyme, as well as the enzymes responsible for assimilating nitrates. These changes in the morphology of the roots can be attributed to possible changes in the cellular energy metabolism that facilitate the proliferation of lateral roots (Jindo *et al.*, 2012). In turn, Van Oosten *et al.* (2017) emphasizes how humic acids help the plant to cope with situations of hydric and saline stress, activating defense mechanisms and increasing the compounds responsible for antioxidant activity, as well as the levels of proline and plant hormones specifically cytokinins.

These benefits can be easily exploited by farmers because HC can be very inexpensive, their price ranges from 40 to 800 dollars per ton, which compared to the prices of top-quality fertilizers can generate a greater cost-benefit, taking into account that it is a complementary product that due to its benefits will minimize the costs of applying fertilizers in the future (Quilty and Cattle, 2011; Rose *et al.*, 2014).

Beneficial fungi and bacteria (BFB)

The use of BFB in agriculture has its first known reports more than 100 years ago, when in 1909 a study showed that a consortium of *Pseudomonas radicicola* and *Azotobacter sp.* improved the growth of oats and barley, in addition to improving the absorption of N. Despite promising results over the years, it was not until 1979 when it gained relevance in agriculture, emphasizing the bacteria that promote plant growth by modifying the soil microflora (Ruzzi and Aroca, 2015). About this topic, two types of groups of functional and ecological bacteria have been studied; Mutualistic endosymbionts of the Rhizobium type and plant growth promoting bacteria (PGPB) found in the rhizosphere, these bacteria belong to several genera such as *Rhizobium, Bradyrhizobium, Azotobacter, Azospirillum, Pseudomonas* and *Bacillus* (Du Jardín, 2015; Van Oosten et al., 2017).

Bacteria have been shown to be multifunctional in terms of their benefits on plants, since they contribute to mitigating both abiotic and biotic stresses, including pathogen control, greater tolerance to salt, greater resistance to heavy metals and other toxins; as well as help increase growth and yield (Alavi *et al.*, 2013; Berg *et al.*, 2014; Du Jardín, 2015).

Among the ways in which bacteria interact with plants are parasitism, mutualism, penetration into the interior of cells, extending to the rhizosphere or rhizoplane and with the participation in biogeochemical cycles that modify the structure of the soil and plant cover. The modes of action are diverse depending on the species. The most common mechanisms presented by the application of bacteria to plants are changes in the hormonal content, increasing the contents of auxins or cytokinins, the production of volatile compounds, the increase in the availability of nutrients through the production of siderophores that facilitate the entry through the root and the production of organic acids that modify the pH, especially affecting the availability of micronutrients (Calvo *et al.*, 2014; Halpern *et al.*, 2015; Du Jardín, 2015; Rouphael and Colla, 2018).

Like bacteria, beneficial fungi also form parasitoid associations and mutualistic symbioses with plants; It is believed that approximately 5000 species of fungi can colonize roots and subsequently providing plants with soil nutrients (Behie and Bidochka, 2014). Within this large group of fungi, the mycorrhizal fungi stand out, which are a heterogeneous group of taxa that establish symbiosis with more than 90% of all plant species. Among the different forms of physical interactions, arbuscular mycorrhizal fungi (AMF) are a widespread type of endomycorrhizae, where the fungal hyphae of *Glomeromycota* species penetrate the cortical cells of the root. Various studies report that AMF contribute to the survival of plants in desert ecosystems and function in soils with low levels of organic matter and phosphorus (Du Jardín, 2015; Osuna-Ávila *et al.*, 2021).

It has also been reported that AMFs are very effective in improving the absorption of nutrients helping the growth of the plant, currently they are of great interest since they have been recognized as a biofertilizer that helps control both biotic and abiotic stress. In addition, mycorrhizal fungi can form hyphal connections between the root systems of two or more host plants, forming networks of mycorrhizae below the ground between various plants (Gilbert and Johnson, 2017; Hussain *et al.*, 2018).

Another genus of fungi that has become very important in the agricultural sector is *Trichoderma sp.* which was widely recognized for its function as a biological control, especially against phytopathogenic fungi. However, recent studies show a relationship between its association with the roots and a greater absorption of nutrients from plants because of a solubilization of macro and micronutrients. Several strains of *Trichoderma* have also been found to stimulate plant growth, improve yield and nutritional quality. Another recently discovered benefit is the ability of this genus to increase the photosynthetic capacity and in turn the content of photosynthetic pigments (Fiorentino *et al.*, 2018; Harman *et al.*, 2019; Lombardi *et al.*, 2020).

