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Antagonist Species to *Streptomyces* sp. that Causes Common Potato Scab

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HIGHLIGHTS

- *Trichoderma* spp. and *Bacillus subtilis* reduced colonial growth of *Streptomyces* sp.
- *Trichoderma* spp. and *Bacillus subtilis* reduced scab damage in potato tuber.

Abstract: Potato scab is one of the main diseases affecting potato tubers during their development, causing great commercial damage to farmers. The use of some antagonistic microorganisms has been shown as a viable alternative to phytosanitary control of several crops. However, this work aimed to verify *in vitro* effects of microorganisms antagonistic against phytopathogenic *Streptomyces* sp. and *in vivo* treatment of scab in potato tubers. *Trichoderma longibrachiatum*; *Trichoderma harzianum*; *Pochonia clamydosporia*; *Bacillus subtilis* (except for *in vivo* test); *Bacillus subtilis* + *Trichoderma longibrachiatum,* and *Bacillus subtilis* + *Enterococcus faecium* were tested for *in vitro* growth inhibition of *Streptomyces* via microorganism radial pairing test, with both volatile and non-volatile metabolites. In parallel, the antagonistic microorganisms were tested *in vivo* in protected environments by cultivating potato plants in pots with soil contaminated with *Streptomyces* sp., then evaluating the number of injured tubers, injured area, severity index, production losses and the frequency of tubers by lesion type. The results indicate major *in vitro* inhibition of *Streptomyces* sp. by *Trichoderma longibrachiatum*, *Trichoderma harzianum,* and *Bacillus subtilis* + *Enterococcus faecium* under the antagonism (48 – 80%) and antibiosis tests, as well as a major reduction of tuber lesions size (34 – 60%), severity index, and production losses in the *in vivo* test.

Keywords: *Solanum tuberosum*; *Trichoderma* spp.; *Bacillus subtilis*; Biological control.

INTRODUCTION

Common potato scab caused by phytopathogenic bacterial species of the genus *Streptomyces* is one of the main problems faced by potato producers in the world [1]. Phytopathogenic strains of scab produce the phytotoxin thaxtomin A that induces symptoms of necrotic lesions in potato tubers when their production is exposed to a site with large bacterial populations [2]. Although the disease does not drastically affect yield, it makes the tubers non-tradable for retail and of less value for processing (chips and fries).

Some factors promote the incidence of the common scab, such as the predominance of more aggressive *Streptomyces* species, use of susceptible potato varieties, dissemination by contaminated potato seed, continuous planting in infested soils, soil pH, soil compaction, and soil microorganism alteration due to the indiscriminate use of pesticides that therefore makes their control difficult for the producer [3].

However, different management strategies can be adopted to reduce scab incidence and severity, including biological control. Biological control consists of reducing the phytopathogenic organism inoculum or biological activity by the natural or introduced presence of a competitor or inhibitor [4].

The advantages of using antagonistic biological agents are reducing chemical pesticide use, which contributes to improving production area sustainability; reducing production costs [5]; while it produces and exudes some compounds that promote plant growth.

Many antagonists are used; however, *Bacillus* spp. and *Trichoderma* spp. are the most studied and used in Brazil for having multiple biocontrol mechanisms, giving them the potential to overcome the phytopathogen defenses.

Bacillus spp. has been shown to have an antagonistic activity due to the production of bactericidal compounds and the ability to colonize and induce systemic resistance in the plant [6]. Some studies have pointed to the effect of *Bacillus subtilis* on the control of pathogens of different crops: garlic [7], tomato [8], even control of potato common scab [9, 10, 6], ratifying its effectiveness in plant diseases control.

Trichoderma species reported as a biocontrol agent are the most used fungi for this purpose and are present in many types of environments. These fungi can grow rapidly, especially on soil organic matter, conferring them high competitiveness by reducing the space of development for phytopathogens, in addition to the capacity to produce antibiotics like chitinases, proteases, cellulases, etc. [11]. A record of *Trichoderma harzianum* was found controlling the common scab, showing the potential influence on the disease [10].

Some studies have indicated positive results of controlling common scabs with other non-phytopathogenic species of *Streptomyces* [12, 13], demonstrating that biological control may be a promising pathway for managing this disease. Thus, the present study aimed to verify the *in vitro* effects of antagonistic microorganisms against phytopathogenic *Streptomyces* sp. and *in vivo* effects of the common scab damage on potato tubers.

MATERIAL AND METHODS

In vitro and *in vivo* antagonism tests were performed from June to December 2017. Tubers with the disease characteristic lesions were collected in a commercial potato production area located in Mucugê down, Bahia State, Brazil (lat -13° 02 '7,92" and long -41° 27 '36.91") for isolation, identification of bacteria, and subsequent use of *in vitro* antagonism assay.

The isolation process followed Loria and coauthors [14]. The tubers were cleaned and sanitized with running water and neutral detergent. Shortly after, small samples were taken among tuber healthy tissue and injured tissue; and heated in a water bath at 55 °C for 30 minutes. After this, the samples were macerated on glass slides and received 2 drops of sterile distilled water. Then the liquid was inoculated in Petri dishes with a basic medium (water and agar) to pH 10.

The Petri dishes with samples were incubated in biochemical oxygen demand chamber (BOD) for 7 days at a temperature of 28 °C, where after the growth period, different colonies with morphological characteristics similar to the *Streptomyces scabies* species were selected from the Petri dishes [15]. The colonies were placed in bacterial suspensions with sterile distilled water, which were subsequently inoculated into Petri dishes containing YME (Yeast Malt Extract) medium for bacterial growth under incubation in BOD for 7 days and 28 °C.

After the growth period, a pathogenicity test was carried out using small potato tuber disks, where the bacterial colony was inoculated on a potato tuber sample. The potato samples were incubated for 120 hours before evaluating the bacterial pathogenicity [16].

We tried to identify the isolated bacterium, by morphological and biochemical characterization. The morphological characterization was made via evaluation of the hyphae micromorphology by optical microscopy, colony coloring, spore coloring, and pigment production [14].

The biochemical characterization was evaluated using different carbohydrates according to Shirling and Gottlieb [17], using varied growth mediums with the following sources of sugars: D-glucose (positive control), D-mannitol, D-raffinose, L-arabinose, D-fructose, L-rhamnose, Myo-inositol, D-xylose, Sucrose, and the basic medium - without sugar (negative control).

