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# Potential Agricultural Uses of Micro/Nano Encapsulated Chitosan: A Review

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**Abstract:** Chitosan is a non-toxic, biodegradable, and biocompatible natural biopolymer widely used as a nanocarrier, emulsifier, flocculant, and antimicrobial agent with potential applications in industry. Recently, chitosan has been used as an encapsulating agent for bioactive plant compounds and agrochemicals by different technologies, such as spray-drying and nanoemulsions, to enhance antimicrobial activity. Chitosan nanocomposites have been shown to increase potential biocidal, antibacterial, and antifungal activity against pathogens, presenting higher stability, decreasing degradation, and prolonging the effective concentration of these bioactive compounds. Therefore, the objective of this work is to review the most outstanding aspects of the most recent developments in the different methods of encapsulation of bioactive compounds (phenolic compounds, essential oils, among others) from plants, as well as the applications on phytopathogenic diseases (fungi and bacteria) in vitro and in vivo in cereal, fruit and vegetable crops. These perspectives could provide information for the future formulation of products with high efficacy against phytopathogenic diseases as an alternative to chemical products for sustainable agriculture.

**Keywords:** chitosan nanocomposites; biocontrol; antimicrobial; plants; sustainable agriculture



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## 1. Introduction

In agriculture, plants are susceptible to environmental damage and pathogen attack, which causes crop diseases and economic losses. Therefore, different strategies have been developed to reduce plant diseases caused by pathogens. In this sense, the use of biological agents has increased [1]. Biological agents comprise a variability group of compounds with antagonist activity against phytopathogenic microorganisms such as phenolics, terpenoids, proteins, and polysaccharides; these compounds have been obtained from bacteria, fungi, plants, or insects [1–3]. Polysaccharides are macromolecules with biological and functional properties used to develop products with potential agricultural applications. In this sense, cellulose, starch, pectin, chitin, and chitosan are polysaccharides with interesting applications in agriculture. Chitin and chitosan are carbohydrate biopolymers that can be isolated from marine waste and microorganisms; these biopolymers are widely studied for high biocompatibility, biodegradability, low toxicity, film-forming ability, antimicrobial activity, and specific interactions with phytopathogenic microorganisms and for improving the productivity of different crops [1,4–8]. Chitin and chitosan have been used as biostimulants and biocontrol in agriculture [2,9]. In this sense, chitosan has demonstrated an effect on crop productivity and potential protection against phytopathogenic agents such

as fungi and bacteria [7,10]. It has been reported that the mechanism of action of chitosan against bacteria, fungi, and viruses could be due to the interactions of the amino groups with the negatively charged groups found in the membrane of pathogens such as some phospholipids, fatty acids, and proteins when disrupting the membrane due to physicochemical changes in it causes the death of pathogens. Likewise, chitosan could induce cell death of phytopathogenic microorganisms by an increase of permeability or inhibit the mRNA expression of proteins for growth and replications by the internal interaction of chitosan [7,11]. Also, it has been demonstrated that chitosan inhibited the crop damage caused by nematodes and oomycetes when used as a fertilizer, seed treatment, foliar applications, and soil conditioner agent [5]. Chitosan induces a natural defense mechanism to crops by enhancing protective proteins, and proteinase inhibitors, stimulating the synthesis of phytoalexins, chitinases, lignin, and hydrogen peroxide; it also increases the production of reactive oxygen species and inhibiting a wide range of bacteria, fungi, and viruses [1,6,12]. For chitosan agriculture application purposes, chitosan must be soluble in neutral aqueous solutions, thermodynamically stable, and have a small particle size. Therefore, the researchers have used different techniques to modify chitosan to increase its presence in crops longer. Likewise, chitosan has been used as a compound carrier that stimulates plant defenses and inhibits microorganisms harmful to plants [9,13–15]. In this sense, micro and chitosan nanoparticles have been obtained by different techniques such as spray-drying, lyophilization, and nanoemulsions to encapsulate bioactive compounds on crops to enhance and protect against biotic and abiotic stress [3,5–7]. Therefore, this manuscript aims to review the current state of knowledge of the potential effect of micro/nano-encapsulated chitosan with different techniques on inhibiting phytopathogenic microorganisms and crop production.

## 2. Exclusion Criteria

This work was compiled from recent literature (2013–2023) to identify relevant information on this research topic from the Scopus and Google Scholar databases to identify relevant literature. Next, we combined the keywords chitosan, phenolic compounds, and essential oils with the following keywords: microencapsulation, nanoencapsulation, nanoemulsions, antifungal antibacterial, and crops. We decided only to include literature in the English language. Finally, we grouped 116 research papers for this manuscript.

## 3. Chitosan as Encapsulation Agent

### *3.1. Chitosan Generalities*

Chitosan is a natural biopolymer obtained by the partially or fully deacetylation form of chitin, which randomly has an N-acetylglucosamine and glucosamine units, being the only polycation biopolymer in nature [1,2,16]. Chitosan is obtained principally by chemical methods, enzymatic methods [17], fermentation, or a combination of biological-chemical processes [18] from marine wastes such as crabs' exoskeleton, shrimp waste, and arthropods; also, it has been isolated from algae and the cell walls of fungi [1,9,12]. Chitosan has important characteristics such as biodegradable, antitumoral, antimicrobial, and anti-inflammatory properties, antioxidant activity, and film form ability; therefore, chitosan applications have been studied for biomedical and pharmaceutical industries, as well as cosmetics, food and feed production, treatment of water, among others [16]. In agriculture, chitosan has been studied for enhanced plant defense systems against phytopathogenic microorganisms by foliar or direct application on the soil as fertilizer, as well as a carrier for bioactive compounds and agrochemical-encapsulation for a controlled release on crops [1,7,12]. In this sense, it has been reported that amine and OH groups of chitosan produce various chemical reactions in the field, as well as form a complex with other compounds by -$NH_2$ or free groups, and nitrogen release for plant nutrients by amino decomposition by soil microorganism demonstrated that chitosan has wide applications on agriculture [9,19]. Chitosan polymeric chain exhibited physicochemical and biological properties according to the deacetylation degree, the acetyl group distribution, pH, and

molecular weight (related to the numbers of acetyl-glucosamine and amine units) [9]. In this sense, various potential fields have studied chitosan with different molecular weights because chitosan solubility is strongly related to high or low molecular weights, being chitosan of low weight is the most used since it can more easily permeate the membranes, being found in the same way that using concentrations between 0.1–5% of chitosan in solution it presents the properties described above [17]. Recently, chitosan structural modifications have been studied for the enhanced drug-delivery potential to obtain nanofibers or nanoparticles for potential applications in the industry [13,14,17,20,21]. In that regard, chitosan microparticles for bioactive compounds carrier for industrial applications have been obtained by different techniques such as spray-drying, ionic gelation, emulsion, and lyophilization [1,9,14,21]. Chitosan micro and nanocapsules have been proven in vitro and in vivo in agriculture. Authors reported inhibition of phytopathogenic microorganisms, as well as an immunomodulatory and biostimulant activity on cereals, vegetables, and fruit plants [5,7,19,22,23].

### *3.2. Encapsulations Techniques for Chitosan Micro/Nanoparticles*

Microencapsulation using chitosan as an encapsulating agent is an emerging technology that protects active compounds from degradation, reduces incompatibility problems, and controls the release of active compounds, providing solutions to different issues in the agricultural field. The microencapsulation products are microparticles, microcapsules, and microspheres with different morphology and internal structure [24,25]. Spray drying and freeze-drying have been reported among the main microencapsulation technologies.

