

**BIOLOGICAL AGENTS WITH FUNGICIDAL ACTIVITY AS AN ALTERNATIVE STRATEGY FOR THE MANAGEMENT OF ANTHRACNOSE (*Colletotrichum* spp.) IN MANGO CROPS (*Mangifera indica* L.)**

**FINAL PROJECT REPORT  
(2020- 2022)**

**I. SAMPLING, ISOLATION AND IN VITRO BIOLOGICAL EFFECTIVENESS TESTS OF BIOLOGICAL AGENTS  
(2020-2021)**

**II. APPLICATIONS OF BIOLOGICAL AGENTS IN THE FIELD AND POST-HARVEST  
(PRODUCTION CYCLE 2021)**

**III. APPLICATIONS OF BIOLOGICAL AGENTS IN THE FIELD PHASE  
(PRODUCTION CYCLE 2022)**

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# I. SAMPLING, ISOLATION AND IN VITRO BIOLOGICAL EFFECTIVENESS TESTS OF BIOLOGICAL AGENTS (2020-2021)

## 1. OBJECTIVES

1. Select virulent strains of *Colletotrichum* spp., isolated from mango fruits of the "Ataulfo, Tommy Atkins, Kent and Keitt" varieties.
2. Carry out soil, root and foliage sampling in mango orchards, and in undisturbed agroecological zones with great biodiversity.
3. Isolate strains of beneficial bacteria of the genera *Bacillus* and *Pseudomonas*, and of the fungus *Trichoderma* spp.
4. Carry out in vitro biological effectiveness tests to detect and select strains of bacteria and fungi with antagonistic activity against virulent strains of *Colletotrichum*.

## 2. MATERIALS AND METHODS

### 2.1. Isolation of *Colletotrichum*

Field visits were carried out in commercial mango orchards of the Ataulfo, Kent, Tommy and Keitt varieties in the State of Nayarit, Mexico, and fruits with anthracnose symptoms were collected. The fruits were taken to the Phytopathology Laboratory of the INIFAP-Santiago Ixcuintla Experimental Field. Fruit peel fragments with anthracnose symptoms were cultured on potato carrot agar (PCA) culture medium. Of the purified isolates, monosporic cultures were made in water-agar (AA), and they will be increased in potato dextrose agar (PDA).

### 2.2. Selection of virulent strains of *Colletotrichum*

From the purified *Colletotrichum* isolates, the strains with the highest speed of sporulation and growth of the mycelium of the fungus were selected. Subsequently, the virulence of the isolates was verified by inoculating fruits with 5  $\mu\text{L}$  of a spore suspension ( $1 \times 10^5$  spores  $\text{mL}^{-1}$ ). The fruits were placed in a humid chamber at a temperature of 27°C until the appearance of symptoms, which occurred 3-4 days after inoculation. Eleven different strains were inoculated, of which 6 were selected. These strains are the ones that were used in the biological effectiveness tests of biological agents.

### **2.3. Field sampling to obtain strains of bacteria and *Trichoderma***

Twenty field trips were made to carry out the collections (from July to December 2020, and from January to July 2021). The samples were taken in the state of Nayarit, in the municipalities of Tepic, San Blas and Santiago Ixcuintla, and in Los Mochis, Sinaloa. Soil samples, roots of wild grasses, aerial roots of wild trees and leaves of wild trees were collected. The samples were taken from areas that have not been disturbed by agricultural activities and/or with abundant vegetation. The samples were transferred to the Phytopathology Laboratory for processing and isolation.

### **2.4. Isolation of *Trichoderma* strains**

The isolation of the *Trichoderma* fungus was carried out from soil samples in PDA culture medium. Likewise, monosporic cultures were made to guarantee the purity of the strains. In total, 31 *Trichoderma* strains were isolated. From the isolated strains, their growth and sporulation capacity (capacity to produce spores) was determined. In total, 12 strains were selected, which showed rapid growth and abundant sporulation (Figure 1).

### **2.5. Isolation of strains of bacteria**

Grass roots, aerial tree roots and tree leaves were isolated. Isolations were performed in PDA and King's B culture media. The dishes were incubated for 72 h at  $28 \pm 1$  °C and growth was observed under a stereoscopic microscope. A bacterial colony counter was used to separate the bacteria. In the case of *Bacillus* spp., the colonies with the typical growth of the genus were selected, while for *Pseudomonas fluorescens* an Accuris® E3000 UV ultraviolet transilluminator, 115 VAC, was used, where the strains that showed fluorescence were selected. In the case of bacteria of the genus *Azotobacter* spp., the isolations were carried out in Ashby-sucrose agar-agar culture medium, while for *Streptomyces* spp., oat agar medium supplemented with 1% nystatin was used.

### **2.6. Selection of antagonistic bacteria by means of in vitro biological effectiveness tests.**

To observe the in vitro antagonistic potential of the bacteria against *Colletotrichum* spp., the phytopathogen was cultured (0.5 cm diameter agar disk) in the center of a Petri dish, and 2 to 4 bacteria were cultured per streak equidistantly. at a distance of 3 cm from the pathogen. Since this test is to visually determine which bacteria have antagonistic capacity against *Colletotrichum*, the tests consisted of three repetitions for each combination of bacteria in PDA culture medium for 7 days at 28°C. In order to make a comparison of the biological effectiveness of the bacteria, *Colletotrichum* isolates without bacteria were used as control (control). About 282 bacterial isolates were made. 36 strains of bacteria with antagonistic potential were selected.

To determine its biological effectiveness, one strain of bacteria was cultivated per dish with three streaks, and *Colletotrichum* was cultivated in the center of the petri dish. The petri dishes were incubated for 7 days at  $26\pm 1$  °C. Five replicates per bacterial strain were used. To determine the antagonism of the bacterial strains, the diameter of mycelial growth and the percentage of mycelial growth inhibition were used. Measurements were made at 3, 4, 5, 6 and 7 days after sowing.

### 3. RESULTADOS

#### 3.1. Confrontations of strains of *Trichoderma* vs *Colletotrichum* spp.

It was found that 12 *Trichoderma* strains (Figure 1) showed antagonism against virulent *Colletotrichum* strains. To determine if the *Trichoderma* fungus inhibited the growth of the pathogen, a confrontation technique was used that consisted of placing a circle of sterile filter paper 2 cm in diameter in the center of a petri dish with culture medium. Subsequently, with a sterile punch, a piece of PDA with *Trichoderma* was placed in the center of the circle, and the dish was incubated at  $26\pm 1$  °C for 7 days. Five replicates were performed for each strain. After seven days, *Trichoderma* had completely covered the Petri dish, including the circle of filter paper. After 7 days, the circle was removed from the dish, and a 0.5 cm diameter slice of *Colletotrichum* was placed in the space that remained, and again the dishes were incubated at  $26\pm 1$  °C for 7 days. As a control, they were planted at *Colletotrichum* in PDA culture medium. The results obtained showed that 12 strains completely inhibited the development of *Colletotrichum* mycelium (Figures: 2-7). Using this technique, it was observed that the *Trichoderma* strains presented a strong antagonism against *Colletotrichum*, these results indicate that the beneficial fungus produces certain metabolites that inhibit the development of *Colletotrichum*.

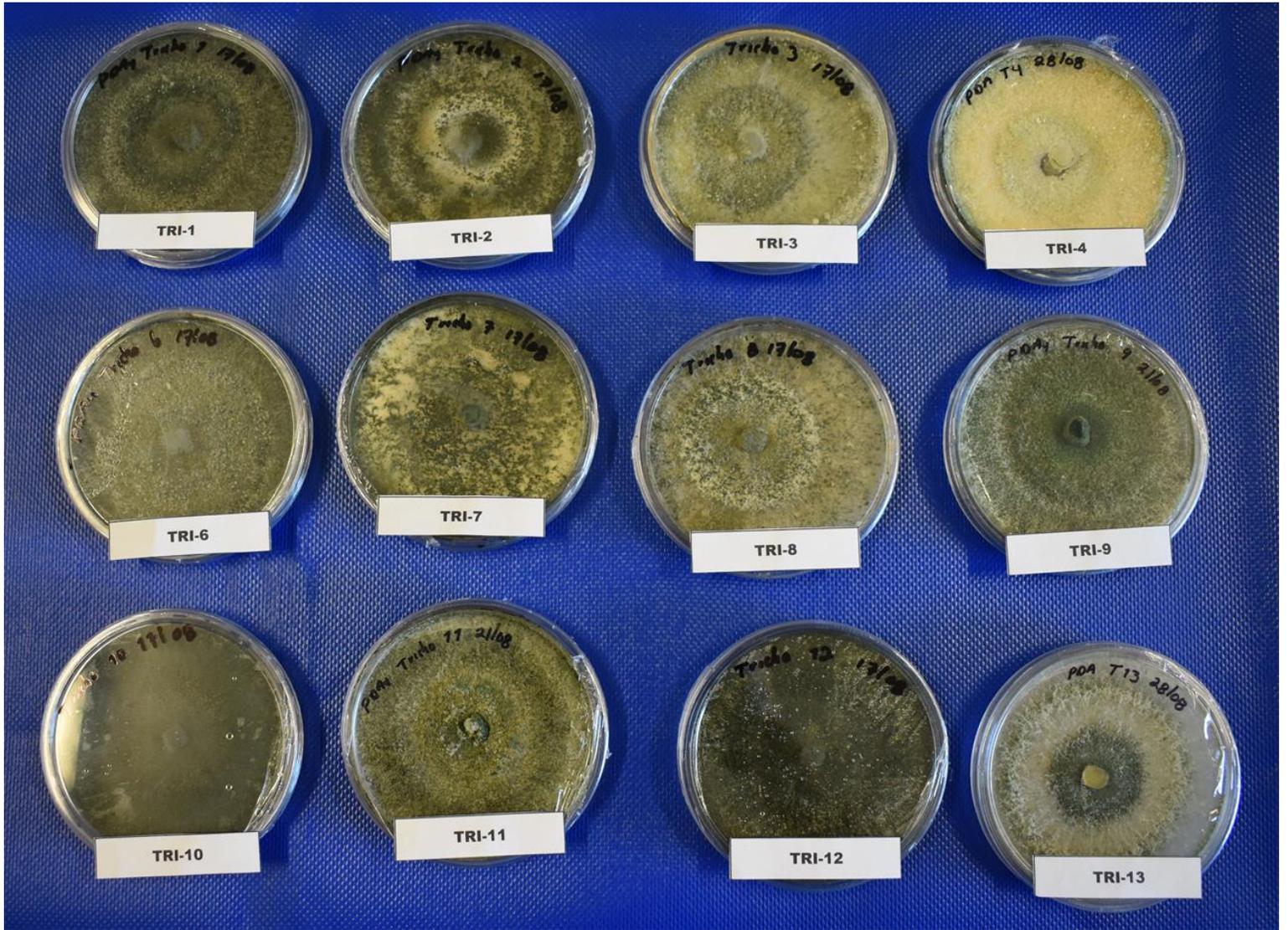


Figura 1. Selección de cepas de *Trichoderma* spp., con capacidad antagónica contra cepas virulentas de *Colletotrichum* spp.

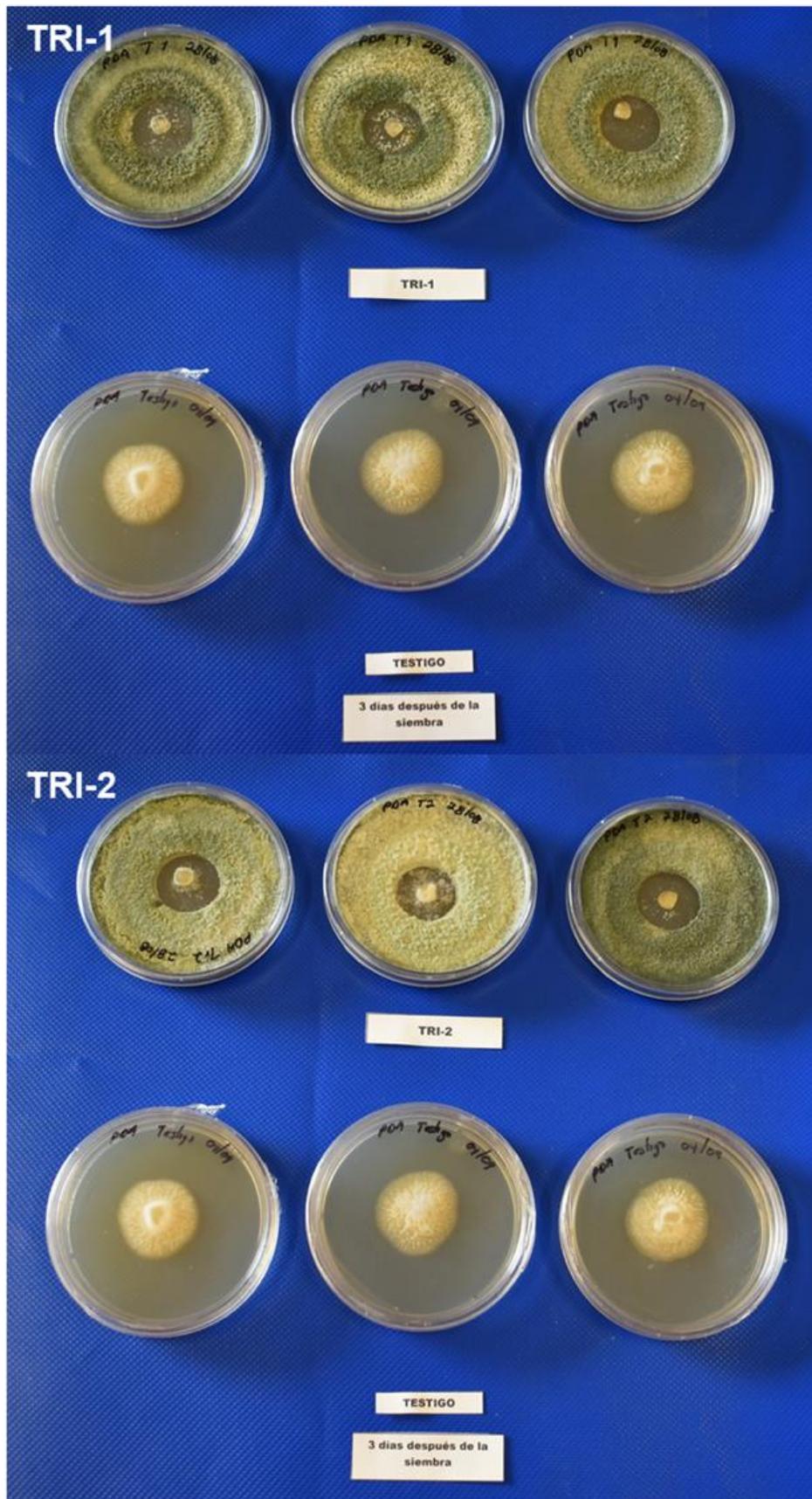


Figura 2. Inhibición del crecimiento de micelio de *Colletotrichum* spp., al confrontarse con cepas antagónicas (TRI-1 y TRI-2) de *Trichoderma* spp.

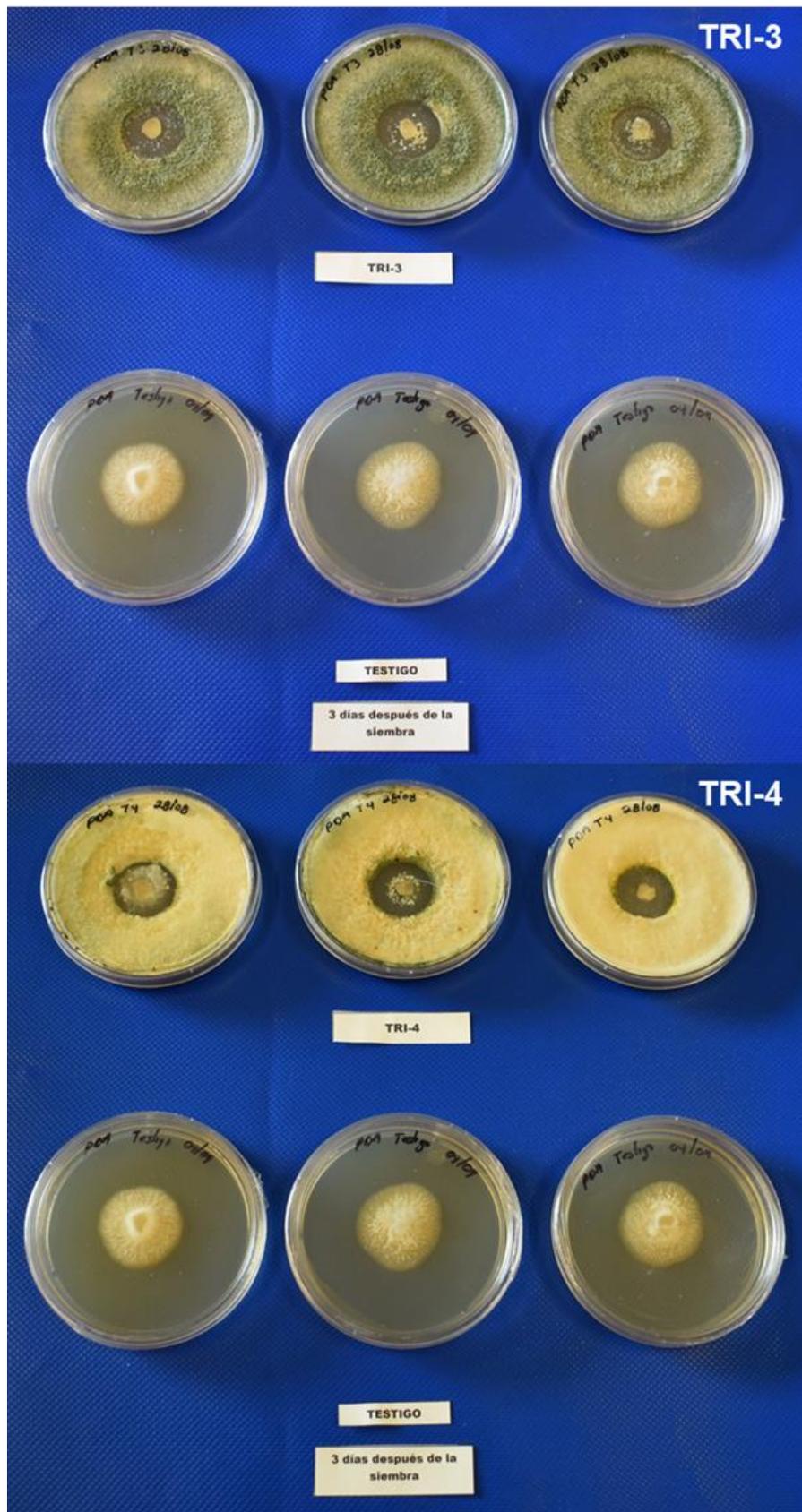


Figura 3. Inhibición del crecimiento de micelio de *Colletotrichum* spp., al confrontarse con cepas antagonicas (TRI-3 y TRI-4) de *Trichoderma* spp.

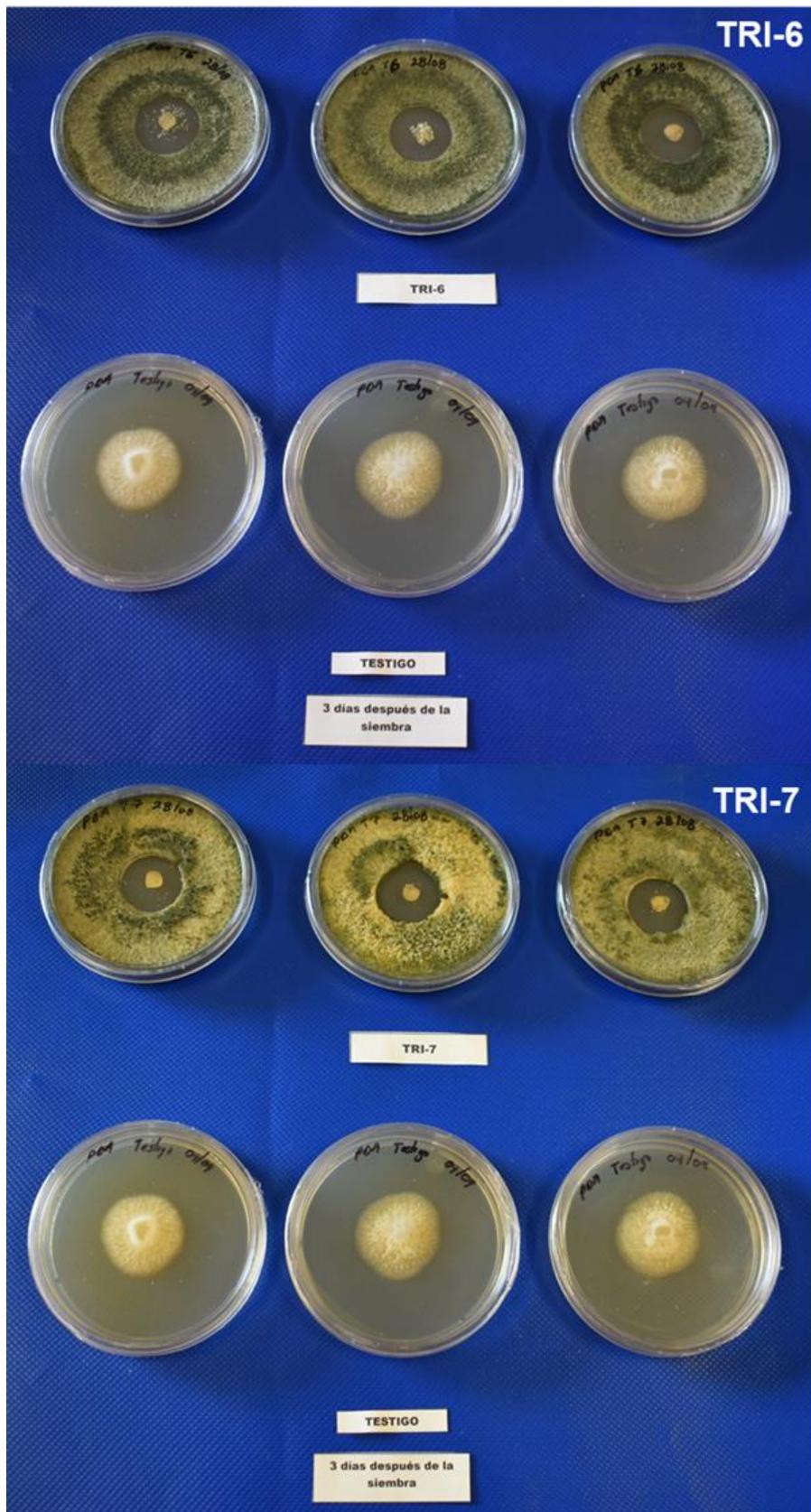


Figura 4. Inhibición del crecimiento de micelio de *Colletotrichum* spp., al confrontarse con cepas antagonicas (TRI-6 y TRI-7) de *Trichoderma* spp.

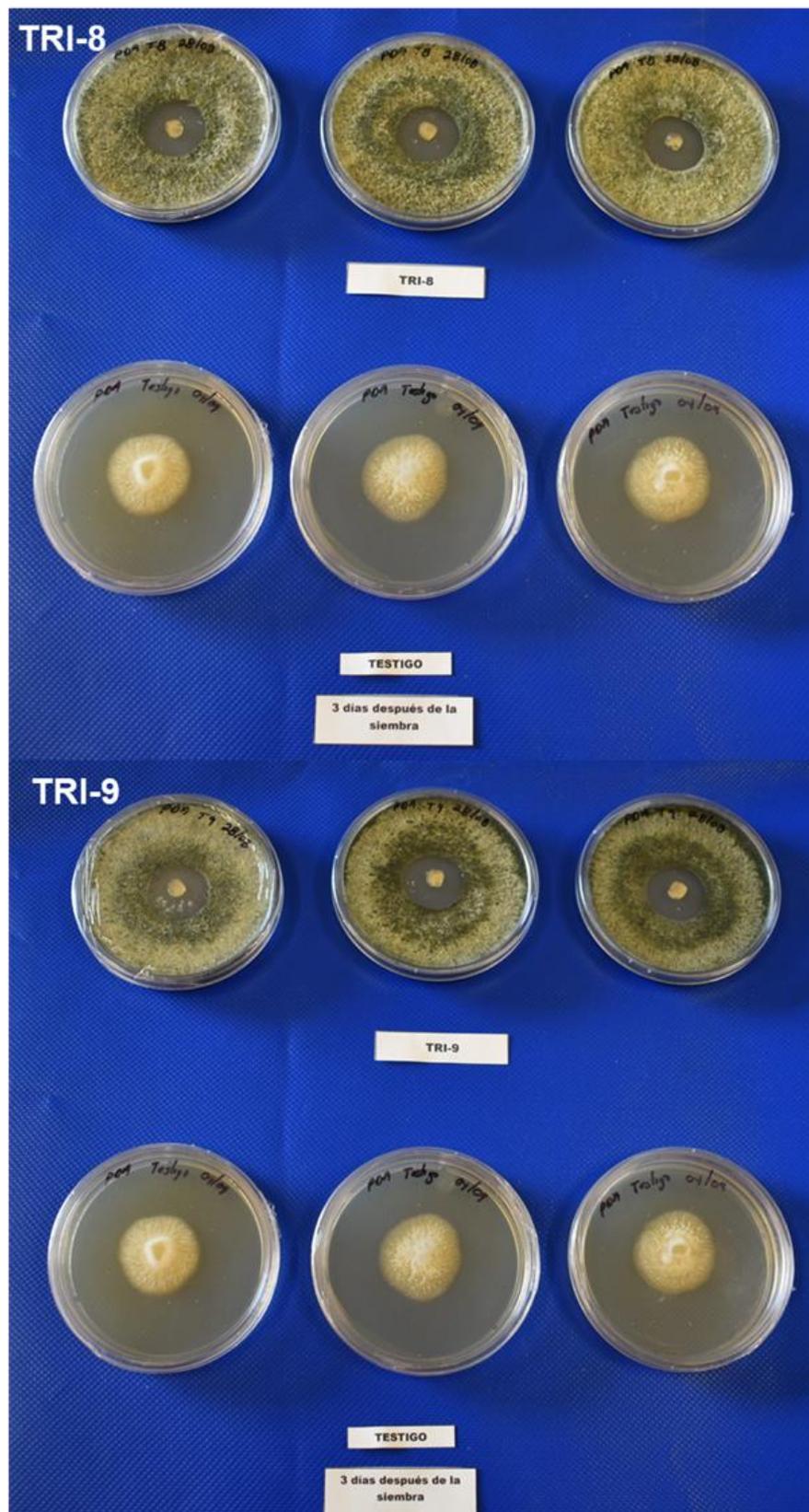


Figura 5. Inhibición del crecimiento de micelio de *Colletotrichum* spp., al confrontarse con cepas antagonicas (TRI-8 y TRI-9) de *Trichoderma* spp.