Seaweed extracts (SE)

The use of seaweed extracts as biostimulants has emerged in the last decade commercially as products that promote plant growth and as stress relievers, such as excess heat, salinity, and drought. However, its use in agriculture dates to ancient times since it was used as compost to improve the soil and directly to improve the productivity of crops, being used commonly by the Roman Empire, Japan, China, France, Spain and the former Great Britain (Craigie, 2011; Van Oosten *et al.*, 2017; Mukherjee and Patel, 2020).

The use of SE has taken on commercial relevance, especially as biofertilizers or purifying the metabolites that make up these extracts, these compounds include the polysaccharides laminarin, alginates and carrageenan's and their degradation products, other components such as sterols and compounds that contain N such as betaines and hormones. Metabolites such as phlorotannin's, fatty acids, halogenated compounds, alkaloids, terpenoids and lectins have also been isolated; many of these being exclusive to its source of algae (Du Jardin, 2015; Mukherjee and Patel, 2020).

There are 2 main groups of algae on the market: macroalgae and microalgae and there are currently more than 47 companies that produce and market various extracts of algae for agricultural use. Most of the formulations come from the brown algae of the *Ascophyllum nodosum* species that are mainly harvested from marine waters, but its quality as a biofertilizer varies according to the availability of nutrients, the harvest time, and the place where it is harvested, so its effectiveness is not as constant as you would like. Based on this, the scientific community has found a promising alternative towards the standardization of the raw material and the reduction of costs in the production of algal biomass with the use of microalgae, which are already on the market in some species, such as *Chlorella* spp., *Dunaliella* spp., *Haematoccocus* spp., *Isochrysis* spp., *Nannochloropsis* spp., *Porphyridium* spp., and *Spirulina* spp. but they are only sold as supplements to traditional fertilization (Van Oosten *et al.*, 2017; Chiaiese *et al.*, 2018).

The effects of the application of algae extracts can be seen both in the soil and in the plant itself. In an edaphic way, the application of these extracts as solid or liquid compost, modify the aeration of the soil and its water retention capacity, it also modifies the cation exchange capacity and some of the compounds have the capacity to capture heavy metals. that could become toxic to plants (Du Jardin, 2015). For their part, SEs have effects on plant growth, increase in photosynthetic activity and chlorophyll content, increases in the activity of enzymes related to nitrogen metabolism, which leads to a higher content of proteins and amino acids. Effects on flowering and the production of compounds related to antioxidant capacity have also been reported. In addition, impacts on germination, seedling establishment and development have been reported that are related to hormonal effects, which have been identified in algae extracts as cytokinin's, auxins, abscisic acid, gibberellins, and other classes of hormone-like compounds, such as sterols. and polyamines (Stirk *et al.*, 2013; Wally *et al.*, 2013; Mukherjee and Patel, 2020).

Another of the positive effects of using SE is the effect it has on the mechanisms responsible for the defense of the plant before stressful situations, several studies show that extracts of seaweed that contain betaines and cytokinin's can help to maintain the stability of the proteins. embedded in the cell wall and keep cells plump for longer in situations of lack of water or excessive heat. Effects on stomatal conductivity and water potential in the leaf have also been reported due to changes in potassium flux in cells (Calvo *et al.*, 2014; Saa *et al.*, 2015; Mukherjee and Patel, 2020).

Inorganic compounds

Inorganic biostimulants are all those elements that can be beneficial for plants but do not fall under the rule of essentiality. The five main beneficial elements are Al, Co, Na, Se and Si, which can be found in the soil solution as inorganic salts and recently being applied as commercial products (Marschner, 2011; Bhupenchandra *et al.*, 2020).

Through the years, these elements have been recognized for their effects on the growth, quality and protection of crops. Its effects on abiotic stress situations have recently been studied and positive effects have been found on cell wall rigidity, osmoregulation, reduction in perspiration under water stress, thermal regulation against temperature stress, antioxidant protection, interactions with symbionts, protection against heavy metal toxicity, in addition to the synthesis and signaling of plant hormones (Du Jardin, 2015; Bhupenchandra *et al.*, 2020).