#### 3.2.1. Spray-Drying

Spray-drying transforms a fluid (emulsion, dispersion, solution) into dry particles by spraying the solution in a heated air stream. The principle of this technology is the elimination of humidity by applying heat to the food product, atomizing the feeding solution in a hot air flow, improving the drying speed, and obtaining as a final product a dry powder in the lower part of the dryer [26]. The spray-drying process involves four steps: atomization of the feeding solution, airflow contact, moisture evaporation, and separation of particles [27–31].

The final product features depend directly on all the stages, including its operating parameters such as feeding speed, inlet and outlet temperatures, initial concentration of solid material, surface tension, and intrinsic properties of the drying material [27,28].

The main advantages of spray-drying are low operating costs, energy-efficient technology and fast processing, high encapsulation efficiency, encapsulated product stability, and control of the size, shape, and morphology of the particles, among others [24,32]. Table 1 shows the advantages and disadvantages of spray-drying.

**Table 1.** Advantages and disadvantages of the spray-drying process.

| Advantages | Disadvantages |
| --- | --- |
| • Low operating costs<br>• Energy-efficient technology and fast processing<br>• High encapsulation efficiency<br>• Encapsulated product stability<br>• Control of particle size, shape, and morphology<br>• Applicable at the industrial level<br>• Process simplicity and operational ease | • Low yield for small batches<br>• Highly sensitive at high temperature |

### 3.2.2. Lyophilization

Freeze-drying is used to preserve heat-sensitive foods and other biological materials since low temperatures are used. In addition, freeze-drying has been used successfully in microencapsulation to maintain nutritional factors and facilitate drying [33]. It is a process that stabilizes materials through four main operations, such as freezing, sublimation, desorption, and finally, storage [34]. First, the material is frozen at a low temperature in freeze-drying, forming ice crystals [35]. Then the frozen solution is subjected to very low pressures, and the formed ice crystals sublime [36]. Finally, sublimation is the main principle of the freeze-drying process. During sublimation, water is directly turned to vapor in a vacuum without going through the liquid state [33]. Generally, a lyophilization cycle consists of three steps: freezing, primary drying, and secondary drying [24,34,35,37,38]. Table 2 shows the advantages and disadvantages of lyophilization.

**Table 2.** Advantages and disadvantages of the freeze-drying process.

| Advantages | Disadvantages |
| --- | --- |
| ▪ Operates at low temperatures<br>▪ Stable products under oxidation conditions<br>▪ A suitable technique for encapsulation of ingredients that are unstable in aqueous media | ▪ Long processing times<br>▪ High energy consumption<br>▪ Expensive operation<br>▪ Poor ingredient protection due to porous coating |

### 3.2.3. Nanoemulsions

Nanoemulsions are colloidal (lipidic) systems in which two immiscible liquids are dispersed with each other. One of the liquids is the solvent, while the other is the dispersed phase. These can be classified into different types depending on the dispersing degree (Figure 1): O/W, the most common in which water is dispersed in oil, and W/O in which water is distributed in oil, W/O/W, is a double emulsion where W/O emulsion is dispersed in another phase of water, O/W/O, where a W/O emulsion is started and re-emulsified with oil. For this emulsion, a high-energy technique is needed. In a bicontinuous emulsion, both oil and water are intercalated. Nanoemulsions comprise particle sizes between 10 and 1000 nm. Since these systems have high stability and interfacial areas, nanoemulsions increase the biocapacity of hydrophobic active ingredients. Besides those mentioned above, some of the advantages of preparing nanoemulsions are protection against oxidation and hydrolysis [39–41]. There are different methods for the formation of nanoemulsions, and these are mainly classified into those that are high and low energy. Table 3 shows the different techniques for formulating nanoemulsions [42–49].

**Table 3.** Classification of high and low energy methods for obtaining nanoemulsions.

| High Energy Methods | Low Energy Methods |
| --- | --- |
| • High-pressure valve homogenization<br>• High-pressure microfluidic homogenization<br>• Ultrasonic homogenization | • Spontaneous emulsification |

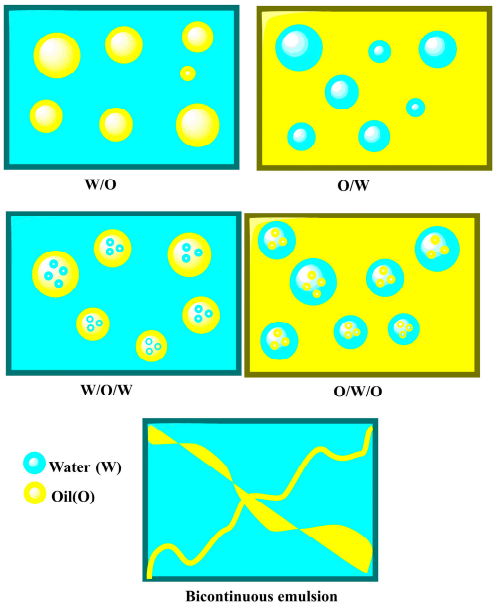


**Figure 1.** Types of nanoemulsions.

### *3.3. Chitosan Micro/Nanoparticles Has Encapsulated Agent of Bioactive Compounds from Plants*

#### 3.3.1. Phenolic Compounds

Phenolic compounds are a large group of compounds that are extracted mainly from the leaves or fruits of plants to which; due to their low solubility in an aqueous medium, alternatives have been sought to improve their solubility, and one of the characteristics that have in its favor is the negative charges due to the large number of -OH groups, encapsulating them in matrices that contain groups capable of protonating/deprotonating, ensuring the encapsulation and release of these compounds and thus having a better bioavailability of them [17,50–52]. The encapsulation of phenolic compounds can not only occur from fruits and leaves but also take advantage of the residues that are had after the harvest of said plantations and give a second use to the waste [53]. It has been determined that the polyphenols extracted from olive leaf, encapsulated in chitosan nanoparticles, have functioned as fungicidal agents when controlling *Verticillium* wilt in tomato plants, with a loading efficiency of around 58% and a size of about 330 nm [54]. Pomegranate (*Punica granatum* L.) peel extract has also been encapsulated, in which loading efficiencies between 26–70% and a particle size between 174–898 nm were obtained. In addition to these NPs, they exhibited antimicrobial activity against gram-positive *S. aureus* [55]. Grape seed polyphenols were encapsulated in lecithin-chitosan liposomes; the efficiency loaded varied between 88.2 ± 4.7% and 99.5 ± 2.3%, with uncoated and chitosan-coated liposomes, respectively, the release of these compounds was performed, finding that when they were coated with chitosan, the release was more controlled [56]. The synthesis of nanoliposomes by micro fluidization of tea polyphenols, obtaining around 78% encapsulation efficiency, as well as the size of 67 nm, where the inhibition of the growth of *S. aureus*, *E. coli*, *S. typhimurium*, and *L. monocytogenes* was lower compared to the extract, but the release of the compounds was a little more than 80% in the compounds that were found encapsulated vs. a little more than 20% of the compounds without encapsulation [57]. Among the investigations carried out by several authors, it can be observed that most of these encapsulated phenolic compounds, in this case, chitosan, can improve their solubility in an aqueous medium and preserve their antifungal and bactericidal activity.

