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Article

In vitro effectiveness of silicon dioxide and graphene nanoparticles combined with extracts of *Bacillus amyloliquefaciens* against phytopathogenic fungi

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Abstract

The use of nanoparticles in agriculture opens the opportunity for the development of agro-products with this technology, aimed at controlling diseases caused by phytopathogenic fungi. This study aimed to evaluate *in vitro* the inhibitory effect of silicon dioxide (SiO_2 NPs) and graphene nanoparticles (Graf NPs) mixed with extracts of *Bacillus amyloliquefaciens* (EcBa) on the mycelial development and formation of reproductive structures of *Fusarium solani*, *Rhizoctonia solani*, *Colletotrichum acutatum*, and *Alternaria alternata*. For the biological effectiveness test, the poisoned medium technique was used under a completely randomized design of two doses (D_E 70 and D_E 90) and absolute control with 20 replications for each treatment. Data were analyzed using an analysis of variance and Tukey's mean test (*p*# 0.05). Effective doses were calculated using a Probit analysis. The treatment that showed the best inhibitory effect was SiO_2 NPs + EcBa since it managed to inhibit mycelium growth and decreased the production of reproductive structures (spores and sclerotia) by 84% to 100% with low doses of *Fusarium solani*, *Rhizoctonia solani*, *Colletotrichum acutatum*, and *Alternaria alternata*, followed by Graf Nps + EcBa, EcBa, at higher doses, they obtained 83.7 to 100% inhibition, respectively.

Keywords:

agronanotechnology, beneficial bacteria, nanofungicides.

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Introduction

The use of nanotechnology (NT) is of great importance in agriculture due to its multiple applications, expanding the possibilities for the development of agro-products, such as nanofertilizers, nanoherbicides and nanopesticides, that improve crop yields and protect the environment (Lira-Saldivar *et al*., 2018). Nanostructured porous silicon has important properties, as its optical, chemical, and electrical characteristics can be modified for the development of chemical and biological sensors (Ríos *et al*., 2020).

The efficiency of porous silicon has been proven for drug development since these molecules can be introduced into silicon nanoparticles (NPs) functioning as drug carriers (Santos *et al*., 2014). Silicon nanoparticles have the potential to revolutionize existing technology used in agriculture and plant biotechnology, they can provide ecological alternatives and have concrete solutions to problems, such as those related to weeds, pathogens, drought and productivity (Rastogi *et al*., 2019).

On the other hand, graphene oxide is a material derived from carbon, which presents itself as a graphene sheet, which could be functional with different oxygenated groups, such as hydroxyl, epoxy, and carbonyl, which are present in the structures of graphene, causing it to be very hydrophilic (Lira-Saldivar *et al*., 2018). Being a two-dimensional, thin, honeycomb-shaped sheet, it gives it remarkable mechanical, electrical, thermal, and barrier properties. Graphene-based nanocomposites have been a hot area of research in the last decade. For these reasons, incorporating graphene into polymers to design nanocomposites has been the goal of countless research efforts (Smith *et al*., 2019).

Studies have revealed graphene's role in antimicrobial activity, laying the groundwork for its use in pathogen control as it exhibits different antimicrobial activities, including antibacterial, antifungal, and antiviral properties (Almardani *et al*., 2019). Of the reports published on the subject of NPs, the following stand out: the use of pesticide nanoformulations for the control of *Phenacoccus solenopsis* (Elabasy *et al*., 2020).

Copper NPs on the control of phytopathogenic fungi (*Fusarium solani*, *Fusarium verticillioides*, *Verticillium dahliae*, *Neofusicoccum* sp. and *Fusarium oxysporum*) (Pariona *et al*., 2018). Graphene in nanoformulations with fungicides showed an inhibitory capacity against *Fusarium graminearum* on growth, mycelial biomass and spore germination (Wang *et al*., 2021).

On the other hand, phytopathogenic fungi are a limiting factor in crop production as they significantly reduce yield, causing damage to plants and fruits; among these are *Fusarium oxysporum*, which comprises more than 120 known strains or special forms. *Fusarium oxysporum* f. sp. Lycopersici causes wilt in tomato. *Fusarium* wilt is a destructive tomato disease in several countries around the world and is of great concern to growers due to its large production loss (Malandrakis *et al*., 2018). For its part, *Rhizoctonia solani* is a fungus that is more virulent and is widely distributed in the soil, it causes severe yield losses of potatoes around the world, and causes damage; for example, to stems and roots (Kiptoo *et al*., 2021).