In vitro antagonism evaluation was performed through radial pairing test between the phytopathogenic bacteria and the antagonistic microorganisms as described by Astorga-Quirós and coauthors [7], using 5 replicates in a completely randomized design and replicated to prove the accuracy of the results. *Trichoderma longibrachiatum* (TL) strain FL1 from TriconemateMax®; *Trichoderma asperellum* (TA) strain FA1 from TricobiolMax®; *Pochonia chlamydosporia* (PC) isolate; *Bacillus subtilis* (BS) from Serenade® (positive control); *Bacillus subtilis* + *Bacillus lincheniformis* + *Trichoderma longibrachiatum* (BSBLTL) from Nem out®; *Bacillus subtilis* + *Enterococcus faecium* + *Lactobacillus plantarum* (BSEFLP) from Compost aid®, were used in the antagonism test. All treatments were obtained from commercial products, except PC.

To prepare the treatment disks for *in vitro* tests, commercial wettable powder-based products (BS, BSBLTL and BSEFLP) were dissolved in water to generate a microbial suspension with a concentration of 1g L^{-1} of the products. Then two suspension drops were placed in Petri dishes containing PDA (Potato Dextrose Agar) medium, spread on the surface of the medium with the Drigalski loop and incubated in BOD for microbial colonies growth for 5 days. In commercial products with microorganisms inoculated in rice as substrate (TL, TA and PC), 1 grain of rice was used, which was inoculated on a Petri dish containing PDA medium, then placed in BOD for colony growth.

Phytopathogenic bacteria (10 days old) were seeded on the whole surface of a Petri dish containing the YME medium, then a disk of growth medium containing the antagonist (6 days old) was inserted in the center of the Petri dish. The Petri dishes were placed in BOD at 28 °C for 96 h; and every 24 h, growth measurements of the colony of the antagonistic microorganism were made. After 96 h, it was obtained the relation between the area of growth or inhibition of the antagonist and the phytopathogen colony area. And then, the percent inhibition of phytopathogenic bacterial growth was calculated.

Antagonism also was measured by antibiosis testing for volatile and non-volatile metabolites for each treatment, using the same design and replicate from pairing test. For antibiosis of volatile metabolites, phytopathogenic bacteria disks were inoculated into dishes with YME medium and disks with the antagonistic microorganisms on dishes with PDA (Potato Dextrose Agar) medium. Both dish bases were placed one on top of the other, sealed with parafilm, and incubated in the BOD at 28 °C for 7 days, after which colony growth diameter was measured [18].

Antibiosis test by non-volatile metabolites followed the methods described by Isaias and coauthors [19], where phytopathogenic bacteria were grown in a YME medium containing the secondary metabolites produced by antagonists. Metabolites were extracted from a suspension of antagonistic microorganisms made with liquid medium PD (Potato Dextrose), a disk was inserted in the growth medium and incubated at 28 °C for 48 h. After 48 h, the suspension was filtered with a millipore filter, 1 mL was removed from filtrate and added in 99 mL of molten YME medium. After 7 days of incubation, the growth diameter of phytopathogenic bacteria was measured.

With the results from bacterial colony growth with volatile and non-volatile metabolites, the percentage of radial growth inhibition - PICR was calculated, using the following equation (1) [20]:

$$PICR= ((R1-R2) /R1)*100 \quad (1)$$

Where: R1 = bigger radius (growth of the control colony – without antagonist), and R2= smaller radius (colony growth under antagonist influence).

In vivo antagonism test was conducted in a protected environment (greenhouse of screen without temperature control) where potato plants were grown in 20 dm^3 pots with contaminated soil collected in a commercial production area with a natural incidence of *Streptomyces* sp. and history of production losses in previous crops. The experiment was performed in a completely randomized design with 5 replicates.

The antagonism of TL (2 x 10^8 CFU g^{-1}), TA (2 x 10^8 CFU g^{-1}), PC (2 x 10^8 CFU g^{-1}), BSBLTL (3.75 x 10^8 CFU g^{-1}), and BSEFLP (3 x 10^8 CFU g^{-1}) to *Streptomyces* sp. in potato tubers during their production were tested. Two controls served for evaluation: sterilized soil - SS (positive control) and soil without treatment (negative control).

The pots were pre-fertilized with 30 g of organic-mineral formulation 06-30-00, 5 g of FTE (formulated micronutrients), and later, during the plant development, 10 g of KCl was divided into 3 applications. Then were planted seed potatoes type II, generation 2 of Ágata cultivar. 0.66 g of the product containing the antagonistic microorganism in 1 L of water were applied directly into the soil weekly, beginning at the planting period.

At the end of 86 days after planting (DAP), the shoot was removed from the plants for tuber maturation. And after 10 days, the following parameters were collected and evaluated: number of lesioned tubers; lesion area, using the James scale for lesion [21]; severity index, calculated by the method of Granja and coauthors [22] modified, using the James scale [21] to evaluate and apply the grades; percentage of losses, obtained by the weight of lesioned tubers in relation to the total weight of plant tubers; and frequency of tuber by lesion type.

All data were recorded in tables and analyzed for variance, and their means were tested by LSD test ($p < 0.05$) via the software Sisvar® 5.6 [23].

RESULTS

Streptomyces characterization

The bacterial isolates were collected in potato tuber lesions from a commercial area and after characterized. Figure 1a shows the bacterial colony after 7 days of incubation and growth.