#### 3.3.2. Essential Oils

Essential oils are present in various aromatic plants. They are considered as secondary metabolism with multiple functions that interact with the environment surrounding the plant as a defense against pathogens and herbivores, but as an attractor for pollinators

to complete their life cycle. Through the natural production of these essential oils, it is possible to extract them from the vegetative parts that contain them and be able to apply them for agricultural improvement purposes due to their high potential as a fungicide, bactericide, nematicide, insecticide, acaricide, among other functions [58]. However, due to their physicochemical properties, these compounds are highly volatile, poorly soluble in water, sensitive to heat, and susceptible to oxidation. This means that they cannot remain for long periods in a place where a microbicidal effect is required. That is why one of the solutions today is the micro/nanoencapsulation of these compounds to improve the prevalence and effectiveness; where chitosan is one of the most suitable wall materials for encapsulation of these compounds, since in addition to protecting them from environmental conditions that, cause volatilization and other disadvantages of these compounds, it also presents antimicrobial properties in combination with essential oils [59].

In Table 4, we can see a great diversity of aromatic plants with high contents of essential oils, which were encapsulated with chitosan of different molecular weights, degree of deacetylation, and percentage of chitosan inclusion. Most of the essential oil is solubilized with a surfactant, normally Tween 80 and 20, where its content depends on the type and concentration of encapsulated oil. Because chitosan is mostly dissolved in an acid medium for its encapsulation, it is more appropriate to use nanoencapsulation techniques such as nanoprecipitation, ionic gelation, and electrospraying. Therefore, parameters that guarantee an optimal encapsulation process are of interest, such as the percentage of encapsulation efficiency (EE) and the rate of loading capacity (LC), since they indicate the amount of essential oil encapsulated.

**Table 4.** Chitosan encapsulation of essential oils.

| Essential Oils | Particle Type | Encapsulation Method | Encapsulation Conditions | Results | Reference |
|---|---|---|---|---|---|
| *Origanum majorana* essential oil (OmEO) | Nanoparticle (CH) | Ionotropic gelation | 1% chitosan<br>85% deacetylation degree<br>1.03% Tween 80<br>1:0 to 1:1 CH: OmEO | 37.25 to 88.06% EE<br>0.5 to 6.73% LC | [60] |
| Garlic essential oil (GEO) | Nanoparticle (CH) | Ionic gelation | 0.2% chitosan<br>50–190 kDa<br>75–85% deacetylation degree<br>1% Tween 80<br>1:0 to 1:1 CH: GEO | 23.8 to 32.8% EE<br>5.2 a 19.4% LC | [61] |
| *Zataria multiflora* essential oil (ZEO) | Nanoparticle (CSNP) | Ionic gelation | 0.3% chitosan<br>684 kDa<br>85% deacetylation degree<br>1:0 to 1:1 CSNP: ZEO | 3.26 to 45.24% EE<br>5.22 to 9.05% LC | [62] |
| *P. atlantica* essential oil (PAHEO) | Nanoparticle (CH) | Ionic gelation | 1% chitosan<br>60–190 kDa<br>80% deacetylation degree<br>0.16% Tween 80<br>1:0 to 1:1.5 CH:PAHEO | 43.3 to 61.5% EE | [63] |
| Hyssop essential oil HEO | Nanoparticle (chitosan-pea protein CHPP) | Nanoprecipitation | 1% chitosan<br>60–190 kDA<br>80% deacetylation degree<br>0.16% Tween 80<br>1:1 to 5:1 CHPP: HEO | 55.2 to 87.1% EE | [64] |
| *Satureja kermanica* essential oil (SKEO) | Nanoparticle (CS) | Ionic gelation | 1% chitosan<br>1:0 to 1:1 CS:SKEO | 45. 18 to 75.88% EE<br>2.89 to 7.15% LC | [65] |
| Lavender and clove Eos | Microspheres | Ionic gelation | 1% chitosan<br>1.9% EO<br>0.2% tween 20 | Clove: 7.62% EE<br>Lavender: 16.48% EE | [66] |
| *Origanum vulgare* essential oil (OEO) | Nanoparticle (CH) | Electrospraying | 1% chitosan<br>70 kDa<br>75–85% deacetylation degree<br>1:0 to 1:0.5 CH: OEO | 70.1 to 79.6% EE | [67] |

**Table 4.** *Cont.*

| Essential Oils | Particle Type | Encapsulation Method | Encapsulation Conditions | Results | Reference |
|---|---|---|---|---|---|
| *Nepeta hormozganica* and *Nepeta dschuprensis* essential oils | Nanoparticle (CH) | Co-precipitation | 0.5% chitosan<br>1.125% Tween 80<br>1:0 to 1:1.25 CH:EO | 32.73 to 75.91% EE | [68] |
| *Carum copticum* essential oil (CEO) | Nanoparticle (CH) | Co-precipitation | 1% chitosan<br>75–85% deacetylation degree<br>0.1% Tween 80 | 80% EE<br>14% LC | [69] |
| *Cymbopogon citratus* essential oil | Minicapsule | Chitosan-agar | 6 mL chitosan<br>3.6 mL EO | 83% EE | [70] |
| *Zingiber zerumbet* essential oil (ZEO) | Nanoparticle (CH) | Ionic gelation | 1.5% chitosan<br>80% deacetylation degree<br>Tween 80<br>1:0 to 1:1 CH: ZEO | 51.98 to 84.16% EE<br>0.53 to 2.16% LC | [71] |

When used as a nano-encapsulant, chitosan is diverse and efficient since it can increase EE and LC by incorporating essential oil into the CH: EO mixture. Such is the case of the nanoencapsulation of *Origanum majorana* L. essential oil (OmEO), where increasing the CH: OmEO ratio up to 1:1, the EE and LC reach maximums of 88.06 and 6.73%, respectively, and how this phenomenon occurs is of interest for future research [60].

In some cases, the increase in the percentage of inclusion of the essential oil in the encapsulation with chitosan is not directly proportional to the EE or the LC. For example, the nanoencapsulation of garlic essential oil (GEO) with chitosan has a maximum EE of 32.8% when the CH: GEO ratio is the lowest (1:0.25) and decreases inversely proportional to the increase in the GEO ratio up to 23.8%. However, the opposite occurs with the LC, since at a higher percentage of GEO, the LC is 19.4% in a CH: GEO of 1:1; this is compared when the CH: GEO ratio is the lowest (1:0.25), with an LC of 5.2% [60]. This is also reported in the encapsulation of *Zataria multiflora* essential oil (ZEO), where the highest EE and LC occur in a CH: ZEO of 1:0.25 with 45.24 and 9.05%, respectively [62]. The same phenomenon occurs in *Zingiber zerumbet* L. essential oil (ZEO) nanoencapsulation. By increasing the CH:ZEO ratio, the EE decreases from 84.13 to 51.98 and then rises to 68.01%. However, the LC increases to 2.16 to the maximum CH: ZEO ratio of 1:1 [62].

The EE might sometimes be somewhere between the essential oil inclusion percentage. Such is the case of the nanoencapsulation of *P. atlantica* essential oil (PAHEO). In contrast, the inclusion of essential oil increases reaches 61.5% of EE at a 1:0.75 CH: PAHEO ratio. However, when the proportion of PAHEO increases, the EE decreases to 47.6% with a 1:1.5 ratio, being very similar to the 1:0.25 ratio with 43.3%, so if the maximum EE is sought, the appropriate inclusion percentage must be chosen. This decrease could be because the chitosan becomes saturated with essential oil [63]. The same phenomenon occurs when it comes to encapsulating *Satureja kermanica* essential oil (SKEO), where the maximum EE and LC occur at a ratio of 1:0.75 CH: SKEO with 75.88 and 7.15%, respectively, and as this ratio increases, the decrease occurs due to the possibility of the saturation of the chitosan with the essential oil [65]. Also, when species of the same genus are compared, the EE is similar due to the similar physical characteristics and chemical composition. In the case of species of the same genus, such as *Nepeta hormozganica* essential oil (NHEO) and *Nepeta dschuprensis* essential oils (NDEO), the EE is better at a ratio of 1:1 with 73.64 and 75.91%, respectively, and decreasing in both cases when increasing the ratio to 1:1.25 [68].

