Agrociencia

FUNGICIDAL ACTIVITY OF CHITOSAN IN SYNERGY WITH *Trichoderma harzianum* AND *Bacillus subtilis* FOR THE CONTROL OF CHILI WILT DISEASE

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ABSTRACT

Chili (*Capsicum annuum* L.) wilt disease is considered one of the main diseases of this crop. It is caused by a complex of phytopathogens such as *Fusarium oxysporum*, *Rhizoctonia solani*, and *Sclerotium rolfsii*. These fungi have developed resistance to fungicides, resulting in environmental contamination that harms human health. These factors motivate the search for environmentally safe and efficient products to control this disease without causing adverse effects to ecosystems. Chitosan, which is innocuous and has antagonistic activity, favors the growth of some beneficial microorganisms. In this study, the compatibility of chitosan at eight concentrations (75, 150, 300, 600, 600, 1200, 2400, 4800, and 9600 ppm) with *Trichoderma harzianum* and *Bacillus subtilis* was evaluated, as well as its combined inhibitory effect against the causal agents of chili wilt disease (*F. oxysporum*, *R. solani*, and *S. rolfsii*). *T. harzianum* and *B. subtilis* were found to be compatible with chitosan. However, at concentrations of 2400 ppm and above, their growth and sporulation were affected. In the inhibition tests, low concentrations (75–600 ppm) stimulated greater mycelial growth of *Trichoderma* and had greater inhibition of mycelial growth of the phytopathogens, while concentrations of 600–1200 ppm stimulated their parasitism against *F. oxysporum* and *R. solani.* Similarly, the presence of chitosan in the culture medium favored greater growth of *B. subtilis* and higher inhibition percentages (91.65, 71.04, and 68.55 %) for *S. rolfsii*, *F. oxysporum*, and *R. solani*, compared to the treatment with *B. subtilis* without chitosan. Therefore, the combination of these microorganisms with chitosan could be considered as an alternative for the control of this disease.

Keywords: Biopolymer, compatibility, phytopathogens, harmless, inhibition, beneficial microorganisms.

Citation: Jiménez-Pérez O, Gallegos-Morales G, Castro-del Ángel E, Hernández-Castillo FD, Castillo-Reyes F. 2025. Fungicidal activity of chitosan in synergy with *Trichoderma harzianum* and *Bacillus subtilis* for the control of chili wilt disease. **Agrociencia.** https://doi.org/ 10.47163/agrociencia.v59i1.3059

Editor in Chief:
Dr. Fernando C. Gómez Merino

Received: August 24, 2023.
Approved: November 4, 2024.
Published in Agrociencia:
January 22, 2025.

INTRODUCTION

In Mexico, chili (*Capsicum annuum* L.) is of cultural, gastronomic, and economic importance (SIAP, 2023). It is affected by several diseases, which considerably reduce its yield. Chili wilt is one of the most important diseases (Andrade-Hoyos *et al*., 2019; Reyes-Tena *et al*., 2019; Villar-Luna *et al*., 2022), affecting several varieties of this crop (Moctezuma-Bautista *et al*., 2021) due to root rot, causing loss of turgor, yellowing, flower abortion, and plant death (Espinoza-Ahumada *et al*., 2019; Villar-Luna *et al*., 2022), and resulting in losses of up to 100 % (Mojica-Marín *et al*., 2009; Villar-Luna *et al*., 2022). This disease is caused by a complex of fungi such as *Fusarium* spp., *Rhizoctonia solani* (Andrade-Hoyos *et al*., 2019; Espinoza-Ahumada *et al*., 2019; Vallejo-Gutiérrez *et al*., 2019), *Sclerotium rolfsii*, and *Verticillium* spp., among others, and oomycetes such as *Pythium* spp. and *Phytophthora capsici* (Pérez-Acevedo *et al*., 2017; Leos-Escobedo *et al*., 2022).