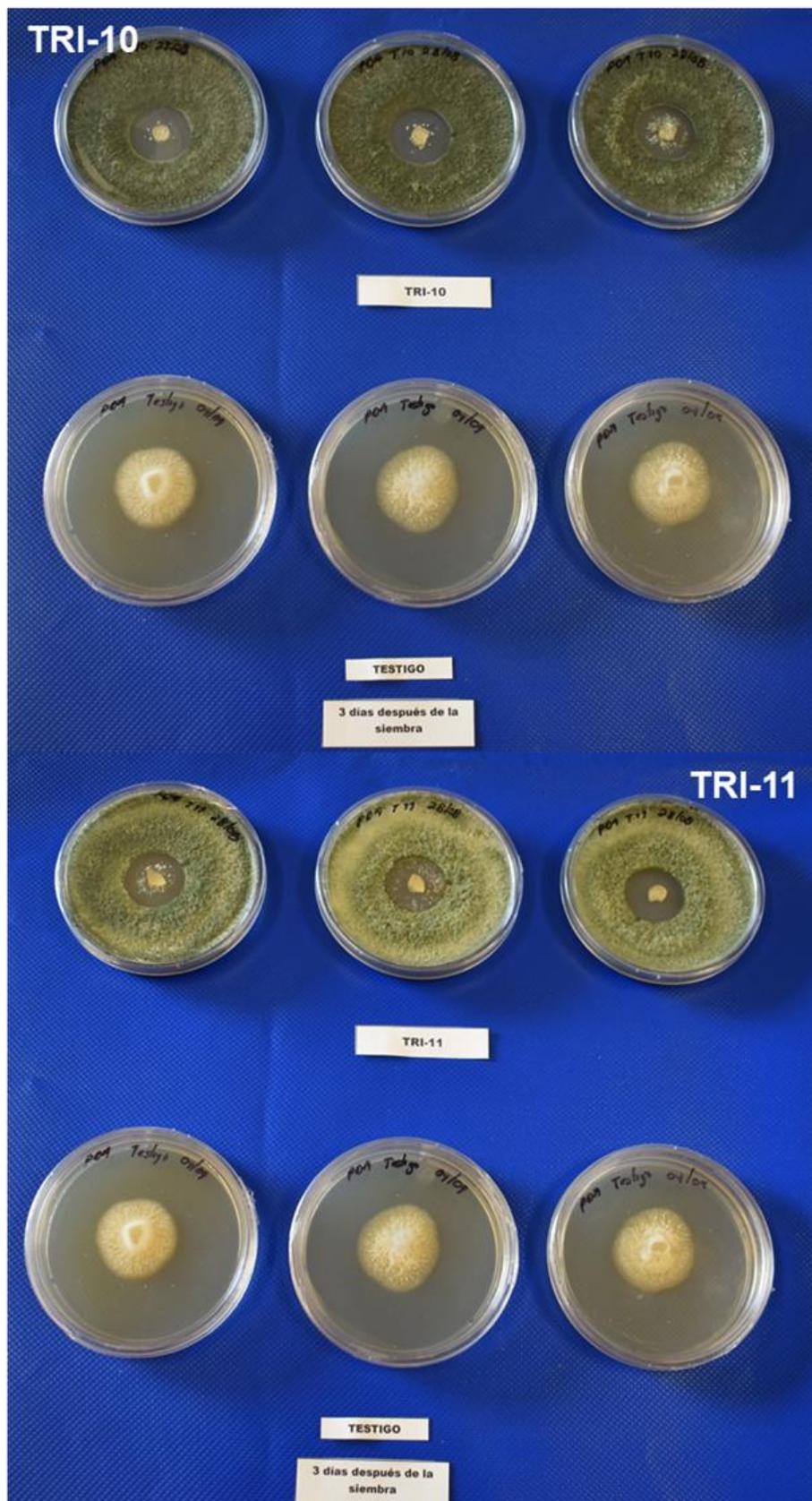


Figura 6. Inhibición del crecimiento de micelio de *Colletotrichum* spp., al confrontarse con cepas antagónicas (TRI-10 y TRI-11) de *Trichoderma* spp.

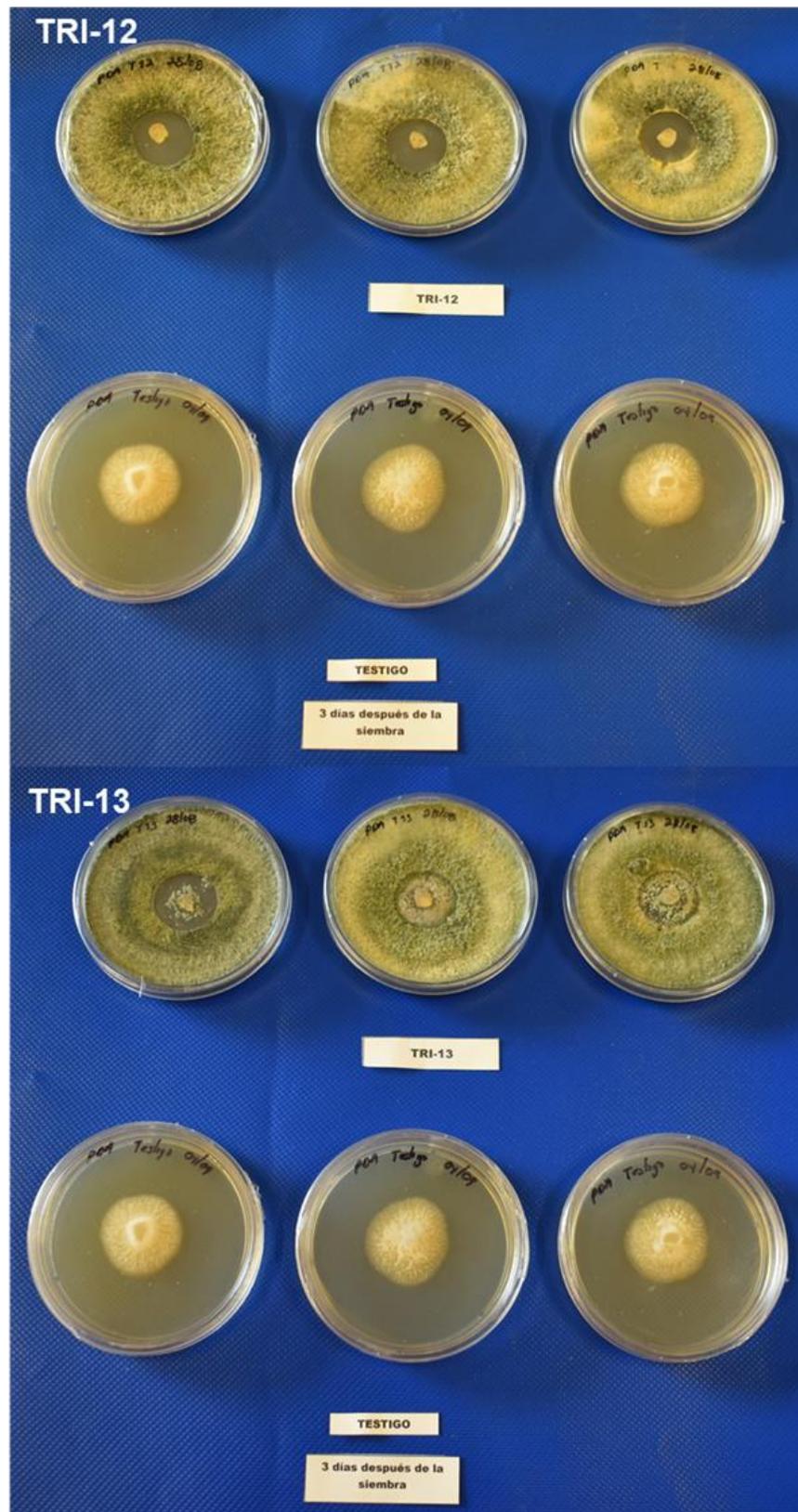
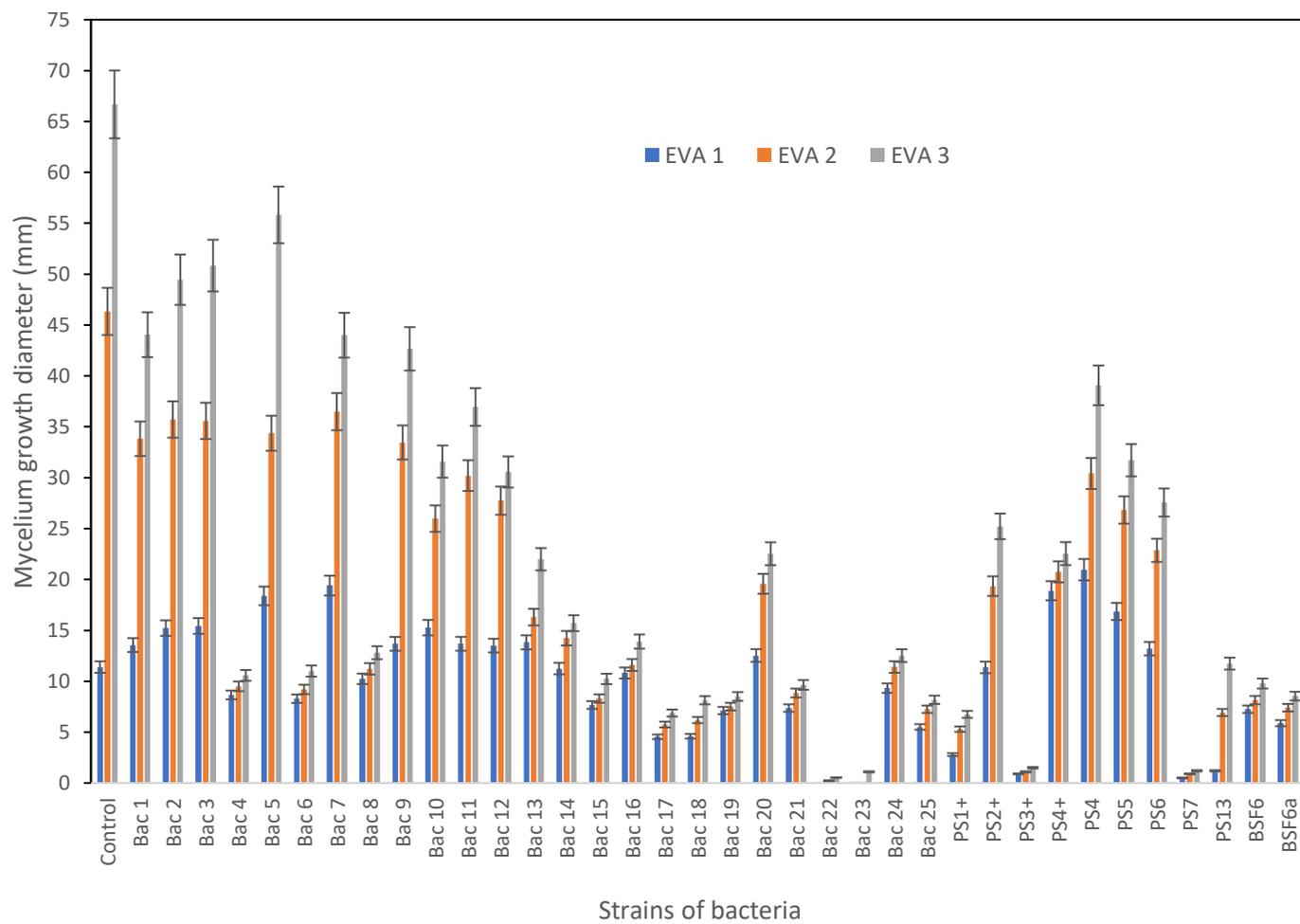


Figura 7. Inhibición del crecimiento de micelio de *Colletotrichum* spp., al confrontarse con cepas antagonistas (TRI-12 y TRI-13) de *Trichoderma* spp.

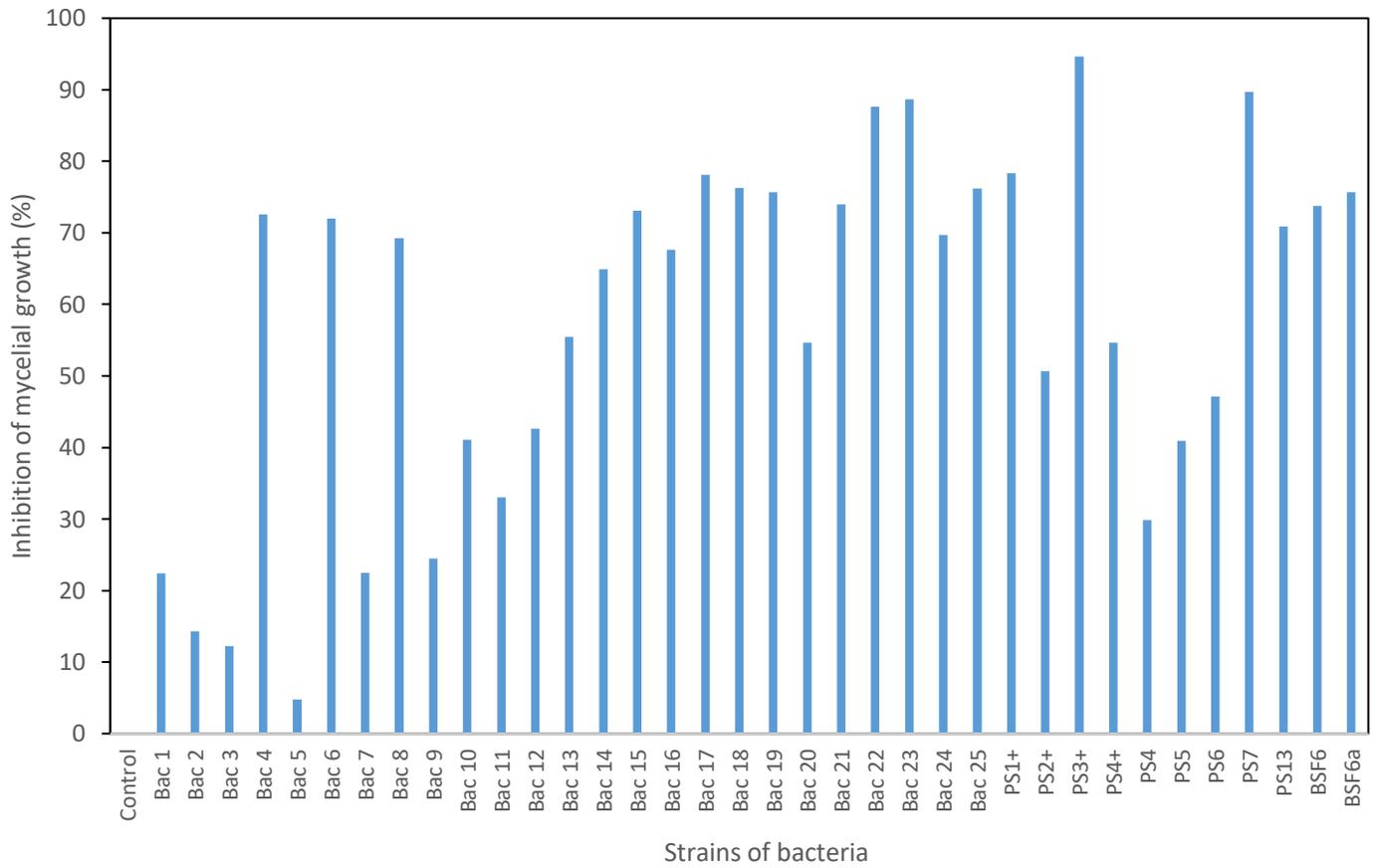
### **3.2. Confrontations of strains of bacteria vs. *Colletotrichum* spp.**

It was determined that several strains of bacteria inhibited the growth of mycelium of the fungus. In the diameter of mycelium growth, after 7 days, it was observed that 19 strains of bacteria decreased mycelium growth, which was less than 2 cm, while the control (without the presence of bacteria) had a diameter of 6.7 cm. Strains BAC 22, BAC 23, PS3 and PS7 almost completely inhibited the growth of mycelium (Figure 8). Regarding the mycelium inhibition percentage, 18 bacterial strains presented inhibition percentages equal to or greater than 70 %. On the other hand, the strains of bacteria BAC 22, BAC 23, PS3 and PS7 obtained inhibition percentages equal to or greater than 90 % (Figure 9). On the other hand, in the 2021 samplings, other bacteria were obtained with outstanding effectiveness in inhibiting the growth of pathogenic strains of *Colletotrichum* spp. (Bac-5 Ch, Bac-2 Ch, Bac-3 Ch, Bac-7 Ch, Bac-6 Ch, Bac-7 Ch, Bac-9 coffee, Bac-10 coffee, Bac-8 Ch and Bac-13 coffee). These bacterial strains were tested in the field in the 2022 production cycle.

Figures (10-20) show some examples of bacterial strains inhibiting the growth of *Colletotrichum* spp. mycelium.



**Figure 8. Mycelium growth of *Colletotrichum* spp., when confronted with antagonistic bacterial strains in three evaluations.**



**Figura 9. Porcentaje de inhibición del crecimiento de micelio de *Colletotrichum* spp., al confrontarse con cepas bacterianas antagonicas.**

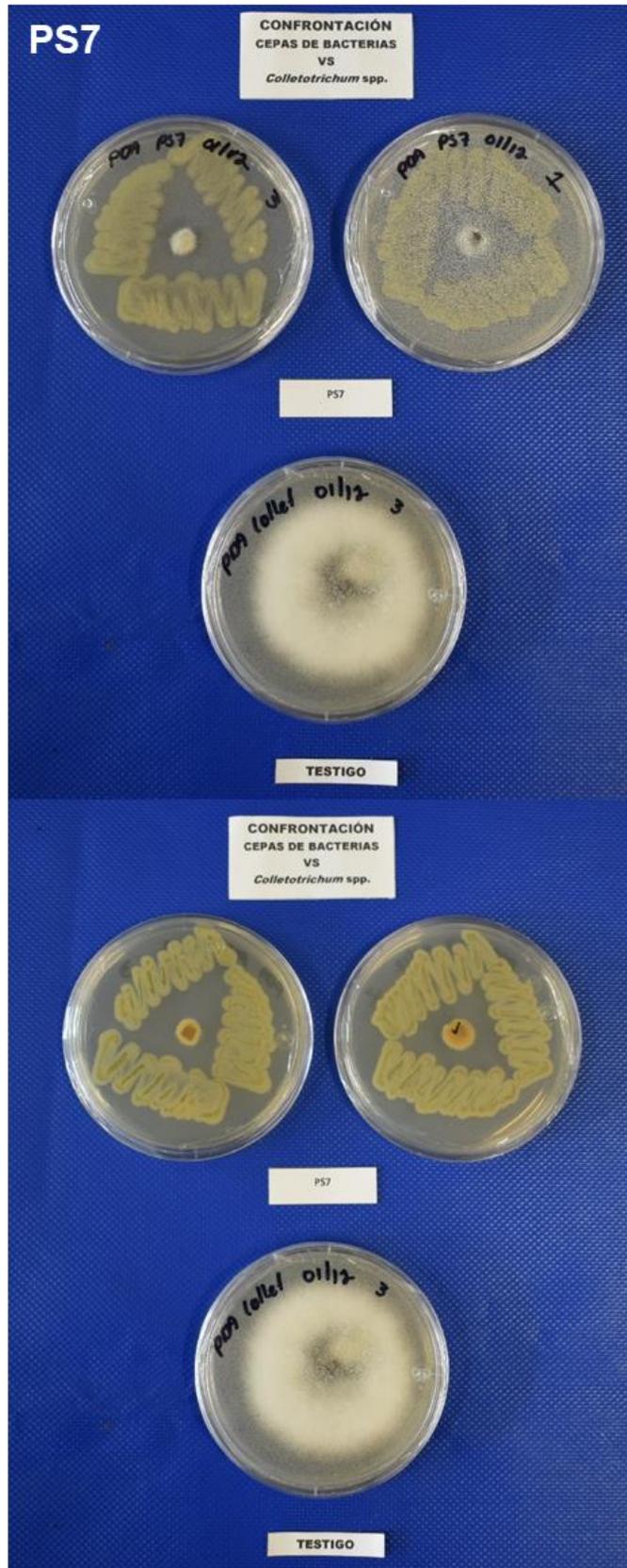


Figure 10. Inhibition of the growth of *Colletotrichum* spp. mycelium, when confronted with an antagonistic bacterial strain (PS7).

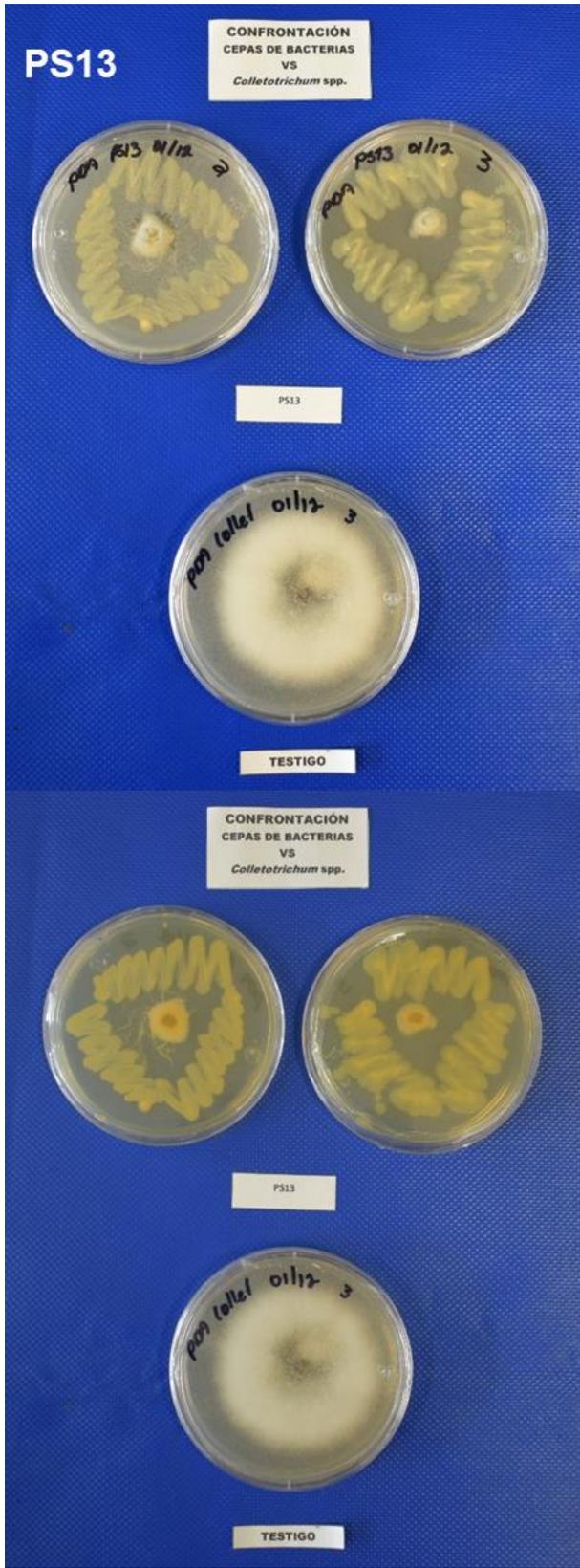


Figure 11. Inhibition of the growth of *Colletotrichum* spp. mycelium, when confronted with an antagonistic bacterial strain (PS13).



Figure 12. Inhibition of the growth of *Colletotrichum* spp. mycelium, when confronted with an antagonistic bacterial strain (PS3+).



Figure 13. Inhibition of the growth of *Colletotrichum* spp. mycelium, when confronted with an antagonistic bacterial strain (PS1+).

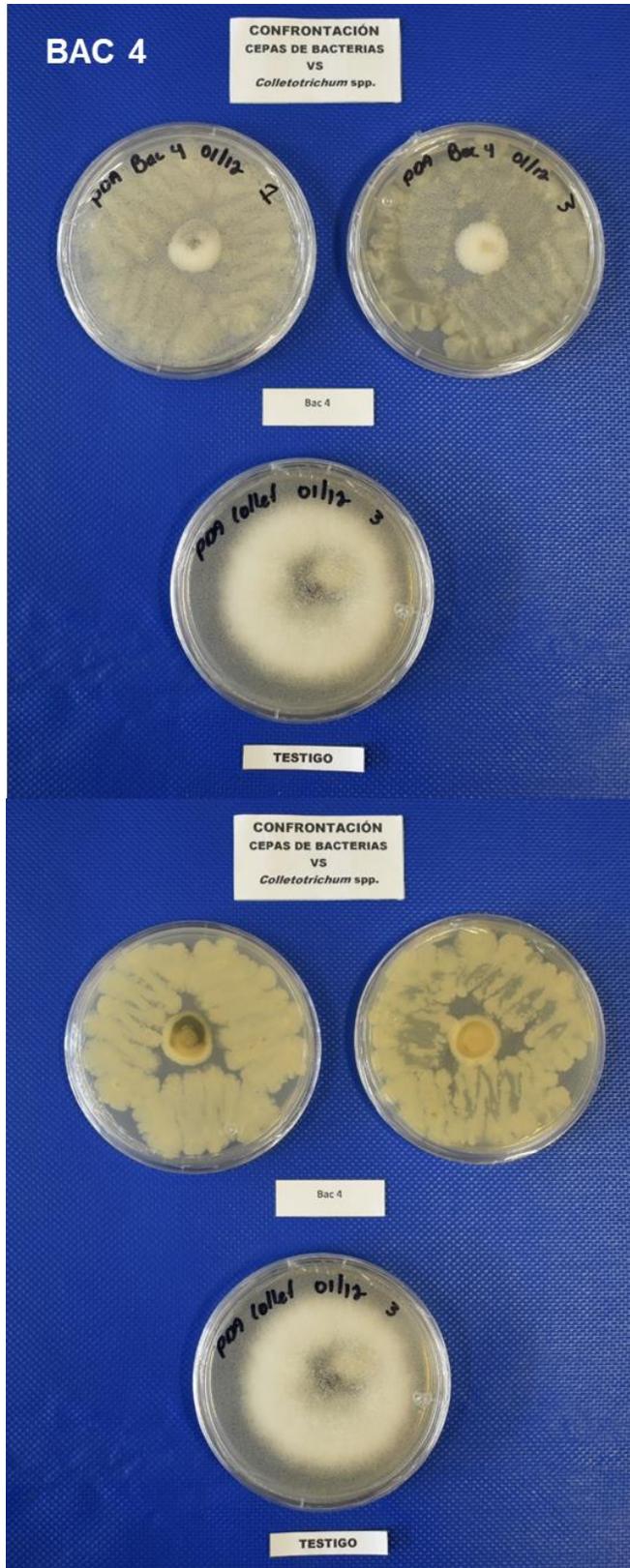


Figure 14. Inhibition of the growth of *Colletotrichum* spp. mycelium, when confronted with an antagonistic bacterial strain (BAC4).