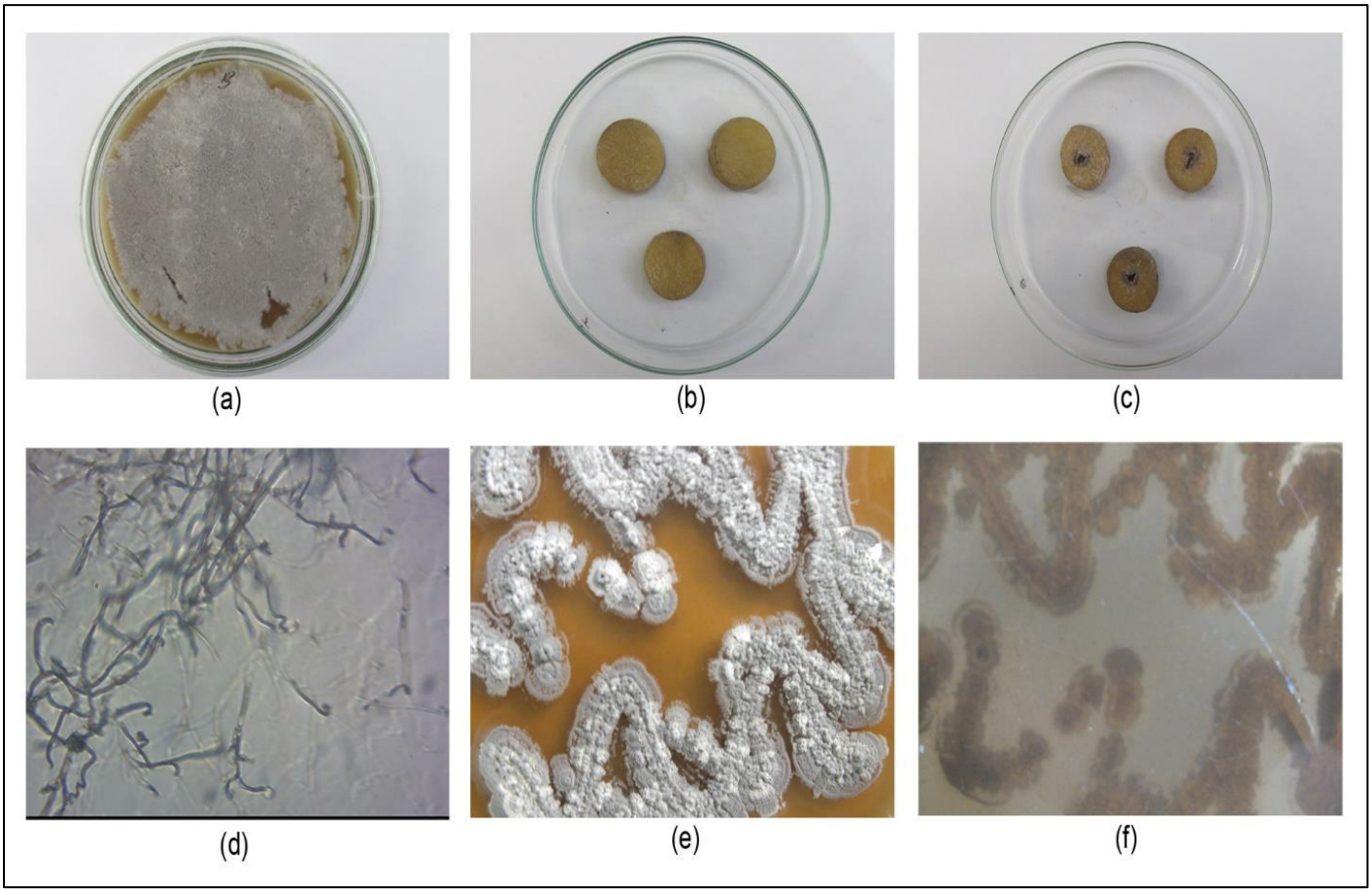

Figure 1. Isolation and morphological characterization of *Streptomyces* sp. (a) Bacterial colony after isolation after 7 days of growth; (b) Pathogenicity test control treatment; (c) Pathogenicity test on lesioned tubers disks after 5 days of incubation; (d) Micromorphology of hyphae – 100x enlarged optical; (e) Coloring of spores after 7 days of growth; and (f) Coloring of colony and pigment.

The pathogenicity of the bacterial isolate was evidenced by absence of lesions in tuber disks without bacterial inoculation (Figure 1b) related to presence of lesions in the disks contaminated with the bacterial isolate (Figure 1c). The lesions had a necrotic area of 6.28 mm^2 and depth of 1.2 mm, with a 100% incidence of disks inoculated with the bacteria; therefore, the species shows high virulence.

Figure 1d shows bacterial cells forming filamentous clusters similar to fungal hyphae; bacteria with these characteristics are microorganisms belonging to the genus *Streptomyces* [14].

The bacteria produced white and gray spores (Figure 1e), colony coloring beige, and produced brown pigments (Figure 1f). The biochemical test (Table 1) indicates that the species uses many carbon sources for food, except the carbohydrate mannitol.

Table 1. Biochemical test of *Streptomyces* isolated from potato tubers lesions.

BM[1]	Gluc	Arab	Fruct	Inos	Man	Raf	Ram	Suc	Xyl
-	+	+	+	+	-	+	+	+	+

[1] BM = Basic medium (negative control); Gluc = Glucose (positive control); Arab = L-arabinose; Fruct = D-Fructose; Inos = Myoinositol; Man = D-Mannitol; Raf = D-Rafinose; Ram = L-Ramnose; Suc = Sucrose; Xyl = D-Xylose; (+), positive use; (+/-), questionable use; (-), negative use.

Comparing the results of this paper with those found by Corrêa [24] about *Streptomyces scabiei*, concerning morphological characteristics, the colony and spore colors have been similar. But concerning the *Streptomyces* isolated biochemical characteristics, we observed a divergence on mannitol biochemical metabolism since *Streptomyces scabiei* metabolizes all types of carbohydrates tested in this work. A molecular characterization of *Streptomyces* was not performed, and we observed a divergence for *Streptomyces scabiei* in the biochemical test. However, after the morphological characteristics and the pathogenicity test, we found that this divergence is an unknown phytopathogenic *Streptomyces*, Therefore, the isolate used in this work will be considered an unknown *Streptomyces* phytopathogenic species. The lack of knowledge about this isolate can be supported by the survey carried out in the thesis of Corrêa [24], that found a new *Streptomyces* genetic group occurring in the Chapada Diamantina Bahia Region, the same place from where the contaminated tubers were collected, which corroborates how to refer the species.

In vitro antagonism test

See below the growth result of the antagonistic microorganisms on the antagonism test performed on phytopathogenic *Streptomyces* sp., causing inhibition of the bacterial population (Figure 2).