To control these phytopathogens, farmers apply chemically synthesized fungicides such as 2-thiocyanomethyl (TCMTB), Azoxystrobin, Carbendazim, Thiabendazole, and Tebuconazole (Espinoza-Ahumada *et al*., 2019), which do not solve the problem and are harmful to people's health and the environment (Andrade-Hoyos *et al*., 2019; Villar-Luna *et al*., 2022). In addition, they cause phytopathogens to acquire resistance to chemical compounds, thus raising production costs (Andrade-Hoyos *et al*., 2019; Espinoza-Ahumada *et al*., 2019). However, there are other control alternatives, such as the use of antagonistic microorganisms (Espinoza-Ahumada *et al*., 2019; Ley-López *et al*., 2022).

Rhizobacteria of the genus *Bacillus* have antagonistic effects against phytopathogens (Leos-Escobedo *et al*., 2022) through various mechanisms of action, such as competition for nutrients, parasitism, and antibiosis by the production of enzymes (chitinases) and antibiotics (bacillomycin, fungimycin, mycosubtilin, and zwittermycin) (Mojica-Marín *et al.*, 2009). Similarly, some rhizospheric fungi, such as *Trichoderma* spp., have been shown to be effective for the control of phytopathogens through various mechanisms of action, such as mycoparasitism, competition for space, antibiosis by enzyme production, plant defense mechanism activation, and promotion of plant growth (Espinoza-Ahumada *et al*., 2019; Gallegos-Morales *et al*., 2022).

In addition to the application of antagonistic microorganisms, there are other control alternatives, such as the use of chitosan, which is a harmless biopolymer (Escudero *et al*., 2017; Pérez-Madruga *et al*., 2019) consisting of glucosamine units and, to a lesser extent, N-acetyl-D-glucosamine, linked together by β1-4 type bonds. It is extracted by deacetylation of arthropod chitin, mainly from the exoskeleton of crustaceans. This polymer exhibits inhibitory activity against a wide variety of phytopathogens such as bacteria, oomycetes (Pérez-Madruga *et al*., 2019), and fungi. Also, it possesses other qualities of agronomic importance, such as improving seed germination, favoring mineral absorption, promoting plant growth, activating plant defense mechanisms (López-Corona *et al*., 2019; Pérez-Madruga *et al*., 2019), and stimulating the growth of some beneficial microorganisms such as *Trichoderma asperellum* (Rautela *et al*., 2019) and *Bradyrhizobium japonicum* (Costales *et al*., 2017).

Since reports that demonstrate the compatibility of chitosan with beneficial microorganisms are scarce, the objectives of this work were to demonstrate the compatibility of chitosan with *Trichoderma harzianum* and *Bacillus subtilis*, as well as to prove their synergism in combination for the control of the complex of phytopathogens causing chili wilt.

MATERIALS AND METHODS

Experiment location

The experiment was carried out at the Microbiology Laboratory of the Department of Agricultural Parasitology of the Antonio Narro Autonomous Agrarian University (UAAAN) in Saltillo, Coahuila, Mexico (25° 21′ 13′′ N, 101° 1′ 56′′ W), at an altitude of 1742 m.

Biological material

Chitosan with a 100 % deacetylation grade extracted from shrimp exoskeletons by bacterial fermentation and 50 % sodium hydroxide (NaOH) was used. The strains of antagonistic microorganisms (*Trichoderma harzianum* and *Bacillus subtilis*) and phytopathogens causing chili wilt disease (*Fusarium oxysporum*, *Sclerotium rolfsii*, and *Rhizoctonia solani*) were taken from the microbial collection of the microbiology laboratory. All fungi were reactivated on potato dextrose-agar (PDA) culture medium (DIBICO, Mexico), and the bacteria on nutrient agar medium (DIBICO, Mexico).

Compatibility of beneficial microorganisms with chitosan

Mycelial growth and sporulation of *T. harzianum* were measured *in vitro* in Petri dishes with PDA culture medium supplemented with chitosan at eight concentrations (Q-75, Q-150, Q-300, Q-600, Q-1200, Q-2400, Q-4800, and Q-9600 ppm) and a control treatment without chitosan, with pH adjusted to 6.5 and with four replicates per treatment. Once the culture medium was solidified, a 5 mm diameter explant of culture medium with 72 h mycelial growth of *T. harzianum* was placed in the center of each Petri dish. They were then incubated at 28±2 °C with a photoperiod of 12 h light and 12 h dark.