Optimizing the EE can greatly influence whether the chitosan is mixed with another encapsulant in addition to the essential oil inclusion ratio. For example, in the encapsulation of Hyssop essential oil HEO when pea-protein is combined with chitosan, the maximum EE is achieved when the chitosan/pea-protein ratio is lower, in addition to the lower CH/pea-protein: HEO ratio with a maximum EE of 87.1%, in contrast to the worst conditions with a high CH/pea-protein: HEO ratio resulting in around 50% EE [64].

3.3.3. Others

In addition to phenolic compounds and essential oils, other bioactive compounds of interest in the technological field for encapsulating chitosan are alkaloids. Alkaloids are a group of nitrogenous substances of plant origin, generally with a complex structure and high molecular weight [72]. In a study by Wang et al. [73], alkaloid-loaded alginate-chitosan microspheres by ionic gelation were prepared. The encapsulated alkaloids were epiberberine, jatrorrhizine, coptisine, palmatine, berberine, evodiamine, and rutaecarpine, isolated from *Coptis chinensis* and *Evodia rutaecarpa*. The microspheres had a particle size of about 114 µm. In another study by Harangozó et al. [74], they formed chitosan nanoparticles (NP) induced by 4-sulfonatocalixarenes as a cross-linking agent and macrocyclic receptor for the encapsulation of coralyne and berberine alkaloids. The charged nanoparticles had a size of approximately 150 to 200 nm and a loose structure, which would facilitate the release of the encapsulated alkaloid. Otherwise, chitosan-collagen nanocapsules loaded with magnoflorine were synthesized to improve their antioxidant potential. The size of the nanocapsules was determined by transmission electron microscopy, showing a small size of approximately 12 ± 2 nm [75].

Also, chitosan-coated bilosomes loaded with berberine (BER-CTS-BLS) were elaborated, optimizing the formulation and obtaining a particle size of 202.3 nm, an entrapment of 83.8% and a surface charge of 30.8 mV [76]. In addition, chitosan-coated poly (D, L-lactide-co-glycolide) nanoparticles loaded with alkaloids (peganin, harmol, and harmine) from *Peganum harmala* were prepared using the oil/water emulsion solvent evaporation technique. The NPs exhibited an average particle size of 202.27 ± 2.44 nm, a polydispersity index of 0.23 ± 0.01, a zeta potential of 9.22 ± 0.94 mV, and a trapping efficiency of 86.77 ± 4.18% [77]. Other important compounds are saponins, which present one or more sugar chains in their structure. They also contain steroids or triterpenoid aglycone [78]. Bernela et al. [79], prepared by ionic complexation, chitosan-gum katira nanoparticles loaded with glycyrrhizic acid, a triterpene saponin obtained from *Glycyrrhiza glabra*. The particles showed a spherical shape and a size of 80 nm. Moreover, Kunjumon et al. [80] nanoencapsulated the madecassoside compound, a saponin extracted from *Centella asiatica*. The nanoparticles were made with alginate-chitosan by ionic gelation, obtaining particles with uniform spherical morphology and size between 200–600 nm. Also, chitosan and alginate capsules have been prepared to improve the stability of *Momordica grosvenorii* saponin, showing sizes of 1687 µm and 80.25% encapsulation efficiency [81]. Different researchers have focused on obtaining bioactive compounds from plants to give them application and technological functionality, as in those encapsulated with chitosan.

## 4. Chitosan Micro/Nanoparticles against Biotic Plant Stress (In Vitro)

### *4.1. Fungi*

After the encapsulation of essential oils, the disadvantages, as mentioned earlier, are reduced due to their physicochemical properties. However, before testing its effectiveness in the field, it must first be tested in the laboratory to determine the effective concentrations in a controlled environment, starting from that concentration as a reference. Various essential oils encapsulated with chitosan have been tested with different phytopathogenic fungi, among which we find genera of important crops such as *Alternaria*, *Aspergillus*, *Botrytis*, *Colletotrichum*, *Fusarium*, *Penicillium*, *Rhizoctonia*, and *Sclerotinia* (Table 5). In the vast majority, the essential oils themselves have a fungicidal effect. However, the encapsulation of these oils with chitosan greatly enhances the fungicidal effect due to the high stability that gives them in the chitosan polymer matrix, being its slower volatilization and/or degradation, prolonging the concentration that is effective in stopping the development of fungi (Table 5).

**Table 5.** Chitosan nanoencapsulates with fungicidal effect in vitro.

| Essential Oil | Nanoparticle | Fungi | Results | References |
|---|---|---|---|---|
| *Origanum majorana* L. essential oil (OmEO) | Nanoparticle (CH) | *A. flavus*, *A. fumigatus*, *A. luchuensis*, *A. niger*, *P. chrysogenum*, *P. italicum*, *C. cladosporioides*, *F. poae*, *A. alternata* | MIC (ppm): OmEO: 2500 and OmEO-CH: 1000 Aflatoxins (ppm) to 1000 ppm: OmEO: 0 ppm and OmEO-CH: 0.24 ppm | [60] |
| Garlic essential oil (GEO) | Nanoparticle (NPHD) | *F. oxysporum* *A. niger*, *A. versicolor* | MIC (mg/mL): GEO: 7.5, NPHD: 10, and GEO-NPHD: 2.5 GEO: 7.5, NPHD: without effect, and GEO-NPHD: 5 GEO: 7.5, NPHD: without effect, and GEO-NPHD: 5 | [61] |
| *Zataria multiflora* essential oil (ZEO) | Nanoparticle (CSNP) | *B. cinerea* | Inhibition (%) to 1500 ppm: ZEO: 55.7, CSNP: 65.15, and ZEO-CSNP: 96.9 | [62] |
| *Zingiber zerumbet* essential oil (ZEO) | Nanoparticle (CH) | *A. flavus* | Inhibition (%) to 1000 ppm: ZEO: 66.8 and ZEO-CH: 100 Aflatoxins (ppm) to 800 ppm: ZEO: 2.65 and ZEO-CH: 0 | [62] |
| *P. atlantica* essential oil (PAHEO) | Nanoparticle (CNP) | *B. cinerea* | Inhibition (%) to 20 ppm: PAHEO: 74, CNP: 70, and PAHEO-CNP: 100 | [63] |
| Hyssop essential oil HEO | Nanoparticule (chitosan-pea protein CHPP) | *B. cinerea* | Inhibition (%) to 2 mg/mL: HEO: 70 and HEO-CHPP: 84.3 | [64] |
| *Satureja kermanica* essential oil (SKEO) | Nanoparticle (CS) | *R. solani*, *A. alternata*, *B. cinérea*, *S. sclerotiorum*, *F. oxysporum* | Inhibition (%) (250 ppm) In all fungi SKEO-CS> SKEO > CS KEO-CS: 100 | [65] |
| Lavender and clove EOs | Microspheres | *B. cinerea* | Inhibition (%) with 1 g de microspheres not dissolved Clove: 6.84 and Lavender: 16.69 | [66] |
| *Origanum vulgare* essential oil (OEO) | Nanoparticle (CH) | *A. alternata* | MIC (% *w*/*v*): CH: 0.02% and OEO-CH: 0.005% | [67] |
| *Nepeta hormozganica* and *Nepeta dschuprensis* essential oils | Nanoparticle (CS) | *R. solani*, *A. alternata* *B. cinerea*, *S. sclerotiorum* and *F. oxysporum* | Inhibition (%) to 500 ppm In all fungi and both essential oils EO-CS > EO > CS EO-CS: 100% inhibition in ambos, both essential oils | [68] |
| *Carum copticum* essential oil (CEO) | Nanoparticle (NCH) | *A. alternata* | Inhibition (%) to 200 ppm: NCH: 14.01, CEO: 88.43, and CEO-NCH: 94.22 | [69] |
| *Cymbopogon citratus* essential oil (CCEO) | Minicapsules (CH) | *C. gloeosporioides* | Inhibition (%): CH: 3.1, CCEO (1156 ppm): 53.9, and CCEO-CH (1370 ppm): 100 Inhibition (%) for 30 d: CCEO: 0 and CCEO-CH: 100 | [70] |