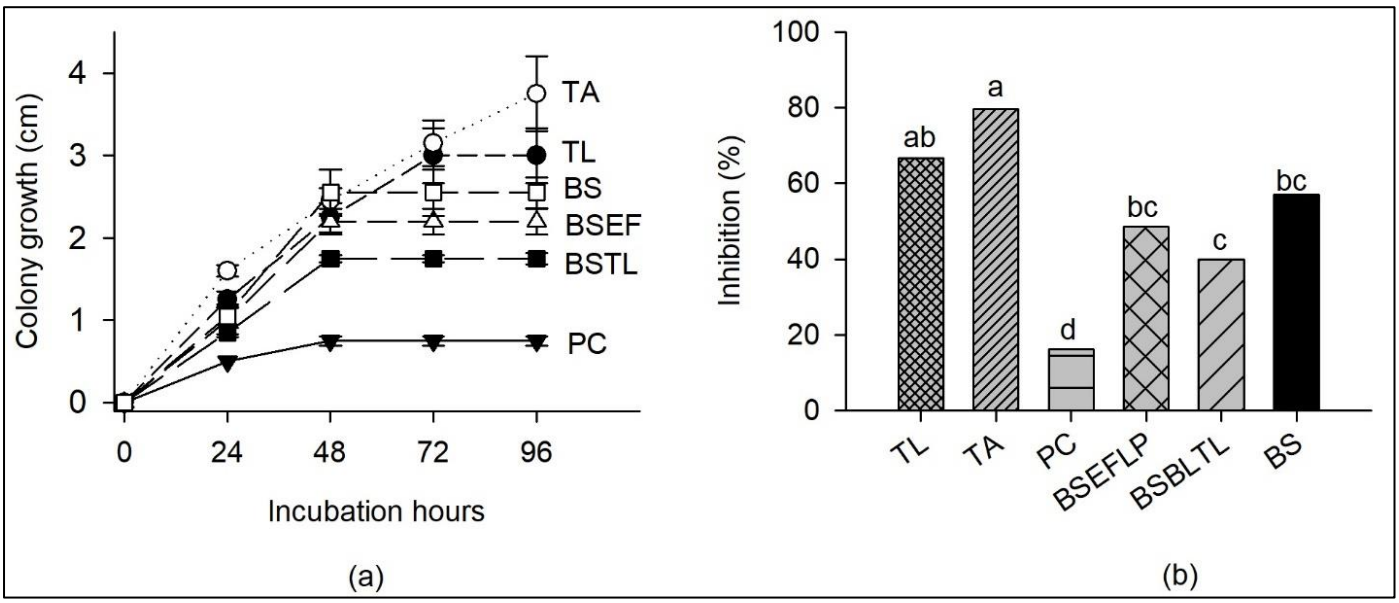

Figure 2. Antagonism test of microorganisms against *Streptomyces* sp. (a) Progression of antagonist colony growth during the incubation period. (b) The inhibitory surface of *Streptomyces* sp. colony growth. TL = *Trichoderma longibrachiatum*, TA = *Trichoderma asperellum*, PC = *Pochonia chlamydosporia*, BS = *Bacillus subtilis*, BSEFLP = *Bacillus subtilis* + *Enterococcus faecium* + *Lactobacillus plantarum*, BSBLTL = *Bacillus subtilis* + *Bacillus licheniformis* + *Trichoderma longibrachiatum*. Mean values followed by the same letters are not significantly different on the LSD test ($p < 0.05$).

All antagonist species tested in this work influenced the growth of the *Streptomyces* colony; however, *Trichoderma* appeared to be more aggressive in the phytopathogens growth inhibition when compared to other tested microorganisms (Figure 2a and 2b). TA showed growth potential until the last day of evaluation (96 hours), with radial growth of 3.75 cm (Figure 2a).

On the other hand, differently from what happened with TA, TL ceased growing at 72 hours after inoculation, with 3 cm of radial growth. As the fungal species growth was noted, we observed the *Streptomyces* colony reaction, beginning to rapidly sporulate, forming a white mass on the colony (Figure 3). After sporulation (formation of the white mass), the microorganisms had no more effect on the bacterium.