Among this group of beneficial elements, the one most used as a biostimulant is silicon (Si), which is the second most abundant element on the earth's surface. Plants take Si in the form of silicic acid [$Si(OH)_4$] from the soil solution and it is easily transported to the different organs of the plant, its essentiality for plants is still under discussion, however, its positive effects on growth and productivity are widely demonstrated (Marschner, 2011; Tubana *et al.*, 2016). One of the most important effects of Si is that it affects the chemical and biological properties of some micronutrients such as P, Al, Fe, Mn and the mobility of heavy metals, microbial activity, the stability of soil organic matter and the formation of polysilicic acids, which have a significant effect on soil texture, water retention capacity, adsorption capacity and stability of soil erosion (Swain and Rout, 2017).

Regarding the effects on plants, there are increased resistance to abiotic stress and resistance to pathogens and diseases. Effects on the absorption of nutrients such as P, Ca, K and N have also been demonstrated, especially when these are found in low amounts in the soil. Another positive effect when applying Si is that they increase the vigor and rigidity of the leaves, thus increasing light absorption and photosynthesis. It has been found that they also modulate the mobility of water, apparently due to the formation of silica gel in the cell walls, which reduces perspiration, thus avoiding the problems caused by water stress (Guntzer et al., 2012; Liang, et al. al., 2015; Albrecht, 2019).

Biopolymers

In recent years, many attempts have been made to substitute petroleum products in materials development, so many biopolymers such as starch, cellulose, collagen, gelatin, alginate, chitin and chitosan have been investigated, due to that they have a functionality applicable in sustainable environmental development (Croisier and Jérôme, 2013).

In agriculture, alternatives are being sought for the use of agrochemicals through green technology or renewable nanomaterials. Synthetic polymers such as polycaprolactone, polyethylene, polyvinyl alcohol, and acrylate-based polymers are used to achieve slow release of fertilizers that allow better absorption and improvement of the soil; However, these synthetic polymers are not biodegradable, and their residues can remain in the soil for a long time, helping to pollution. It has been reported that the biopolymers, mentioned above, due to their properties have been used as covers for fertilizers and slow-release pesticides, in addition to their use as hydrogels for moisture retention in places where the climate is very arid (Sampathkumar *et al.*, 2020).

The term biopolymer is generally understood as an organic polymer that is produced naturally by living organisms or that is synthesized through waste from living things. These compounds have their main advantage in their easy degradation and that they transform into compounds that are easily found in the environment such as water, carbon dioxide or methane (Tănase *et al.*, 2014). Various studies have proven that they act as inducers that can activate the defense responses of plants and induce plants to produce disease-resistant compounds, they have also been shown to have various properties as protectors against abiotic stress. Within this group, one of the biopolymers with the greatest number of beneficial properties is chitosan (Du Jardin, 2015; Zheng *et al.*, 2020).

Chitosan

Origin and uses of chitosan

Chitosan is a crystalline linear polymer and a deacetylated form of chitin (Figure 2), which is a linear copolymer of 2-acetamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-D- glucopyranose. Being the second most abundant renewable polymer in nature, after lignocellulosic biomass, it can be found in the exoskeletons of crustaceans, fungal cell walls and in the cuticle of insects (Croisier and Jérôme, 2013; Piras *et al.*, 2014; Malerba and Cerana, 2016; Choudhary *et al.*, 2017).

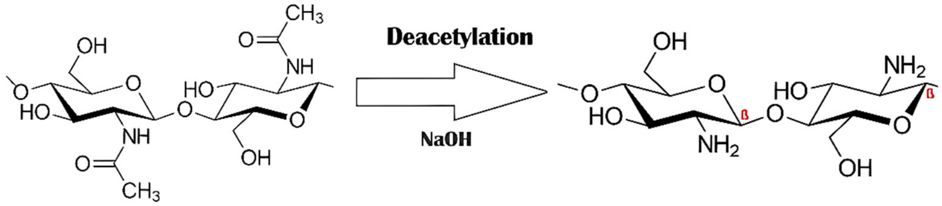

Figure 2. Conversion of chitin to chitosan

To become chitosan, chitin must have at least 60 degrees of deacetylation, which is done by chemical hydrolysis under severe alkaline conditions or by enzymatic hydrolysis in the presence of enzymes such as chitin deacetylase. Chitosan is identified based on its degree of deacetylation, which can vary between 60% and 90% and average molecular weight between 50 and 2000 KDa, these variations are due to its different ways of obtaining and production (Riva *et al.*, 2011; Mármol *et al.*, 2011; Croisier and Jérôme, 2013). These variations generate chitosan preparations in terms of the degree of deacetylation, molecular mass, degree of polymerization, viscosity, pKa value, so when talking about chitosan we do not speak of a single compound but of a group of biopolymers that are currently found. commercially available. In addition, the presence of (acetyl) amino groups within chitosan structures has been reported that would be very beneficial for chemical modifications to build sophisticated molecular architectures with wide potential for use in industry (Malerba and Cerana, 2016; Zheng *et al.*, 2020).