Every 24 h, with the help of a digital Vernier (Truper, Mexico), mycelial growth was measured (in millimeters) until one of the control treatments completely filled the Petri dish. Subsequently, at 96 h, the percentage of sporulation was measured, for which 10 mL of sterile distilled water was added to each Petri dish, and scraping was performed with the help of a sterile glass rod. The contents with spores were placed in a 50 mL Falcon tube. Spore counting was performed with the aid of a Neubauer chamber and a compound microscope at 40 X, and the number of spores per milliliter was determined.

The compatibility of *B. subtilis* with chitosan was evaluated in Erlenmeyer flasks of 250 mL capacity, with 100 mL of nutrient broth culture medium supplemented with chitosan at the same concentrations mentioned above, plus a control treatment without chitosan, for a total of nine treatments with three replicates. The treatments were adjusted to 6.5 pH and incubated at 28±2 °C with a photoperiod of 12 h light and 12 h dark, under constant agitation at 150 rpm for 72 h. Colony-forming units (CFUs) were then counted in the same way as for *T. harzianum*.

Synergism of beneficial microorganisms in combination with chitosan as an inhibitor of *in vitro* mycelial growth of phytopathogens

The combination of *T. harzianum* and chitosan in dual confrontation against phytopathogens was performed in Petri dishes with PDA culture medium supplemented with chitosan at six concentrations, medium without chitosan, and a control (the phytopathogen without confrontation), adjusted to pH 6.5, for a total of eight treatments (Q-75 ppm + *Trichoderma*, Q-150 ppm + *Trichoderma*, Q-300 ppm + *Trichoderma*, Q-600 ppm + *Trichoderma*, Q-1200 ppm + *Trichoderma*, Q-2400 ppm + *Trichoderma*, *Trichoderma,* and control) for each phytopathogen, with four replicates per treatment. Once the culture medium had solidified, a 5 mm diameter portion of culture medium with mycelial growth of *T. harzianum* was placed at one end of the Petri dish, and at the other end of the dish, the phytopathogen with 96 h of growth. The dishes were incubated at 28±2 °C with a photoperiod of 12 h light and 12 h dark until the control treatment completely full the Petri dish.

Inhibition was assessed using a five-level visual scale, where 1 = *Trichoderma* completely covers the phytopathogen on the Petri dish; 2 = *Trichoderma* covers at least two-thirds of the Petri dish; 3 = *Trichoderma* and the phytopathogen colonize half of the Petri dish; 4 = the phytopathogen covers at least two-thirds of the Petri dish and appears to resist invasion by *Trichoderma*; and 5 = the phytopathogen completely covers *Trichoderma* and the Petri dish. *Trichoderma* was considered an antagonist of the phytopathogen when level 3 was not exceeded, this level being the lowest antagonism level and level 1 the highest. It was considered to have no antagonism capacity when the phytopathogen reached levels 4 and 5 (Bell *et al*., 1982).

The combination of *B. subtilis* and chitosan was carried out in a similar way, using the same concentrations and treatments, substituting *Trichoderma* for *B. subtilis*. Once the culture medium was solidified, *B. subtilis* was inoculated with a bacteriological loop in each of the four cardinal points of the Petri dishes and a control only with the phytopathogen, with four replicates per treatment. Then, a 5 mm diameter portion of culture medium with mycelial growth of one of the phytopathogens was placed in the center of each box and incubated at 28±2 °C with a photoperiod of 12 h light and 12 h dark.

Every 24 h, mycelial growth was measured in millimeters with a digital Vernier (Truper, Mexico) until the control treatment completely filled the Petri dish. With the data obtained, the percentages of inhibition (% *in*) of each phytopathogen were obtained using the following formula:

$$\%\ inhibition = \frac{C - T}{C} * 100$$

where C is the diameter of phytopathogen growth in the control treatments and T the diameter of phytopathogen growth in the treatments with *B. subtilis*, with and without chitosan (Castillo-Reyes *et al*., 2015).