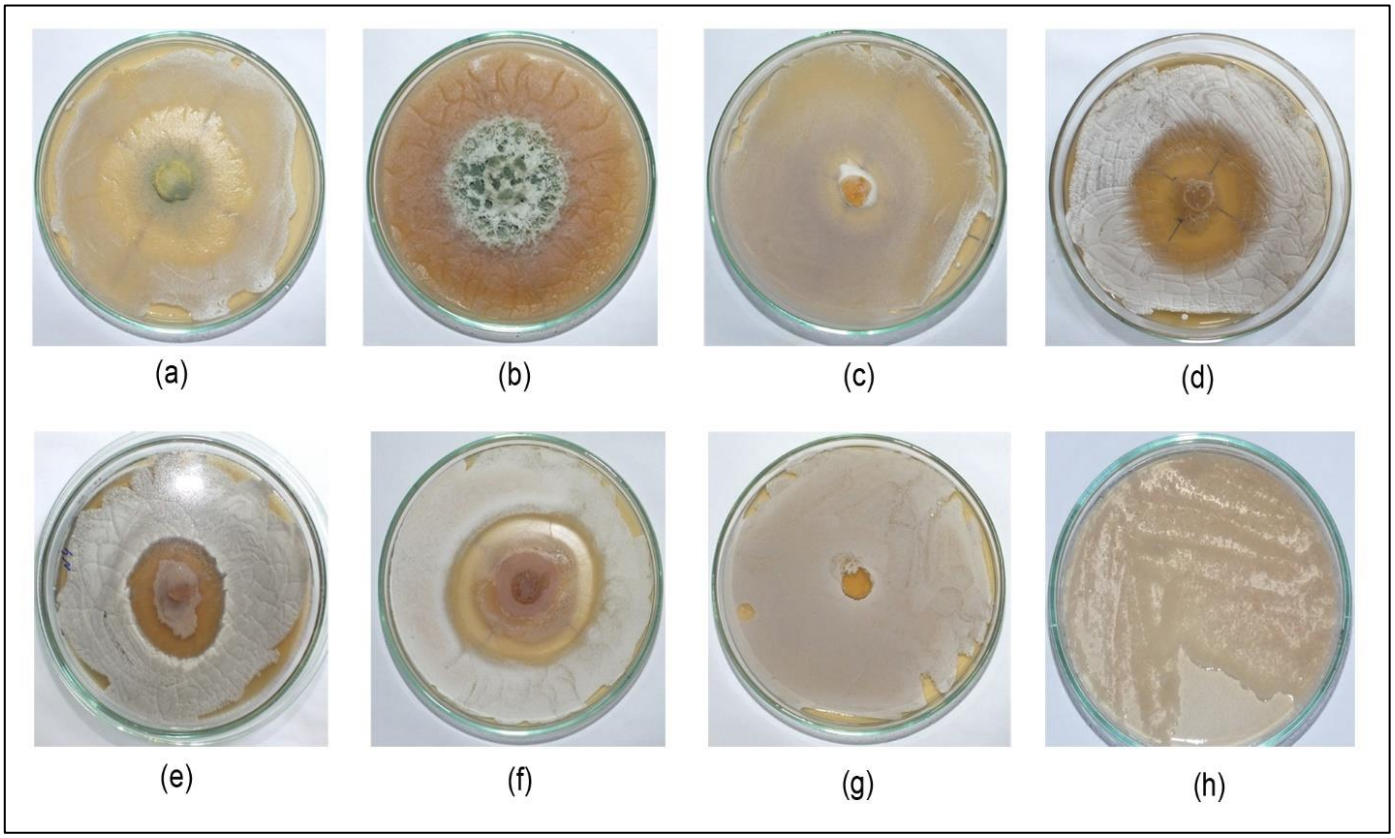

Figure 3. Antagonism test after 96 hours of incubation.; (a) TL on *Streptomyces* sp.; (b) TA on *Streptomyces* sp.; (c) PC on *Streptomyces* sp.; (d) BS on *Streptomyces* sp.; (e) BSBLTL on *Streptomyces* sp.; (f) BSEFLP on *Streptomyces* sp.; and (g) control - without the antagonist, (h) Non-sporulated Streptomyces colonies. TL = *Trichoderma longibrachiatum*, TA = *Trichoderma asperellum*, PC = *Pochonia chlamydosporia*, BS = *Bacillus subtilis*, BSEFLP = *Bacillus subtilis* + *Enterococcus faecium* + *Lactobacillus plantarum*, BSBLTL = *Bacillus subtilis* + *Bacillus licheniformis* + *Trichoderma longibrachiatum.*

Among the antagonistic bacteria, BS stood out to other microorganisms, being close to the control exerted by the *Trichoderma* species, but its growth ceased in 48 hours. We observed the same result in the other treatments that contained bacteria (Figure 2a and 2b).

Regarding the inhibition of *Streptomyces* sp. by antagonistic microorganisms in the *in vitro* test (Figure 2b), the best results were observed in TA and TL, with 79.62 and 66.67% inhibition of *Streptomyces* sp., respectively. Among the treatments tested, BS had intermediate inhibitory performance, obtaining a value of inhibition of 57% in the growth of *Streptomyces* sp. colony.

TA, TL, and BS in the antibiosis test by volatile metabolites (Figure 4a) obtained good performance inhibiting 34, 34, and 39% of the population growth of *Streptomyces* sp., respectively. In parallel, the treatment with the lowest result using this antagonism strategy was the BSEFLP, which inhibited 18% of bacterial growth, that is, about 50% inhibition less than the microorganisms with superior results.

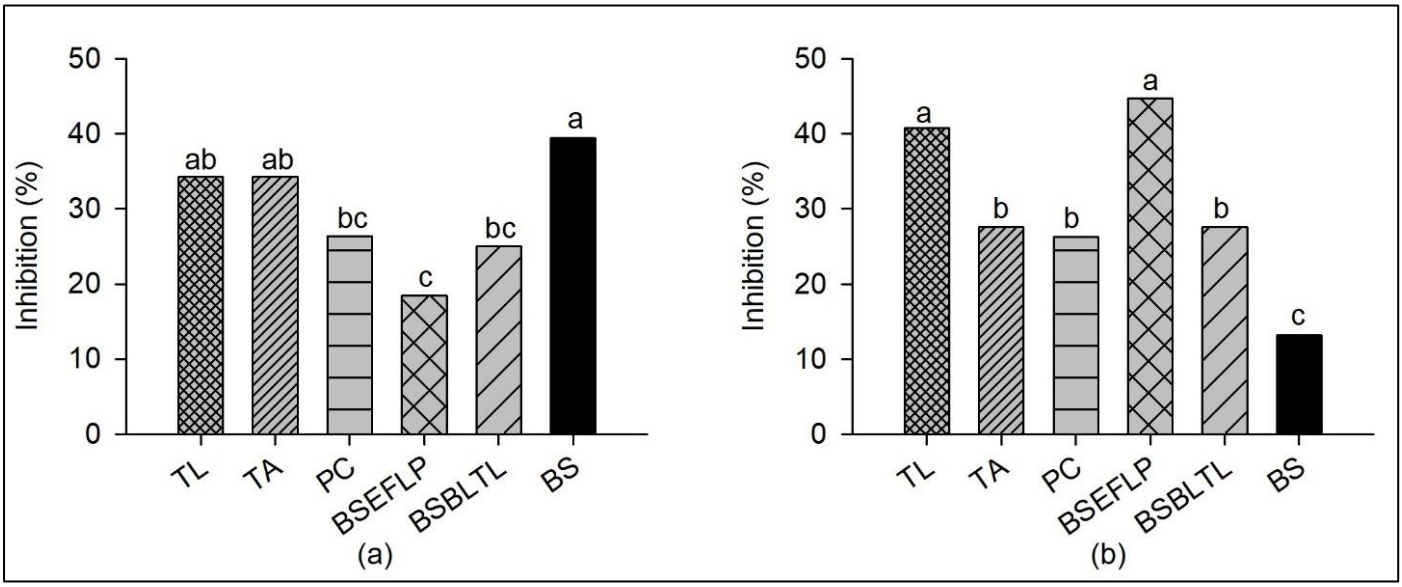

Figure 4. Antibiosis test on *Streptomyces* sp. (a) Volatile metabolites (b) Non-volatile metabolites. TL = *Trichoderma longibrachiatum*, TA = *Trichoderma asperellum*, PC = *Pochonia chlamydosporia*, BS = *Bacillus subtilis*, BSEFLP = *Bacillus subtilis* + *Enterococcus faecium* + *Lactobacillus plantarum*, BSBLTL = *Bacillus subtilis* + *Bacillus licheniformis* + *Trichoderma longibrachiatum*. Mean values followed by the same letters are not significantly different on the LSD test ($p < 0.05$).