Chitin was first isolated in 1811, when Professor Henri Braconnot obtained it from a fungus and named it fungin. After that, Auguste Odier in 1821, gave it the name chitin after isolating it from the exoskeleton of insects. For its part, chitosan was discovered in 1859 by Charles Roughet, when he made chitin soluble in water, however, the term was used until 1870 and the great discoveries about these biopolymers did not arrive until 1930 and despite these Discoveries the true interest of the industry came in the 70s, when alternatives were sought to better use the biological waste of marine crustaceans (Crini, 2019; Amine *et al.*, 2021).

Its main utility is due to its antimicrobial properties, its charge density and its film-forming or coating properties. Currently, chitosan is used in the form of solutions, suspensions, and particles, for example nanoparticles, beads, resins, sponges, hydrogels, foams, protective films, fibers, and microscopic threads. Chitosan has been commonly used in the biomedical industry, due to its proven medicinal effects. Also, for decades, its use in the food industry is well known as a thickener, gelling agent, and emulsifier, it is used in edible protective films, as a functional ingredient that provides dietary fiber due to its hypocholesterolemic effect and in industrial processes. In addition, one of the most relevant areas is its use as an adjuvant in water treatment, due to its favorable characteristics with the environment; Various studies demonstrate its role as a chelator of heavy metals and pesticides (Mármol *et al.*, 2011; Morin-Crini *et al.*, 2019).

Chitosan applications in agriculture

Because it is a friendly compound with the environment, due to its rapid degradation, low toxicity and easy obtaining, the use of chitosan in agriculture has increased in recent years. The use of chitosan in agriculture began approximately in the 90's, since bactericidal properties were found, or in some cases it hindered the growth of bacteria, delaying their effects on plants. Also, due to its chelating activity, it has been proven as an excellent fungicide and antiviral. Although its main use was to retard the release of fertilizers and pesticides (Vasconcelos, 2014; Morin-Crini *et al.*, 2019).

The application of chitosan has been studied in many species of crops, including cereals, ornamental, fruit and medicinal plants, and its effectiveness depends on the structure and concentration of this, the plant species and the phenological stage in which plant is found. Several studies have demonstrated its property as an antiperspirant, in turn its effect as a plant growth promoter and defense system promoter in stress situations has been studied (Abu-Muriefah, 2013; Pichyangkura and Chadchawan, 2015; Saharan *et al.*, 2016; Deshpande *et al.*, 2017).

Chitosan and its derivatives are used in agriculture in various ways (Table 1); One of these uses is its role as a seed coating in which its antifungal role generates the ability to protect the seedlings, they also have a positive effect on the germination rate, the growth parameters, and the vigor of these. Another of its uses is as an amendment to improve the structure of soils and it was found that chitosan successfully reduces *Fusarium* wilt and against infections by *Cylindrocladium floridanum, Alternaria solani*, and *Aspergillus flavus*. Its uses also include the application as a foliar spray, supplement in hydroponic solutions and as a supplement in plant tissue culture medium, finding improvement in crop performance, induction of the defensive system and promotion of plant growth (Orzali *et al.*, 2016; Morin-Crini *et al.*, 2019).

Physiological and biochemical effects of chitosan on crops

As mentioned above, various studies have shown that chitosan is a natural molecule that induces numerous physiological responses in plants, however, these responses depend on its structure and concentration when applied, in addition to the species and stage of development. of the plant. The mode of action of chitosan is not completely revealed, various authors suggest that the physiological effects of the application of chitosan in plants are the result of the ability of this polycationic compound to bind to a wide range of cellular components such as DNA, the plasma membrane and the constituents of the cell wall, but also to bind to specific receptors involved in the activation of defense genes, in a similar way to the defense inducers of plants (Du Jardín, 2015; Malerba and Cerana, 2016).