Experimental design

Each experiment was established in a completely randomized experimental design. The data obtained were subjected to an analysis of variance (ANOVA), and a Tukey's test for comparison of means ($p \leq 0.05$) was performed using the statistical program InfoStat version 2019.1.2.0.

RESULTS AND DISCUSSION

Compatibility of *Trichoderma harzianum* and *Bacillus subtilis* with chitosan

Mycelial growth of *T. harzianum* at different concentrations of chitosan in PDA culture medium showed that concentrations from 75 to 1200 ppm (Figure 1A–E) did not affect fungal growth because at these concentrations there were no significant differences with the control (Figure 1I) (Table 1). The fungi in these treatments filled the Petri dishes at 72 h. However, when the concentration increased to 2400 ppm, mycelial growth of *T. harzianum* was impaired (Figure 1F). In the sporulation evaluation, concentrations of 75 and 150 ppm favored fungal sporulation with 1.5 x 10^8 and 5.2 x 10^8 spores per mL, respectively, which were statistically ($p > 0.05$) different from the control (1.2 x 10^8) and the rest of the treatments (Table 1).

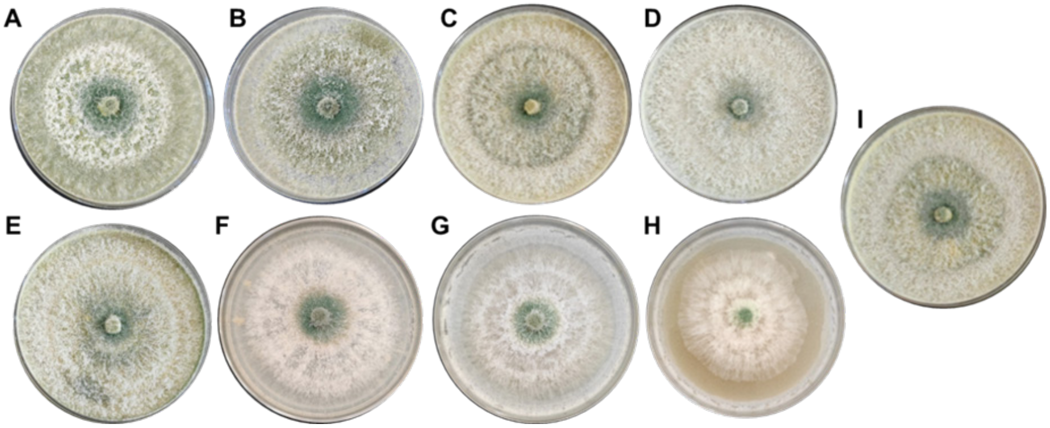

Figure 1. Growth of *Trichoderma harzianum* in potato dextrose-agar (PDA) culture medium supplemented with chitosan at different concentrations. A: 75 ppm; B: 150 ppm; C: 300 ppm; D: 600 ppm; E: 1200 ppm; F: 2400 ppm; G: 4800 ppm; H: 9600 ppm; I: PDA without chitosan.

Table 1. Growth of *Trichoderma harzianum* and *Bacillus subtilis* in culture medium supplemented with chitosan at different concentrations.

Treatment	*Trichoderma harzianum*		*Bacillus subtilis*
	Growth (mm) Mean ± EE	Spores per mL Mean	UFC per mL Mean
Q-75 ppm	85 ± 0.00 A	1.5 x 10^8 B	1.7 x 10^9 BC
Q-150 ppm	85 ± 0.00 A	5.2 x 10^8 A	1.9 x 10^9 BC
Q-300 ppm	85 ± 0.00 A	1.1 x 10^8 C	2.8 x 10^9 A
Q-600 ppm	85 ± 0.00 A	6.8 x 10^7 E	3.1 x 10^9 A
Q-1200 ppm	85 ± 0.00 A	7.9 x 10^7 D	1.95 x 10^9 B
Q-2400 ppm	59.02 ± 1.15 B	4 x 10^2 F	9.3 x 10^8 D
Q-4800 ppm	58.11 ± 0.94 B	3.3 x 10^2 F	8.6 x 10^6 E
Q-9600 ppm	48.49 ± 0.61 C	2.8 x 10^2 F	1.3 x 10^5 E
Control	85 ± 0.00 A	1.2 x 10^8 C	1.5 x 10^9 C

*Means with the same letter are not significantly different ($p > 0.05$). UFC: colony forming units; EE: standard error.