In the non-volatile metabolites antibiosis (Figure 4b), BSEFLP obtained one of the best results among the other treatments (44.73% of inhibition), whereas BS showed the lowest result (13.15% of inhibition). The relationship of these two treatments to the type of antibiosis was inversely proportional, indicating that when dealing with the exudation of antimicrobial compounds, BS may preferentially use gaseous compounds to inhibit the growth of the *Streptomyces* sp. population, while BSEFLP may use preferably non-gaseous compounds (liquid, plasma, etc.) to control *Streptomyces* sp.

In the antibiosis test by non-volatile metabolites, TL obtained an inhibition value of 40.79%, similar to BSEFLP; however, TA had a low performance (27.63% of inhibition). According to high TA result in the antagonism test and antibiosis by volatile metabolites and its low result in the antibiosis test by non-volatile metabolites, it can be suggested that besides antibiosis, another strategy of antagonism may have a strong influence on the inhibition of *Streptomyces* sp.

In vivo antagonism test

For evaluating of *in vivo* antagonism test in potato tubers, Figure 5a shows the number of injured tubers by *Streptomyces* sp. as a function of the antagonistic microorganisms tested. We observed that the *Trichoderma* species and BSEFLP had the best results, with lesions occurring in only 1, 2, and 1 tuber for the treatments TL, TA, and BSEFLP, respectively.

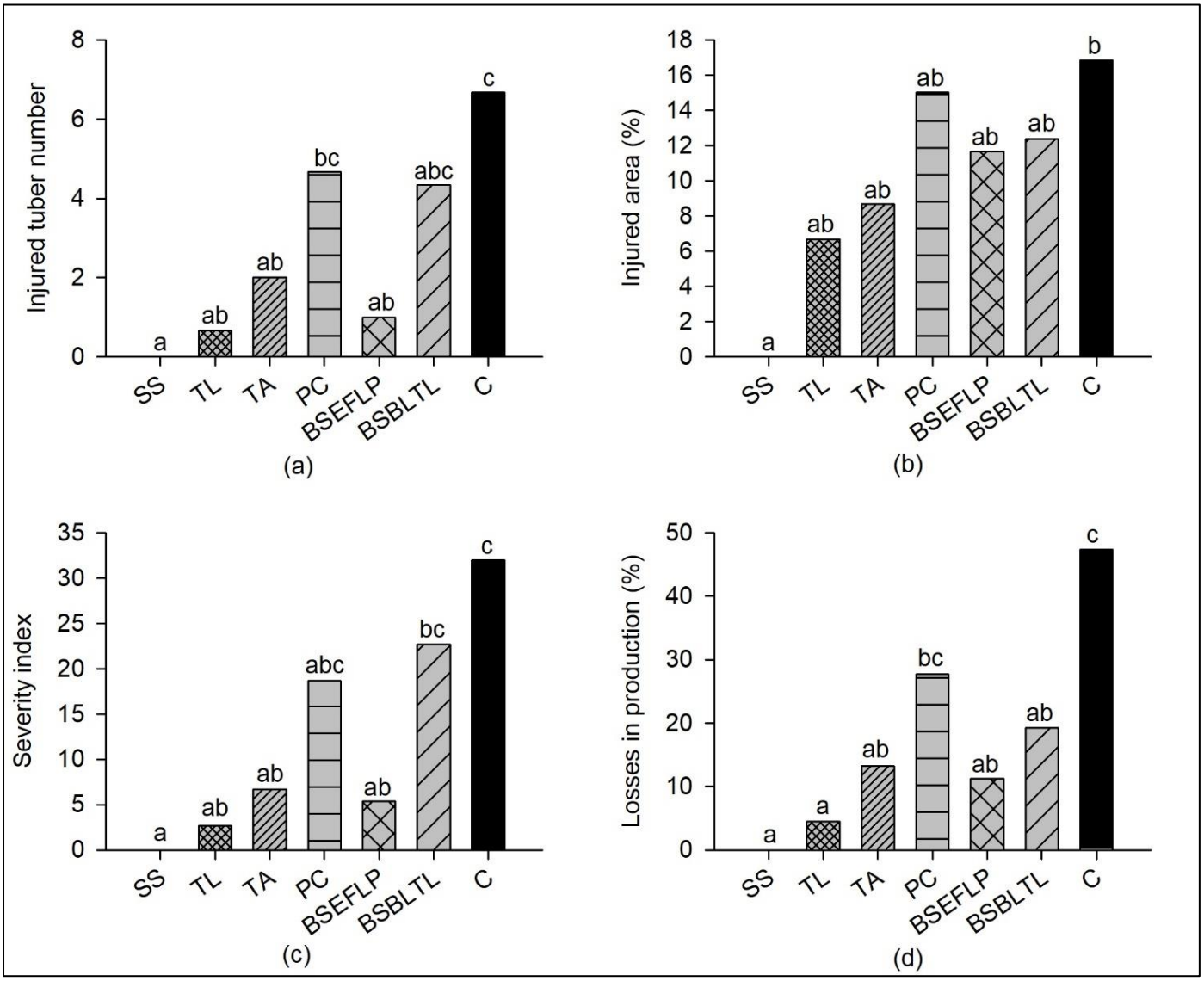

Figure 5. Antagonism *in vivo* of microorganisms on *Streptomyces*. (a) Injured tubers number of lesioned tubers, (b) Injured area on the tuber, (c) Scab severity index on potato tubers, and (d) Losses of tubers by scab. SS = Sterilized soil (Positive control), TL = *Trichoderma longibrachiatum*, TA = *Trichoderma asperellum*, PC = *Pochonia chlamydosporia*, BSEFLP = *Bacillus subtilis* + *Enterococcus faecium* + *Lactobacillus plantarum*, BSBLTL = *Bacillus subtilis* + *Bacillus licheniformis* + *Trichoderma longibrachiatum*, and C = Control (Negative control). Mean values followed by the same letters are not significantly different on the LSD test ($p < 0.05$).