The effectiveness of encapsulated essential oils lies mainly in the type of oil, its concentration, and the phytopathogenic fungus, where some are more susceptible than others. Such is the case of the effectiveness of GEO in spore germination, where GEO-NPHD is more effective than chitosan and essential oil, with a MIC of 1.11, 3.33, and 1.66 mg/mL, respectively, for *F. oxysporum*; with 0.37, 1.11, and 0.56 mg/mL respectively for *A. niger*; and 3.33, >3.33 and 15 mg/mL respectively for *A. versicolor*. We can highlight that the most susceptible fungus is *F. oxysporum,* followed by *A. niger* and *A. versicolor,* and that the encapsulation is more effective, followed by the essential oil and finally the chitosan, the encapsulation being greater than the chemical control tebuconazole, for what this type of encapsulation could reduce the uses of chemical fungicides [61]. The same phenomenon occurs with PAHEO, where at a concentration of 20 ppm, PAHEO-CNP achieves 100%

inhibition of *B. cinerea*, the cause of gray mold, with PAHEO being less effective with 74% inhibition and CNP with 70% of inhibition. These results can be explained due to the essential oil's slow release during the fungus incubation time [72].

Different types of oil in the same phytopathogenic fungus may represent less inhibition. In this case, in the evaluation of the fungicidal effect of an HEO nanoencapsulation, we observed that the encapsulation is superior to the essential oil. However, to achieve inhibition of 84.3% and 70%, respectively, a concentration of 2 mg/mL is needed, 100 times greater than PAHEO-CNP [64]. This can be explained by the different chemical compositions of the essential oils, PAHEO being rich in α-pinene (91.47%) and HEO in pinocamphone (iso 47.95% and trans 14.49%). Similar results in concentration to inhibit the growth of *B. cinerea* with ZEO encapsulation, at a concentration of 1500 ppm, with ZEO-CSNP being superior with 96.9% inhibition, followed by CSNP with 65.15% and ZEO with 55.7%. This greater inhibition in ZEO-CSNP also, like other studies, may be due to the low release and prolonged contact between the fungus with the chemical compounds of the essential oil [82]. The fact of changing essential oil is also verified in what was evaluated by [65,68], where the fungicidal effect encapsulated with chitosan is tested against various phytopathogenic fungi such as *A. alternata*, *B. cinerea*, *F. oxysporum*, *R. solani*, and *S. sclerotiorum* with SKEO, NHEO, and NDEO packages; Resulting for the three oils in all the fungi higher the EO-CS > EO > CS; however, SKEO-CS requires 250 ppm and NHEO-CS:NDEO-CS require 500 ppm to achieve similar inhibitions. This can be explained by the high concentration of thymol and carvacrol (46.54 and 30.54%, respectively) that SKEO presents, while NHEO and NDEO are abundant in nepetalactone compounds and in comparison, it is known that thymol and carvacrol are the essential oils with the greatest effective fungicide.

Same case when OEO and CEO encapsulated oils are evaluated against the fungus *A. alternata*, EO-CH is higher than EO and CH in both cases, but with 50 ppm to inhibit 100% with OEO-CH and with 200 ppm to inhibit 94.22% with CEO-NCH; where the concentration of carvacrol or thymol can explain the difference since both essential oils present these compounds, however, OEO presents 84.5% carvacrol and CEO 29.7% thymol, being OEO almost three times higher in this type of compound, which could explain the four times more effective, this added to the fact that they are different encapsulation methods, being OEO-CH by electrospraying and CEO-NCH by co-precipitation [67,69].

### *4.2. Bacteria*

Both chitin and chitosan have demonstrated antiviral, antibacterial, and antifungal properties in the agricultural sector. Due to its biocompatibility, biodegradability, and bioactivity, chitosan is an effective option in controlling bacterial plant diseases [83]. Chitosan reduces bacterial diseases by two main mechanisms. The first is the direct function against bacteria: which includes the mechanisms of plasma membrane damage, electrostatic interactions with bacterial DNA and RNA, and the ability to chelate nutrients and minerals such as calcium, zinc, and magnesium, which are required for transcription and translation. While the second mechanism is the induction of plant defense responses: when chitosan interacts with receptors on the cell surface (PPRs receptors), activation of specific signal transduction pathways occurs, and these signals are transmitted inward from cells to the sites where gene transcription and translation are regulated to generate the appropriate defense response (expression of proteins PR1 and PR5) (Figure 2) [1]. The systemic response induced by chitosan includes activating enzymes, producing secondary metabolites, biosynthesis of phytohormones, and expressing unique genes for early response related to plant defense [84,85]. While the direct antimicrobial effect of chitosan derives from the interaction of positively charged chitosan with negatively charged residues, such as carbohydrates, proteins, and lipids, present in the microbial membrane. These interactions change the cell membrane's permeability and cause cytoplasmic contents leakage, ultimately leading to cell death [1,83].

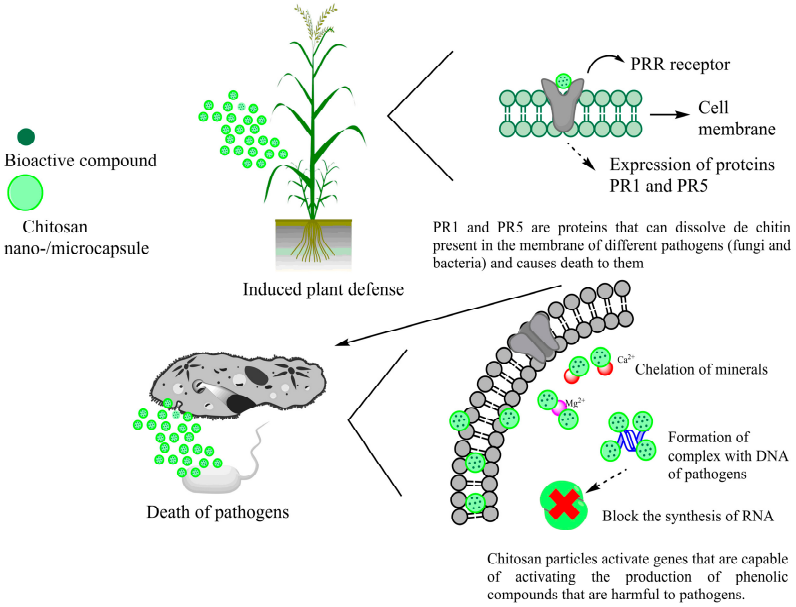


**Figure 2.** Mechanism from the interaction of micro/nano-encapsulates of chitosan with plants and pathogens.

The different proportions of glucosamine and N-acetyl glucosamine give place to the different primary physicochemical properties of chitosan, such as the degree of deacetylation, molecular weight, viscosity, and structural shape (hydrogel, chemically modified chitosan, high weight chitosan, low molecular weight chitosan, conjugated chitosan, as well as chitosan nanoparticles). The degree of deacetylation and the molecular weight of chitosan mainly determine its antimicrobial properties. Chitosan, with the highest degree of deacetylation (>70%), has the best antimicrobial and antibiofilm properties [83,85].