Likewise, among the fungi that cause damage in the postharvest stage is *Colletotrichum acutatum*, affecting, among others, avocado fruits (Barroso *et al*., 2021). There is also *Alternaria alternata*, which severely affects tomato fruits, decreasing the quality of this agricultural product with considerable economic losses (Coromoto and Reyes, 2018). In relation to the control of these pathogens, it is commonly carried out through the use of chemical fungicides, which cause damage to health, pollute the environment and generate resistance in microorganisms, as in the case of benzimidazoles, where numerous cases of resistance generation in various special forms of *F. oxysporum* have been reported (Arie, 2019).

Because of this, it is necessary to develop ecologically friendly strategies for the control of phytopathogenic fungi. The use of antagonistic bacteria such as *Bacillus subtilis* and *B. amyloliquefaciens* has been reported. Bacteria in the *Bacillus* group are well known as producers of a wide range of antagonist compounds, such as peptides and lipopeptides, polyketide compounds, bacteriocins, and siderophores (Fira *et al*., 2018). Habe *et al*. (2017) indicated that *B. subtilis* produced circular lipopeptides, such as surfactin and iturin, which are considered to be antifungal compounds that affect target cells at the membrane level.

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There are few scientific reports on the fungicidal activity of the use of silicon and graphene NPs in combination with *B. amyloliquefaciens*. The present study aimed to evaluate the inhibitory effect of these compounds and the bacterial extract on the mycelial development and formation of reproductive structures of the fungi *F. solani*, *R. solani*, *C. acutatum*, and *A. alternata in vitro*. Seeking an alternative for the management of fungal diseases with inputs with less environmental impact but with high effectiveness.

Materials and methods

Biological material

Phytopathogenic fungi *F. solani*, *R. solani*, *A. alternata*, and *C. acutatum* were isolated, purified, and identified in the Toxicology Laboratory of the Antonio Narro Autonomous Agrarian University (UAAAN), for its acronym in Spanish, which were cultured in potato dextrose agar (PDA) culture medium (BD Bioxon®) for subsequent use.

Preparation of extracts of *B. amyloliquefaciens* enriched with silicon dioxide (SiO_2) and graphene (Graf) NPs

The strain of *B. amyloliquefaciens* (EcBa) previously identified in the Toxicology Laboratory of the UAAAN was used; this strain was cultured in inclined tubes with nutrient agar (TM Media®) for its growth. Subsequently, 500 ml of liquid culture medium was prepared for the production of iturins by fermentation using the procedure described by Mckeen *et al*. (1986). pH was adjusted to 6 and it was autoclaved for 15 min at 121 °C.

It was left to cool to room temperature and inoculated with 1 ml of $1x10^6$ CFU bacterial suspension, leaving in incubation at 30 °C, and in constant stirring in an incubator with stirring (150 rpm) for three days. After this time, the extract was carried out, eliminating the bacteria by centrifugation at 5 000 rpm for 20 min and the use of filters with a pore diameter of 0.22 µm (Linktor® Syringe filters). Once the EcBa extract was obtained, the products formulated with silicon dioxide (SiO 2 NPs) and graphene NPs (Graf NPs) provided by the company Culta, SA de CV (Ciudad Mante, Tamaulipas) were made.

For this purpose, 2 g of NPs per 100 ml of bacterial extract was mixed; the solution was sonicated by immersing the ultrasound probe (Branson® Sonifier 450 USA) at 35% constant power for 10 min at a temperature of 60 °C for better dispersion; it was stored at 4 °C protected from light.

In vitro evaluation of *B. amyloliquefaciens* extracts with SiO_2 and Graf NPs on the mycelial development of phytopathogenic fungi

The fungus *F. solani* was used to determine the biological window corresponding to bioassay one of mycelial inhibition, where five treatments were evaluated: extract of *B. amyloliquefaciens* (EcBa) alone, SiO_2 NPs and mixed in sterile distilled water (SiO_2 NPs + H_2O), SiO_2 NPs and mixed with extract of *B. amyloliquefaciens* (SiO_2 NPs + EcBa), Graf NPs and mixed in sterile distilled water (Graf NPs + H_2O), Graf NPs and mixed with *B. amyloliquefaciens* extract (Graf NPs + EcBa), with nine doses (0.1, 0.5, 1, 2.5, 5, 8, 16, 32, and 40 ml L^{-1}, and the control) per treatment, with 10 replications.