Table 1. Uses and benefits of the application of chitosan in agricultural crops

Crops	Application from	Results	References
Bell pepper (*Capsicum annuum* L.) Cv. 'Yolo Wonder'	Foliar in leaves and fruits	Increase in weight, diameter and fruit yield	Mahmood *et al.*, 2017
Bell pepper (*Capsicum annuum* L.) Cv. 'California Wonder'	Priming in seeds	Acceleration of germination and lower incidence of fungal attacks	Samarah *et al.*, 2016
Chilli pepper (*Capsicum annuum* L.)	Foliar	Increase in number of fruits, leaves and chlorophyll content. In addition, it reduced the incidence of attacks by *Phytophthora capsici*	Esyanti *et al.*, 2019
Tomato (*Lycopersicon esculentum* L.) Cv. 'PKM1'	*In vitro*	Protection against *Alternaria solani*	Sathiyabama *et al.*, 2014
Tomato (*Solanum lycopersicum* L.) Cv. 'Marglobe'	Soil irrigation	Increase in root colonization by *Pochonia chlamydosporia*, a parasitic fungus of *Meloidogyne spp.*	Escudero *et al.*, 2017
Tomato (*Solanum lycopersicum* L.) Cv. 'BINAtomato-6'	Foliar	Increase in variables related to the yield and activity of the nitrate reductase enzyme	Mondal *et al.*, 2016
Tomato (*Lycopersicon esculentum* L.)	Foliar	Increase in biomass, yield and chlorophyll content, low water stress	Hassnain *et al.*, 2020
Cucumber (*Cucumis sativus* L.)	Postharvest, as a protective cover	Maintained quality and reduced carbon dioxide production	Olawuyi *et al.*, 2019
Cucumber (*Cucumis sativus* L.) Cv. 'Celebrity F1'	Foliar	Increased quality, yield and vegetative growth for two consecutive years	Shehata *et al.*, 2012
Cucumber (*Cucumis sativus* L.)	Foliar	Maintained growth and production rates under temperature stress	Ali *et al.*, 2020
Bean (*Phaseolus vulgaris* L.)	Foliar	Increase in variables related to growth and yield under water stress	Abu-Muriefah *et al.*, 2013
Bean (*Phaseolus vulgaris* L.) Cv. 'Giza 3'	Priming	Reduced the incidence of damping-off and root rot	El-Mohamedy *et al.*, 2017
Bean (*Phaseolus vulgaris* L.) Cv. 'Nebraska'	Foliar	Inconsistent results on yield parameters under temperature stress	Ibrahim and Ramadan, 2015
Potato (*Solanum tuberosum* L.) Cv. 'Agria'	*In vitro*	Increased fresh tuber weight and yield	Amini, 2015
Potato (*Solanum tuberosum* L.) Cv. 'Spunta'	*In vitro* and in hydroponic solution	Significantly reduced the attack of various species of *Fusarium*	Mejdoub-Trabelsi *et al.*, 2020
Watermelon (*Citrullus lanatus* L.) Cv. 'Jubilee'	Hydrogel on the substrate	Increased root size and stoma width	Gonzalez-Gomez *et al.*, 2017
Maize (*Zea mays* L.) Cv. 'Suria local'	*In vitro* and foliar	Increase in antioxidant activity and variables associated with yield	Choudhary *et al.*, 2017b
Maize (*Zea mays* L.) Cv. 'Pioneer 3906' and 'SR03'	Foliar	Reduced the effects of salt stress	Al-Tawaha *et al.*, 2018
Maize (*Zea mays* L.) Cv. 'Giza 9'	Priming and foliar	Increased chlorophyll content and yield, low salt stress	ALKahtani *et al.*, 2020
Rice (*Oryza sativa* L.)	Edaphic solution	Increase in yield	Nguyen and Tran, 2013
Rice (*Oryza sativa* L.)	*In vitro*	*Rhizoctonia solani* growth reduction	Liu *et al.*, 2012

More specifically, chitosan produces positive effects in plants because it induces the activity of enzymes involved in oxidative metabolism such as superoxide dismutase, peroxidase, and catalase; in addition to increasing chlorophyll levels and in turn a better photosynthetic activity (Pichyangkura and Chadchawan, 2015). It is also attributed beneficial properties in situations of water scarcity because it induces stomal closure, thus reducing perspiration and therefore water loss (Kashyap *et al.*, 2015).