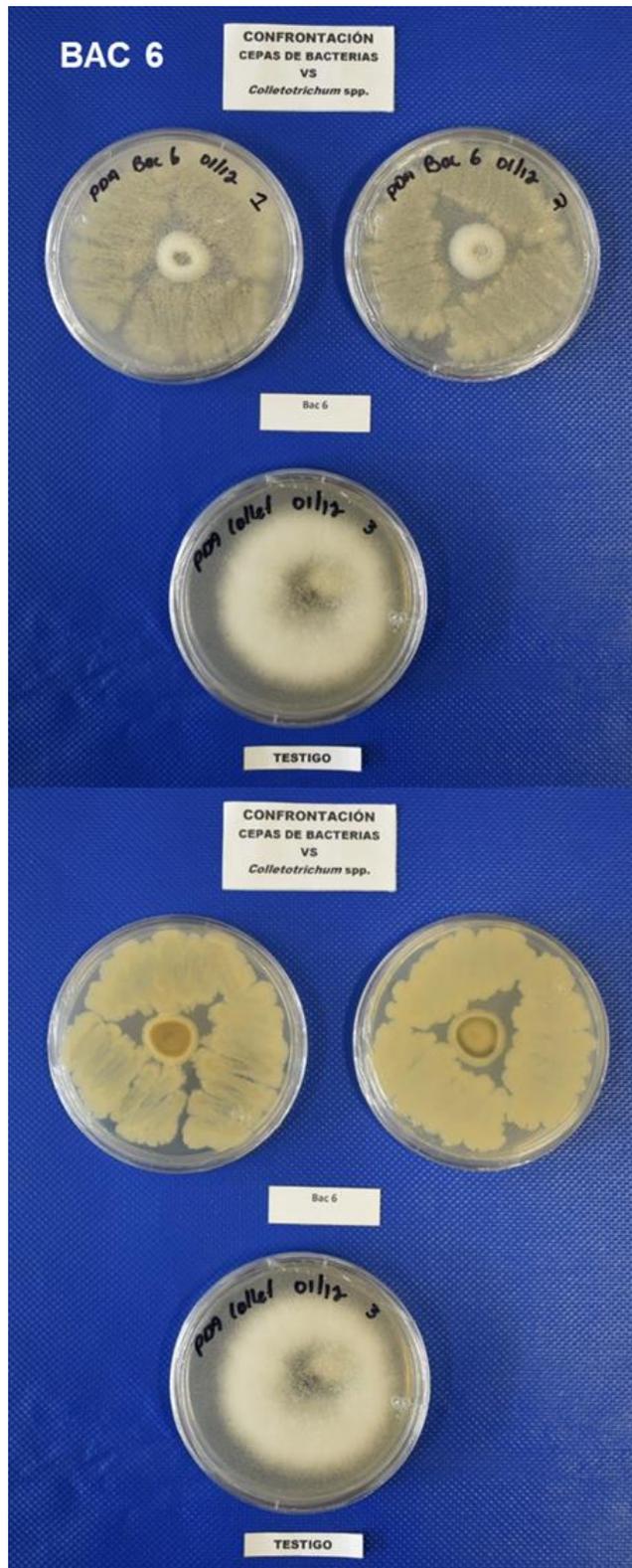


Figure 15. Inhibition of the growth of *Colletotrichum* spp. mycelium, when confronted with an antagonistic bacterial strain (BAC6).

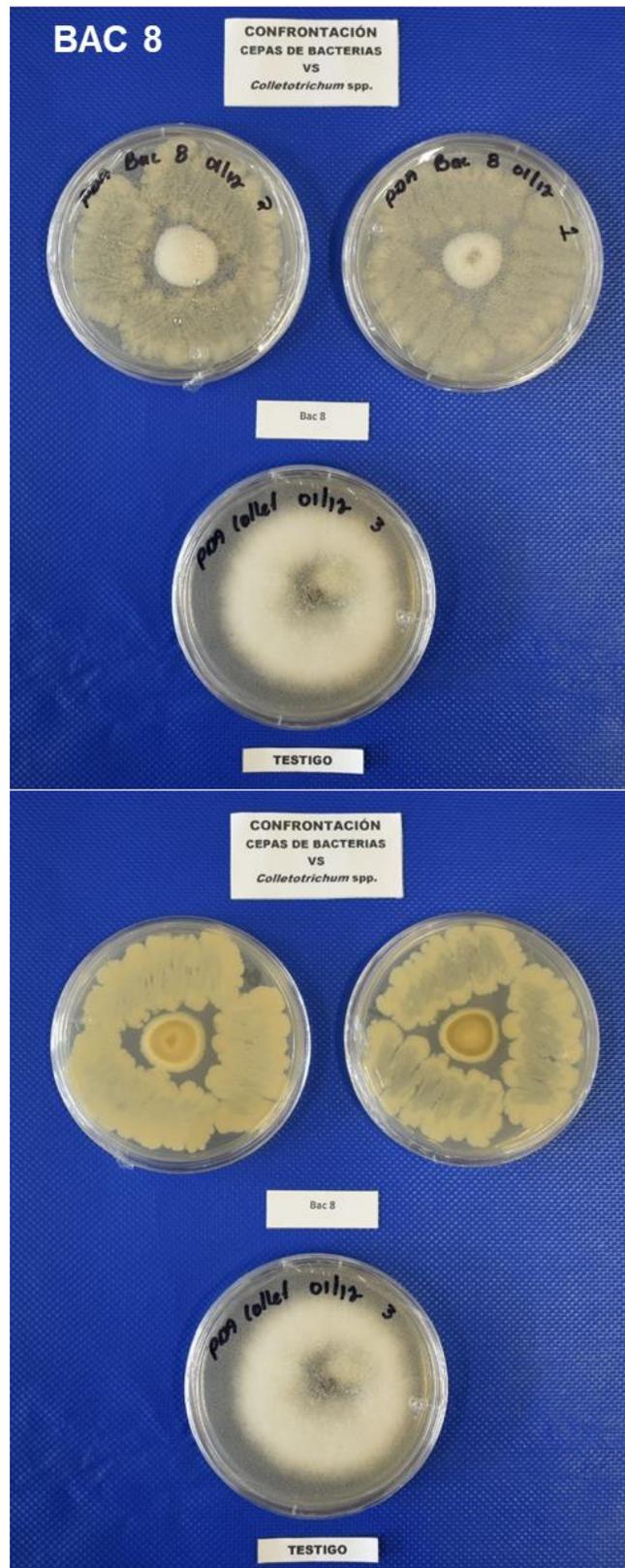


Figure 16. Inhibition of the growth of *Colletotrichum* spp. mycelium, when confronted with an antagonistic bacterial strain (BAC8).

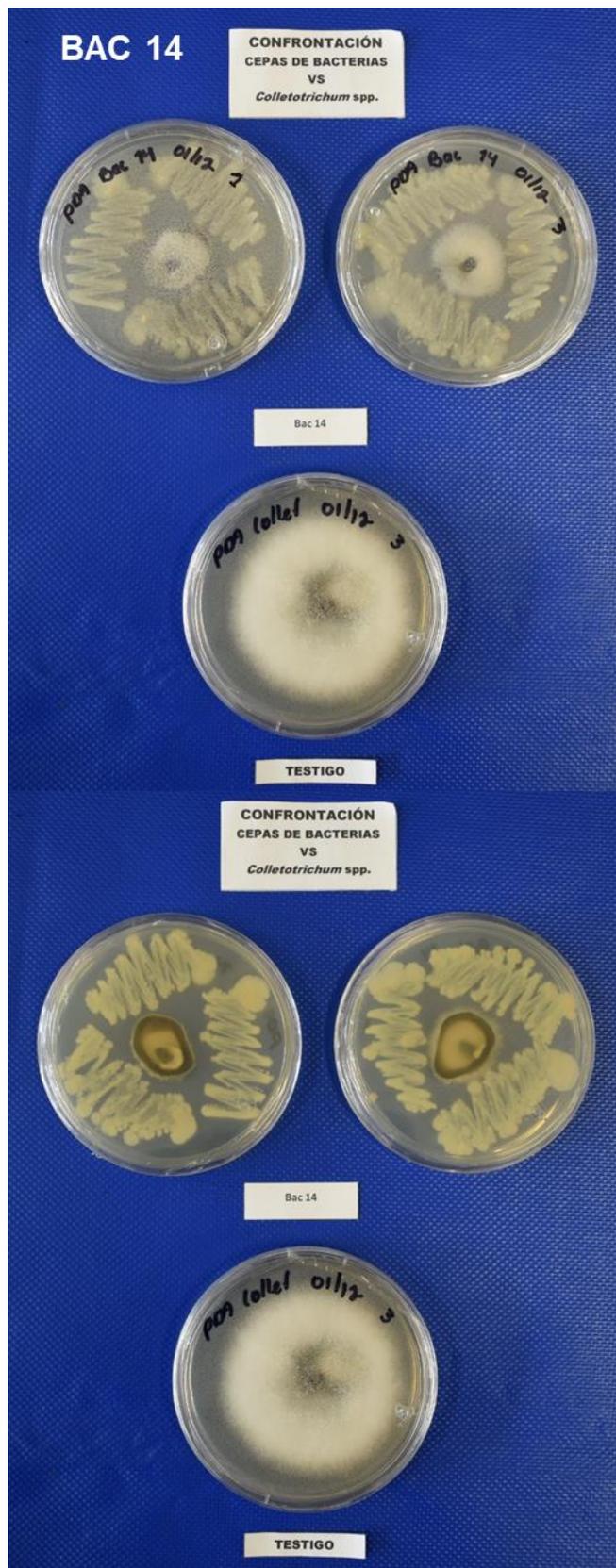


Figure 17. Inhibition of the growth of *Colletotrichum* spp. mycelium, when confronted with an antagonistic bacterial strain (BAC14).

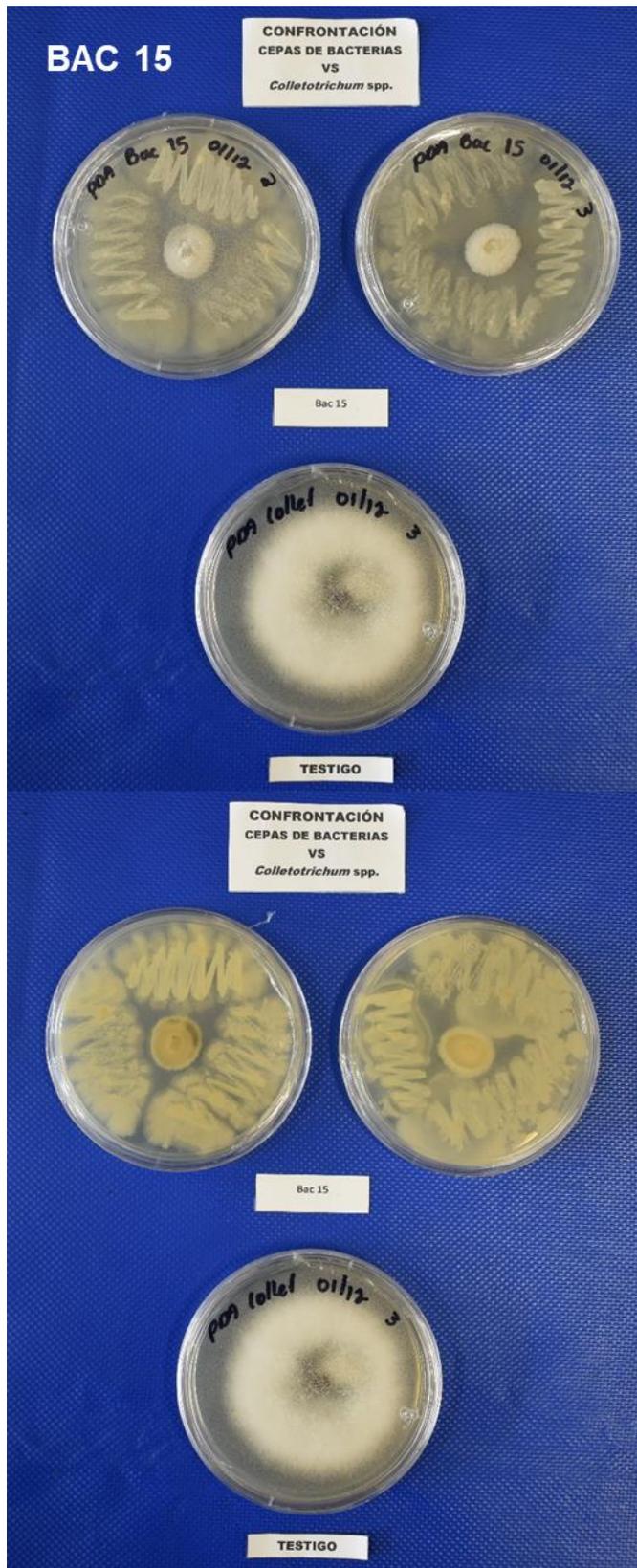


Figure 18. Inhibition of the growth of *Colletotrichum* spp. mycelium, when confronted with an antagonistic bacterial strain (BAC15).

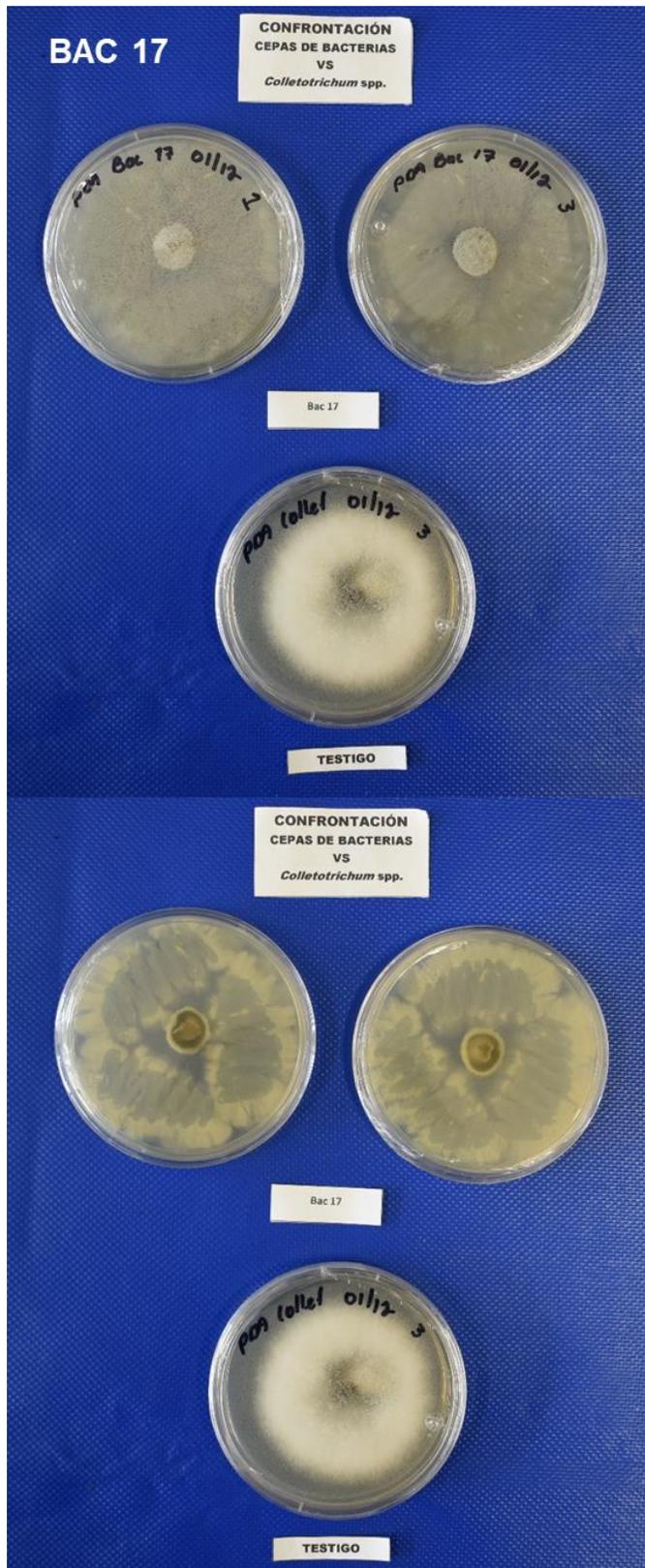


Figure 19. Inhibition of the growth of *Colletotrichum* spp. mycelium, when confronted with an antagonistic bacterial strain (BAC17).



Figure 20. Inhibition of the growth of *Colletotrichum* spp. mycelium, when confronted with an antagonistic bacterial strain (BAC20).

## 4. CONCLUSIONS

1. *Bacillus*, *Pseudomonas*, *Streptomyces* and *Azotobacter* strains with antagonistic capacity against virulent strains of the fungus *Colletotrichum* spp, whose phytopathogen induces anthracnose in mango fruits, were isolated.
2. At least 31 strains of the beneficial fungus *Trichoderma* spp. were isolated, of which 12 strains showed greater speed of growth, sporulation and antagonistic capacity against virulent strains of *Colletotrichum* spp.
3. It was observed that the greatest diversity and quantity of biological agents were found in little disturbed areas, where human intervention was very limited.
4. It was determined that different strains of bacteria and fungi of the *Trichoderma* genus have antagonistic capacity against *Colletotrichum*, so they could be an alternative to control anthracnose in mango crops.

**II. APPLICATIONS OF BIOLOGICAL AGENTS IN THE FIELD AND POST-HARVEST**  
**III. (PRODUCTION CYCLE 2021)**

**1. OBJETIVES**

1. To evaluate the biological effectiveness of biological agents in the control of anthracnose (*Colletotrichum* spp.) from flowering to harvest in mango orchards of the "Ataulfo" variety.
2. To evaluate the biological effectiveness of biological agents in the control of anthracnose (*Colletotrichum* spp.) in postharvest in mango fruits of the varieties "Ataulfo, Tommy Atkins, Kent and Keitt".

**2. MATERIALS AND METHODS**

**2.1. Applications of Biological Agents in the Field Phase  
(2021 production cycle)**

**2.1.1. Establishment of the experiment**

Two experiments were established in commercial mango orchards of the "Ataulfo" variety in the last week of January and the first week of February 2021. One orchard was located in the Ejido (common) de Estación Nanchi, Municipality of Santiago Ixcuintla, Nayarit, Mexico, in 8-year-old trees. The other orchard was located in the Ejido (common) de Valle Lerma, Municipality of Santiago Ixcuintla, Nayarit, Mexico, with 9-year-old trees. The selection of the aforementioned orchards was made based on the incidences of anthracnose that they had presented in the 2020 production cycle.

**2.1.2. Application of treatments**

Biological agents (antagonistic bacteria and fungi of the genus *Trichoderma*) were used based on the results of the in vitro tests. Tables 1 and 2 present the treatments for each of the orchards. The applications were made every 15 days, with a total of 8 applications from flowering to harvest. The equipment used was Hyundai® Liquid/Powder HYD4514L engine sprayers.

**2.1.3. Evaluations and variables**

The evaluations were carried out to determine the incidence of anthracnose in panicles and fruits. The variables to be evaluated were: a) Incidence of anthracnose in panicles; b) Incidence of anthracnose in fruits between 3 and 5 cm in length; c) Incidence of anthracnose in fruits of more than 5 cm in length; d) Incidence of anthracnose in fruits with physiological maturity ready for harvest; e) Effectiveness of control of biological agents. Four evaluations were carried out, one in flowering and the other three during the development of the fruit. To determine the incidence of anthracnose, the fruits that showed two or more lesions per fruit equal to or greater than 3 millimeters in diameter were considered.

The incidence was expressed as a percentage, for which the following formula was used:

$$\text{Incidence of anthracnose (\%)} = \frac{\text{Fruits with anthracnose symptoms}}{\text{Total fruits}} \times 100 \dots$$

Control efficacy was calculated using the following formula:

$$\text{EC (\%)} = \frac{\text{IAC} - \text{IAt}}{\text{IAC}} \times 100 \dots$$

Where:

EC = Control effectiveness of biological agents (%)

IAC = Incidence of anthracnose in the control

IAt = Incidence of anthracnose in treatment

**Table 1. Treatments with biological agents to control anthracnose in a commercial mango orchard of the "Ataulfo" variety, located in the Estación Nanchi, Municipality of Santiago Ixcuintla, Nayarit, Mexico, in the 2021 production cycle.**

NUMBER	TREATMENT	MICROORGANISMS
T1	BAC 22	Bacterium
T2	BAC23	Bacterium
T3	PS3	Bacterium
T4	PS7	Bacterium
T5	PS1	Bacterium
T6	BAC17	Bacterium
T7	BAC18	Bacterium
T8	BSF6A	Bacterium
T9	BAC4	Bacterium
T10	BAC15	Bacterium
T11	CESIX	Fungus <i>Trichoderma</i>
T12	PINO	Fungus <i>Trichoderma</i>
T13	16 LOS MOCHIS	Fungus <i>Trichoderma</i>
T14	6 VOLCAN	Fungus <i>Trichoderma</i>
T15	CESIX1	Fungus <i>Trichoderma</i>
T16	FUNGIFREE™	<i>Bacillus subtilis</i>
T	CONTROL	No application

**Table 2. Treatments with biological agents for the control of anthracnose in a commercial mango orchard of the "Ataulfo" variety, located in Valle Lerma, Municipality of Santiago Ixcuintla, Nayarit, Mexico, in the 2021 production cycle.**

NÚMERO	TRATAMIENTOS	MICROORGANISMOS
T17	BAC 22 + CESIX	Bacterium + <i>Trichoderma</i>
T18	BAC23 + PINO	Bacterium + <i>Trichoderma</i>
T19	PS3 + LOS MOCHIS16	Bacterium + <i>Trichoderma</i>
T20	PS7 + VOLCAN6	Bacterium + <i>Trichoderma</i>
T21	PS1 + CESIX1	Bacterium + <i>Trichoderma</i>
T22	BAC17 + CESIX2	Bacterium + <i>Trichoderma</i>
T23	BAC18 + CESIX3	Bacterium + <i>Trichoderma</i>
T24	BSF6A + CESIX4	Bacterium + <i>Trichoderma</i>
T25	BAC4 + HULE5	Bacterium + <i>Trichoderma</i>
T26	BAC15 + HULE6	Bacterium + <i>Trichoderma</i>
T27	CESIX2	Fungus <i>Trichoderma</i>
T28	CESIX3	Fungus <i>Trichoderma</i>
T29	CESIX4	Fungus <i>Trichoderma</i>
T30	HULE5	Fungus <i>Trichoderma</i>
T31	HULE6	Fungus <i>Trichoderma</i>
T32	FUNGIFREE™	<i>Bacillus subtilis</i>
T	CONTROL	No application

#### 2.1.4. Treatment design

A completely randomized design with five replicates per treatment (five trees) was used. In each tree, 10 fully developed panicles (50 panicles per treatment) were selected and marked. In the case of anthracnose in fruits, all the fruits of each tree were taken as a sample, where the experimental unit was a fruit. An analysis of variance (ANOVA) and comparison of means (Tukey;  $p \leq 0.05$ ) were made using SAS (SAS Institute, Inc., 2010).

## **2.2. Applications of Biological Agents in the Postharvest Phase (2021 production cycle)**

### **2.2.1. Biological agents**

Biological agents (antagonistic bacteria and fungi of the genus *Trichoderma*) were used based on the results of the in vitro tests. Table 3 shows the treatments for each of the varieties used. Likewise, the biological effectiveness of biological agents of commercial brands available in the market was evaluated (Table 3).

### **2.2.2. Inoculation, dose and immersion time of biopesticide formulations**

The inoculation of the fruits was carried out by making an "X"-shaped wound 1 mm deep and 3-4 mm long with a sterile scalpel; eight wounds were made per fruit. *Colletotrichum* spp. was inoculated in each wound with a spore suspension ( $1 \times 10^5$  spores mL<sup>-1</sup>); the inoculation was carried out with a 120 mL atomizer, and the spore suspension was sprayed on the face of the fruit where the wounds were made. The inoculated fruits were incubated for 8 hours in a humid chamber in plastic bags and sterile absorbent paper. The fruits were treated by immersion for 5 minutes with the treatments in table three. The doses used were 5,000 and 10,000 ppm (5 and 10 mL/L of water). The treated fruits were incubated in a humid chamber at a temperature of  $27 \pm 1$  °C for 10 days.

### **2.2.3. Biological effectiveness tests on postharvest fruits of the main mango varieties.**

Healthy fruits in physiological maturity of the varieties "Ataulfo, Keitt, Kent and Tommy Atkins" were used. The fruits were harvested in different orchards in the state of Nayarit, Mexico, in the period from June to September 2021. The order of the evaluations with the varieties was as follows: "Ataulfo, Tommy Atkins, Kent and Keitt".