These results coincide with those reported by other authors, who mention that *Trichoderma* is tolerant to chitosan at certain concentrations. Hernández-Domínguez *et al*. (2021), when using chitosan (Sigma-Aldrich at 85 % distillation) at different concentrations (1, 4, 6, 8, 10, and 12 mg mL^{-1}), showed that *Trichoderma parareesei* did not present significant differences with the control; however, there was a decrease in growth at concentrations of 6, 10, and 12 mg mL^{-1}.

Chittenden and Singh (2009) reported that mycelial growth of *T. harzianum* was not affected by low molecular weight chitosan (50–190 kDa) at concentrations of 0.5 and 0.75 % (5000 and 7500 ppm). In contrast to this study, *T. harzianum* was more sensitive, since concentrations from 2400 to 9600 ppm (2.4 to 9.6 mg mL^{-1}) did affect its growth, as they used chitosan with different properties, such as deacetylation percentage (the higher this value is, the more active chitosan is). Zavala-González *et al*. (2016) suggest that the tolerance of *Trichoderma* species to this biopolymer is related to a low linoleic acid content and low fluidity of their cell membrane. Escudero *et al.* (2017) mention that some fungi, such as *Pochonia chlamydosporia*, use chitosan as a source of nutrients for their growth and that low concentrations of this biopolymer increase their mycelial growth.

In the case of compatibility of *B. subtilis* with chitosan, at 72 h of incubation, the bacteria presented the highest number of UFC at concentrations of 300 and 600 ppm, with values of 2.8 x 10^9 and 3.1 x 10^9, respectively, which was statistically ($p > 0.05$) higher than the other treatments and the control (Table 1). However, at concentrations higher than 2400 ppm, the UFC of the bacteria decreased.

These results are similar to those reported by Costales *et al*. (2017), who tested the *in vitro* compatibility of *Bradyrhizobium japonicum* with chitosan. At a concentration of 10 mg mL^{-1} in the culture medium, the bacteria increased their number of UFC compared to the control, while at concentrations of 500 mg mL^{-1} they decreased. In this experiment, low concentrations (75–600 ppm) of chitosan increased the UFC of *B. subtilis*, and decreased at high concentrations (1200–9600 ppm). These results are of great relevance because the information related to the compatibility of beneficial bacteria with chitosan is very scarce, especially the combination of bacteria antagonistic to phytopathogens with the biopolymer.

Combination of chitosan and *Trichoderma harzianum* against phytopathogens

In the combination of chitosan with *T. harzianum* against *S. rolfsii* (Figure 2), as the concentration of chitosan increased, the growth of the phytopathogenic fungus decreased; however, the growth of *Trichoderma* was not affected, since it presented inhibition levels of 2 (Figure 2, Aa–Ac) and 1 (Figure 2, Ad–Af). The latter had the highest inhibition compared to the *T. harzianum* treatment without chitosan (Figure 2, Ag), which presented a level 2 on the evaluation scale.