The severity index (Figure 5a) as a consequence of the size of lesions and their frequency due to the attack of phytopathogens, points to results similar to the number of lesioned tubers and the lesioned tuber surface, where the lowest severity of the disease was observed in tubers of plants treated with TL, TA, and BSEFLP, with mean severity of 4.88, that is, 85% less than the severity index of negative control treatment.

The treatment with lower losses was the TL (Figure 5d), which obtained only 4.47% loss of production due to scabs. This result represents significative loss reduction (90%) compared to the control treatment.

Table 2 shows the frequency of scab lesions in tubers. This evaluation is relevant because it considers the different types of lesions (superficial, eruptive, or deep level) since tubers with superficial lesions may still be marketable depending on the place of sale or low supply of potatoes on the market.

Table 2. Cumulative frequency of scabs lesion by type in potato tubers

Treatment	Type 1[1]	Type 2	Type 3	Type 4	Type 5
Control treatment	8	3	0	0	10
TL	2	0	0	0	0
TA	4	2	0	0	0
PC	4	3	1	0	6
BSBLTL	5	6	0	0	2
BSEFLP	2	1	0	0	0
Sterilized soil	0	0	0	0	0

[1]Type 1 = Superficial lesions <10 mm in diameter; Type 2 = superficial lesions > 10 mm in diameter; Type 3 = elevated lesions <10 mm in diameter; Type 4 = elevated lesions > 10 mm in diameter, and Type 5 = deep lesions. TL = *Trichoderma longibrachiatum*, TA = *Trichoderma asperellum*, PC = *Pochonia chlamydosporia*, BSEFLP = *Bacillus subtilis* + *Enterococcus faecium* + *Lactobacillus plantarum* and BSBLTL = *Bacillus subtilis* + *Bacillus licheniformis* + *Trichoderma longibrachiatum*.

Therefore, we can see in Table 2 that the best treatments consisted of TL, TA, and BSEFLP, since they had their contaminated tubers mostly with type 1 and 2 lesions, which are superficial, especially TL, which only presented lesioned tubers with type-1 lesions.

In all parameters evaluated *in vitro* and *in vivo*, PC was shown to be less effective treatment, probably because this microorganism is more related to control of phytonematodes, suggesting that it has no significant effects on the control of *Streptomyces* sp.

DISCUSSION

To control bacterial development and damage caused by *Streptomyces* sp. in potato tubers, we tested different antagonistic microorganisms already reported by literature to control other phytopathogenic agents [7, 19, 25, 26].

Bacillus spp. has been recently documented in literature controlling phytopathogenic *Streptomyces* [9, 27, 28, 6]; however, the results found here also show the influence that *Trichoderma* can perform on the population growth of phytopathogenic bacteria, where TA and TL had inhibition of 28 and 14.46%, respectively, higher than BS in pairing test.

According to Yendyo and coauthors [25], in their research about *in vitro* antagonism of different *Trichoderma* species, including *Trichoderma harzianum*, they observed an antagonistic effect of fungal species against *Ralstonia solanacearum* in tomatoes. Astorga-Quirós and coauthors [7] also verified the effect of *Trichoderma* sp. on the inhibition of growth of *Pseudomonas marginalis* isolated from garlic. These results reinforce that the inhibitory effect of *Trichoderma* can not only reduce the growth of phytopathogenic fungi but also of bacteria, as verified in this work.

The strategies used by antagonistic microorganisms to inhibit other biological agents are, among others, the release of volatile compounds or diffuse chemical compounds in solid or liquid media. In this work, the verified inhibition of secondary metabolites by BS, TL, TA, and BSEFLP demonstrated that each microorganism can use different antagonist strategies to control *Streptomyces* sp.

The expressiveness of BS volatile metabolites to control *Streptomyces* sp. (Figure 4a) evidences an important tool used by microorganisms checking out competitive advantages to other biological agents present in the environment. Gao and coauthors [29] demonstrated that volatile compounds produced by *Bacillus subtilis* controlled the mycelial growth of *Botrytis cinerea*, *Colletotrichum gloeosporioides*, *Penicillium expansum*, *Monilinia fructicola*, and *Alternaria alternata*, with an average inhibition rate of 59.97%.

Caulier and coauthors [30] listed at least 14 groups of volatile compounds with antimicrobial action produced by *Bacillus subtilis*, being inorganic (NO, NH_3, HCN, and H_2S), as a product of their action with the medium (solubilization and metabolism), or organic (nitrogenous organic compounds; sulfur and metals; terpenoids; alcohols, ketones, alkanes, aldehydes, alkenes, and acids), from the bacteria metabolism, altering the performance of several other microorganisms that cohabit in the same environment [31].

TA and TL showed a level of control of *Streptomyces* sp. by volatile metabolites close to the BS, demonstrating that it can also use this antibiosis strategy to compete with other microorganisms in the environment. In an antagonism test for volatile metabolites, *Trichoderma* spp. obtained 30% reductions in the mycelial growth of *Cladosporium* spp. [32]. In addition, control of 25% of the mycelial growth of *Fusarium Oxysporum* (NRRL38499) by *Trichoderma harzianum* was verified in volatile metabolite antibiosis test and associated this control to at least 15 volatile compounds emitted by *Trichoderma* [33].

Regarding antibiosis by non-volatile metabolites (Figure 4b), the genus *Bacillus* may also present relevant microbial control through its non-volatile compounds. BSEFLP obtained among the tested bacteria groups the best result for controlling *Streptomyces* sp. Lin and coauthors [27] have already observed the

control of *Bacillus amyloliquefaciens* for *Streptomyces scabiei* from surfactin, iturin A and fergicin compounds produced by the beneficial microorganism in a diffuse medium.

In addition to *Bacillus subtilis*, BSEFLP contains *Enterococcus faecium* and *Lactobacillus plantarum*, both are gram-positive bacterium that produces lactic acid, which in turn is present in the intestinal tract of animals and dairy foods. The potential use of *Enterococcus faecium* antagonism is more related to microorganisms that break down foods, mainly of dairy origin, but can also occur in foods of plant origin [26]. Therefore, *Enterococcus faecium* may also have contributed to higher responses between bacterial treatments in the pairing test and antibiosis by non-volatile metabolites.