### **2.2.4. Effect of biopesticide formulations on anthracnose in mango fruits:**

Fruits were inoculated with the virulent strains of *Colletotrichum* in the aforementioned varieties. For each of the treatments, including the control (fruits treated with sterile distilled water), the incidence and severity of the disease was evaluated at 4, 6 and 8 days after immersion (DAI) of the fruits in the treatments. In the biopesticide control efficacy variable, two evaluations were made, at 6 and 8 DAI of the fruits.

**Table 3. Treatments with biological agents for the control of anthracnose in postharvest conditions in mango fruits of the "Ataulfo, Tommy Atkins, Kent and Keitt" varieties harvested in Nayarit, Mexico, in the 2021 production cycle.**

NUMBER	TREATMENT	MICROORGANISMS
T1	BAC 22	Bacterium
T2	BAC23	Bacterium
T3	PS3	Bacterium
T4	PS7	Bacterium
T5	PS1	Bacterium
T6	BAC17	Bacterium
T7	BAC18	Bacterium
T8	BSF6A	Bacterium
T9	BAC4	Bacterium
T10	BAC15	Bacterium
T11	BAC6	Bacterium
T12	PS13	Bacterium
T13	BAC19	Bacterium
T14	BAC21	Bacterium
T15	BAC25	Bacterium
T16	CESIX	Fungus <i>Trichoderma</i>
T17	PINO	Fungus <i>Trichoderma</i>
T18	LOS MOCHIS16	Fungus <i>Trichoderma</i>
T19	VOLCAN6	Fungus <i>Trichoderma</i>
T20	CESIX1	Fungus <i>Trichoderma</i>
T21	CESIX2	Fungus <i>Trichoderma</i>
T22	CESIX3	Fungus <i>Trichoderma</i>
T23	CESIX4	Fungus <i>Trichoderma</i>
T24	HULE5	Fungus <i>Trichoderma</i>
T25	HULE6	Fungus <i>Trichoderma</i>
T26	FUNGIFREE™	<i>Bacillus subtilis</i>
T27	SERENADE™	<i>Bacillus subtilis</i>
T28	STARGUS™	<i>Bacillus amyloliquefaciens</i>
T	CONTROL	No application

### 2.2.5. Variables evaluated in the fruits

To determine the biological effectiveness of biological agents on the control of anthracnose, the following variables were used:

- I. **Incidence of anthracnose in wounds:** the number of incisions (wounds) with the presence of anthracnose on the fruit was counted, and expressed as a percentage. Anthracnose incidence was considered when dark brown to black lesions were observed with a length of more than 2 mm on or to the side of the incisions.
- II. **Diameter of the lesion due to anthracnose:** in the incisions that presented symptoms of anthracnose, two measurements per wound were made, with a digital vernier, of the diameter of the lesion, whose diameter was expressed in millimeters.
- III. **Number of anthracnose lesions outside the incision:** the number of dark brown to black lesions with more than 2 mm in length that formed on the epidermis of the fruits after being sprayed with the spore suspension was counted.

### 2.2.6. Experiment design

A completely randomized unifactorial design was used with seven repetitions per treatment, with incidence, diameter of the anthracnose lesion and number of lesions outside the incision as response variables, where one wound was the experimental unit. An analysis of variance (ANOVA) and comparison of means (Tukey;  $p \leq 0.05$ ) were made using SAS (SAS Institute, Inc., 2010).

### 3. RESULTADOS

#### 3.1. In vitro biological effectiveness tests

Ten new strains of *Trichoderma* were isolated, six of which showed abundant growth of mycelium and rapid sporulation. It was observed that the *Trichoderma* strains presented a strong antagonism against the *Colletotrichum* fungus, these results indicate that the beneficial fungus produces certain metabolites that inhibit the development of the phytopathogen. Regarding antagonistic bacteria, 15 new strains were isolated, of which four showed strong antagonism (decreased growth and development of the phytopathogen) against virulent strains of *Colletotrichum*.

#### 3.2. Applications of Biological Agents in the Field Phase in Estación Nanchi (Orchard 1)

In all the evaluated treatments, no anthracnose symptoms were observed in panicles, the incidence was only observed in fruits. In the results of the first evaluation in the Estación Nanchi, no incidences of anthracnose greater than 9 % were observed in fruits of more than 1.5 cm in diameter in control fruits. The treatments with antagonistic bacteria that showed lower incidences (equal to or less than 3%) were T3, T4, T9 and T10. On the other hand, the treatments with the *Trichoderma* fungus that showed incidence percentages similar to that of the aforementioned bacteria were T13 and T14. The fruits treated with the commercial product Fungifree™ presented incidences of less than 3 % (Figure 1). In the second evaluation, the incidence in control fruits was around 12 %. The fruits treated with antagonistic bacteria that showed incidences of less than 5 % were T3, T4, T6 and T10. While the shoots with *Trichoderma* T14 and T15 presented incidences close to 5%. The fruits treated with the Fungifree™ product presented incidences close to 4 % (Figure 2). For the third evaluation, the control fruits obtained the highest incidence with 14 % of anthracnose (Figure 3). In the general evaluation, the control fruits presented an incidence of anthracnose of 9.5 %, while the fruits of the treatments with bacteria (T3, T4, T6 and T10), *Trichodermas* (T14 and T15) and Fungifree™ showed lower incidences of anthracnose than 5.5% (Figure 4).

Regarding the efficacy of anthracnose control, it was determined that the treatment with T6 bacteria had an efficacy of 70 %, while T3, T4 and T10 showed efficiencies close to 65 %. On the other hand, the treatments with *Trichodermas* T14 and T15 had efficiencies of around 60 %. While the fruits treated with Fungifree™ presented an efficacy of 67 %. The rest of the treatments showed efficiencies of less than 55 % (Figure 5).

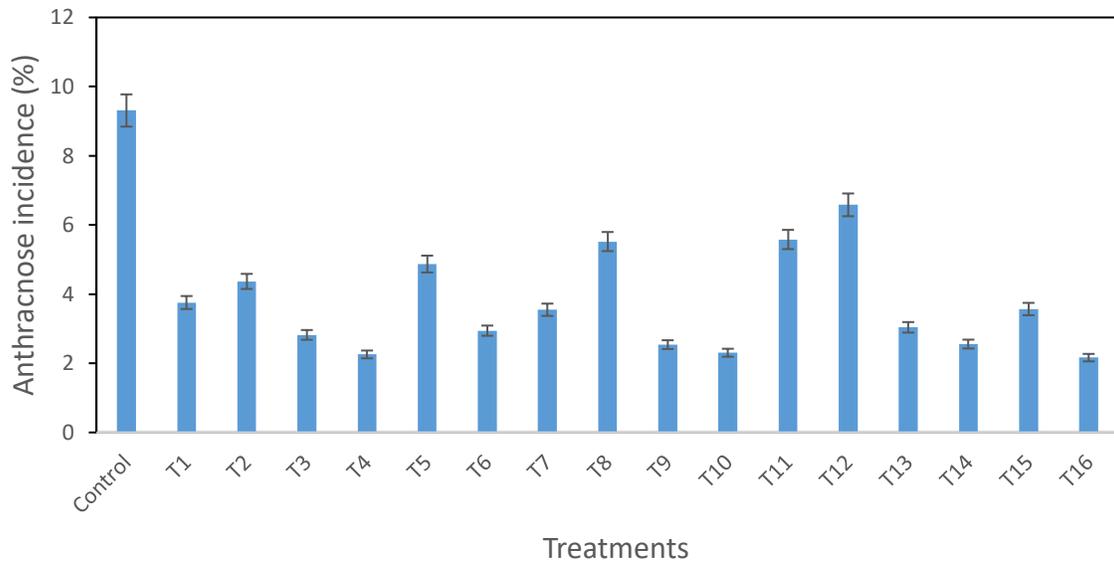


Figure 1. First evaluation of the incidence of anthracnose in fruits of a commercial "Ataulfo" mango orchard in the common of Estación Nanchi, Municipality of Santiago Ixcuintla, Nayarit. Orchard 1. Treatments: Control; T1= Bac-22 (10 mL/L); T2= Bac-23 (10 mL/L); T3= Bac-Ps3 (10 mL/L); T4= Bac-Ps7 (10 mL/L); T5= Bac-Ps1 (10 mL/L); T6= Bac-17 (10 mL/L); T7= Bac-18 (10 mL/L); T8= Bac-Bsf6a (10 mL/L); T9= Bac-4 (10 mL/L); T10= Bac-15 (10 mL/L); T11= Tricho-Cesix (10 mL/L); T12= Tricho-Pino (10 mL/L); T13= Tricho-16 Los Mochis (10 mL/L); T14= Tricho-6 Volcán (10 mL/L); T15= Tricho-1 Cesix (10 mL/L) T16= Fungifree (10 g/L).

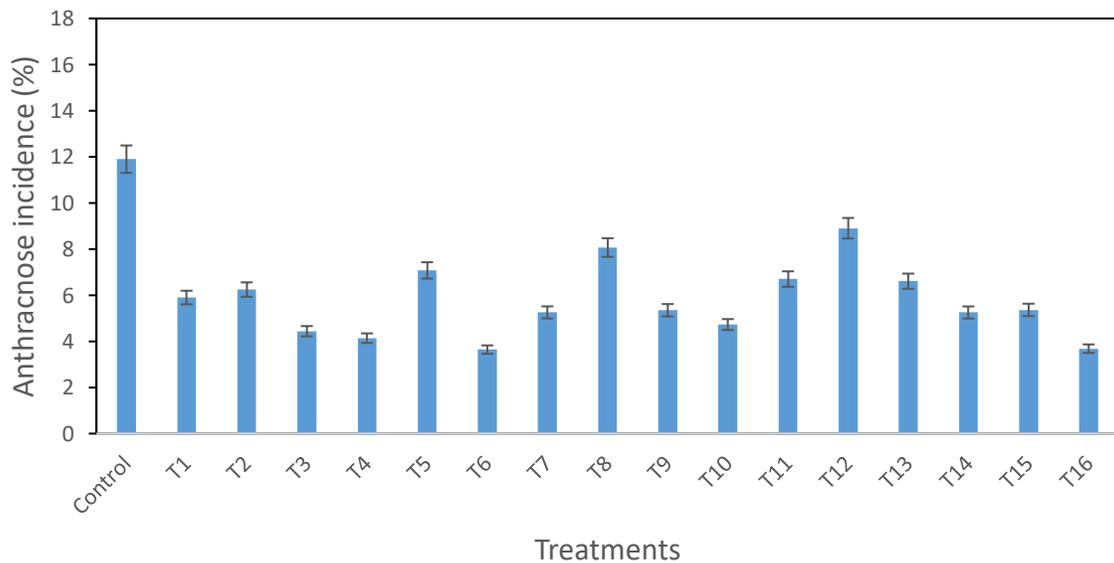


Figure 2. Second evaluation of the incidence of anthracnose in fruits of a commercial "Ataulfo" mango orchard in the common land of Estación Nanchi, Municipality of Santiago Ixcuintla, Nayarit. Orchard 1. Treatments: Control; T1= Bac-22 (10 mL/L); T2= Bac-23 (10 mL/L); T3= Bac-Ps3 (10 mL/L); T4= Bac-Ps7 (10 mL/L); T5= Bac-Ps1 (10 mL/L); T6= Bac-17 (10 mL/L); T7= Bac-18 (10 mL/L); T8= Bac-Bsf6a (10 mL/L); T9= Bac-4 (10 mL/L); T10= Bac-15 (10 mL/L); T11= Tricho-Cesix (10 mL/L); T12= Tricho-Pino (10 mL/L); T13= Tricho-16 Los Mochis (10 mL/L); T14= Tricho-6 Volcán (10 mL/L); T15= Tricho-1 Cesix (10 mL/L) T16= Fungifree (10 g/L).

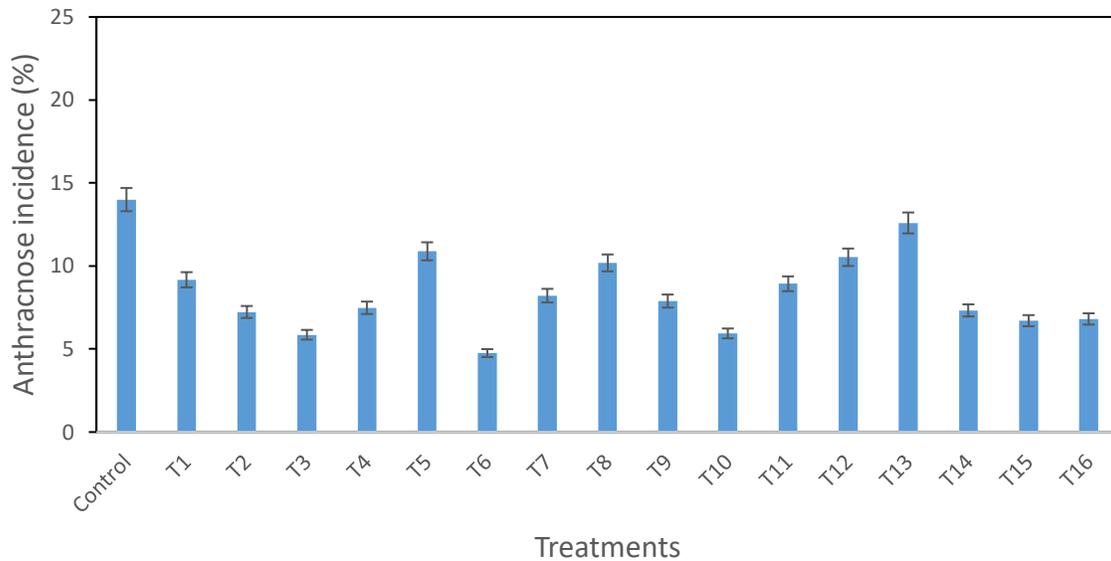


Figure 3. Third evaluation of the incidence of anthracnose in fruits of a commercial "Ataulfo" mango orchard in the common of Estación Nanchi, Municipality of Santiago Ixcuintla, Nayarit. Orchard 1. Treatments: Control; T1= Bac-22 (10 mL/L); T2= Bac-23 (10 mL/L); T3= Bac-Ps3 (10 mL/L); T4= Bac-Ps7 (10 mL/L); T5= Bac-Ps1 (10 mL/L); T6= Bac-17 (10 mL/L); T7= Bac-18 (10 mL/L); T8= Bac-Bsf6a (10 mL/L); T9= Bac-4 (10 mL/L); T10= Bac-15 (10 mL/L); T11= Tricho-Cesix (10 mL/L); T12= Tricho-Pino (10 mL/L); T13= Tricho-16 Los Mochis (10 mL/L); T14= Tricho-6 Volcán (10 mL/L); T15= Tricho-1 Cesix (10 mL/L) T16= Fungifree (10 g/L).

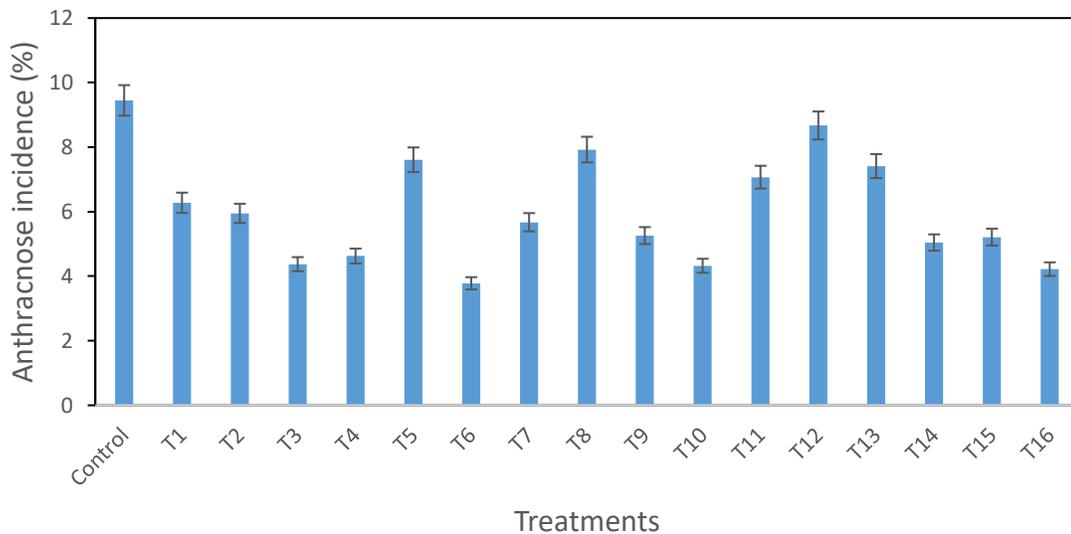


Figure 4. General evaluation of the incidence of anthracnose in fruits of a commercial "Ataulfo" mango orchard in the common of Estación Nanchi, Municipality of Santiago Ixcuintla, Nayarit. Orchard 1. Treatments: Control; T1= Bac-22 (10 mL/L); T2= Bac-23 (10 mL/L); T3= Bac-Ps3 (10 mL/L); T4= Bac-Ps7 (10 mL/L); T5= Bac-Ps1 (10 mL/L); T6= Bac-17 (10 mL/L); T7= Bac-18 (10 mL/L); T8= Bac-Bsf6a (10 mL/L); T9= Bac-4 (10 mL/L); T10= Bac-15 (10 mL/L); T11= Tricho-Cesix (10 mL/L); T12= Tricho-Pino (10 mL/L); T13= Tricho-16 Los Mochis (10 mL/L); T14= Tricho-6 Volcán (10 mL/L); T15= Tricho-1 Cesix (10 mL/L) T16= Fungifree (10 g/L).

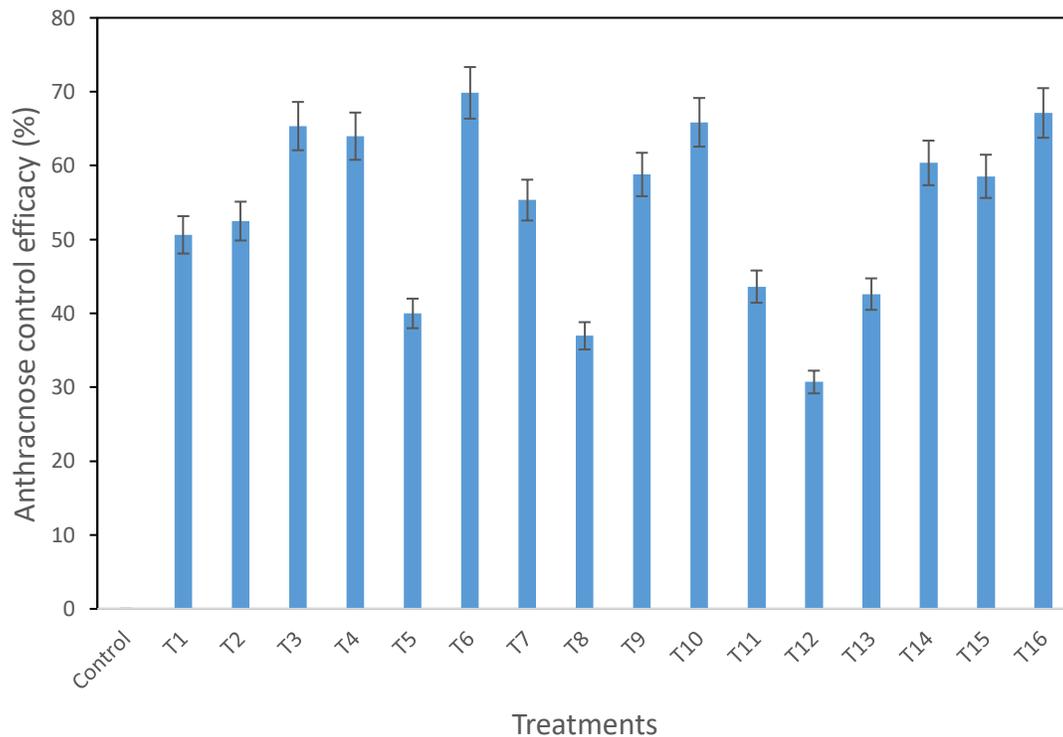


Figure 5. Efficacy of control of anthracnose in fruits of a commercial "Ataulfo" mango orchard in the common land of Estación Nanchi, Municipality of Santiago Ixcuintla, Nayarit. Orchard 1 (general evaluation). Treatments: Control; T1= Bac-22 (10 mL/L); T2= Bac-23 (10 mL/L); T3= Bac-Ps3 (10 mL/L); T4= Bac-Ps7 (10 mL/L); T5= Bac-Ps1 (10 mL/L); T6= Bac-17 (10 mL/L); T7= Bac-18 (10 mL/L); T8= Bac-Bsf6a (10 mL/L); T9= Bac-4 (10 mL/L); T10= Bac-15 (10 mL/L); T11= Tricho-Cesix (10 mL/L); T12= Tricho-Pino (10 mL/L); T13= Tricho-16 Los Mochis (10 mL/L); T14= Tricho-6 Volcán (10 mL/L); T15= Tricho-1 Cesix (10 mL/L) T16= Fungifree (10 g/L).

### 3.3. Applications of Biological Agents in the Field Phase in Valle Lerma (Orchard 2)

In all the evaluated treatments, no anthracnose symptoms were observed in panicles, the incidence was only observed in fruits. In the results of the first evaluation in Valle Lerma, no incidences of anthracnose greater than 11 % were observed in the control fruits. In the mixed treatments with antagonistic bacteria and *Trichoderma* that showed lower incidences (equal to or less than 2%) were T19, T22, T23, T24 and T25. On the other hand, the treatments with the *Trichoderma* fungus that showed similar incidence percentages were T27, T28, T29 and T31. Likewise, the treatments with only bacteria that showed similar incidences to the aforementioned treatments were T33, T34, T35 and T37. The fruits treated with Fungifree™ presented incidences of 2.4 % (Figure 6). In the second evaluation, the incidence in control fruits was around 14 %. The fruits treated with antagonistic bacteria and *Trichoderma* that showed incidences of less than 5 % were T17, T19, T20, T21 and T26, while the treatments with only *Trichoderma* T29, T30 and T31 presented incidences close to 5.5 %. On the other hand, the treatments with T36 and T33 bacteria presented incidences of 3.8 and 5.5 % respectively. The fruits treated with the Fungifree™ product presented incidences close to 7 % (Figure 7). For the third evaluation, the control fruits obtained the highest incidence with 17 % of anthracnose. The fruits treated with antagonistic bacteria and *Trichoderma* T17, T20 and T26 presented lower incidences with 5.5, 6.5 and 8.7 % respectively. In turn, the treatments with *Trichoderma* T30 and T27 showed incidences of 7.7 to 9.6 %. While the treatments with T36, T34 and T33 bacteria presented incidences of 5.1, 7.7 and 8.0 % respectively. The fruits treated with the Fungifree™ product presented an incidence of 11 % (Figure 8). In the general evaluation, the control fruits presented an anthracnose incidence of 12.5 %. The fruits of the treatments with bacteria and *Trichoderma* (T17, T20 and T26), *Trichoderma* (T29, T30 and T31) and bacteria alone (T36, T33 and T35) showed incidences of anthracnose less than 6 %. The commercial product Fungifree™ had an incidence of 7% (Figure 9).

Regarding the effectiveness of anthracnose control, it was determined that the treatment with *Trichoderma* T36, and the mixture of bacteria with *Trichoderma* T36 presented control efficiencies of 69 and 70% respectively. Other treatments that had efficiencies of 55 to 60% were T20, T26, T29, T30, T32, T31, T33, and T34. While the fruits treated with Fungifree™ presented an efficacy of 46 %. The rest of the treatments showed efficiencies of less than 53 % (Figure 10).

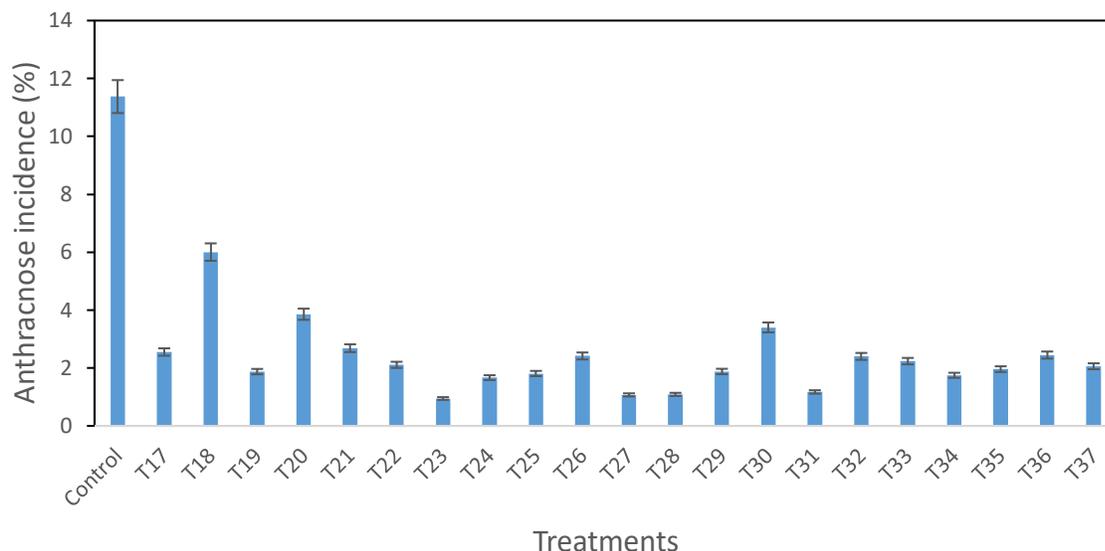


Figure 6. First evaluation of the incidence of anthracnose in a commercial “Ataulfo” mango orchard in the Valle Lerma ejido, Municipality of Santiago Ixcuintla, Nayarit. Orchard 2. Treatments:Control; T17= Bac-22 y Tricho-Cesix (10 + 10 mL/L); T18= Bac-23 y Tricho-Pino (10 + 10 mL/L); T19= Bac-Ps3 y Tricho-16 Los Mochis (10 + 10 mL/L); T20= Bac-Ps7 y Tricho-6 Volcán (10 + 10 mL/L); T21= Bac-Ps1 y Tricho-Cesix 1 (10 + 10 mL/L); T22= Bac-17 y Tricho-Cesix 2 (10 + 10 mL/L); T23= Bac-18 y Tricho-Cesix 3 (10 + 10 mL/L); T24= Bac-Bsf6a y Tricho-Cesix 4 (10 + 10 mL/L); T25= Bac-4 y Tricho-5 hule (10 + 10 mL/L); T26= Bac-15 y Tricho-6 hule (10 + 10 mL/L); T27= Tricho-Cesix 2 (10 mL/L); T28= Tricho-Cesix 3 (10 mL/L); T29= Tricho-Cesix 4 (10 mL/L); T30= Tricho-5 hule (10 mL/L); T31= Tricho-6 hule (10 mL/L) T32= Fungifree (10 g/L) T33= Bac-6 (10 mL/L); T34= Bac-Ps13 (10 mL/L); T35= Bac-19 (10 mL/L); T36= Bac-21 (10 mL/L);T37= Bac-25 (10 mL/L).