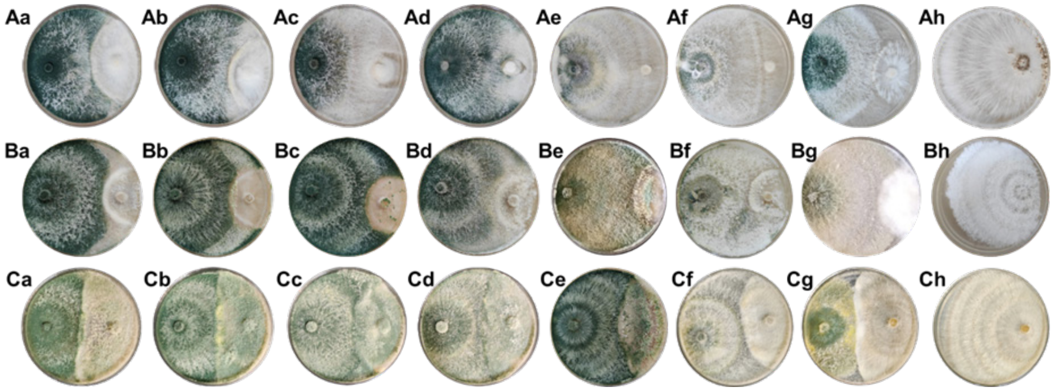

Figure 2. *In vitro* inhibitory effect of the combination of chitosan and *Trichoderma harzianum* against *Sclerotium rolfsii* (A), *Fusarium oxysporum* (B), and *Rhizoctonia solani* (C). Combinations of *T. harzianum* with chitosan at concentrations of 75 (a), 150 (b), 300 (c), 600 (d), 1200 (e), 2400 ppm (f), *T. harzianum* without chitosan (g), and controls (h).

In the assay against *F. oxysporum* (Figure 2, Ba–Bh), the presence of chitosan in the culture medium stimulated greater growth of *Trichoderma* (Figure 2, Ba–Bf). At chitosan concentrations of 75 to 300 ppm (Figure 2, Ba–Bc), *Trichoderma* ranked at level 2 inhibition of *Fusarium*; however, at concentrations of 600 to 2400 ppm (Figure 2, Bd–Bf), it ranked at level 1 inhibition, as it favored parasitism of *T. harzianum* against the phytopathogen, which was not present in the rest of the treatments or in the *Trichoderma* treatment without chitosan (Figure 2, Bg), which was located at level 2 of the scale.

In the bioassay of *R. solani* (Figure 2, Ca–Ch), it was observed that the presence of chitosan in the culture medium favored a greater growth of *Trichoderma* (Figure 2, Ca–Cf) in the treatments with concentrations of 150 to 1200 ppm (Figure 2, Cb–Ce), since they increased the parasitism of *T. harzianum* against the phytopathogen, which grew on *Rhizoctonia* and managed to fill the Petri dish. This placed these treatments at level 1 on the scale, while the *Trichoderma* treatment without chitosan presented the lowest level of inhibition (level 3), as both microorganisms filled half of the Petri dish, indicating no dominance of one over the other (Figure 2, Cg).

These results coincide with those reported by some authors; for example, Chittenden and Singh (2009) demonstrated the synergistic effect of the *in vitro* combination of chitosan at 0.05 and 0.075 % plus *T. harzianum*, which completely inhibited the mycelial growth of *Leptographium procerum* and *Sphaeropsis sapinea*. This was better than the treatment with chitosan alone, where *L. procerum* presented growth of approximately 45 and 25 mm, and *S. sapinae* was not affected in its growth. Hernández-Domínguez *et al*. (2021) refer to the fact that chitosan induces a greater production of metabolites such as chitinases in *Trichoderma* and that these showed greater control against *F. oxysporum*.

In turn, Rautela *et al*. (2019) demonstrated that the presence of chitosan (500 ppm) improved the enzymatic activity (cellulases and chitosanases) and the production of secondary metabolites (tetradecanoic acid and 1,2-benzenedicarboxylic acid) of *Trichoderma* compared to the treatment of *Trichoderma* without chitosan. This could be beneficial to increase its antagonistic activity against phytopathogens. An increase in the antagonistic capacity of *T. harzianum* against the three phytopathogens was noted with the presence of chitosan, behaving differently according to its concentration. At low concentrations (75–300 ppm), it stimulated a greater growth of *Trichoderma*, and at intermediate concentrations (600–1200 ppm), it stimulated its parasitism against *F. oxysporum* and *R. solani*, with which a greater control of the phytopathogens was obtained compared to the treatment without chitosan.