However, for antibiosis by non-volatile metabolites, TL obtained the best result among the two beneficial fungi tested. The efficiency of *Trichoderma* as an antagonist of phytopathogenic agents has been reported in the work of Isaias and coauthors [19], who demonstrated that *Trichoderma harzianum* (CEN 725) and *Trichoderma koningiopsis* (CEN 768) was able to inhibit the development of *Sclerotium rolfsii* and *Verticillium dahliae* by non-volatile metabolites released in growth medium. However, less inhibition was observed in *Trichoderma harzianum* metabolites when compared to *Trichoderma koningiopsis* in the *Verticillium dahliae* control, ratifying the antibiosis response dependence on several factors, including the species to be controlled, because antibiosis depends on the ability of the antagonist to circumvent the defenses of phytopathogens.

The main secondary non-volatile compounds associated with antibiosis produced by *Trichoderma* are epipolythiodioxopiperazines, peptaibols, butenolides, pyridones, azaphilones, koninginins, steroids, lactones, trichothecenes, and anthraquinones [34].

According to Caulier and coauthors [30], antagonism can be mediated by several compounds of microbial origin (bacteriocins, enzymes, toxic substances, volatile metabolites, etc.). Therefore, other studies may identify and characterize these compounds released by antagonistic microorganisms since this result seems to be promising for greenhouse and field tests or even the development of new antibiotic products.

The results of the *in vivo* antagonism (Figure 5) reinforce the evidence of control over *Streptomyces* sp. by the microorganisms used in this work. The number of contaminated tubers and the injured area decreased with the application of TL, TA, and BSEFLP (Figures 5a and 5b), indicating its antagonistic effect on the pathogen. Han and coauthors [9], working with *Bacillus subtilis* to control *Streptomyces scabiei* in potato tubers, found reductions in the area of the infection caused by the pathogen.

Trichoderma species have also been shown to be effective in reducing infections caused by phytogenic bacterial agents. Chien and Huang [35] showed a significant reduction of the spots caused by *Xanthomonas perflorans* in tomato leaves after applying *Trichoderma asperellum* to the plants.

The results of tuber severity and production losses (Figures 5c and 5d) reflect the control performed by *Trichoderma* and *Bacillus subtilis* that reduced the damage caused by *Streptomyces* to tubers (Figure 6). In the literature, many studies point out the reduction of the severity of phytopathogenic diseases by *Trichoderma* and *Bacillus subtilis* [9, 25, 36] on a broad phytopathogen and crops spectrum, including potato scab, on which Wang and coauthors [10] recorded a reduction of approximately 70% in disease severity with 300 kg of a product containing *Bacillus subtillis* and *Trichoderma harzianum* in the first year of cultivation and approximately 55% in the second year.

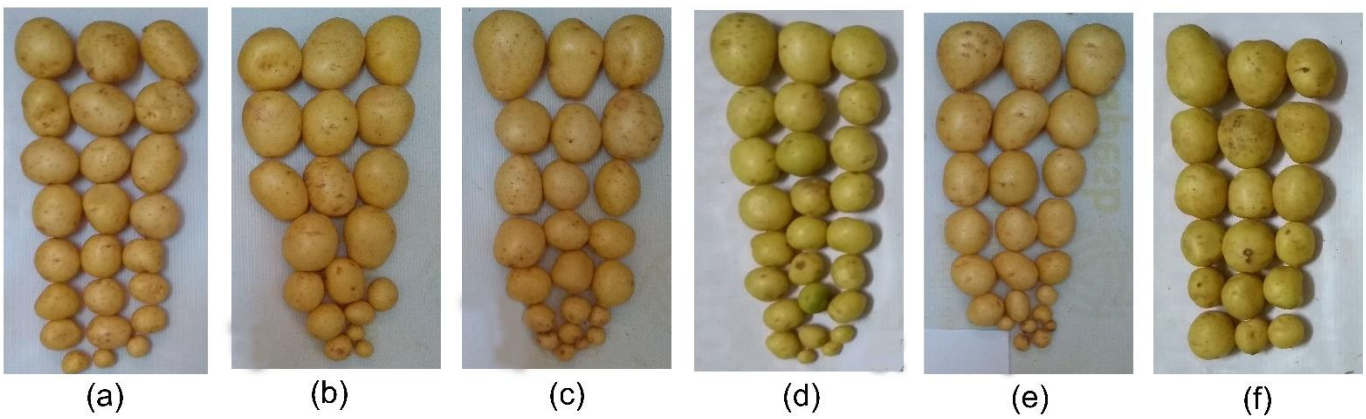

Figure 6. Damage of common scab in potato tuber cultivated with different antagonist microorganism. (a) TL = *Trichoderma longibrachiatum*, (b) TA = *Trichoderma asperellum*, (c) PC = *Pochonia chlamydosporia*, (d) BSEFLP = *Bacillus subtilis* + *Enterococcus faecium* + *Lactobacillus plantarum*, (e) BSBLTL = *Bacillus subtilis* + *Bacillus licheniformis* + *Trichoderma longibrachiatum* and (f) Control.

Bacillus subtilis acts on the soil forming a bacterial biofilm involving the entire root of the crop of economic interest, inhibiting the contact of phytopathogenic microorganisms with the root, or it may also induce plant resistance to the phytopathogen [8]. Therefore, these effects can be propagated in plants and potato tubers.

Trichoderma spp. has high interactivity with different plant parts, mainly roots. These fungi are fast-growing, so they can rapidly colonize the root region and inhibit phytopathogenic competition for space and nutrients, even via parasitism, reducing the damage caused by plant pathological agents or antibiosis activity [34].

The results presented here show the efficacy of the biological control of *Streptomyces* sp. using *Trichoderma* and *Bacillus* species. However, there are still needed in-field studies testing the efficiency of these biocontrol agents against *Streptomyces* sp., to confirm the results obtained under controlled conditions, as well as to test other variables such as antagonist strains, phytopathogenic strains, plant genetic material, and environment.

CONCLUSION

Trichoderma longibrachiatum, *Trichoderma asperellum*, and *Bacillus subtilis* inhibited the growth of the *Streptomyces* sp. colony *in vitro*, with antibiosis exerted by volatile metabolites and non-volatile metabolites to control the phytopathogen. *Trichoderma* species and *Bacillus subtilis* + *Enterococcus faecium* + *Lactobacillus plantarum* also attained *in vivo* antagonism in potato tubers, reducing disease severity and phytopathogen losses.

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