The poisoned medium technique (Ochoa *et al*., 2012) was used, which consisted of placing explants of 0.5 cm in diameter in the center of the Petri dish of 9 cm in diameter with each phytopathogen of interest and incubating them at 25 ±2 °C in darkness until the mycelium growth of the control (only PDA) reached the measurements of the plate. Mycelial growth was measured every 24 h with a vernier; growth data were used to calculate inhibition percentages applying the following formula: (PRGI= [(R1-R2)/R1] x 100). Where: PRGI= percentage of radial growth inhibition, R1= mycelial growth of the control and R2= mycelial growth of the treatment.

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With the data obtained from the PRGI, the doses (D_E 30, D_E 50, D_E 70, and D_E 90) were calculated using Probit analysis. A biological effectiveness test (bioassay two) was performed using the recommended doses for (D_E 70 and D_E 90) of EcBa, SiO_2 NPs + EcBa, Graf NPs + EcBa, and control treatments, with 20 replications (Petri dish) on the phytopathogens *F. solani*, *R. solani*, *A. alternata*, and *C. acutatum*.

In vitro evaluation of *B. amyloliquefaciens* extracts with SiO_2 and Graf NPs on the production of reproductive structures of phytopathogenic fungi

A third bioassay was performed, which evaluated the effective doses (D_E 30, D_E 50, and D_E 70) of EcBa, SiO_2 NPs + EcBa, Graf NPs + EcBa, and control, with three replications for each of the phytopathogenic fungi using the poisoned medium technique (Ochoa *et al*., 2012). For this, 10 days after the fungus was seeded in the Petri dish, 5 ml of sterile distilled water was added to the Petri dish. A 1 ml aliquot was taken and the spores were counted in triplicate in a Neubauer chamber under a 40X optical microscope (Nikon® Japan 449193).

In the fungi *F. solani*, *A. alternata*, and *C. acutatum*, the spores were transferred to test tubes and homogenized in a vortex. Twenty microliters of the suspension were taken and counted as previously described (Barroso *et al*., 2021). In the case of *R. solani*, a sclerotia count was performed. In this case, the Petri dish was divided into four quadrants and the sclerotia were counted under a stereo microscope (Olympus® SZ2-LGB).

Experimental design

In mycelial inhibition (bioassay one), a completely randomized design was used in each treatment with six doses: EcBa (2.5, 5, 8, 16, 32, and 40 ml L^{-1}), SiO_2 NPs + EcBa, Graf NPs + EcBa, SiO_2 NPs + H_2O, and Graf NPs + H_2O (1, 2.5, 5, 8, 16, and 32 ml L^{-1}), plus a control (0 ml L^{-1}) PDA without treatment as an absolute control; each dose had 10 replications, giving a total of 70 experimental units for each treatment evaluated on *F. solani*, respectively.

In the biological effectiveness tests (bioassay two), a completely randomized design was used for each phytopathogenic fungus (*F. solani*, *R. solani*, *A. alternata*, and *C. acutatum*), evaluating the effective doses D_E 70 and D_E 90, plus a control (0) with 20 replications, giving a total of 60 experimental units for each treatment evaluated (EcBa, SiO_2 NPs + EcBa, Graf NPs + EcBa).

Regarding the production of reproductive structures (bioassay three), it was carried out in a completely randomized design of three effective doses: 1= D_E 30, 2= D_E 50, 3= D_E 70, and a control (0) with three observations, giving a total of 12 experimental units for each treatment and phytopathogenic fungus evaluated.

Data analysis

In the variables evaluated, a completely randomized design was used in each treatment. An analysis of variance and comparison of means of the least significant difference by Tukey ($p \leq 0.05$) were used by means of the Statistical Analysis System version 9.0. In the determination of the effective doses, the PRGI data obtained by means of the formula were used and the doses were calculated by Probit analysis with the same statistical program.