Combination of chitosan and *Bacillus subtilis* against phytopathogens

The combination of chitosan with *B. subtilis*, as with *Trichoderma*, favored the growth of the bacteria, which was visualized in a higher percentage of inhibition of the phytopathogens. In the experiment against *S. rolfsii* (Figure 3, Aa–Ah), the presence of chitosan in the culture medium inhibited the mycelial growth of the fungus, while it stimulated the growth of the bacteria. It presented an inhibition of 91.35 % at a concentration of 2400 ppm (Figure 3, Af); furthermore, the presence of 75 ppm of chitosan in the culture medium with *Bacillus* obtained 75.26 % inhibition of the phytopathogen (Figure 3, Aa), presenting an increase of 15.64 % compared to the treatment of *B. subtilis* without chitosan (Figure 3, Ag), which was the treatment that showed the lowest inhibition of the fungus with 59.62 % (Table 2).

In the assay against *F. oxysporum* (Figure 3, Ba–Bh), the presence of chitosan stimulated a higher percentage of inhibition by *B. subtilis* against the phytopathogen, where it

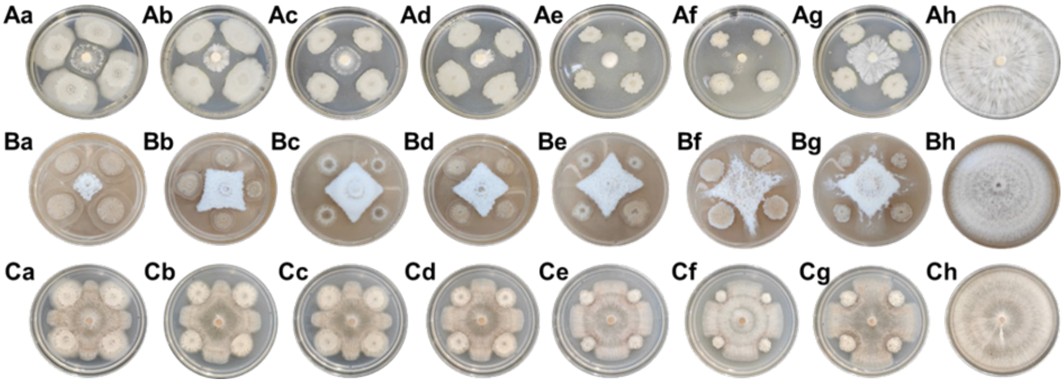

Figure 3. *In vitro* inhibitory effect of the combination of chitosan and *Bacillus subtilis* against *Sclerotium rolfsii* (A), *Fusarium oxysporum* (B), and *Rhizoctonia solani* (C). Combinations of *B. subtilis* with chitosan at concentrations of 75 (a), 150 (b), 300 (c), 600 (d), 1200 (e), 2400 ppm (f), *B. subtilis* without chitosan (g), and controls (h).

Table 2. *In vitro* inhibition of phytopathogens by the combination of *Bacillus subtilis* and chitosan at different concentrations.

Treatment	*Sclerotium rolfsii* Mean ± EE	*Fusarium oxysporum* Mean ± EE	*Rhizoctonia solani* Mean ± EE
Q-75 ppm + *Bacillus*	75.26 ± 0.72 C	71.04 ± 0.53 A	63.84 ± 0.94 AB
Q-150 ppm + *Bacillus*	72.10 ± 1.04 C	64.58 ± 0.19 B	68.55 ± 2.81 A
Q-300 ppm + *Bacillus*	74.30 ± 0.50 C	65.82 ± 0.30 BC	59.16 ± 0.79 BC
Q-600 ppm + *Bacillus*	81.77 ± 0.36 B	63.70 ± 0.53 BC	58.20 ± 0.28 CD
Q-1200 ppm + *Bacillus*	84.72 ± 0.16 B	63.15 ± 0.50 C	53.90 ± 0.16 DE
Q-2400 ppm + *Bacillus*	91.35 ± 0.52 A	60.63 ± 0.50 D	52.43 ± 0.23 E
Bacillus	59.62 ± 1.17 D	56.85 ± 0.85 E	52.51 ± 0.63 E
Control	0.00 ± 0.00 E	0.00 ± 0.00 F	0.00 ± 0.00 F

*Means with the same letter are not significantly different ($p > 0.05$). EE: standard error.

obtained 71.04 % inhibition with the concentration of 75 ppm chitosan (Figure 3, Ba), a value statistically superior to the other treatments.