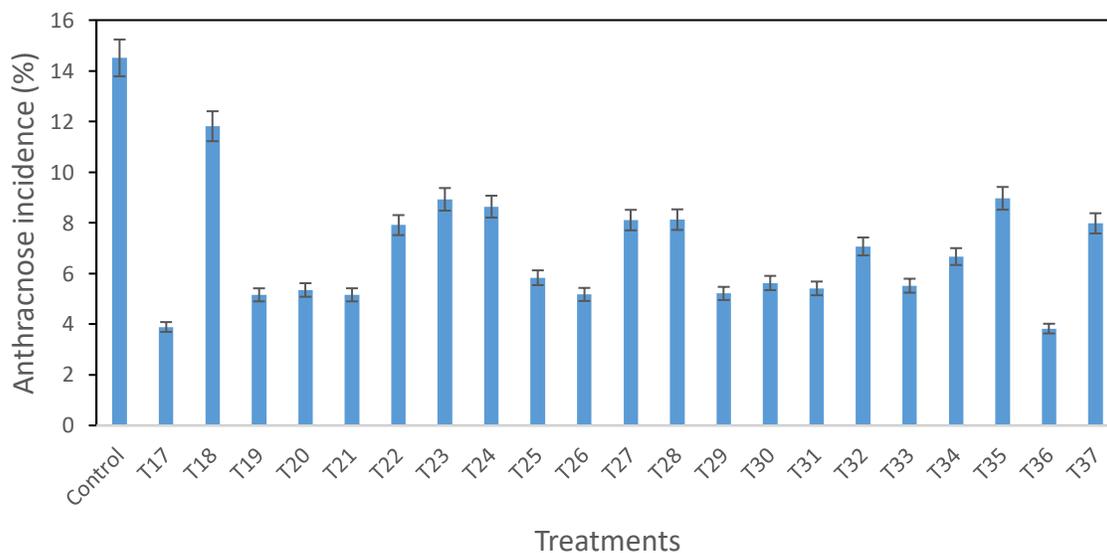


Figure 7. Second evaluation of the incidence of anthracnose in a commercial “Ataulfo” mango orchard in the Valle Lerma ejido, Municipality of Santiago Ixcuintla, Nayarit. Orchard 2. Treatments:Control; T17= Bac-22 y Tricho-Cesix (10 + 10 mL/L); T18= Bac-23 y Tricho-Pino (10 + 10 mL/L); T19= Bac-Ps3 y Tricho-16 Los Mochis (10 + 10 mL/L); T20= Bac-Ps7 y Tricho-6 Volcán (10 + 10 mL/L); T21= Bac-Ps1 y Tricho-Cesix 1 (10 + 10 mL/L); T22= Bac-17 y Tricho-Cesix 2 (10 + 10 mL/L); T23= Bac-18 y Tricho-Cesix 3 (10 + 10 mL/L); T24= Bac-Bsf6a y Tricho-Cesix 4 (10 + 10 mL/L); T25= Bac-4 y Tricho-5 hule (10 + 10 mL/L); T26= Bac-15 y Tricho-6 hule (10 + 10 mL/L); T27= Tricho-Cesix 2 (10 mL/L); T28= Tricho-Cesix 3 (10 mL/L); T29= Tricho-Cesix 4 (10 mL/L); T30= Tricho-5 hule (10 mL/L); T31= Tricho-6 hule (10 mL/L) T32= Fungifree (10 g/L) T33= Bac-6 (10 mL/L); T34= Bac-Ps13 (10 mL/L); T35= Bac-19 (10 mL/L); T36= Bac-21 (10 mL/L);T37= Bac-25 (10 mL/L).

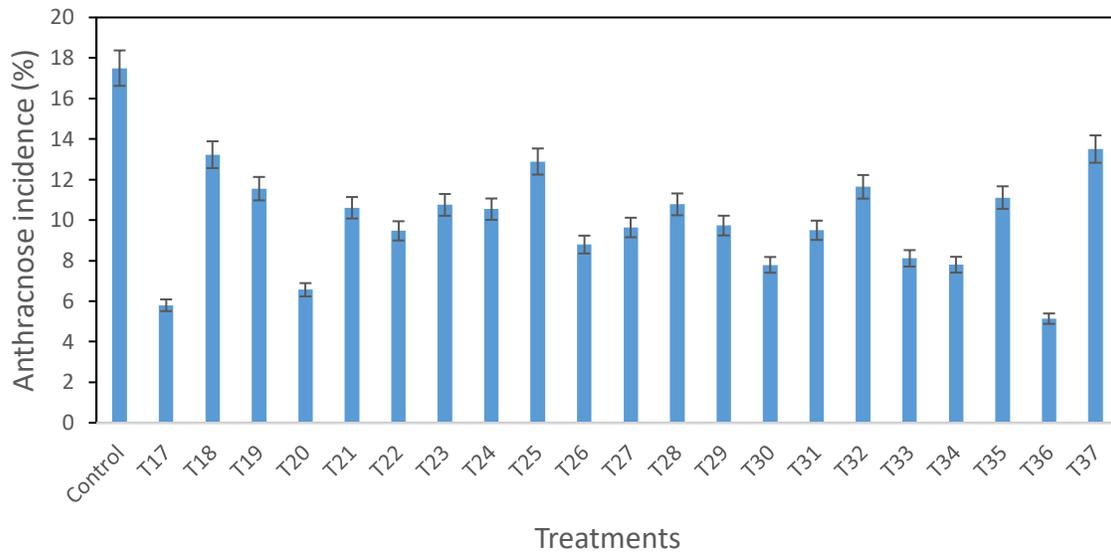


Figure 8. Third evaluation of the incidence of anthracnose in a commercial “Ataulfo” mango orchard in the Valle Lerma ejido, Municipality of Santiago Ixcuintla, Nayarit. Orchard 2. Tratamientos: Control; T17= Bac-22 y Tricho-Cesix (10 + 10 mL/L); T18= Bac-23 y Tricho-Pino (10 + 10 mL/L); T19= Bac-Ps3 y Tricho-16 Los Mochis (10 + 10 mL/L); T20= Bac-Ps7 y Tricho-6 Volcán (10 + 10 mL/L); T21= Bac-Ps1 y Tricho-Cesix 1 (10 + 10 mL/L); T22= Bac-17 y Tricho-Cesix 2 (10 + 10 mL/L); T23= Bac-18 y Tricho-Cesix 3 (10 + 10 mL/L); T24= Bac-Bsf6a y Tricho-Cesix 4 (10 + 10 mL/L); T25= Bac-4 y Tricho-5 hule (10 + 10 mL/L); T26= Bac-15 y Tricho-6 hule (10 + 10 mL/L); T27= Tricho-Cesix 2 (10 mL/L); T28= Tricho-Cesix 3 (10 mL/L); T29= Tricho-Cesix 4 (10 mL/L); T30= Tricho-5 hule (10 mL/L); T31= Tricho-6 hule (10 mL/L) T32= Fungifree (10 g/L) T33= Bac-6 (10 mL/L); T34= Bac-Ps13 (10 mL/L); T35= Bac-19 (10 mL/L); T36= Bac-21 (10 mL/L); T37= Bac-25 (10 mL/L).

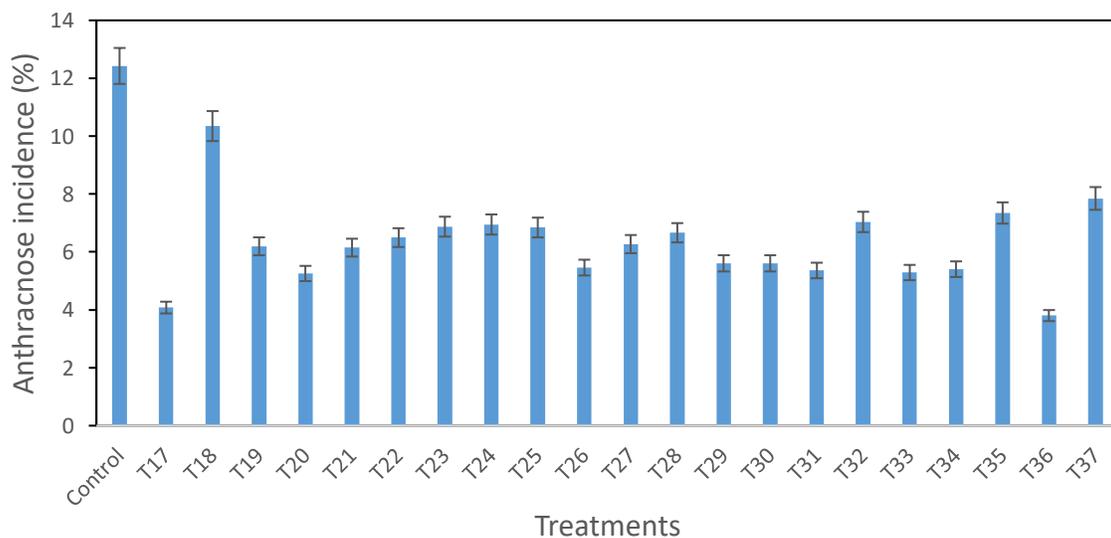


Figure 9. General evaluation of the incidence of anthracnose in a commercial “Ataulfo” mango orchard in the Valle Lerma ejido, Municipality of Santiago Ixcuintla, Nayarit. Orchard 2. Treatments: Control; T17= Bac-22 y Tricho-Cesix (10 + 10 mL/L); T18= Bac-23 y Tricho-Pino (10 + 10 mL/L); T19= Bac-Ps3 y Tricho-16 Los Mochis (10 + 10 mL/L); T20= Bac-Ps7 y Tricho-6 Volcán (10 + 10 mL/L); T21= Bac-Ps1 y Tricho-Cesix 1 (10 + 10 mL/L); T22= Bac-17 y Tricho-Cesix 2 (10 + 10 mL/L); T23= Bac-18 y Tricho-Cesix 3 (10 + 10 mL/L); T24= Bac-Bsf6a y Tricho-Cesix 4 (10 + 10 mL/L); T25= Bac-4 y Tricho-5 hule (10 + 10 mL/L); T26= Bac-15 y Tricho-6 hule (10 + 10 mL/L); T27= Tricho-Cesix 2 (10 mL/L); T28= Tricho-Cesix 3 (10 mL/L); T29= Tricho-Cesix 4 (10 mL/L); T30= Tricho-5 hule (10 mL/L); T31= Tricho-6 hule (10 mL/L) T32= Fungifree (10 g/L) T33= Bac-6 (10 mL/L); T34= Bac-Ps13 (10 mL/L); T35= Bac-19 (10 mL/L); T36= Bac-21 (10 mL/L); T37= Bac-25 (10 mL/L).

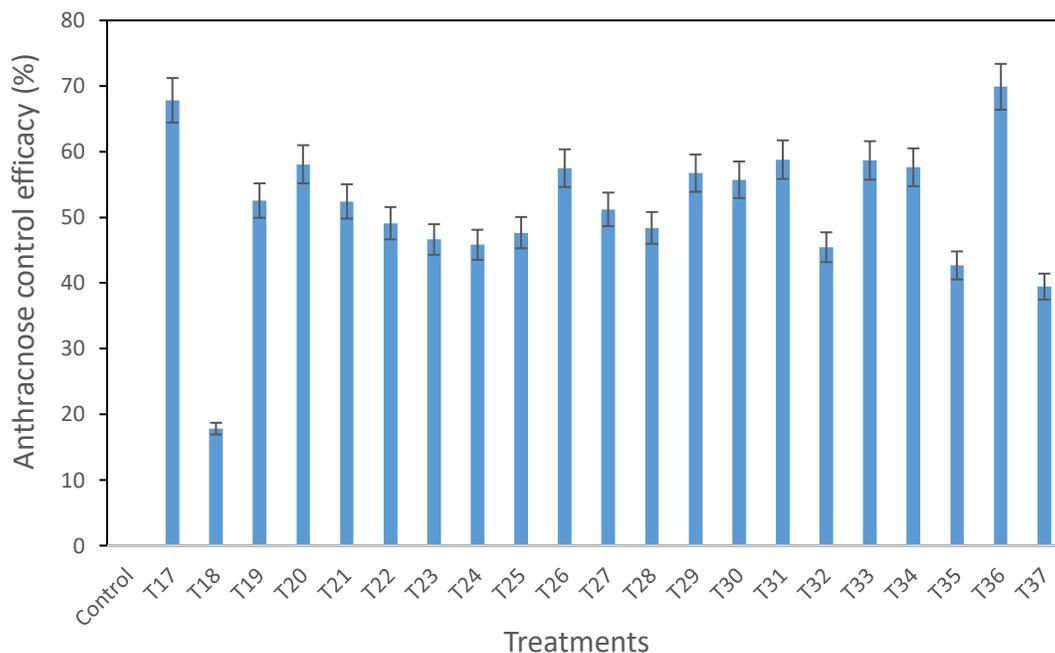


Figure 10. Efficacy of control of anthracnose in fruits of commercial mango orchard "Ataulfo" in the ejido of Valle Lerma, Municipality of Santiago Ixcuintla, Nayarit. Orchard 2. Treatments: Control; T17= Bac-22 y Tricho-Cesix (10 + 10 mL/L); T18= Bac-23 y Tricho-Pino (10 + 10 mL/L); T19= Bac-Ps3 y Tricho-16 Los Mochis (10 + 10 mL/L); T20= Bac-Ps7 y Tricho-6 Volcán (10 + 10 mL/L); T21= Bac-Ps1 y Tricho-Cesix 1 (10 + 10 mL/L); T22= Bac-17 y Tricho-Cesix 2 (10 + 10 mL/L); T23= Bac-18 y Tricho-Cesix 3 (10 + 10 mL/L); T24= Bac-Bsf6a y Tricho-Cesix 4 (10 + 10 mL/L); T25= Bac-4 y Tricho-5 hule (10 + 10 mL/L); T26= Bac-15 y Tricho-6 hule (10 + 10 mL/L); T27= Tricho-Cesix 2 (10 mL/L); T28= Tricho-Cesix 3 (10 mL/L); T29= Tricho-Cesix 4 (10 mL/L); T30= Tricho-5 hule (10 mL/L); T31= Tricho-6 hule (10 mL/L); T32= Fungifree (10 g/L); T33= Bac-6 (10 mL/L); T34= Bac-Ps13 (10 mL/L); T35= Bac-19 (10 mL/L); T36= Bac-21 (10 mL/L); T37= Bac-25 (10 mL/L).

### **3.4. Applications of biological agents in the postharvest phase in mango fruits of the "Ataulfo" variety.**

#### **Incidence of anthracnose**

The highest incidence was registered by the control fruits with 98 % of the incisions with anthracnose symptoms. Of the treatments with bacteria that showed the lowest incidence were T2 at 10 mL/L (32 %), T8 at 5 mL/L (37 %), and T12 at both doses (40 and 48 %). Regarding *Trichoderma*, the lowest incidences occurred in T18 at 5 mL (35 %), T25 in both doses (37 and 38%), and T19 at 10 mL (43%). The incidence in commercial products in both doses (5 and 10 mL) was 34-37% for Fungifree™, 37-22 % in Serenade™, and 50-35% for Stargus™ (Figure 11).

#### **Anthracnose lesion diameter**

The control fruits registered the largest diameter of the lesion with 7.3 mm. The treatments with bacteria that presented the smallest diameters were T2 at 10 mL/L (2.5 mm), T8 at 5 mL/L (2.8 mm), T12 at 5 mL (2.8 mm) and T7 at 10 mL (3.2 mm). While the treatments with *Trichoderma* that showed smaller diameters were T18 at 5 mL (2.6 mm) and T25 in both doses (3.3-2.9 mm). On the other hand, the diameter of the lesion in commercial products in the two doses was 2.5 to 2.7 mm for Fungifree™, 3 to 5 mm in Serenade™, and 3.0 to 2.4 cm for Stargus™ (Figure 12).

#### **Number of lesions outside the incision**

The control fruits registered the highest number of anthracnose lesions on the epidermis with an average of 165 lesions per fruit. The treatments with bacteria were those that presented the least number of lesions in the 10 mL/L dose; T10 (14 lesions), T12 (26 lesions), T14 (26 lesions), T13 (33 lesions), T8 (44 lesions) and T9 (45 lesions). In the fruits treated with *Trichoderma*, the treatments T19, T21 and T25 at 10 mL obtained the lowest number of lesions with 65, 74 and 78 respectively. Regarding the commercial products in both doses, Fungifree™ presented from 103 to 109 lesions, Serenade™ (75 to 137 lesions) and from 73 to 82 lesions for Stargus™ (Figure 13).

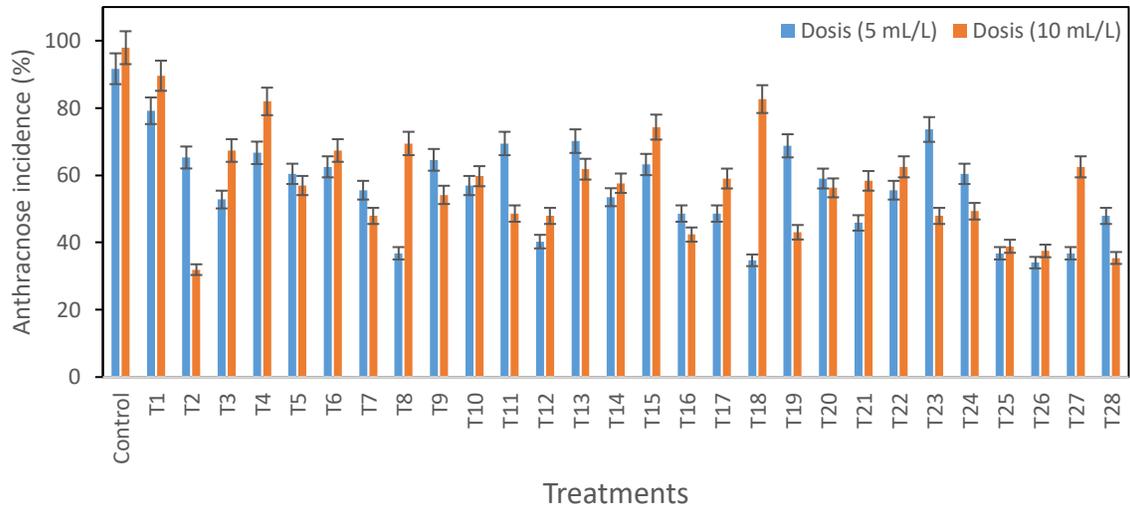


Figure 11. Incidence of anthracnose in postharvest "Ataulfo" mango fruits. Treatments: Control; T1= Bac-22; T2= Bac-23; T3= Bac-Ps3; T4= Bac-Ps7; T5= Bac-Ps1; T6= Bac-17; T7= Bac-18; T8= Bac-Bsf6a; T9= Bac-4; T10= Bac-15; T11= Bac-6; T12= Bac-Ps13; T13= Bac-19; T14= Bac-21; T15= Bac-25; T16= Tricho-Cesix; T17= Tricho-Pino; T18= Tricho-16 Los Mochis; T19= Tricho-6 volcán; T20= Tricho-Cesix 1; T21= Tricho-Cesix 2; T22= Tricho-Cesix 3; T23= Tricho-Cesix 4; T24= Tricho-5 hule; T25= Tricho-6 hule; T26= Fungifree; T27= Serenade; T28= Stargus.

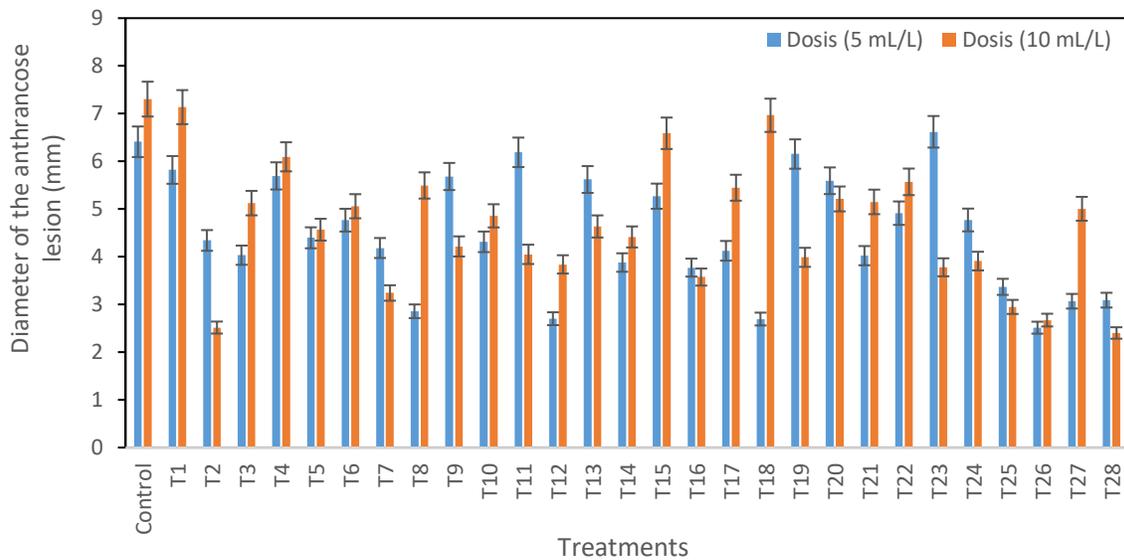


Figure 12. Diameter of the anthracnose lesion in postharvest "Ataulfo" mango fruits. Treatments: Control; T1= Bac-22; T2= Bac-23; T3= Bac-Ps3; T4= Bac-Ps7; T5= Bac-Ps1; T6= Bac-17; T7= Bac-18; T8= Bac-Bsf6a; T9= Bac-4; T10= Bac-15; T11= Bac-6; T12= Bac-Ps13; T13= Bac-19; T14= Bac-21; T15= Bac-25; T16= Tricho-Cesix; T17= Tricho-Pino; T18= Tricho-16 Los Mochis; T19= Tricho-6 volcán; T20= Tricho-Cesix 1; T21= Tricho-Cesix 2; T22= Tricho-Cesix 3; T23= Tricho-Cesix 4; T24= Tricho-5 hule; T25= Tricho-6 hule; T26= Fungifree; T27= Serenade; T28= Stargus.

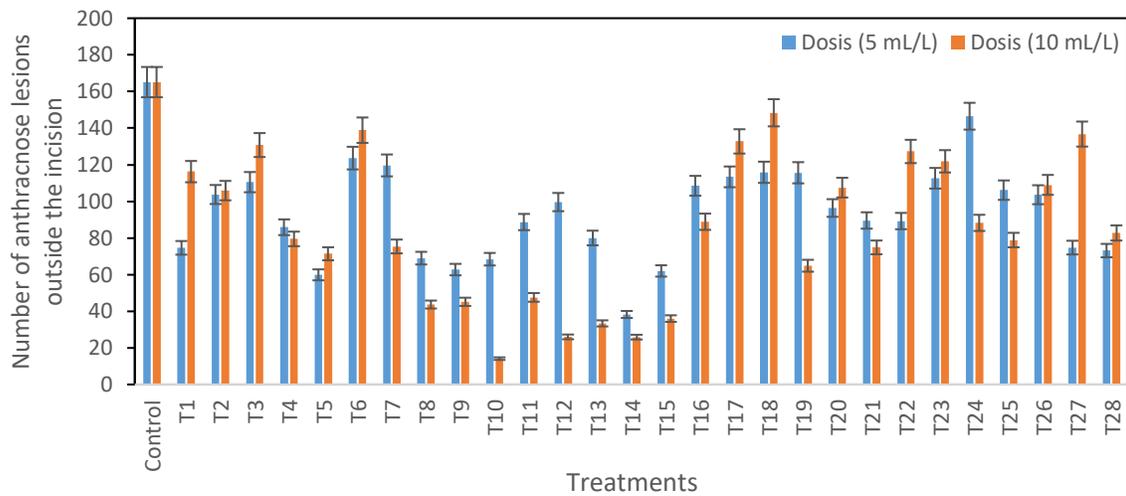


Figure 13. Number of anthracnose lesions outside the incision in postharvest "Ataulfo" mango fruits. Treatments: Control; T1= Bac-22; T2= Bac-23; T3= Bac-Ps3; T4= Bac-Ps7; T5= Bac-Ps1; T6= Bac-17; T7= Bac-18; T8= Bac-Bsf6a; T9= Bac-4; T10= Bac-15; T11= Bac-6; T12= Bac-Ps13; T13= Bac-19; T14= Bac-21; T15= Bac-25; T16= Tricho-Cesix; T17= Tricho-Pino; T18= Tricho-16 Los Mochis; T19= Tricho-6 volcán; T20= Tricho-Cesix 1; T21= Tricho-Cesix 2; T22= Tricho-Cesix 3; T23= Tricho-Cesix 4; T24= Tricho-5 hule; T25= Tricho-6 hule; T26= Fungifree; T27= Serenade; T28= Stargus.