The antibiofilm property of chitosan is also attributed to its polycationic nature given by the functional amino groups ($NH_2$) of the N-acetyl glucosamine units since the positive charge of chitosan reacts with the negatively charged components of the biofilm, such as substances extracellular polymeric cells, proteins, and DNA, resulting in an inhibitory effect on the bacterial biofilm [83].

The bactericidal efficacy of chitosan depends, in addition to the characteristics of chitosan, on other microbial and environmental factors, such as the bacterial species, temperature, pH, and ionic power of the medium. Depending on the species, the minimum concentrations inhibitory to the growth of bacteria vary between 10 to 1000 ppm. Based on available evidence, chitosan also prevents the growth of various plant pathogenic bacteria such as *Xanthomonas*, *Pseudomonas syringae*, *Agrobacterium tumefaciens*, and *Erwinia carotovora* [1,86,87].

The bacterial inhibition property differs between different structural forms, such as chitosan nanoparticles (NPs) can be easily prepared by adding polyanions such as tripolyphosphate (TPP) to the chitosan solution under continuous agitation. These NPs have more significant antimicrobial activity than the parent chitosan molecule [86].

Abdahallah et al. (2020) reported a greater antibacterial activity of the NPs in the growth of *Xanthomonas oryzae* pv. *oryzae* compared to chitosan alone [88]. In addition, the ability of chitosan to electrostatically interact with other cell membrane components can be enhanced by the conjugation of chitosan with other compounds since the availability of functional groups along the chitosan structure allows the combination of chitosan with other polymers or elements to form nanocomposites [9].

Among the chitosan-based nanocomposites, we can mention N-succinyl chitosan (NSC), N-benzyl chitosan (NBC), and carboxymethyl chitosan (CMC) [89,90].

N-succinyl chitosan (NSC) is a water-soluble chitosan derivative with excellent biocompatibility and biodegradability, which has been studied for drug delivery but has been

used as a carrier for hematoporphyrin in plant disease control. Hematoporphyrin (HP) is a first-generation photosensitizer used in antimicrobial photodynamic inactivation, which is an alternative strategy to combat resistant plant pathogens through the use of light-sensitive molecules (photosensitizers) that produce reactive oxygen species (ROS) capable of killing pathogens upon illumination. This novel antibacterial nanosystem, prepared by linking the HP to the NSC through ester bonds, improves the photostability of the HP. Breakage of ester bonds by enzymatic activity at the site of bacterial infection frees HP to perform its photodynamic inactivation [90]. Reductive amination of chitosan obtained n-benzyl chitosan (NBC) derivatives with different benzaldehyde analogs. The antimicrobial activity of chitosan, chitosan nanoparticles (chitosan + TPP), and NBC (NBC + TPP) was evaluated against plant pathogenic bacteria (*Erwinia carotovora* subsp. *atroseptica*, *Erwinia carotovora* subsp. *Carotovora*, and *Ralstonia solanacearum*). The antimicrobial effect of NBC nanoparticles (NBC + TPP) was higher than the activity of chitosan nanoparticles (chitosan + TPP) and chitosan alone [89].

CMC has been used as a carrier biomaterial for daphnetin (DA), a representative monomer of coumarin compounds). In this way, DA nanocomposites with CMC were synthesized to induce the systemic response in tobacco plants against *Ralstonia solanacearum* by increasing the activity of defense enzymes and the overexpression of proteins related to pathogenesis, thus managing to suppress the effective development of bacterial wilt caused by *R. solanacearum* [91].

### *4.3. Nematodes*

Unlike fungi and bacteria, nematodes have not been studied very well, so the possibility of testing chitosan-encapsulated extracts has not been explored. A reported case of the use of chitosan nanoencapsulation of two compounds purified from onion against the nematode *Bursaphelenchus xylophilus*, which causes wilt in pine and other conifers. These compounds are dipropyl trisulfide (DPTS) and methyl propyl trisulfide (MPTS), which were evaluated shortly after being applied. There was no great difference between the pure compounds and these same encapsulations. However, when evaluating their prevalence over time until after 14 d, it is observed that the LC50 is 28.12 ppm for encapsulated DPTS and >50 ppm for the pure compound, which tells us that the use of the encapsulation fulfills the purpose, which is that the compound prevails over time and prolongs its effectiveness [92].

### *4.4. Others*

From other phytopathogens, different from fungi, bacteria, and nematodes, oomycetes cause great losses when the relative humidity conditions are very high, being two genera of great importance that are *Pythium* and *Phytophthora*, where, like the nematodes, these have been little studied in terms of the use of extracts encapsulated with chitosan. In the case of *Phytophthora*, it has been reported that chitosan nanoencapsulation (CSN) of *Cinnamomum zeylanicum* essential oil (CZEO) has had a good effect on the management of *P. drechsleri*, being CZEO-CSN > CNS > CZEO in concentrations of 1500 to 188 ppm with inhibition of 100, 75.5 and 67.15% respectively at 1500 ppm, where this difference could be due to the fact that nanoencapsulation preserves the compounds in the culture medium, managing to inhibit *P. drechsleri* with a higher concentration of dissolved oil and in contact with this phytopathogen [82].

In the case of *Pythium*, the effect of three different nanoencapsulated essential oils, SKEO-CS, NHEO-CS, and NDEO-CS, was evaluated against *Pythium aphanidermatum*. As in the fungi in which these nanoencapsulates were evaluated, the same phenomenon occurs against this oomycete, where EO-CS > EO > CS; 250 ppm of SKEO-CS being necessary to inhibit 100% and 500 ppm of NHEO-CS:NDEO-CS for the same inhibition. This indicates that, as in fungi, the phenolic essential oils thymol and carvacrol affect both types of phytopathogens [65,68].

## 5. Chitosan Micro/Nanoparticle Potential on Crops Susceptible to Diseases Caused by Pathogens (In Vivo)

### *5.1. Cereals*

Cereals are important crops susceptible to abiotic and biotic factors such as temperature, soil salinity, and phytopathogenic diseases [5]. In this sense, different methods have been studied to reduce the impact of external factors on cereal production, such as using fertilizers, antitranspirant formulations, and growth promoters [5,7,9]. Chitosan has been used as an alternative to commercial fertilizers because it has been reported to decrease the transpiration of cereals, as well as promote plant growth, enhance germination, and activate the defense system [93–96]. Also, encapsulation strategies have been studied to improve the potential application of chitosan because it could be used as an encapsulated agent of bioactive compounds such as phenolic compounds and essential oils, among others, that capsules improve the quality of cereals [1,5,9]. In this sense, nanoemulsions and ionotropic gelation are the most reported encapsulation chitosan strategies on cereals (Table 6). Nanoemulsions using chitosan have been applied on barley on foliar application, seed treatment, and soil (as fertilizer); authors reported that nanoemulsions treatment promotes plant growth, leaf elongation, and increased grain yield [92,96]. Also, Zayed et al. [95] found that nanoemulsions of chitosan enhance germination and promote bean plant growth by seed treatment with nanocapsules. Likewise, nanoemulsions and ionotropic gelation chitosan have been applied on cereals such as chickpeas, maize, rice, and wheat. It has been reported that foliar and soil applications and seed treatment nanocapsules induce a defense system, promote plant growth, enhance seed germination, potassium and phosphorus content, and increase grain yield [94,95,97–99].