Effects of chitosan on plant defense inducers

For the induction of defense responses in plants, chitosan can affect gene expression by generating changes in the interaction with chromatin, and it can also bind to specific receptors. One of these receptors is a binding protein belonging to the lectin family of glycoproteins. The presence of chitosan receptors is also suggested by the rapid activation of the plasma membrane H^{+-}ATPase. Once recognized by a receptor, the signal is translated by a secondary messenger and physiological responses begin to activate (Malerba and Cerana, 2016; Hidangmayum *et al.*, 2019).

Among the best-known responses of chitosan to cell receptors, the accumulation of hydrogen peroxide, reactive oxygen species (ROS), nitric oxide (NO) and an increase in the content of Ca^{2+} inside the cell have been demonstrated (Figure 3). The production of these compounds triggers a series of responses related to defense mechanisms in plants (Du Jardin, 2015; Pichyangkura and Chadchawan, 2015; Malerba and Cerana, 2016).

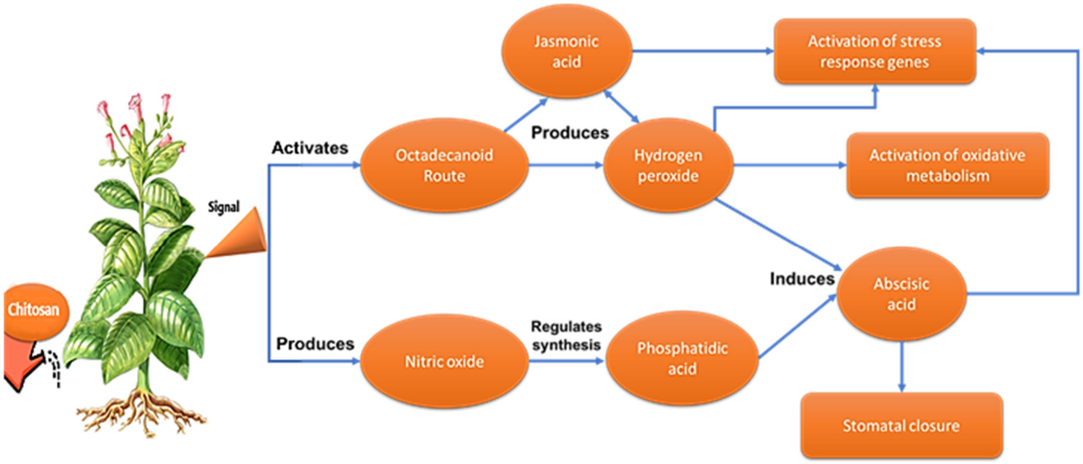

Figure 3. Processes involved in chitosan signaling

In the case of ROS, the plant treated with chitosan induces a greater activity of the enzymes related to oxidative metabolism such as superoxide dismutase (SOD), peroxidase (POX) and catalase (CAT) which, in addition to reducing the species reactive, they promote the production of malondialdehyde which in turn is responsible for reducing lipid peroxidation. Another enzyme that is affected by the application of chitosan is phenylalanine ammonium lyase (PAL), responsible for the biosynthesis of most bioactive compounds in plants; Likewise, various studies report an increase in the concentration of proline. These processes are positively related to the positive effects of chitosan in plants subjected to some type of abiotic stress, such as stress due to salinity and drought (Pichyangkura and Chadchawan; Hidangmayum *et al.*, 2019). While the Ca^{2+} content is responsible for regulating the activity of the enzyme callose synthase, which is responsible for producing callose, a substance responsible for repairing wounds in cells caused by mechanical damage or attack by pathogens (Malerba and Cerana, 2016; Hidangmayum *et al.*, 2019).

On the other hand, the accumulation of NO induces the production of phosphatidic acid, which is positively related to the production of abscisic acid (ABA), a phytohormone that can produce stomatal closure and thus reducing the rate of perspiration. altering the turgor of the occlusive cells and consequently causing their closure. In turn, the production of ABA also induces the synthesis of solutes such as proline, glycine, betaine, and trehalose; which decrease the water potential of the cell, allowing it to take in water that surrounds it or retain what it already has (Wilson *et al.*, 2014; Pichyangkura and Chadchawan, 2015). Another positive effect that chitosan produces in plants is the ability to activate plant defense genes through the octadecanoid pathway, increasing the synthesis of jasmonic acid (JA), a key compound for plants to protect themselves from attacks by pathogens, mainly insects. (Pichyangkura and Chadchawan, 2015; Hidangmayum *et al.*, 2019).