### **3.5. Applications of biological agents in the postharvest phase in mango fruits of the "Tommy Atkins" variety.**

#### **Incidence of anthracnose**

The highest incidence was registered by the control fruits with 100 % of the incisions with anthracnose symptoms. Of the treatments with bacteria that showed the lowest incidence were T13 at 10 mL/L (35 %), T14 at both doses (46 and 43 %), T8 at 5 mL (52 %), and T9 at 5 mL (52%). The rest of the treatments with bacteria, *Trichoderma* and the commercial products Fungifree™, Serenade™ and Stargus™ showed incidences greater than 60 % (Figure 14).

#### **Anthracnose lesion diameter**

The control fruits registered a lesion diameter of 8 mm, however, the treatments T16, T22 and T23 presented diameters similar to or greater than the control fruits. The treatments with bacteria that presented the smallest diameters were T13 at 10 mL/L (1.9 mm), T14 in the two doses (2.7 and 2.9 mm), T11 at 10 mL (3 mm), T8 and T9 at 5 mL (3.5 mm) and T10 to 10 mL (3.9 mm). While the *Trichoderma* treatments that showed diameters less than 5 mm were T22, T24 and T25. On the other hand, the diameter of the lesion in commercial products in the two doses was 6.2 to 4.3 mm for Fungifree™, 7.0 to 6.7 mm in Serenade™, and 6.0 to 4.0 mm for Stargus™ (Figure 15).

#### **Number of lesions outside the incision**

The control fruits registered the highest number of anthracnose lesions on the epidermis with an average of 74 lesions per fruit, however, the treatments T8, T9, T10, T11, T17, T19, T23 and T26 had more than 60 lesions per fruit. On the other hand, the treatments that obtained less than 45 lesions per fruit were the treatments with T13, T14 and T15 bacteria, and with *Trichoderma* T21 and T24. Regarding the commercial products in both doses, the number of lesions was equal to or greater than 50 (Figure 16).

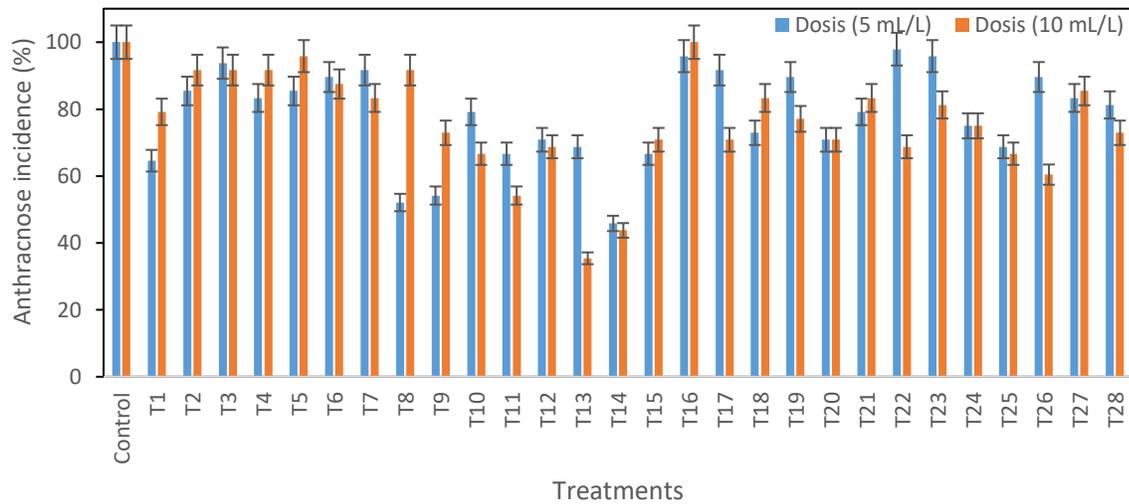


Figure 14. Incidence of anthracnose in postharvest "Tommy Atkins" mango fruits. Treatments: Control; T1= Bac-22; T2= Bac-23; T3= Bac-Ps3; T4= Bac-Ps7; T5= Bac-Ps1; T6= Bac-17; T7= Bac-18; T8= Bac-Bsf6a; T9= Bac-4; T10= Bac-15; T11= Bac-6; T12= Bac-Ps13; T13= Bac-19; T14= Bac-21; T15= Bac-25; T16= Tricho-Cesix; T17= Tricho-Pino; T18= Tricho-16 Los Mochis; T19= Tricho-6 volcán; T20= Tricho-Cesix 1; T21= Tricho-Cesix 2; T22= Tricho-Cesix 3; T23= Tricho-Cesix 4; T24= Tricho-5 hule; T25= Tricho-6 hule; T26= Fungifree; T27= Serenade; T28= Stargus.

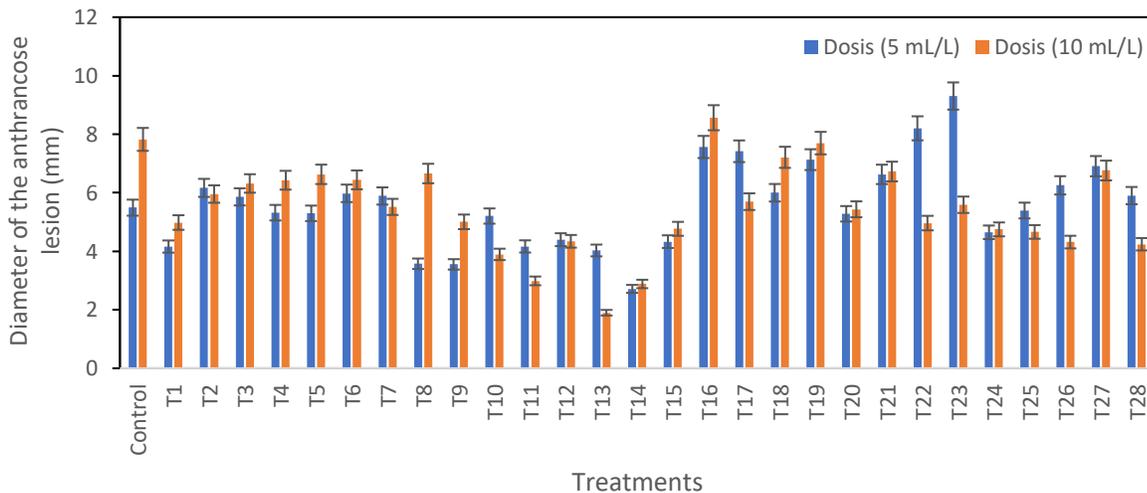


Figure 15. Diameter of the anthracnose lesion in "Tommy Atkins" mango fruits in postharvest. Tratamientos: Control; T1= Bac-22; T2= Bac-23; T3= Bac-Ps3; T4= Bac-Ps7; T5= Bac-Ps1; T6= Bac-17; T7= Bac-18; T8= Bac-Bsf6a; T9= Bac-4; T10= Bac-15; T11= Bac-6; T12= Bac-Ps13; T13= Bac-19; T14= Bac-21; T15= Bac-25; T16= Tricho-Cesix; T17= Tricho-Pino; T18= Tricho-16 Los Mochis; T19= Tricho-6 volcán; T20= Tricho-Cesix 1; T21= Tricho-Cesix 2; T22= Tricho-Cesix 3; T23= Tricho-Cesix 4; T24= Tricho-5 hule; T25= Tricho-6 hule; T26= Fungifree; T27= Serenade; T28= Stargus.

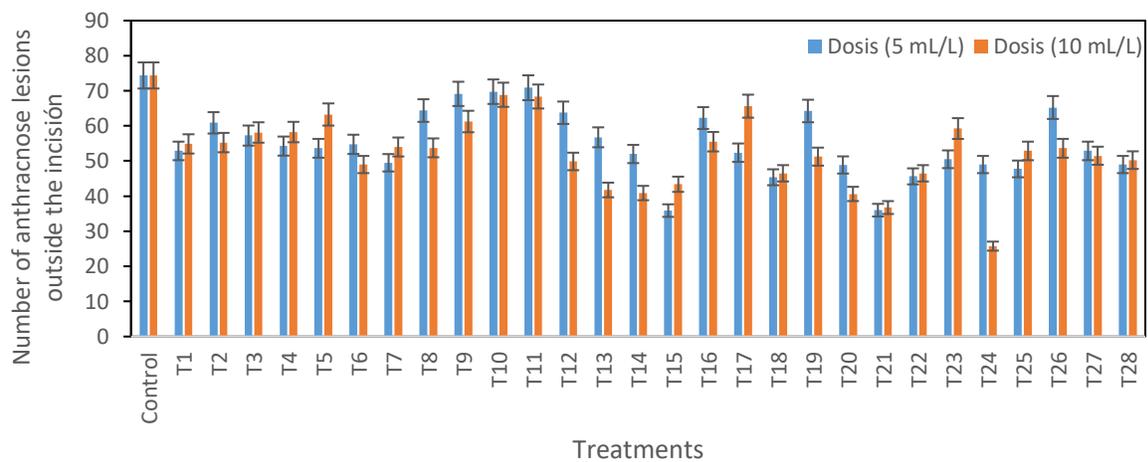


Figure 16. Number of anthracnose lesions outside the incision in postharvest "Tommy Atkins" mango fruits. Treatments: Control; T1= Bac-22; T2= Bac-23; T3= Bac-Ps3; T4= Bac-Ps7; T5= Bac-Ps1; T6= Bac-17; T7= Bac-18; T8= Bac-Bsf6a; T9= Bac-4; T10= Bac-15; T11= Bac-6; T12= Bac-Ps13; T13= Bac-19; T14= Bac-21; T15= Bac-25; T16= Tricho-Cesix; T17= Tricho-Pino; T18= Tricho-16 Los Mochis; T19= Tricho-6 volcán; T20= Tricho-Cesix 1; T21= Tricho-Cesix 2; T22= Tricho-Cesix 3; T23= Tricho-Cesix 4; T24= Tricho-5 hule; T25= Tricho-6 hule; T26= Fungifree; T27= Serenade; T28= Stargus.

### **3.6. Applications of biological agents in the postharvest phase in mango fruits of the "Keit" variety.**

#### **incidence of anthracnose**

The highest incidence was registered by the control fruits with more than 80 % of the incisions with anthracnose symptoms, however, some treatments showed incidences equal to or greater than 70 %, such is the case of T6, T7, T17, T18, T19 and T20. Of the treatments with bacteria that showed the lowest incidence were T12 at 10 mL/L (14 %) and T15 at 5 mL (41 %). The rest of the treatments, including bacteria, *Trichoderma* and the commercial products Fungifree™, Serenade™ and Stargus™ showed incidences greater than 50 % (Figure 17).

#### **Anthracnose lesion diameter**

The control fruits registered a lesion diameter of 4.5 mm, however, most of the treatments presented lesion diameters equal to or greater than the control fruits, reaching a maximum of 7 mm. Except for treatment T12 at 10 mL that presented 1 mm and T15 at 5 mL with 4 mm diameter lesions. On the other hand, the commercial products Fungifree™, Serenade™ and Stargus™ showed lesion diameters very similar to those of most treatments, between 4 and 5 mm (Figure 18).

#### **Number of lesions outside the incision**

The control fruits registered anthracnose lesions on the epidermis with an average of 152 lesions per fruit, however, there were treatments that showed the same or more lesions per fruit than the control fruits, such is the case of T13, T16, T17, T18 and T19. On the other hand, the treatments that presented the least number of lesions were *Trichoderma* T21 at 5 mL/L (57 lesions), T24 at both doses (59 to 61 lesions) and T25 at 5 mL (67 lesions). In the case of the bacteria that showed fewer lesions, in at least one of their doses, and that were in the range of 80 to 100 lesions, they were T5, T6, T7 and T11. Regarding the commercial products in both doses, the number of lesions was in the range of 90 to 140 lesions per fruit (Figure 19).

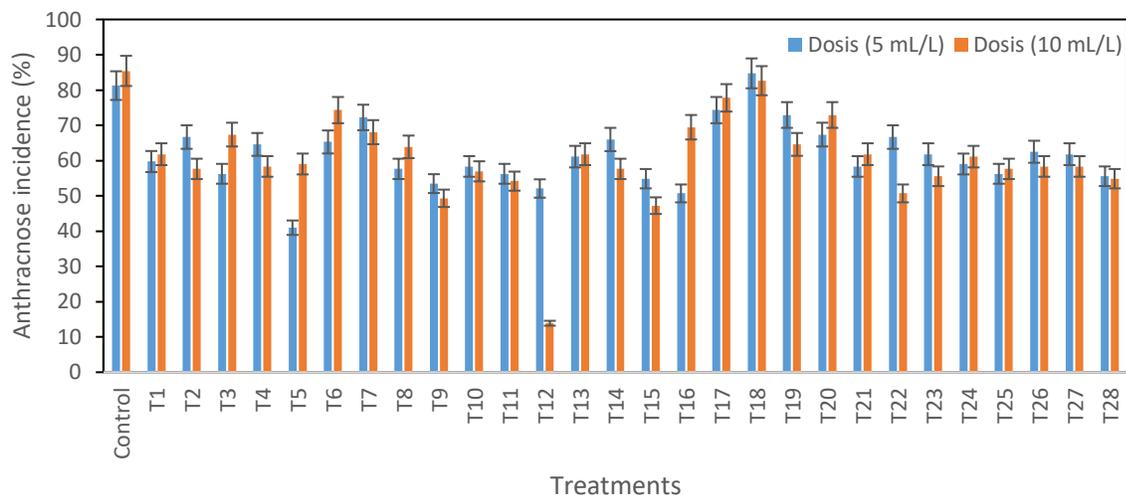


Figure 17. Incidence of anthracnose in “Keitt” mango fruits in postharvest. Treatments: Control; T1= Bac-22; T2= Bac-23; T3= Bac-Ps3; T4= Bac-Ps7; T5= Bac-Ps1; T6= Bac-17; T7= Bac-18; T8= Bac-Bsf6a; T9= Bac-4; T10= Bac-15; T11= Bac-6; T12= Bac-Ps13; T13= Bac-19; T14= Bac-21; T15= Bac-25; T16= Tricho-Cesix; T17= Tricho-Pino; T18= Tricho-16 Los Mochis; T19= Tricho-6 volcán; T20= Tricho-Cesix 1; T21= Tricho-Cesix 2; T22= Tricho-Cesix 3; T23= Tricho-Cesix 4; T24= Tricho-5 hule; T25= Tricho-6 hule; T26= Fungifree; T27= Serenade; T28= Stargus.

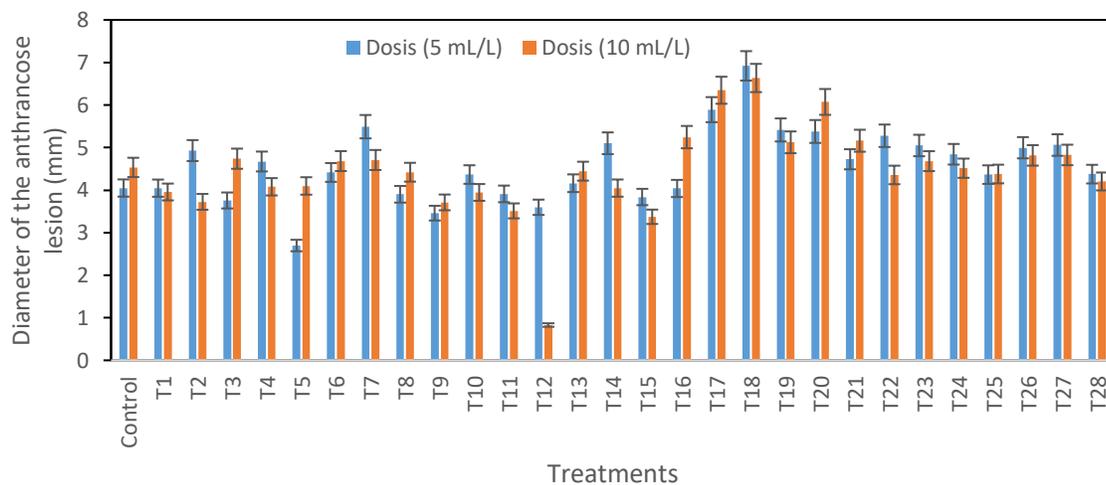


Figure 18. Diameter of the anthracnose lesion in “Keitt” mango fruits in postharvest. Tratamientos: Control; T1= Bac-22; T2= Bac-23; T3= Bac-Ps3; T4= Bac-Ps7; T5= Bac-Ps1; T6= Bac-17; T7= Bac-18; T8= Bac-Bsf6a; T9= Bac-4; T10= Bac-15; T11= Bac-6; T12= Bac-Ps13; T13= Bac-19; T14= Bac-21; T15= Bac-25; T16= Tricho-Cesix; T17= Tricho-Pino; T18= Tricho-16 Los Mochis; T19= Tricho-6 volcán; T20= Tricho-Cesix 1; T21= Tricho-Cesix 2; T22= Tricho-Cesix 3; T23= Tricho-Cesix 4; T24= Tricho-5 hule; T25= Tricho-6 hule; T26= Fungifree; T27= Serenade; T28= Stargus.

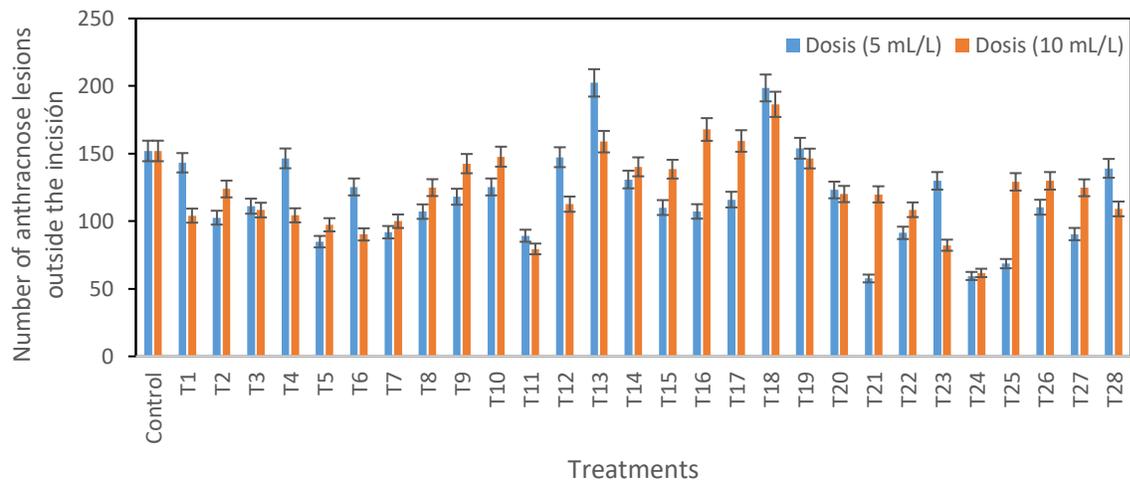


Figure 19.- Number of anthracnose lesions outside the incision in “Keitt” mango fruits in postharvest. Treatments: Control; T1= Bac-22; T2= Bac-23; T3= Bac-Ps3; T4= Bac-Ps7; T5= Bac-Ps1; T6= Bac-17; T7= Bac-18; T8= Bac-Bsf6a; T9= Bac-4; T10= Bac-15; T11= Bac-6; T12= Bac-Ps13; T13= Bac-19; T14= Bac-21; T15= Bac-25; T16= Tricho-Cesix; T17= Tricho-Pino; T18= Tricho-16 Los Mochis; T19= Tricho-6 volcán; T20= Tricho-Cesix 1; T21= Tricho-Cesix 2; T22= Tricho-Cesix 3; T23= Tricho-Cesix 4; T24= Tricho-5 hule; T25= Tricho-6 hule; T26= Fungifree; T27= Serenade; T28= Stargus.

### 3.7. Applications of biological agents in the postharvest phase in mango fruits of the "Kent" variety.

#### Incidence of anthracnose

The control fruits showed an incidence close to 100 % of the incisions with anthracnose symptoms, however, most of the treatments showed incidences equal to or greater than 80 %. Of the treatments that showed the lowest incidence were the treatment with *Trichoderma* T22 in both doses (5 and 10 mL/L) with 68 and 70 % respectively. T12 at 10 mL/L (14%) and T15 at 5 mL (41 %). On the other hand, the incidences in the commercial products in both doses were Fungifree™ (47 to 70 %), Serenade™ (79 to 68 %) and Stargus™ (94 to 85 %) (Figure 20).

#### Anthracnose lesion diameter

The control fruits registered the largest diameter of the lesion with 10 mm, however, most of the treatments presented diameters of the lesions in the ranges of 6 to 8 mm. Except for some treatments with bacteria and *Trichoderma* that recorded lesion diameters between 5 and 6 mm in at least one of their two doses, such is the case of T8, T10, T11, T12 and T14 (bacteria), and T18 and T22 (*Trichoderma*). On the other hand, the commercial products Fungifree™, Serenade™ and Stargus™ showed lesion diameters very similar to that of most treatments, except for Fungifre™ at 5 mL, whose lesion diameter was 3.5 mm (Figure 21).

#### Number of lesions outside the incision

The control fruits registered the highest number of anthracnose lesions on the epidermis with an average of 278. On the other hand, the treatments with bacteria that presented the least number of lesions were T9 at 10 mL/L (80 lesions), T13 in both doses (5 and 10 mL) with 76 and 94 lesions respectively, T12 at 5 mL (92 lesions), T14 in both doses (103 and 98 lesions). On the other hand, the *Trichoderma* with the lowest number of lesions were T19 at 5 mL (66 lesions), T22 5 mL (78 lesions), T18 at 10 mL (89 lesions), and T24 at both doses with 108 and 110 lesions, respectively. Within the treatments with commercial products, Fungifree™ at a dose of 5 mL registered the lowest number of lesions with 93, the rest of the treatments and doses showed an average of 112 to 176 lesions per fruit (Figure 22).

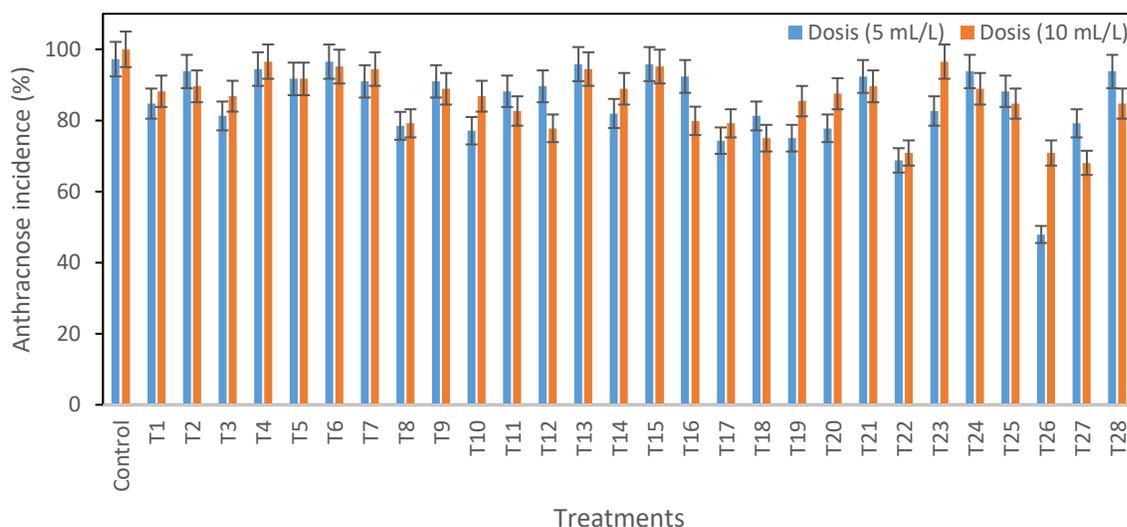


Figure 21. Incidence of anthracnose in postharvest "Kent" mango fruits. Treatments: Control; T1= Bac-22; T2= Bac-23; T3= Bac-Ps3; T4= Bac-Ps7; T5= Bac-Ps1; T6= Bac-17; T7= Bac-18; T8= Bac-Bsf6a; T9= Bac-4; T10= Bac-15; T11= Bac-6; T12= Bac-Ps13; T13= Bac-19; T14= Bac-21; T15= Bac-25; T16= Tricho-Cesix; T17= Tricho-Pino; T18= Tricho-16 Los Mochis; T19= Tricho-6 volcán; T20= Tricho-Cesix 1; T21= Tricho-Cesix 2; T22= Tricho-Cesix 3; T23= Tricho-Cesix 4; T24= Tricho-5 hule; T25= Tricho-6 hule; T26= Fungifree; T27= Serenade; T28= Stargus.

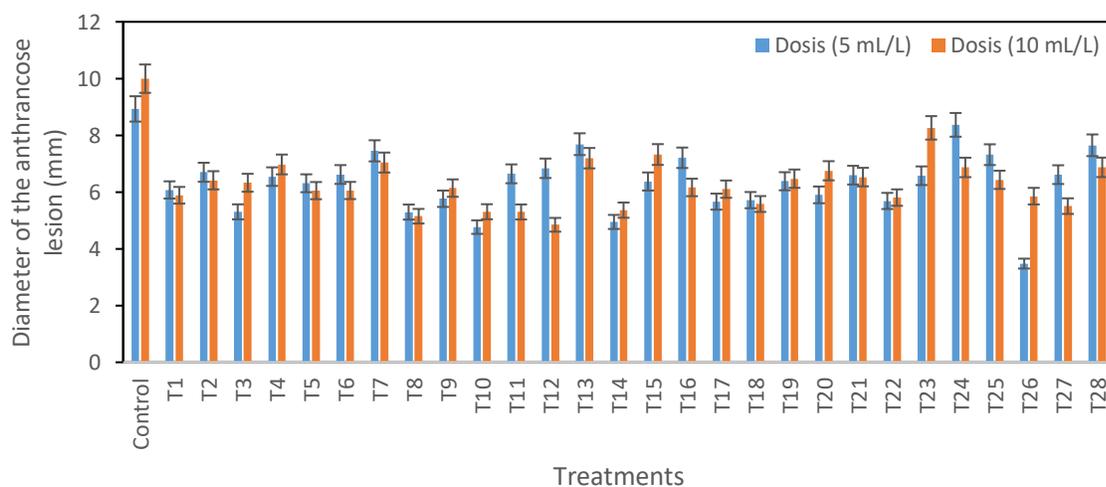


Figure 22. Diameter of the anthracnose lesion in postharvest "Kent" mango fruits. Treatments: Control; T1= Bac-22; T2= Bac-23; T3= Bac-Ps3; T4= Bac-Ps7; T5= Bac-Ps1; T6= Bac-17; T7= Bac-18; T8= Bac-Bsf6a; T9= Bac-4; T10= Bac-15; T11= Bac-6; T12= Bac-Ps13; T13= Bac-19; T14= Bac-21; T15= Bac-25; T16= Tricho-Cesix; T17= Tricho-Pino; T18= Tricho-16 Los Mochis; T19= Tricho-6 volcán; T20= Tricho-Cesix 1; T21= Tricho-Cesix 2; T22= Tricho-Cesix 3; T23= Tricho-Cesix 4; T24= Tricho-5 hule; T25= Tricho-6 hule; T26= Fungifree; T27= Serenade; T28= Stargus.