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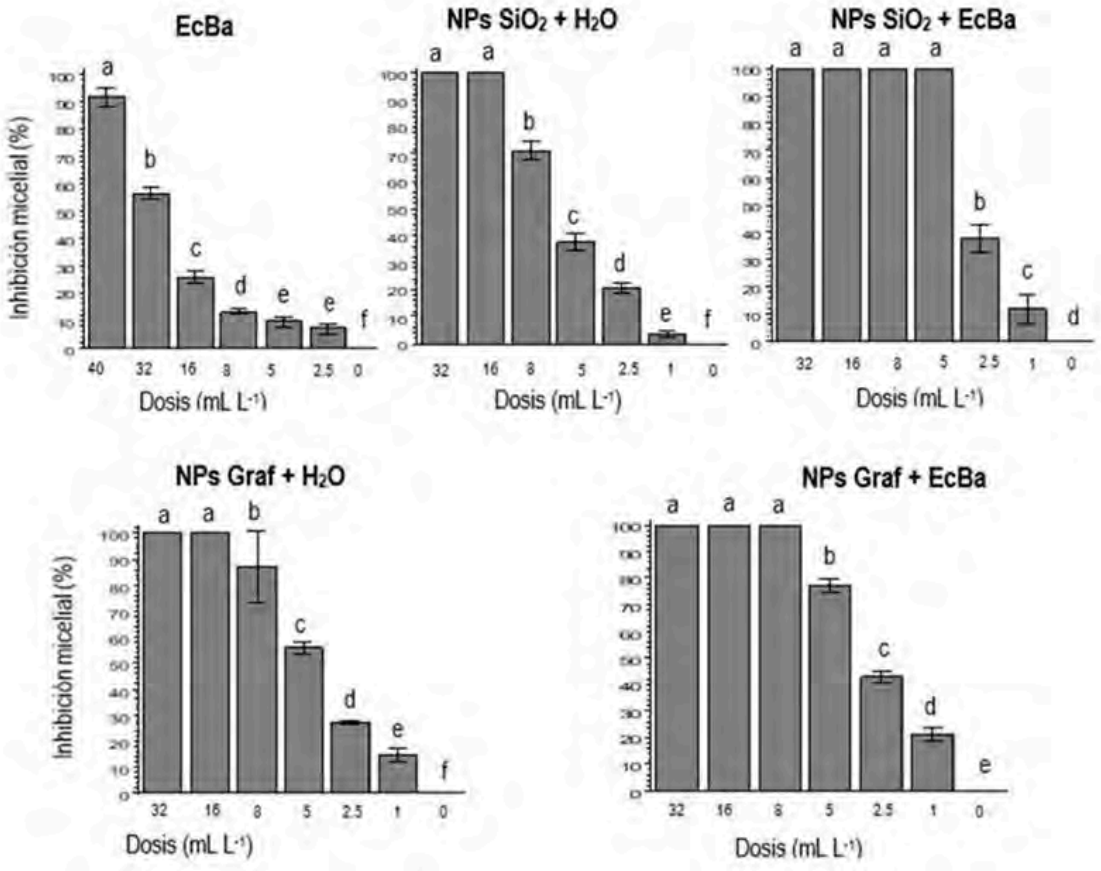
Results and discussion

In vitro evaluation of *B. amyloliquefaciens* extracts with SiO_2 and Graf NPs on the mycelial growth of phytopathogenic fungi

In the results of bioassay 1 (biological window) with the fungus *F. solani*, NPs treatments showed inhibitory effect from 1 to 40 ml L^{-1} (Figure 1). The SiO_2 NPs + EcBa treatment showed 100% mycelial inhibition at concentrations of 5 to 32 ml L^{-1}, followed by the Graf NPs + EcBa treatment with inhibitions of 100 at concentrations of 8 to 32 ml L^{-1}, while the treatments of SiO_2 NPs + H_2O and Graf NPs + H_2O showed an inhibitory effect of 100% at concentrations16 and 32 ml L^{-1}.

Figure 1. Mycelial inhibitory effect of different concentrations of *B. amyloliquefaciens* extracts with silicon oxide and graphene NPs on *F. solani*. EcBa= *B. amyloliquefaciens* extract; SiO_2 NPs + EcBa= silicon dioxide NPs and mixed with EcBa; SiO_2 NPs + H_2O= silicon dioxide NPs and suspended in sterile distilled water; Graf NPs + EcBa= graphene NPs and mixed with EcBa and Graf NPs + H_2O= graphene NPs and suspended in sterile distilled water.