Chitosan as a biofertilizer

In recent years, the use of biostimulants as fertilizers in plants has generated many investigations, chitosan has stood out among this series of compounds due to its rapid degradation through enzymatic processes without affecting the beneficial rhizosphere microbiome of the soil and induces symbiotic exchange between the plant and microorganisms (Sharif *et al.*, 2018). The large number of articles published in the last year shows that chitosan is a unique product available in large quantities and at a very economical price and has a bright future in the development of sustainable agricultural practices, and its application as a biofertilizer has been shown to have positive effects on the absorption of other nutrients without negatively affecting the environment (Malerba and Cerana, 2016; Sharif *et al.*, 2018).

It is known that one of the main problems in agriculture is the low absorption in fertilizers, it is estimated that plants cannot absorb between 40% and 70% of nitrogen, 80-90% of phosphorus, and between 50% % and 70% of the potassium applied to the soil, this problem can be reduced by including nutrients in matrices based on chitosan; Chitosan nanoparticles combined with N, P and K were shown to delay the release, improving absorption and without negative effects on the soil (Malerba and Cerana, 2018). For their part, Ha *et al.* (2019) prepared chitosan nanoparticles by ionic gelling with NPK, generating a slow and controlled release nanofertilizer, resulting in a strong effect on nutrient absorption, chlorophyll content, photosynthetic activity, and growth of coffee seedlings. in a greenhouse.

Abdel-Aziz *et al.* (2016), used similar nanoparticles mixed with NPK, but their application was carried out in a foliar way, finding that chitosan facilitated absorption through the stomata and a mobility study using electron transmission microscopy showed that this type of fertilizer moved through the phloem from the source (leaves) to the sink organ (fruit, shoots or roots), in addition significant increases were found in the harvest index, crop index and index of the yield variables in wheat, shortening the life cycle 23.5% of the plants compared to normally fertilized plants, although this effect is related more to the application of nanoparticles than to the use of chitosan. In turn, Hussain et al. (2012), in their laboratory work, found that a combination of urea with chitosan microspheres delayed the release of the fertilizer, making this a promising mixture to analyze its effects in a field work.

Chitosan and its interaction with the use of iron and zinc

It is likely that the beneficial effects attributed to chitosan on plant development are due to a possible role as a carrier of nutrients and its metal chelating power. This phenomenon occurs because the structure of this compound facilitates the union of metal ions through ion exchange, complex formation and intra and intermolecular encapsulation (Liu *et al.*, 2014; Vasconcelos, 2014; Deshpande *et al.*, 2017).

This process has been presented in conjunction with the inclusion of metallic nanoparticles in agriculture and, as chitosan is the only natural polycationic polysaccharide, it allows an interaction with positively charged elements, which is why it has taken on great relevance within the scientific field. The implementation of these chitosan systems as nanoparticle carriers have proven to be an excellent alternative to the problem of low absorption of fertilizers and environmental pollution caused by the excessive use of agrochemicals (Velásquez, 2015).

It is known that transition metals, such as zinc (Zn), iron (Fe) and copper (Cu) are very important for the correct development of crops in general. Zn has a role as an enzymatic cofactor, participating in the reactions of key enzymes such as superoxide dismutase and alcohol dehydrogenase, it is also necessary to carry out the metabolism of nucleic acids, it has a central role in protein synthesis and is essential for photosynthesis to occur and carbohydrate metabolism in plants. For its part, Fe is a constituent of several enzymes, in addition to being a key element for the formation of chlorophyll, a fundamental compound to carry out photosynthesis. However, the absorption of these elements is limited by various factors, its application in the form of nanoparticles being a viable alternative and the chelating properties of chitosan emerge to maximize their potential (Marschner, 2011; Choudhary *et al*., 2017b).

There are a large number of research works that relate the use of these two micronutrients and chitosan (Table 2), finding a greater emphasis on the use of Zn because its combination has shown positive effects on production, biomass and mechanisms of plant defense (Salimi *et al*., 2019). In their study, Deshpande *et al.* (2017), showed that chitosan nanoparticles complexed with Zn, proved to be a good transporter for foliar application in wheat, in addition to that their release was slow and controlled, thus preventing the loss of nutrients. For their part, Mirbolook *et al.* (2020), found that when Zn chelated with chitosan and applied it both foliar and edaphic, the plants had better Zn absorption and greater root growth. Similarly, Choudhary *et al.* (2019), obtained a strong antifungal activity and an increase in antioxidant activity, in addition to a greater accumulation of Zn in corn plants, when applying this nutrient in a foliar way complexed with chitosan in the form of nanoparticles.