**Table 6.** Summary chitosan nanoparticles as a cereal growth promoter.

| Encapsulation Chitosan Strategy | Cereal Study | Application Type | Effect | References |
|---|---|---|---|---|
| Nanoemulsion | Wheat (*Triticum aestivum* L.) | Seed treatment | Promote plant growth and leaves elongation | [92] |
| Nanoemulsion | Barley (*Hordeum vulgare*) | Seed treatment | Promote plant growth and leaves elongation | [93] |
| Nanoemulsion | Chickpea (*Cicer arietinum*) | Seed treatment | Enhance germination and promotes a defense system | [94] |
| Ionotropic gelation | Maize (*Zea mays*) | Foliar application | Enhance germination, and promote plant growth, stem diameter, and root length | [95] |
| Nanoemulsion | Bean (*Phaseolus vulgaris*) | Seed treatment | Enhance germination and promote plant growth | [96] |
| Nanoemulsion | Barley (*Hordeum vulgare* cv. Reyhan) | Foliar and soil applications | Increase grain yield and promote plant growth | [97] |
| Ionotropic gelation | Maize (*Zea mays*) | Seed treatment and foliar application | Promote plant growth and increase grain yield | [98] |
| Nanoemulsion | Rice (*Oryza sativa* L.) | Seed treatment | Enhance germination | [99] |
| Nanoemulsion | Wheat (*Triticum aestivum* L.) | Foliar and soil applications | Increase grain yield and promote plant growth | [100] |
| Nanoemulsion | Chickpea (*Cicer arietinum*) | Seed treatment | Enhance germination | [101] |
| Nanoemulsion | Maize (*Zea mays*) | Seed treatment | Enhance germination, promote plant growth, and leaves elongation | [102] |

**Table 6.** *Cont.*

| Encapsulation Chitosan Strategy | Cereal Study | Application Type | Effect | References |
|---|---|---|---|---|
| Nanoemulsion | Rice (*Oryza sativa* L.) | Seed treatment and soil application | Promote plant growth and increase grain yield | [103] |
| Nanoemulsion | Wheat (*Triticum aestivum* L.) | Foliar application | Increase grain yield, as well as enhance the potassium and phosphorus content | [104] |
| Nanoemulsion | Wheat (*Triticum aestivum* L.) | Foliar application | Promote plant growth and increase grain yield | [105] |

### *5.2. Fruits*

An edible coating represents an alternative method to prolong the shelf life of some fruits. Chitosan and chitosan-based edible films are biodegradable materials with high antimicrobial properties that can prolong food's shelf life by protecting them from microbial spoilage [105].

Using chitosan and chitosan derivatives increases the shelf life of fruits by altering enzymatic activity. For example, chitosan (350 kDa) and oligochitosan (6 kDa) protect pear fruits against *Alternaria kikuchiana* and *Physalospora pipicola* fungi through the activity of different enzymes such as chitinase and β-glucanase in the case of oligochitosan, and peroxidase in the case of chitosan [106].

Chitosan nanoparticles obtained by ionic gelation were evaluated for their effect on the ripening process of bananas. The observations of the physical ripening characteristics of the bananas showed that the fruits with the coating of chitosan nanoparticles presented a slower discoloration of the skin (2–3 days) compared to the control (fruits without coating). In addition, the skin of the coated fruit was smoother than the skin of the control fruit when observed in an electron microscope [107].

Sahraei et al. [108] evaluated the effect of a coating based on chitosan nanoparticles on apple cv's quality and shelf life. For this, the fresh fruits were coated with a chitosan emulsion (<100 nm). After 9 weeks of evaluation, the results showed that the coating based on chitosan nanoparticles significantly reduced respiration rate, weight loss, enzymatic activity, and ethylene production in coated fruits, so it was possible to verify the potential use of chitosan nanoparticles to prolong the useful life of apple fruits.

Chitosan is an attractive alternative due to its biofilm-forming characteristics, biodegradability, and antimicrobial activity. However, its preservative properties can be improved by adding compounds, which have synergistic effects to limit the growth of microbial agents and biochemical and physical damage, thus improving the quality and useful life of the products in which it is applied [83].

Thus, to prolong the shelf life of apricots (stored at 2 °C), an edible coating of chitosan and soy protein isolate was used, which allowed a significant reduction in weight loss and firmness in the fruits, in addition, the coating could inhibit pectin degradation thus preserving the quality of apricots [109]. While metallic nanoparticles formulated based on chitosan-citric acid—bismuth/zirconium showed significant antifungal activity against *Botrytis cinerea* in cherry fruits sprayed with these nanoparticles. The treatment with nanoparticles extended the shelf life of the fruits up to 10 days under normal environmental conditions [110].

The effect of two edible coatings to prolong the shelf life of blueberries (stored at 0 °C) was evaluated; the first coating was made from chitosan and titanium dioxide, while the second consisted only of chitosan. The results showed that combining chitosan with titanium dioxide not only maintained the firmness of the fruits but also improved the post-harvest quality of blueberries [111].

Chitosan-silica nanocomposites were tested on table grapes (Italia and Benitaka cvs.) to assess their antifungal efficacy against the fungus *Botrytis cinerea*, which causes gray

mold disease. The chitosan-silica nanocomposites were the most effective treatment by reducing the fungus development between 59 and 83% compared to the fruits to which only water was sprayed (control). In addition, the chitosan-silica treatment had no negative effects on grape quality, so using nanocomposites based on chitosan-silica could be an alternative for controlling gray mold disease in table grapes [112].

### *5.3. Vegetables*

Once the microbicidal effectiveness against fungi, bacteria, nematodes, and oomycetes in vitro has been demonstrated, the concentrations at which it was most effective against these phytopathogens can be taken as a starting point. Therefore, it can be shown whether encapsulation under in vivo conditions may improve the protective effect of vegetables such as potatoes, cucumber, pepper, tomato, and eggplant.

As in vitro studies, the factors that greatly affect the effectiveness of encapsulation with microbicidal extracts are concentration, compound profile, type of pathogen, type of plant, and time, among others (Table 7).

**Table 7.** Nanoencapsulated with microbicidal effect in vegetables.

| Essential Oil | Nanoparticle | Vegetable | Fungi | Results | References |
|---|---|---|---|---|---|
| *Carum copticum* essential oil (CEO) and *Peganum harmala* extract (PE) | Nanoparticle (NCH) | Tomato plant | *A. alternata* | Severity: NCH: 35.78 ± 4.40%, CEO: 52.35 ± 3.71%, PE: 30.80 ± 2.06%, CEO-NCH: 18.55 ± 2.11% and NPE-CEO: 6.48 ± 3.71% | [69] |
| *Cymbopogon citratus* essential oil (CCEO) | Minicapsules (CH) | Topito pepper plants | *C. gloeosporioides* | MIC: 255 µL of CCEO-CH | [70] |
| *Cinnamomum zeylanicum* essential oil (CEO) | Nanoparticles (CH) | Cucumber | *P. drechsleri* | Incidence(1.5 g/L): CSNs: 38.66%, CEO: 75.84% and CEO-CSNs: 0% Severity day 9 (1.5 g/L) CSN: 30%, CEO: 74% and CEO-CSNs: 0% Decay day 21 CSN: 44.87% and CEO-CSNs: 26.1% | [82] |
| *Mentha piperita* essential oil (MEO) | Nanogel chitosan—cinnamic acid (CS-CI) | Tomato | *A. flavus* | MIC 4 weeks: MEO: 2100 ppm, CS-CI: 1000 ppm and CS-CI-MEO: 500 ppm | [113] |
| Chitosan | Chitosan nanoparticle | Eggplant | *Meloidogyne incognita* *Tobacco mosaic tobamovirus* (TMV) | Only nematode: Reduction J2 (Effectiveness): 64.50% Reduction of gall (Effectiveness): 67.87% Nematode + virus Reduction of J2 (Effectiveness): 66.61% Reduction of galls (Effectiveness): 30.71% | [114] |
| Eugenol and thymol | Nanoparticles (CH) | Potato | *Ralstonia solanacearum* | Severity: 10.3 to 90 ppm | [115] |