Regarding the bacterial extract EcBa, 91.71% was obtained using the highest concentration (40 ml L^{-1}). The combination of NPs with *B. amyloliquefaciens* extract produced a synergistic effect, which potentiates the fungicidal effect from the lowest concentrations. In this regard, we can mention that the bioactive molecules of *B. amyloliquefaciens* are the circular lipopeptides of the surfactin, iturin and fengycin families, they affect cells at the membrane level, causing physical degradation and growth inhibitory activity, inducing systemic resistance in plants, and competing for ecological niches with plant pathogens (Ngalimat *et al*., 2021).

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In the case of silicon oxide nanoparticles, they disrupt cellular functions such as differentiation, increased wall permeability, deactivation of protein molecules, and affect the transmembrane energy cycle (Derbalah *et al*., 2018). In relation to graphene nanoparticles, they induce the alteration of the cell membrane, damage DNA, influence the energetic metabolic pathways to inactivate microorganisms and have photochemical activity by causing oxidative stress (Fernando *et al*., 2018).

These results are consistent with Duan *et al*. (2021), where they report an inhibitory effect (86.7, 84.2, 72.8 and 74%) on *Fusarium proliferatum*, *Fusarium solani*, *Fusarium verticillioides* and *Fusarium oxysporum*, respectively. For their part, Peng *et al*. (2022) reported that 50, 100, and, 150 mg L^{-1} of silicon oxide nanoparticles increased ginger rhizome firmness, water loss, increased antioxidant enzyme activity, total phenolic and flavonoid contents, and inhibited *Fusarium solani* by preventing the penetration of hyphae into cells.

Likewise, El-Abeid *et al*. (2020) mention that graphene nanoparticles with copper have higher antifungal activity with only 1 mg L^{-1} than the conventional fungicide with 2.5 g L^{-1}, making holes and pores in the cell membranes of fungi, which induces cell death, to reduce the severity of *Fusarium* wilt diseases.

The purpose of determining the inhibitory doses (bioassay 1) was to know the amount of treatment to inhibit the development of the phytopathogen, similar to what was reported by Ochoa *et al*. (2012), where the mean effective dose (ED_{50}) was determined. Therefore, in this study, data obtained from PRGI were used to calculate the effective doses (D_E) of the treatments (Table 1). It was observed that the SiO_2 NPs + EcBa treatment showed the lowest concentrations to control *F. solani in vitro*, with D_E 30= 1.75 ml L^{-1}, D_E 50= 2.3 ml L^{-1}, D_E 70= 3.01 ml L^{-1}, and D_E 90= 4.46 ml L^{-1}, a similar effect was shown by the treatment of Graf NPs + EcBa.

Table 1. Determination of effective doses of *B. amyloliquefaciens* extracts with silicon oxide and graphene NPs on *F. solani*.

Treatment	Effective dose	Probable dose (ml L^{-1})	Lower fiducial limit 95%	Upper fiducial limit 95%
SiO_2 NPs + H_2O	D_E 30	3.58	3.36	3.79
	D_E 50	5.36	5.08	5.65
	D_E 70	8.03	7.57	8.56
	D_E 90	14.4	13.21	15.88
SiO_2 NPs + EcBa	D_E 30	1.75	1.51	1.98
	D_E 50	2.3	2.03	2.59
	D_E 70	3.01	2.67	3.46
	D_E 90	4.46	3.84	5.44
Graf NPs + H_2O	D_E 30	1.46	0.93	2.05
	D_E 50	2.8	1.98	3.9
	D_E 70	5.4	3.88	8.03
	D_E 90	13.88	9.15	25.49
Graf NPs + EcBa	D_E 30	1.37	0.9	1.87
	D_E 50	2.33	1.69	3.17
	D_E 70	3.98	2.94	5.75
	D_E 90	8.59	5.92	14.98
EcBa	D_E 30	15.51	13.16	17.58
	D_E 50	23.45	20.94	26.39
	D_E 70	35.46	31.03	42.53
	D_E 90	64.43	51.81	89.52

SiO_2 NPs + H_2O= silicon dioxide NPs and suspended in sterile distilled water; SiO_2 NPs + EcBa= silicon dioxide NPs and mixed with EcBa; Graf NPs + H_2O= graphene NPs and suspended in sterile distilled water; Graf NPs + EcBa= graphene NPs and mixed with EcBa and EcBa= extract of *B. amyloliquefaciens*.