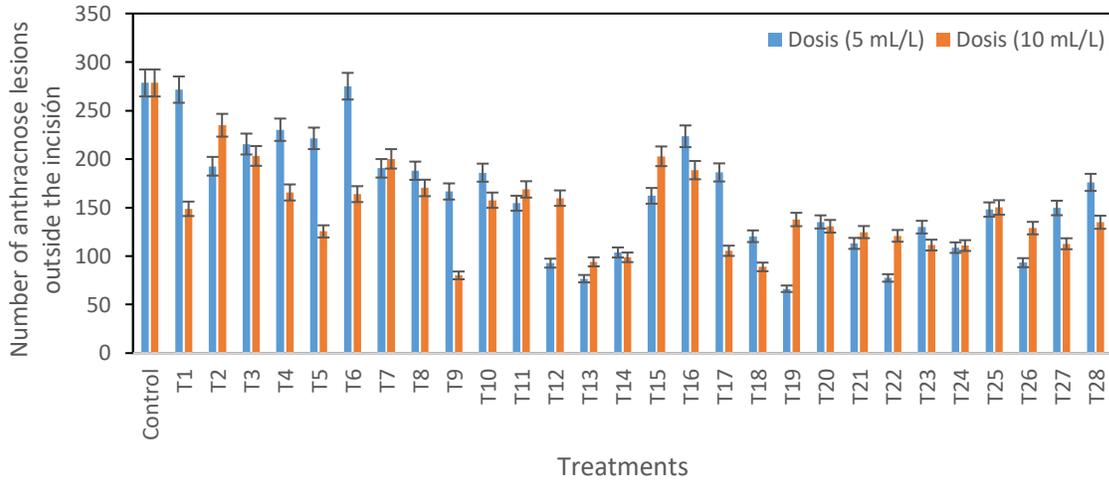


Figure 23. Number of anthracnose lesions outside the incision in postharvest "Kent" mango fruits. Treatments: Control; T1= Bac-22; T2= Bac-23; T3= Bac-Ps3; T4= Bac-Ps7; T5= Bac-Ps1; T6= Bac-17; T7= Bac-18; T8= Bac-Bsf6a; T9= Bac-4; T10= Bac-15; T11= Bac-6; T12= Bac-Ps13; T13= Bac-19; T14= Bac-21; T15= Bac-25; T16= Tricho-Cesix; T17= Tricho-Pino; T18= Tricho-16 Los Mochis; T19= Tricho-6 volcán; T20= Tricho-Cesix 1; T21= Tricho-Cesix 2; T22= Tricho-Cesix 3; T23= Tricho-Cesix 4; T24= Tricho-5 hule; T25= Tricho-6 hule; T26= Fungifree; T27= Serenade; T28= Stargus.

## 4. CONCLUSIONS

1. The weather conditions that prevailed in the selected "Ataulfo" mango orchards were not the most suitable for a high incidence of anthracnose to occur on the fruits in their different phenological stages. Due to the above, the maximum potential of the treatments formulated with antagonistic bacteria strains, *Trichoderma* strains and a mixture of bacteria plus *Trichoderma* could not be observed.
2. No presence of anthracnose was found in the inflorescences of treatment trees, including control trees.
3. Although the incidence of anthracnose was not high, it was possible to observe that control fruits showed a higher incidence of anthracnose. It was observed that some strains of bacteria and *Trichoderma* could have potential for the control of anthracnose in the preharvest stage. It was also noted that there were no differences between the applications with the formulations of biological agents and the commercial product Fungifree™.
4. The biopesticide formulations in their different concentrations in the postharvest stage did not have a significant effect in stopping the incidence and severity (diameter of the lesion) of anthracnose in wounds, as well as in the number of wounds outside the incisions. Therefore, it is concluded that none of the treatments with biological agents, including the commercial fungicides Fungifree™, Serenade™ and Stargus™, completely stop the infection process once the pathogen is already inside the fruit.
5. Despite the fact that none of the treatments stopped the advance of postharvest anthracnose on the fruits in the different mango varieties, it was possible to determine that some strains of bacteria and *Trichoderma* had a control effect on the disease when compared with the fruits. control.
6. Some strains of antagonistic bacteria and *Trichoderma* strains showed greater biological effectiveness in the control of postharvest anthracnose than the commercial products Fungifree™, Serenade™ and Stargus™.

## 5. PHOTOGRAPHIC APPENDIX



Figure 24. Incidence and severity of anthracnose in postharvest "Ataulfo" mango fruits, treated with strains of the antagonist fungus *Trichoderma* spp. Treatments: (1) T19= Tricho-6 volcán; (2) T20= Tricho-Cesix 1; (3) T21= Tricho-Cesix 2; (4) T22= Tricho-Cesix 3; (5) T23= Tricho-Cesix 4; (6) T24= Tricho-5 hule; (7) T25= Tricho-6 hule; (8) T16= Tricho-Cesix; (9) T17= Tricho-Pino; (10) T18= Tricho-16 Los Mochis.

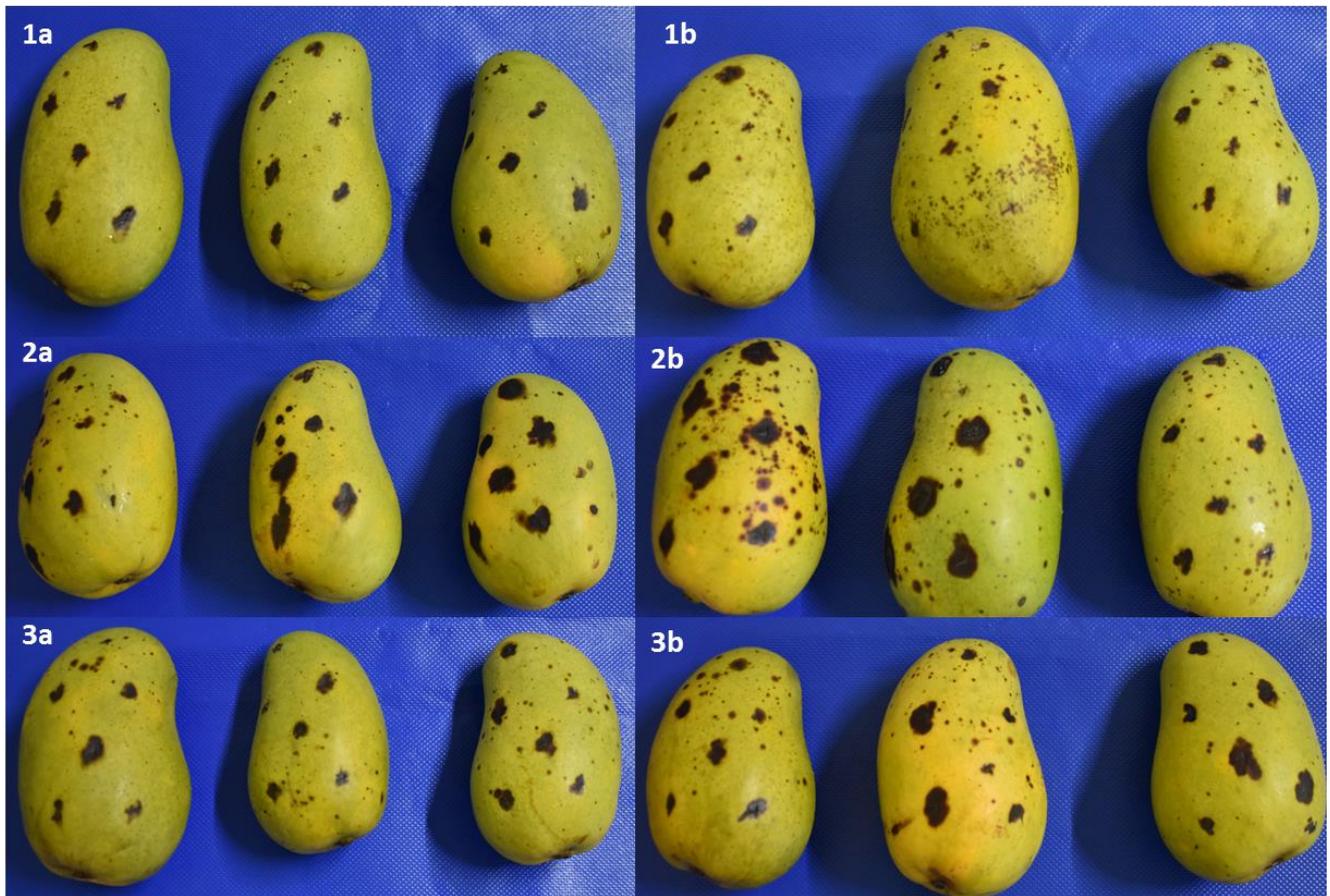


Figure 25. Incidence and severity of anthracnose in postharvest "Ataulfo" mango fruits, treated with commercial bacteria. Treatments: (1a y 1b) T26= Fungifree; (2a y 2b) T27= Serenade; (3a y 3b) T28= Stargus.



Figure 26. Incidence and severity of anthracnose in postharvest "Ataulfo" mango control fruits.



Figure 27. Incidence and severity of anthracnose in postharvest "Ataulfo" mango fruits, treated with antagonistic bacteria strains. Treatments: (1) T1= Bac-22; (2) T2= Bac-23; (3) T3= Bac-Ps3; (4) T4= Bac-Ps7; (5) T5= Bac-Ps1; (6) T6= Bac-17; (7) T7= Bac-18; (8) T8= Bac-Bsf6a.

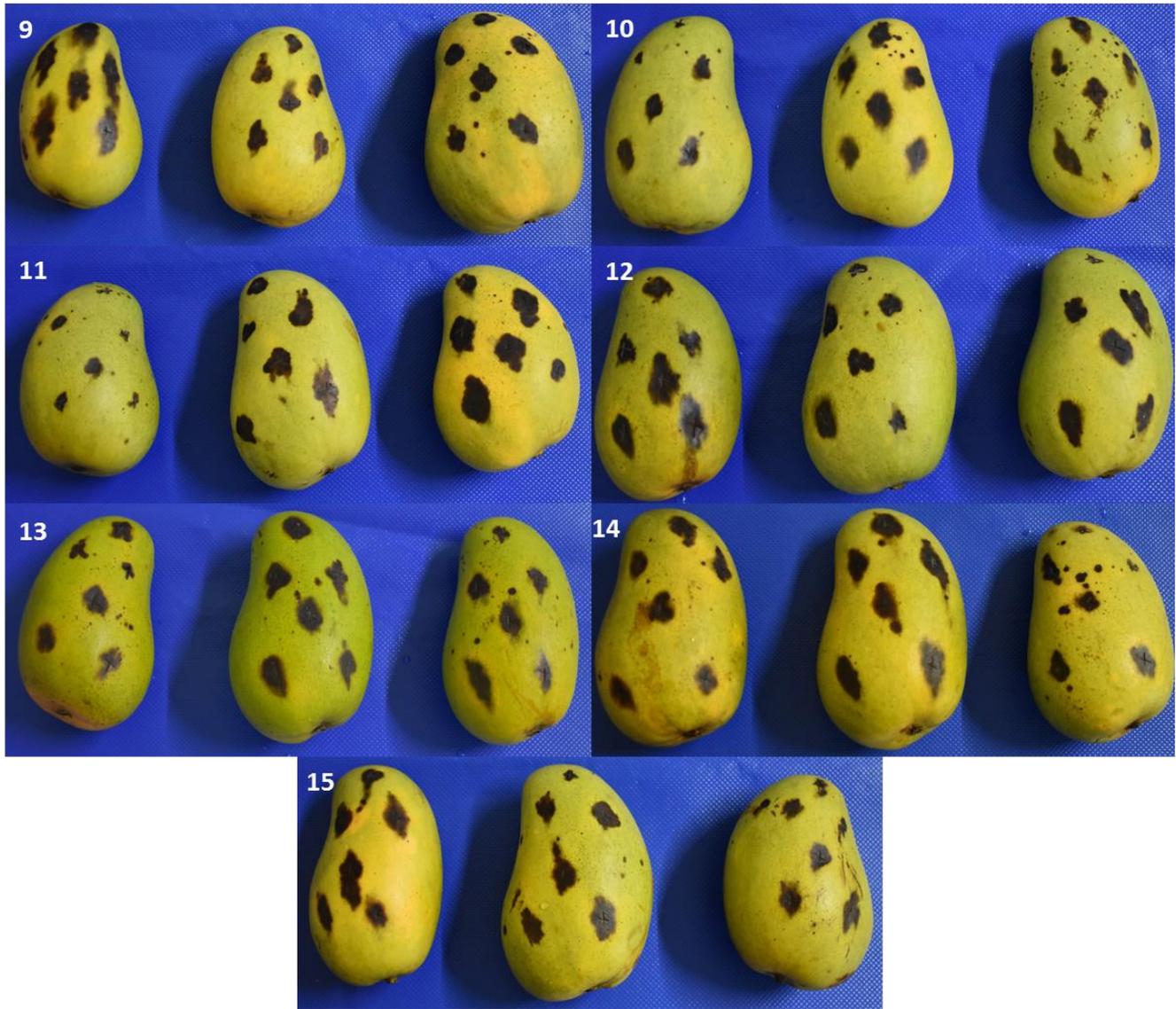


Figure 28. Incidence and severity of anthracnose in postharvest "Ataulfo" mango fruits, treated with antagonistic bacteria strains. Treatments: (9) T9= Bac-4; (10) T10= Bac-15; (11) T11= Bac-6; (12) T12= Bac-Ps13; (13) T13= Bac-19; (14) T14= Bac-21; (15) T15= Bac-25.



Figure 29. Incidence and severity of anthracnose in postharvest "Keiit" mango fruits, treated with strains of the antagonistic fungus *Trichoderma* spp. Treatments: (1) T19= Tricho-6 volcán; (2) T20= Tricho-Cesix 1; (3) T21= Tricho-Cesix 2; (4) T22= Tricho-Cesix 3; (5) T23= Tricho-Cesix 4; (6) T24= Tricho-5 hule; (7) T25= Tricho-6 hule; (8) T16= Tricho-Cesix; (9) T17= Tricho-Pino; (10) T18= Tricho-16 Los Mochis.

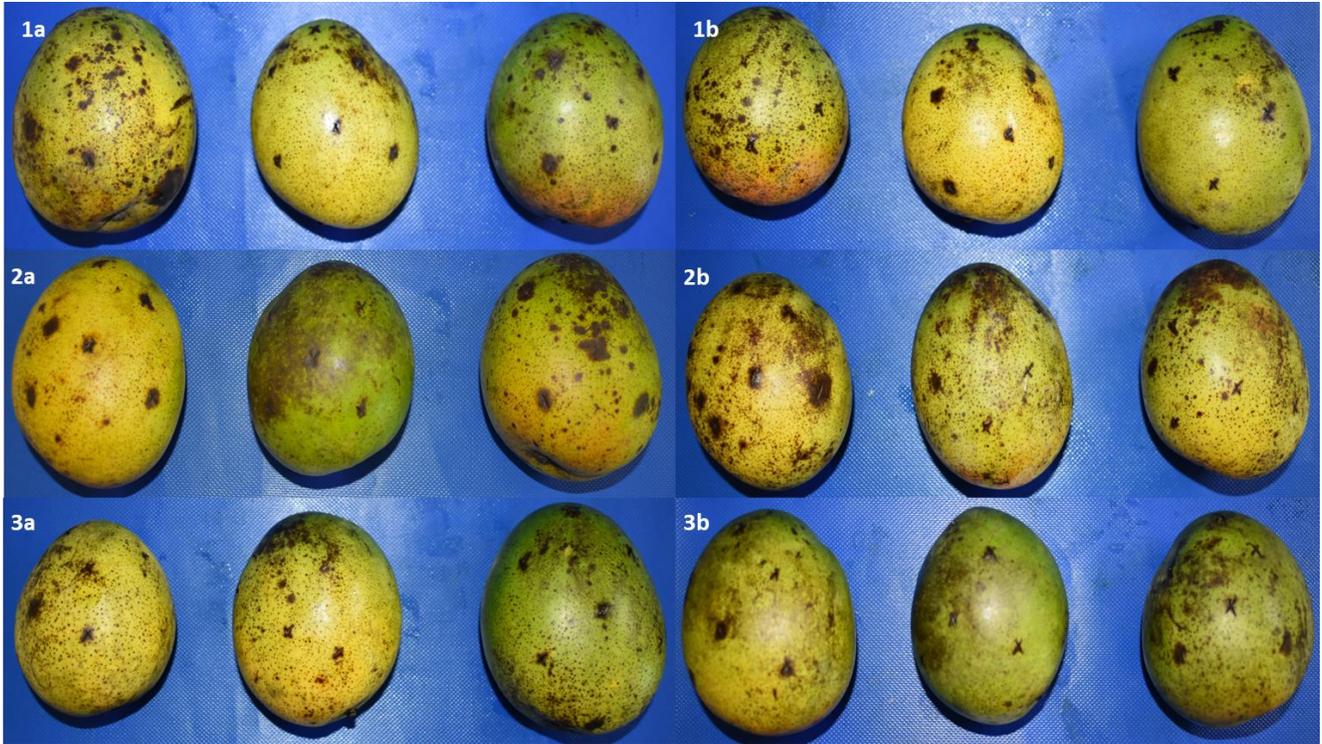


Figure 30. Incidence and severity of anthracnose in postharvest "Keit" mango fruits, treated with commercial bacteria. Treatments: (1a y 1b) T26= Fungifree; (2a y 2b) T27= Serenade; (3a y 3b) T28= Stargus.



Figure 31. Incidence and severity of anthracnose in postharvest “Keit” mango control fruits.



Figure 32. Incidence and severity of anthracnose in postharvest "Keiit" mango fruits, treated with antagonistic bacteria strains. Treatments: (1) T1= Bac-22; (2) T2= Bac-23; (3) T3= Bac-Ps3; (4) T4= Bac-Ps7; (5) T5= Bac-Ps1; (6) T6= Bac-17; (7) T7= Bac-18; (8) T8= Bac-Bsf6a.

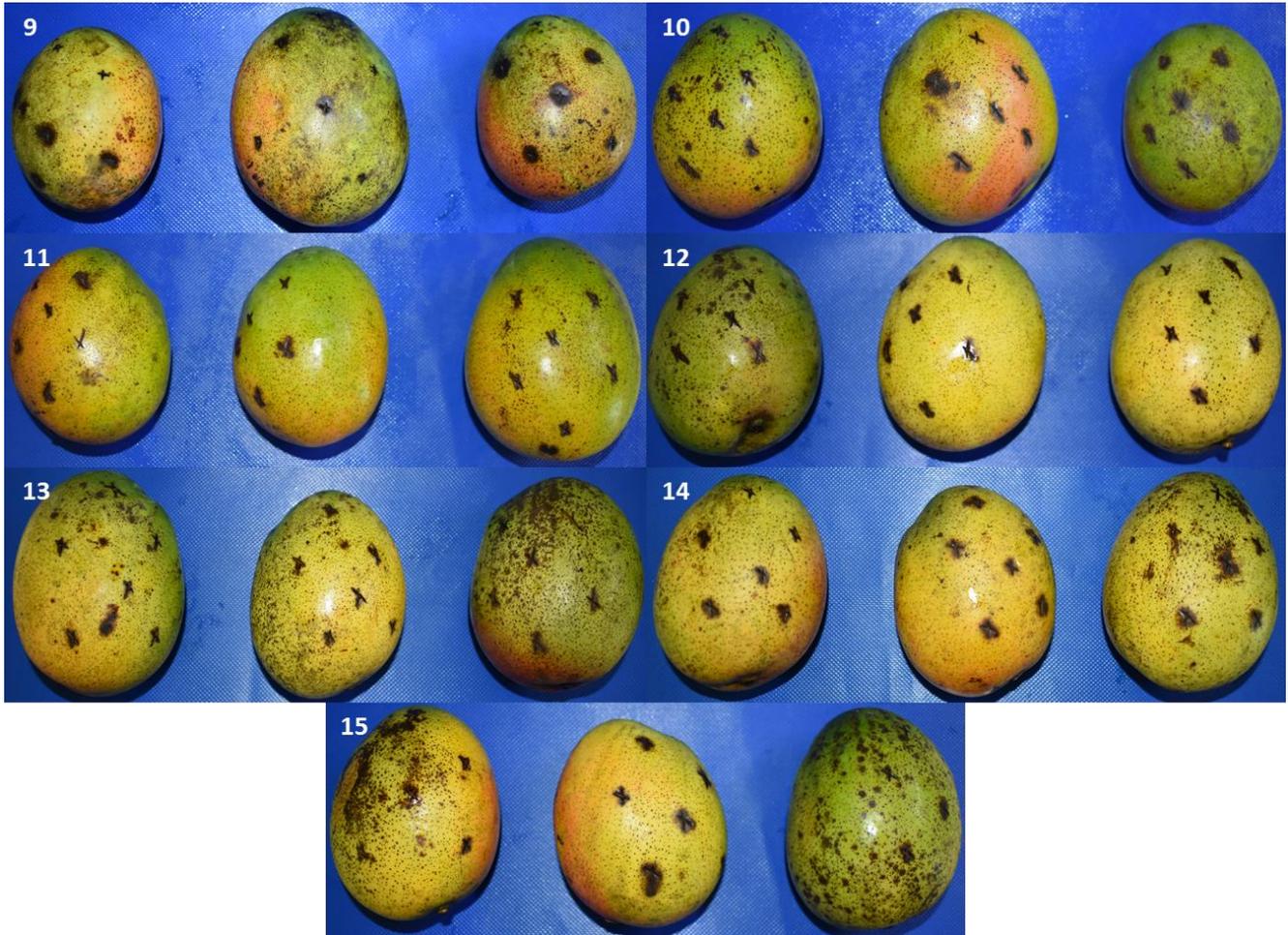


Figure 33. Incidence and severity of anthracnose in postharvest "Keit" mango fruits, treated with antagonistic bacteria strains. Treatments: (9) T9= Bac-4; (10) T10= Bac-15; (11) T11= Bac-6; (12) T12= Bac-Ps13; (13) T13= Bac-19; (14) T14= Bac-21; (15) T15= Bac-25.



Figure 34. Incidence and severity of anthracnose in postharvest "Kent" mango fruits, treated with strains of the antagonistic fungus *Trichoderma* spp. Treatments: (1) T19= Tricho-6 volcán; (2) T20= Tricho-Cesix 1; (3) T21= Tricho-Cesix 2; (4) T22= Tricho-Cesix 3; (5) T23= Tricho-Cesix 4; (6) T24= Tricho-5 hule; (7) T25= Tricho-6 hule; (8) T16= Tricho-Cesix; (9) T17= Tricho-Pino; (10) T18= Tricho-16 Los Mochis.

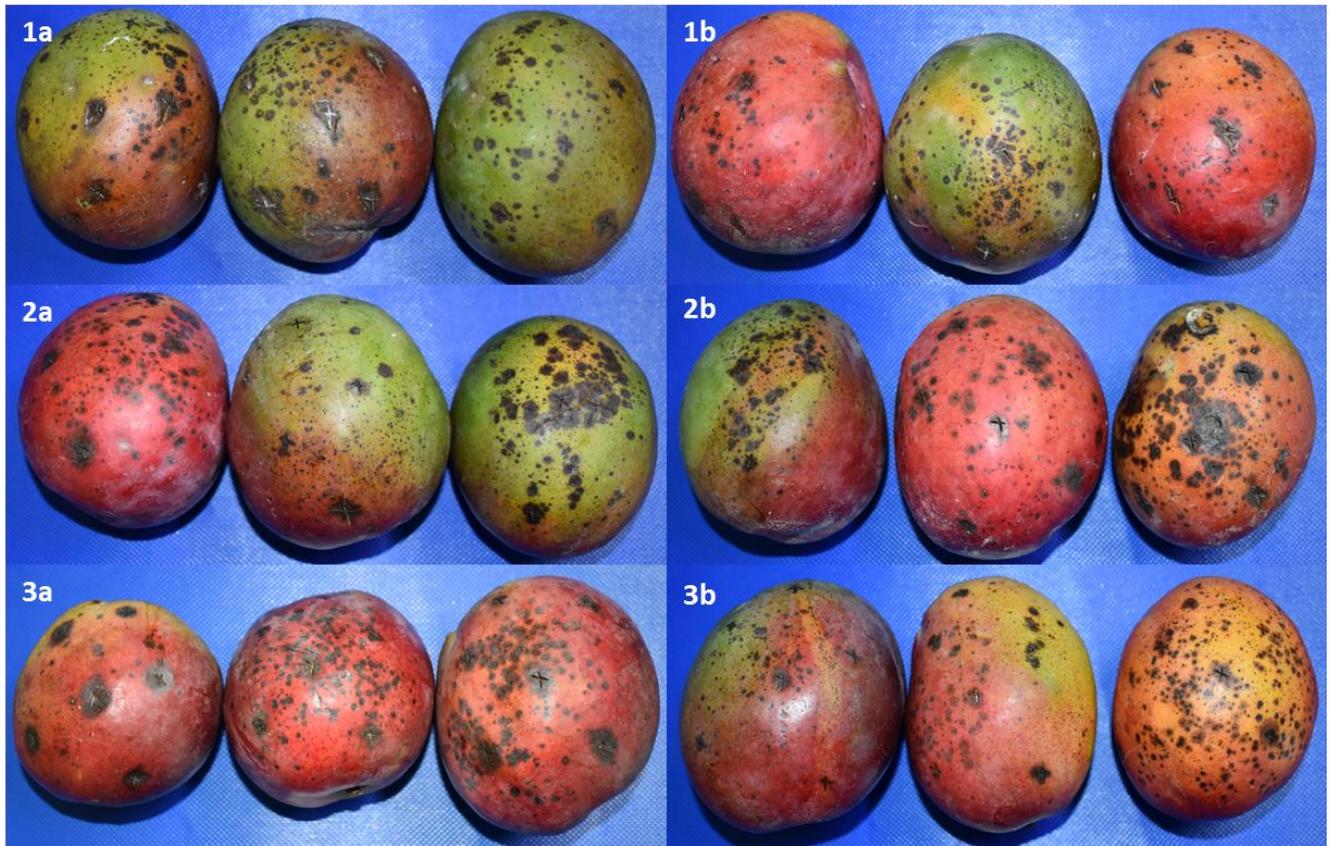


Figure 35. Incidence and severity of anthracnose in postharvest "Kent" mango fruits, treated with commercial bacteria. Treatments: (1a y 1b) T26= Fungifree; (2a y 2b) T27= Serenade; (3a y 3b) T28= Stargus.