In the case of fungi, inhibition can be tested both in fruit and in plants, varying the environmental conditions to which the plants are subjected to the post-harvest fruits. Tofiño-Rivera et al. [70], demonstrated that not only chitosan nanoparticles are effective but also microcapsules loaded with *Cymbopogon citratus* essential oil (CCEO) against the fungus that causes anthracnose *C. gloeosporioides* in topito pepper plants within 45 days of the experiment, achieving a MIC of 255 µL of CCEO inside CH microcapsules. This is due to the prolonged effect of the encapsulation. It maintains an amount of essential oil over time that takes effect as the inoculum is released, preventing the incidence from increasing.

The interaction between compounds of different polarities, such as essential oils and hydrophilic compounds, can synergistically affect the control of phytopathogenic fungi evaluated in plants. Izadi et al. [69] showed that chitosan nanoparticles loaded with *Carum copticum* essential oil (CEO) and *Peganum harmala* extract (PE) affect the control *A. alternata*, which causes tomato early blight disease. After 21 days of the experiment, the percentage of severity at 200 ppm of CEO was obtained: 52.35 ± 3.71%, NCH: 35.78 ± 4.40%, PE: 30.80 ± 2.06%, CEO-NCH: 18.55 ± 2.11%, NPE-CEO: 6.48 ± 3.71%; managing to observe that the nonencapsulated essential oil is the one that had the worst effect and the encapsulated oil and the mixture of encapsulated essential oil and extract being the best. This shows that in both cases, the encapsulation achieves the prevalence of the compounds under environmental conditions that are unfavorable to them, in addition to the fact that the mixture of essential oil with hydrophilic compounds is more effective, presenting a synergistic effect. The evaluation in fruit has other different conditions, so the results vary in effective concentration, in addition to being prolonged for a longer time. Beyki et al. [113] evaluated the effect of *A. flavus* on tomato fruits with chitosan-cinnamic acid (CS-CI) nanogels loaded with *Mentha piperita* essential oils (MEO), obtaining a MIC at 4 weeks of an experiment for MEO, CS-CI, and MEO-CS-CI of 2100, 1000 and 500 ppm, respectively, attributed to the prevalence of essential oils during long periods of experiments, so encapsulation is an option if the quality of post-harvest vegetables is to prevail. Oomycetes such as *P. drechsleri* are also susceptible to chitosan nanoparticles loaded with *Cinnamomum zeylanicum* essential oil, managing to protect vegetables such as cucumbers. Regarding the incidence and severity during 9 days at 1500 ppm, CEO > CSN > CEO-CNS with approximately 75, 30, and 0%, respectively, in addition to 21 days decay of 44.87% for CSN and 26.1% for CEO-CSN. The effect of CSN added to the prevalence of the compounds encapsulated for longer periods has a greater inhibitory effect and preserves the quality of the cucumber [82].

Chitosan nanoparticles without extracts have been tested against nematodes such as *Meloidogyne incognita* in combination with the *tobacco mosaic tobamovirus* (TMV) evaluated in eggplants for 4 weeks. The results indicate reduced effectiveness when it only nematode of 64.50% of J2 and 67.87% of galls; however, when the nematode and the virus coexist, there is a 66.61% effectiveness in reducing J2 and 30.71% in galls. Various effects occur in the efficacy of chitosan nanoparticles for the control of nematodes and viruses, among which one of them is that it increases soil biocontrol organisms which are chitinolytic, so they feed on chitin/chitosan, and their proliferation is considerable higher. Consequently, this reduces the prevalence of other pathogenic microorganisms, in addition to releasing nitrogen in the form of ammonia through the metabolism of chitosan, which causes toxicity in pathogens. Another reason may be that chitosan has been shown to increase the systemic resistance of plants, thus releasing several enzymes and secondary metabolites that help eradicate pathogens, including nematodes [114].

The phytopathogenic bacteria of the conductive vessels of the plants, such as *R. solanacearum*, are a challenge to avoid losses due to the wilting symptom that infected plants present. However, using chitosan nanoparticles loaded with two of the most microbicidal compounds such as thymol and eugenol can reduce the severity of the disease in potatoes, managing to reduce it to only 10.3% at 90 ppm, which is considered a low concentration [115].

## 6. Perspectives

The increase in pests and diseases caused by phytopathogenic microorganisms reduced the crops' productivity and caused economic problems; therefore, biopesticides have been on the rise to reduce the use of chemicals [1]. In this sense, it has been reported that chitosan is a non-toxic biopolymer with potential applications in agriculture because it has been demonstrated have antimicrobial properties and protects the plants against phytopathogenic microorganisms, as well as acts as a biostimulant on crops [1,9]; also, chitosan micro and nanoparticles could be used for a control-release of bioactive compounds and could be a strategy for phytopathogenic control caused plant diseases, as well as enhanced

the productivity of the crops [17,19]. Furthermore, some methods for micro and nanoencapsulation of chitosan change the functional properties and stability, enhancing chitosan's antimicrobial and immunomodulatory activity for potential agricultural applications [1,9]. Hence, technologies such as spray-drying, lyophilization, and nanoemulsions have demonstrated that micro and nano-encapsulated chitosan, also bioactive compounds, have been used as potentially the chitosan particles to induce plant system defense by primary and secondary metabolites. Moreover, it has been demonstrated that these particles promote plant growth and induce gene expression for adaptative abiotic and biotic factors [1,7,12,19]. Therefore, more studies for developing micro and nanoencapsulation chitosan strategies are needed to enhance bioactive compounds' encapsulation and more efficient release that improves the defense mechanism of plants against phytopathogenic microorganisms and pests [9,12,14]. Furthermore, additional research is needed to elucidate the micro and nanoencapsulation of chitosan interaction with phytopathogenic microorganism mechanisms for developing target strategies for inhibited and reduced plant diseases [9,22,116].

## 7. Conclusions

In conclusion, it has been found that most of the compounds encapsulated with chitosan, whether they are phenolic compounds, essential oils, alkaloids, etc., have been made by nanoemulsions. Unlike having emulsions only with chitosan, by having smaller particle sizes, they can more easily penetrate the membranes of either the plants or the pathogens present in them and be able to exert their bioactivity. It has been shown that there is an increase in their solubility in water and the controlled release of these compounds. Therefore, they have been used for different purposes, among them, the coating of fruits and seeds, helping them not to be attacked by different types of pathogens, as growth promoting agents, and avoiding their rapid maturation of the fruits in the same way, it has been seen that the application of these encapsulated compounds can modify the production of genes and enzymes within the plant and thus be able to create resistance against pathogens or other factors abiotic. Therefore, the influence of the micro/nano encapsulations of different compounds of vegetable or animal origin is rising since it is proposed to reduce the use of chemicals that can harm health in the short or long term. This will raise new regulations for using these compounds and determine the positive or negative effects that these could have when used over the years.

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