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For their part, the treatments of SiO_2 NPs + H_2O and Graf NPs + H2O showed a D_E 90 of 14.4 and 13.88 ml L^{-1}, considered as intermediate doses compared to the highest dose in EcBa, which presented a D_E 90= 64.43 ml L^{-1}. The results obtained differ from those reported by Lee *et al*. (2017), where *B. amyloliquefaciens* DA12 extract inhibited the germination of *F. graminearum* with an inhibition rate of 83% at a concentration of 31.3 µg ml^{-1} and 100% at a concentration of 250 µg ml^{-1}. It is important to mention that there are no scientific studies that provide information on the determination of effective doses of bacterial extracts with silicon and graphene NPs in the mycelial inhibition of phytopathogenic fungi.

In the biological effectiveness tests of the effective doses in Table 2, there are significant differences. It was observed that the EcBa treatment inhibited 100% of the four phytopathogenic fungi with a D_E 90 (64.43 ml L^{-1}), which is observed in Figure 2 (T1.2). The inhibitory effect prevailed from the first 24 h to 168 h.

Table 2. Biological effectiveness test of effective doses of *B. amyloliquefaciens* extracts with silicon oxide and graphene NPs on the mycelial development of phytopathogenic fungi.

Treatment	Effective dose	*F. solani*	*R. solani*	*C. acutatum*	*A. alternata*
	Mycelial inhibition (%)				
SiO_2 NPs + EcBa	D_E 70	70.65^{c}	66.25^{b}	61.12^{c}	63.25^{e}
	D_E 90	90.71^{b}	100^{a}	95.06^{a}	72.68^{d}
Graf NPs + EcBa	D_E 70	70.46^{c}	19.65^{c}	44.71^{d}	50.4^{f}
	D_E 90	91.62^{b}	100^{a}	73.4^{b}	79.21^{c}
EcBa	D_E 70	100^{a}	100^{a}	93.87^{a}	83.75^{b}
	D_E 90	100^{a}	100^{a}	100^{a}	100^{a}
Control	0	0^{d}	0^{d}	0^{e}	0^{g}

Means with different letters within each column indicate significant differences between treatments (Tukey, $p \leq 0.05$).

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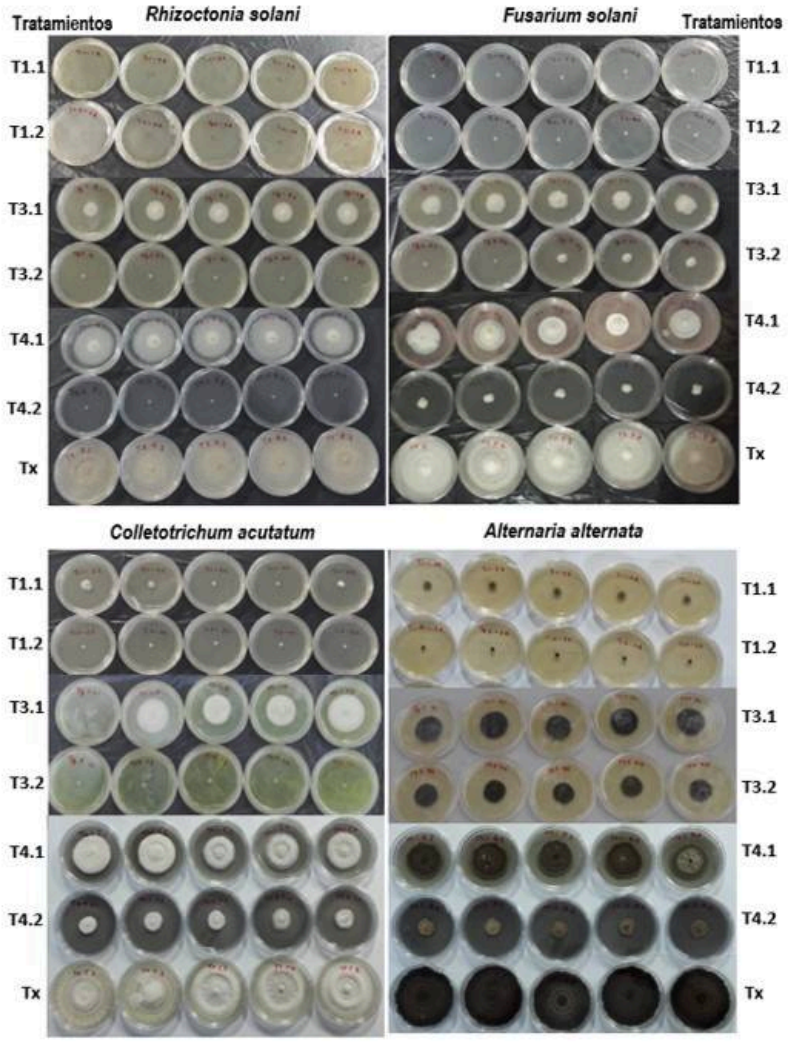
Figure 2. ***In vitro*** **inhibitory activity of** ***B. amyloliquefaciens*** **extracts with silicon oxide and graphene NPs on phytopathogenic fungi. T1.1 and T1.2= D_E 70 and D_E 90 of EcBa; T3.1 and T3.2= D_E 70 and D_E 90 of SiO_2 NPs + EcBa; T4.1 and T4.2= D_E 70 and D_E 90 of Graf NPs + EcBa and T= control.**