In the combination of chitosan and *B. subtilis* against *R. solani* (Figure 3, Ca–Ch), the bacteria did not present inhibition of the fungus by antibiosis, but by competition. Greater bacterial growth was observed in the treatments with chitosan (Figure 3, Ca–Cf), with a higher percentage of inhibition (68.55 %) in the treatment of chitosan at 150 ppm plus *Bacillus*. On the other hand, the *B. subtilis* treatment without chitosan (Figure 3, Cg) only reached 52.51 % inhibition (Table 2).

It is worth mentioning that there are few studies that demonstrate the compatibility of chitosan with antagonistic bacteria used against phytopathogens. However, there are some studies related to the combination of this biopolymer with plant growth-promoting rhizobacteria. Ortega-García *et al*. (2021) demonstrated that by combining *Bacillus amyloliquefasciens* with chitosan (65 % deacetylation) at a concentration of 50 and 100 %, they obtained higher germination, growth, and production of *Asparagus officinalis* compared to the application of these treatments individually. Holguin-Peña *et al*. (2020) promoted sorghum growth by the combined application of chitosan, *B. subtilis*, *B. polymyxa*, and *B. amyloliquefasciens*. Similarly, Agbodjato *et al*. (2016) demonstrated this in maize by combining chitosan with *Azospirillum lipoferum*, *Pseudomonas fluorescens*, and *Pseudomonas putida*.

Costales-Menéndez *et al*. (2021) demonstrated that chitosan favors the survival of *Bradyrhizobium* in soybean seeds, in addition to nodulation in the plant, which causes greater growth. Álvarez-Sánchez *et al*. (2021), through the combined application of chitosan with *B. japonicum*, promoted seed germination, growth, and turnip production when these treatments were applied individually; in addition, there was a lower incidence of *Peronospora brassicae*. These results are like those obtained in this research, since the mixtures of chitosan with *B. subtilis* achieved greater inhibition of phytopathogens compared to the treatment where only the bacteria were applied.

Sawaguchi *et al*. (2015) demonstrated that the application of chitosan to silty soil increased the bacterial population up to 20 times, where chitinolytic microorganisms such as *Kitasatospora* and *Streptomyces*, which are capable of degrading chitosan, predominated. Therefore, the inclusion of microorganisms such as *Bacillus*, *Streptomyces*, and *Trichoderma* sp. could be considered since they are capable of producing chitinases and chitosanases that are compatible with this biopolymer (Castañeda-Ramírez *et al*., 2011). This is considered a possible reason why the treatments of *B. subtilis* with chitosan achieved a greater inhibition of phytopathogens; however, the characterization of the compounds produced by these microorganisms in culture medium with and without chitosan will be considered for future studies to understand the effect produced by chitosan on these bacteria.

CONCLUSIONS

The compatibility of *Trichoderma harzianum* and *Bacillus subtilis* with chitosan was demonstrated. The presence of this biopolymer at low concentrations favored the growth of these microorganisms. Similarly, growth and inhibition by *Trichoderma* were stimulated at chitosan concentrations of 75–300 ppm, while at 600–1200 ppm, parasitism against *Fusarium oxysporum* and *Rhizoctonia solani* was promoted. The presence of chitosan in the culture medium caused greater growth of *B. subtilis*, with higher inhibition percentages for *Sclerotium rolfsii*, *F. oxysporum*, and *R. solani* compared to the treatment without chitosan. *T. harzianum* and *B. subtilis*, mixed with chitosan, exhibited a synergistic effect resulting in a greater inhibition of phytopathogens, so these combinations can be considered as bioalternatives for the control of phytopathogens causing chili wilt disease.

ACKNOWLEDGEMENTS

The authors thank the Antonio Narro Autonomous Agrarian University (UAAAN) for the use of materials and facilities, as well as the National Council of Humanities, Sciences, and Technologies (CONAHCYT) for the support provided to the first author for his postgraduate studies.

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