On the other hand, the combination of Fe with chitosan has not been widely studied, among the few studies where this combination was used, Salimi *et al.* (2019), found negative effects when they applied chitosan to the culture medium that contained Fe nanoparticles, however, when compared with the control, the results were higher in root and aerial growth.

Table 2. Effects of chitosan applied in combination with Zn and/or Fe

Nutrient	Application form	Crops	Results	References
Zn	Encapsulated in chitosan nanoparticles (priming and foliar)	Maize (*Zea mays* L.) Cv. Suria local	Antifungal effects, increased Zn content and increased plant growth	Choudhary *et al*., 2019
	Chitosan Nanoparticles and TPP * (Foliar)	Wheat (*Triticum durum* L.) Cv. MACS 3125 and UC 1114	Increase in the concentration of Zn in grain	Deshpande *et al*., 2017
	Foliar in combination with chitosan and/or humic acids	Bean (*Phaseolus vulgaris* L.) Cv. Nebraska	Inconsistent results on performance parameters under temperature stress	Ibrahim and Ramadan, 2015
	In the form of nanoparticles of ZnO plus chitosan	Chickpea (*Cicer arietinum L.*) Cv. GJ-62	Antifungal activity against *Fussarium oxysporum* and promotion of plant growth	Kaur *et al*., 2018
	Directly to the ground in combination with chitosan	Sunflower (*Helianthus* L.) Cv. Pioneer Hybrid 6946	Reduced nickel levels in contaminated soil and increased antioxidant levels in plants	Turan *et al*., 2018
	Foliar in combination with chitosan	Tomato (*Lycopersicon esculentum* L.)	Increase in biomass and enzymatic activities of SOD and PAL	Salimi *et al*., 2019
	In combination with amino acids and chitosan	Bean (*Phaseolus vulgaris* L.)	Better Zn absorption and higher root growth	Mirbolook *et al*., 2020
Fe and Zn	In culture medium plus chitosan	Pepper (*Capsicum annuum* L.) Cv. LJ-King	The application of chitosan favored growth when combined with Zn, but did not obtain positive results when combined with Fe	Zhao *et al*., 2019
Fe	New fertilizer with chitosan and silica	N/A	A stable fertilizer with a slow release was obtained, but it is not yet tested on plants	Mangallo *et al*., 2020

* Sodium tripolyphosphate

In general, these studies position chitosan as one of the most suitable compounds to be used together with macro and micronutrients, due to its wide range of characteristics that include biocompatibility, biodegradability, high permeability, cost-benefit ratio, non-toxicity, and excellent film-forming ability to be used as covers, plus their uses can be expanded with pesticides, herbicides, genetic material, and plant hormones. However, there are still many challenges to make this compound an accepted commercial product, due to the continuous rejection of new technologies by producers since the production process is not yet standardized, generating a material that has different characteristics and that in turn time causes variable results, so it is necessary to continue with these studies (Mujtaba *et al.*, 2020).

Conclusions

In the last 10 years. The advances in the use of chitosan are impressive, as it is a unique, inexpensive product that can be incorporated into sustainable production systems. Its physiological effects are proven and the benefits on growth, production and especially in the induction of defense of plants under stress conditions are promising for use in situations where production conditions are unfavorable, or to face the problems that the climatic situation presents us. Also, its use as a carrier of nutrients is an alternative that can be used to improve the efficiency of fertilization and achieve better absorption, especially with micronutrients that present difficulties for their absorption such as Zn, this would avoid excess fertilization that causes contamination of soils and aquifers. In addition, its effects as a fungicide, bactericide and antiviral can also be used to reduce the use of agrochemicals. However, despite these positive effects, many differences are still observed in terms of its production and the forms of application, which generates a discrepancy in the results, so it is necessary to expand the investigations to seek a homogenization in these aspects and reach the maximum potential that chitosan possesses.

Authors' Contributions

Conceptualization: A.P.-M., E.S., D.L.O.-B.; Data Curation: A.P.-M., C.A.R.-E.; Formal analysis: A.P.-M., E.S., D.L.O.-B.; Writing original draft: A.P.-M., C.A.R.-E.; Writing-review and editing: E.S., D.L.O.-B., C.C.-M., J.P.S.-A, P.P.-R.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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