These results differ from those reported by Ahumada *et al*. (2019) with extracts of *B. amyloliquefaciens*, they had inhibition levels of 37.8 to 55.2% in different strains of *Fusarium*. On the other hand, Maslennikova *et al*. (2023) show the inhibition of *R. solani* by the mixture of *B. amyloliquefaciens* and *B. subtilis*; the inhibitory activity of treatment with a mixture of bacteria on the fungus was 81%.

Authors such as Es-Soufi *et al*. (2020) mention that, when evaluating the isolate against seven strains of *Colletotrichum acutatum*, they showed the ability to inhibit the mycelial growth of the pathogen between 37 and 72%. Jia *et al*. (2023) mention that with isolates of *A. alternata*, the percentage of inhibition at seven days of incubation was from 60.6 to 72.72%. In contrast, using a D_E 90 (4.46 ml L^{-1}) of SiO_2 NPs + EcBa inhibited 100, 95.06, 90.71, and 72.68% of the phytopathogens *R. solani*, *C. acutatum*, *F. solani*, and *A. alternata*. A similar effect was observed with the Graf NPs + EcBa treatment, which had 100, 91.62, 79.21, and 73.4% on *R. solani*, *F. solani*, *A. alternata*, and *C. acutatum*, under a D_E 90 of 8.59 ml L^{-1}.

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Therefore, it was shown that the doses of *B. amyloliquefaciens* extract with silicon dioxide and graphene NPs were lower compared to the bacterial extract alone, achieving a similar and efficient control. Other studies have demonstrated the antifungal activity of NPs on phytopathogenic fungi; for example, Koka *et al*. (2019) mentioned that MgO NPs at 0.5 mg ml^{-1} achieved an inhibition zone of 16.33 mm in *A. alternata* in Petri dishes of approximately 18.14%, and 14.33 mm in *R. solani* 15.92%; if these doses are compared with those used in the present research where 89.2 mg L^{-1} or 0.08 mg ml^{-1} of the SiO_2 NPs + EcBa treatment was applied, it was observed that when using very low doses of silicon dioxide, excellent percentages of mycelial inhibition were obtained.

For their part, Pariona *et al*. (2018) indicated that copper NPs (Cu-NPs) presented 87 and 90% growth inhibition of *F. solani* mycelium with doses of 0.75 and 1 mg ml^{-1}, respectively. Correa *et al*. (2018) reported 100% mycelial inhibition in the fungi: *A. alternata*, *C. gloeosporioides*, *C. fragariae*, and *Rhizopus stolonifer* using chitosan NPs and thyme essential oil (NPs-TEO-Np 3 and 5%).

In vitro assessment of *B. amyloliquefaciens* extracts with silicon oxide and graphene NPs on the production of reproductive structures of phytopathogenic fungi

The results of the production of reproductive structures of phytopathogenic fungi showed a significant reduction ($p \leq 0.05$) between treatments (Table 3). In general, the SiO_2 NPs + EcBa treatment presented the lowest values in conidia production in *F. solani*, *R. solani*, and *C. acutatum* with the three doses evaluated (D_E 30, D_E 50 and D_E 70), with an inhibition of 96.89, 84.06, 92.45 and 89.28% in *F. solani*, *R. solani*, *C. acutatum*, *A. alternata*, respectively. A similar effect was observed with Graf NPs + EcBa, with inhibition values of 82.17, 75.93, 86.79, and 96.54% in *F. solani*, *R. solani*, *C. acutatum*, *A. alternata* under the highest dose (D_E70), followed by the EcBa treatment, compared to the control, in which the fungi produced a high number of conidia and sclerotia with 0% inhibition.

Table 3. Inhibition of reproductive structures of phytopathogenic fungi with extracts of *B. amyloliquefaciens* with silicon oxide and graphene NPs.

Treatment	D_E	*F. solani*		*R. solani*		*C. acutatum*		*A. alternata*	
		Sp	In (%)	Scle	In (%)	Sp	In (%)	Sp	In (%)
SiO_2 NPs + EcBa	D_E 30	1.49^{c}	78.29^{bc}	67.33^{c}	36.87^{c}	0.42^{d}	84.9^{a}	42.66^{a}	7.83^{f}
	D_E 50	0.64^{c}	90.69^{ab}	20^{e}	81.25^{a}	0.21^{d}	92.45^{a}	13.92^{cde}	69.93^{bc}
	D_E 70	0.21^{c}	96.89^{a}	17^{e}	84.06^{a}	0.32^{d}	88.68^{a}	4.96^{f}	89.28^{a}
Graf NPs + EcBa	D_E 30	4^{b}	41.86^{d}	89^{b}	16.56^{d}	2.34^{ab}	16.98^{d}	28.16^{b}	39.17^{e}
	D_E 50	0.9^{c}	86.82^{abc}	64^{c}	40^{c}	1.54^{c}	45.28^{bc}	18.72^{c}	59.56^{d}
	D_E 70	1.22^{c}	82.17^{bc}	25.66^{de}	75.93^{ab}	0.37^{d}	86.79^{a}	1.6^{f}	96.54^{a}
EcBa	D_E 30	3.3^{b}	51.93^{d}	88.33^{b}	17.18^{d}	1.76^{bc}	37.74^{c}	12.37^{de}	73.27^{bc}
	D_E 50	1.54^{c}	77.52^{c}	60^{c}	43.75^{c}	1.17^{c}	58.49^{b}	16.85^{cd}	63.59^{cd}
	D_E 70	1.06^{c}	84.49^{abc}	33.66^{d}	68.44^{b}	0.48^{d}	83.02^{a}	10.88^{e}	76.49^{b}
Control	0	6.88^{a}	0^{e}	106.66^{a}	0^{e}	2.82^{a}	0^{e}	46.29^{a}	0^{g}

Values with different letters within each column indicate significant differences between treatments (Tukey, $p \leq 0.05$); Sp= spores x 10^{6} ml^{-1}; Scle= sclerotia produced in Petri dishes and In (%)= percentage of spore inhibition.

Various antimicrobial compounds reduce the production and germination of phytopathogenic fungi spores, which is beneficial as they limit their reproduction and dissemination. In a study conducted by Ley *et al*. (2018), they indicated 88.15% inhibition in the germination of zoospores of *Phytophthora capsici* using *B. amyloliquefaciens*, perhaps for this reason the extract used significantly decreased the production of the reproductive structures of the fungi.

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For their part, Jiao *et al*. (2021) showed, in *in vitro* studies with tobacco fungi, that the inhibition of spore germination is due to antimicrobial lipopeptides, especially bacillomycin D and fengycin. On the other hand, Yan *et al*. (2020) determined that the component with antifungal activity was the antibiotic lipopeptide iturin, which can inhibit the growth of mycelia and the germination of spores of the fungus *C. gloeosporioides*, inducing an increase in cell membrane permeability and a decrease in protein content in the fungal cell.

Conclusions

The extracts of *B. amyloliquefaciens* in combination with the NPs (SiO_2 NPs + EcBa and Graf NPs + EcBa) were effective in the *in vitro* control of the phytopathogenic fungi evaluated since a synergistic effect was observed. In general, they inhibited the growth of mycelium and reduced the production of reproductive structures (spores and sclerotia); in this case, in range of 84% to 100%. Therefore, these treatments could be considered as an alternative in the control of diseases caused by phytopathogenic fungi. But first, it is recommended to evaluate them under greenhouse and field conditions to confirm their effectiveness.

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