Figure 36. Incidence and severity of anthracnose in postharvest "Kent" mango control fruits.

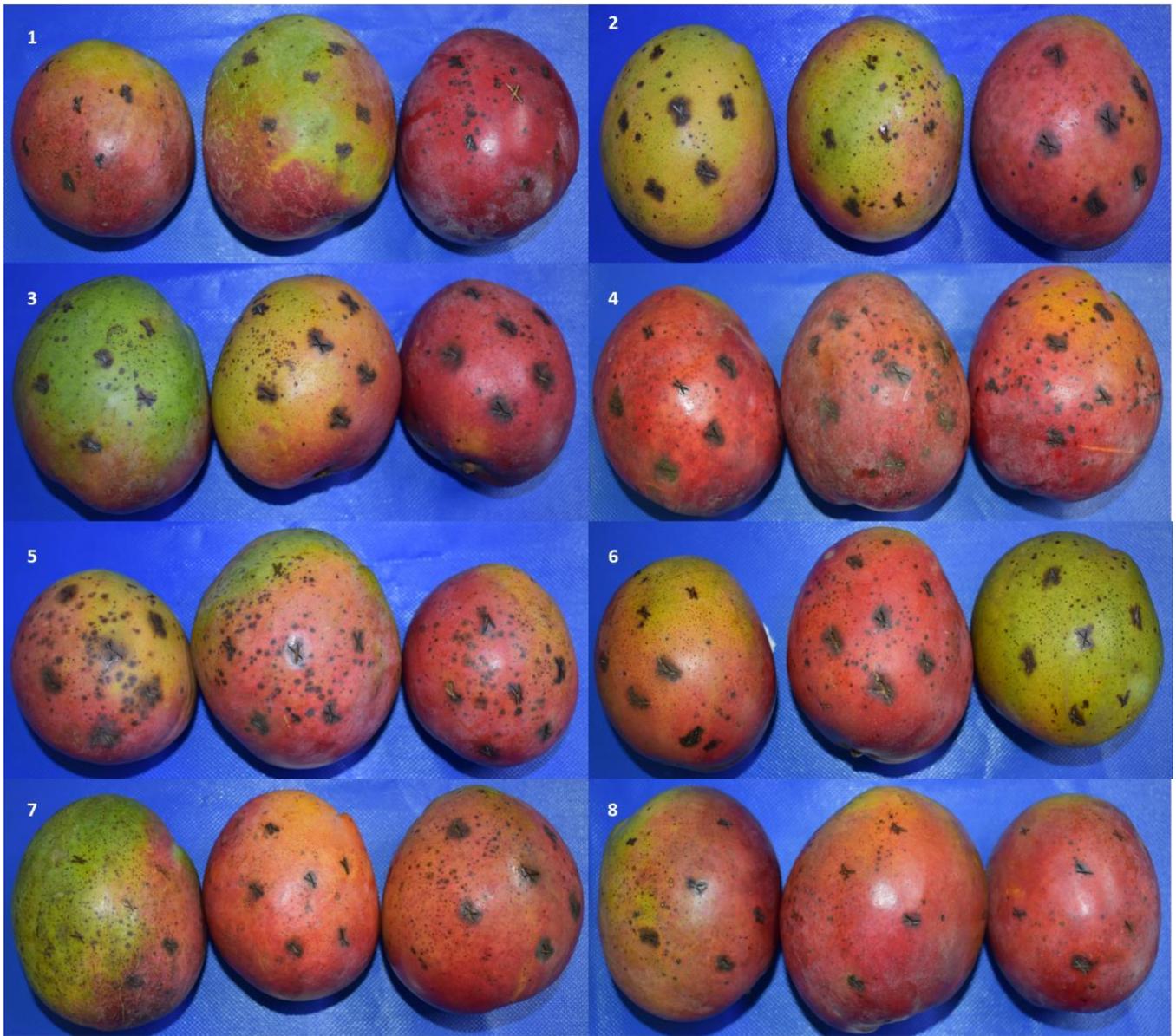


Figure 37. Incidence and severity of anthracnose in postharvest "Kent" mango fruits, treated with antagonistic bacteria strains. Treatments: (1) T1= Bac-22; (2) T2= Bac-23; (3) T3= Bac-Ps3; (4) T4= Bac-Ps7; (5) T5= Bac-Ps1; (6) T6= Bac-17; (7) T7= Bac-18; (8) T8= Bac-Bsf6a.

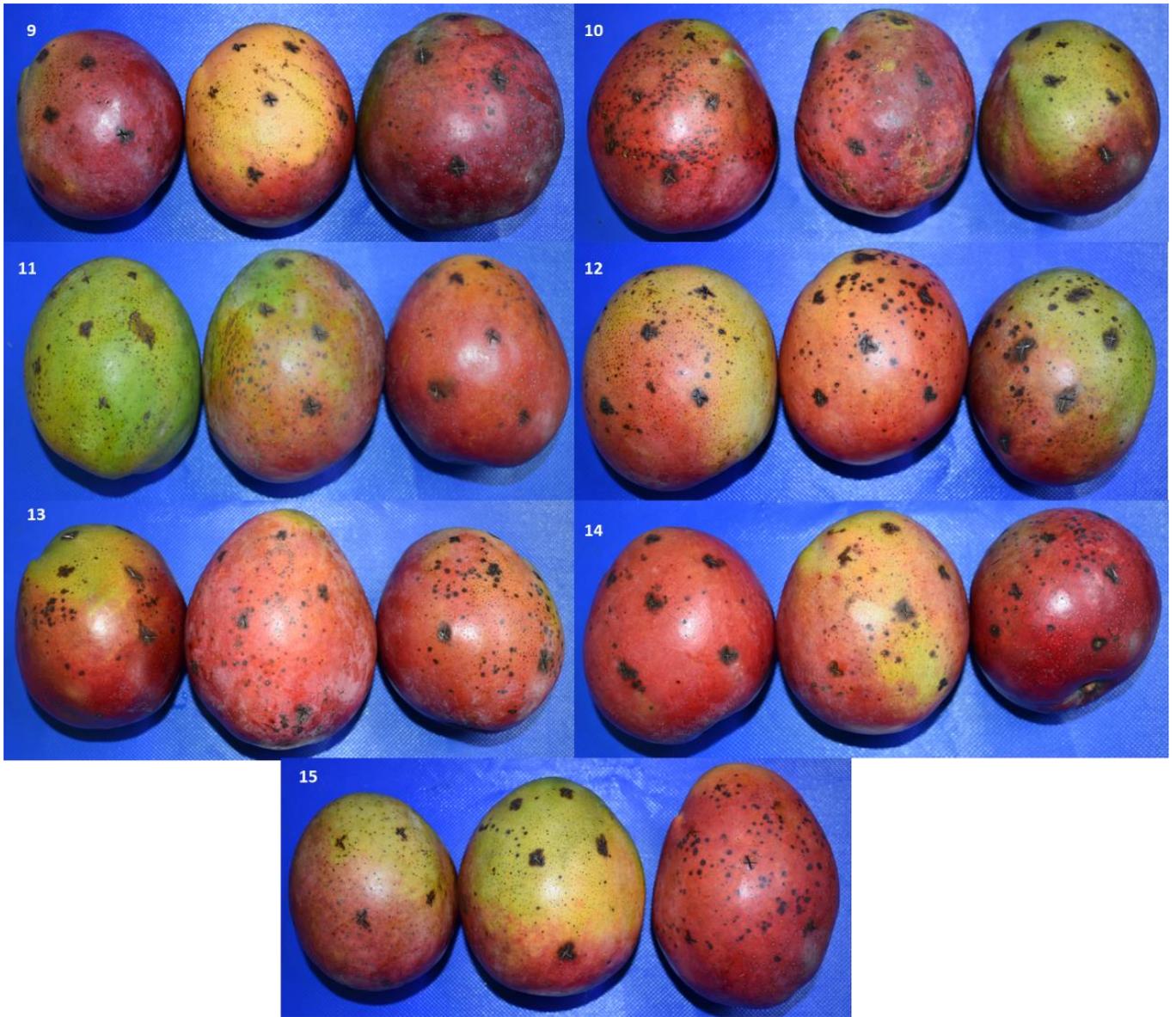


Figure 38. Incidence and severity of anthracnose in postharvest "Kent" mango fruits, treated with antagonistic bacteria strains. Treatments: (9) T9= Bac-4; (10) T10= Bac-15; (11) T11= Bac-6; (12) T12= Bac-Ps13; (13) T13= Bac-19; (14) T14= Bac-21; (15) T15= Bac-25.

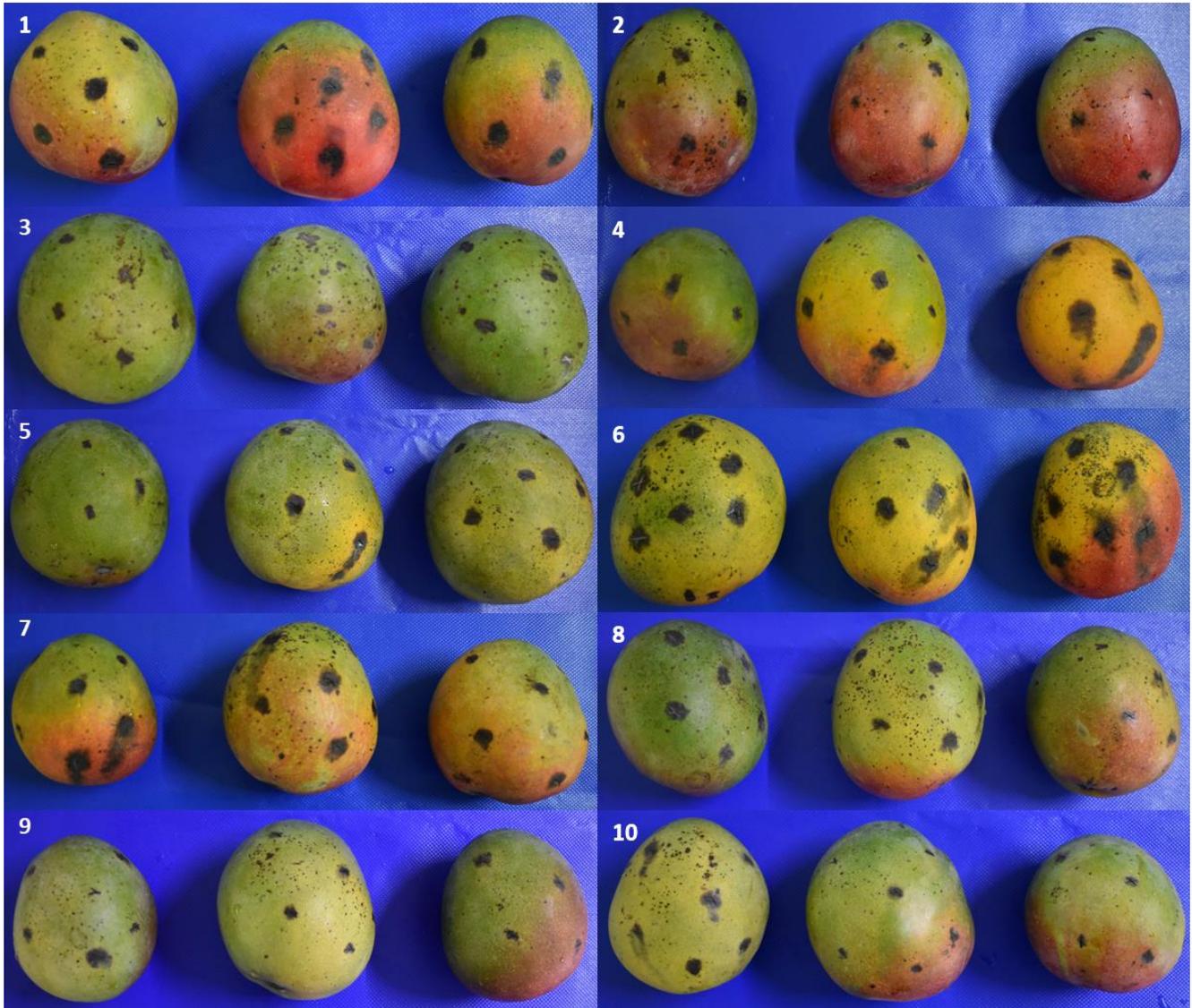


Figure 39. Incidence and severity of anthracnose in postharvest "Tommy Atkins" mango fruits, treated with strains of the antagonistic fungus *Trichoderma* spp. Treatments: (1) T19= Tricho-6 volcán; (2) T20= Tricho-Cesix 1; (3) T21= Tricho-Cesix 2; (4) T22= Tricho-Cesix 3; (5) T23= Tricho-Cesix 4; (6) T24= Tricho-5 hule; (7) T25= Tricho-6 hule; (8) T16= Tricho-Cesix; (9) T17= Tricho-Pino; (10) T18= Tricho-16 Los Mochis.

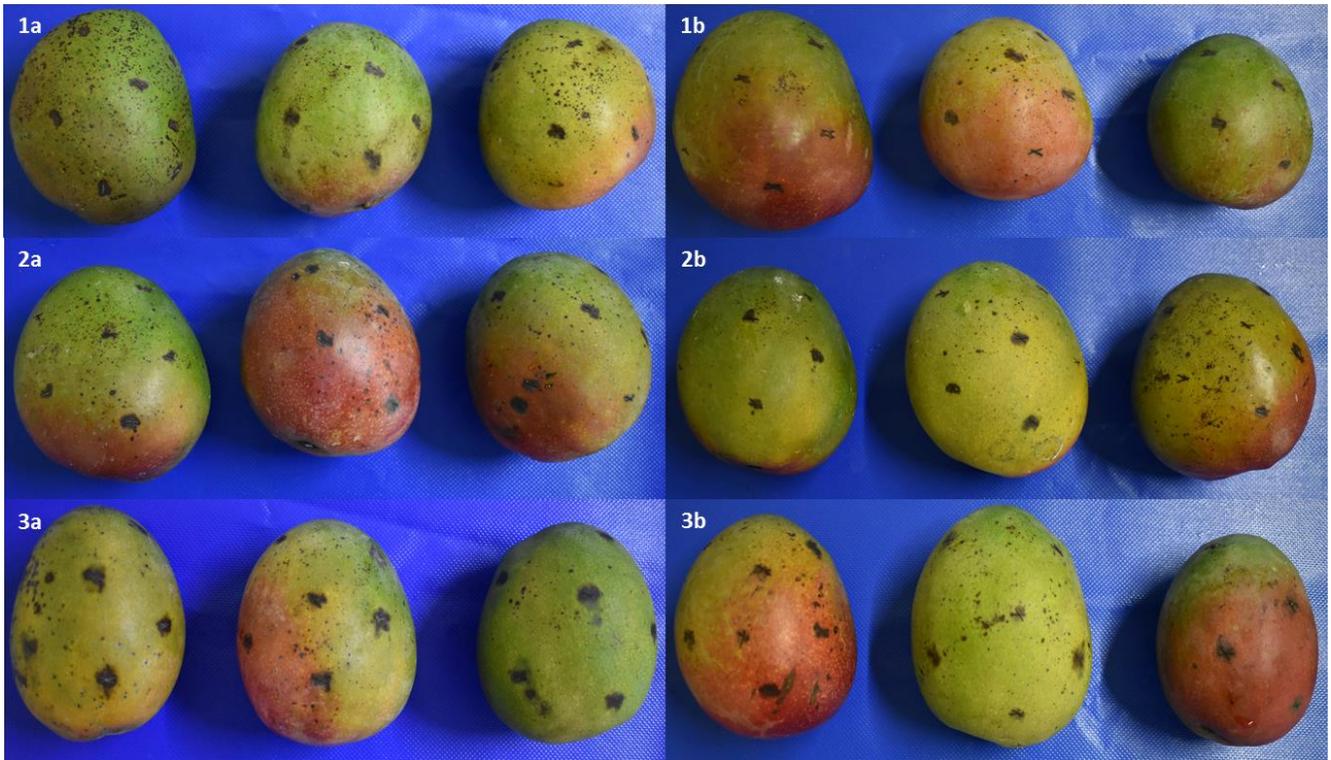


Figure 40. Incidence and severity of anthracnose in postharvest "Tommy Atkins" mango fruits, treated with commercial bacteria. Treatments: (1a y 1b) T26= Fungifree; (2a y 2b) T27= Serenade; (3a y 3b) T28= Stargus.



Figure 41. Incidence and severity of anthracnose in postharvest "Tommy Atkins" mango control fruits.

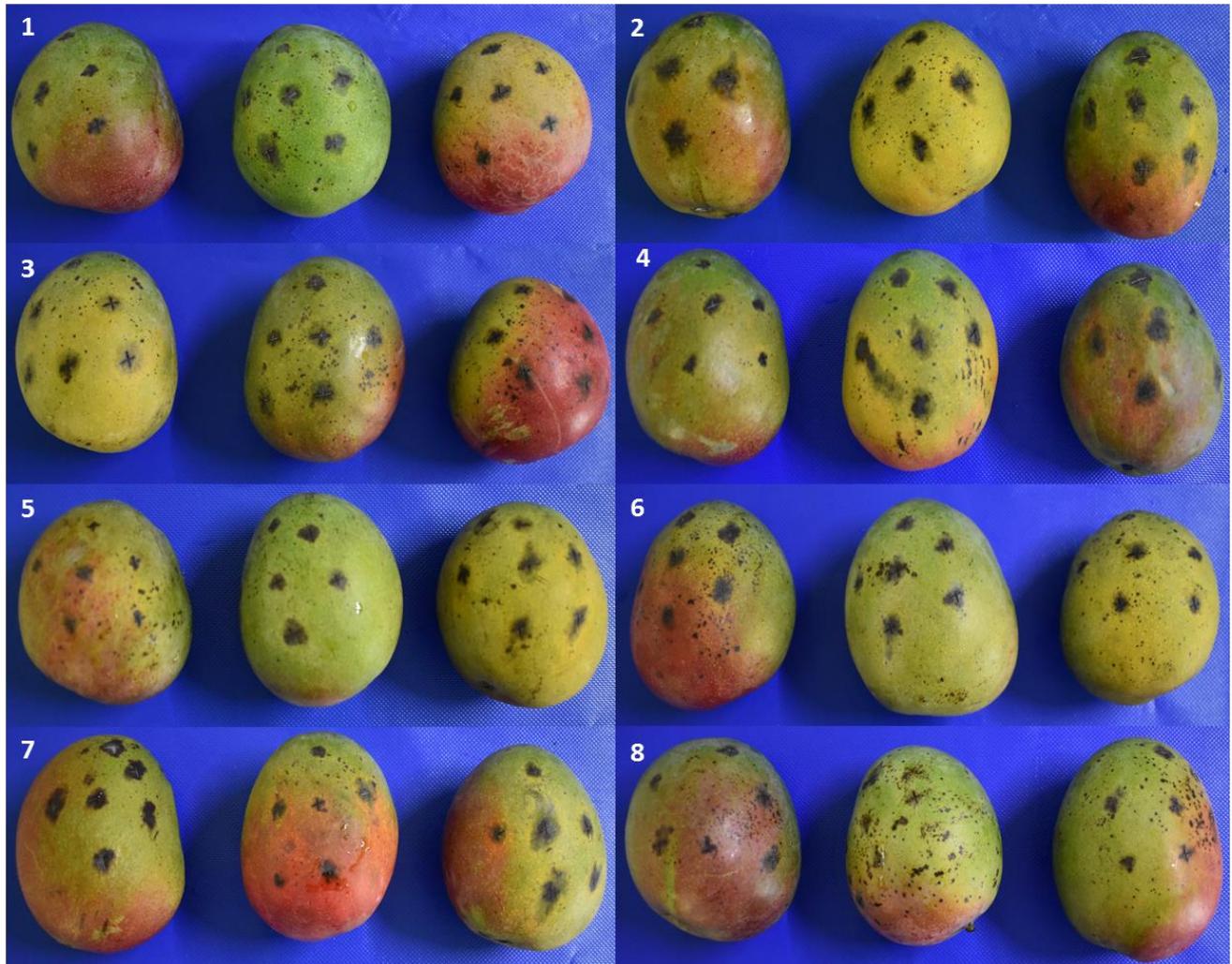


Figure 42. Incidence and severity of anthracnose in postharvest "Tommy Atkins" mango fruits, treated with antagonistic bacteria strains. Treatments: (1) T1= Bac-22; (2) T2= Bac-23; (3) T3= Bac-Ps3; (4) T4= Bac-Ps7; (5) T5= Bac-Ps1; (6) T6= Bac-17; (7) T7= Bac-18; (8) T8= Bac-Bsf6a.

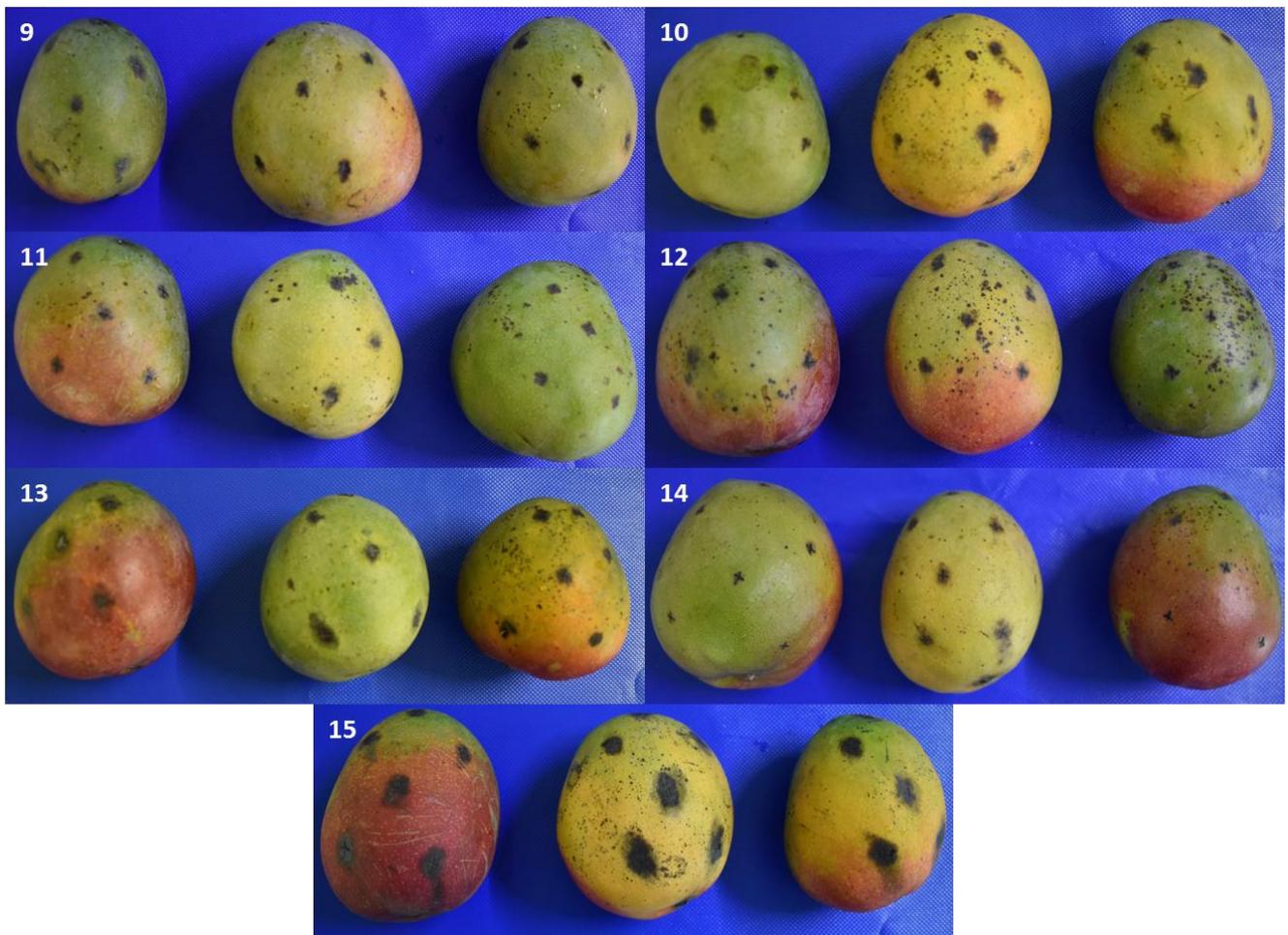


Figure 43. Incidence and severity of anthracnose in postharvest "Tommy Atkins" mango fruits, treated with antagonistic bacteria strains. Treatments: (9) T9= Bac-4; (10) T10= Bac-15; (11) T11= Bac-6; (12) T12= Bac-Ps13; (13) T13= Bac-19; (14) T14= Bac-21; (15) T15= Bac-25.

### III. APPLICATIONS OF BIOLOGICAL AGENTS IN THE FIELD PHASE (PRODUCTION CYCLE 2022)

#### 1. OBJECTIVE

1. To evaluate the biological effectiveness of biological agents in the control of anthracnose (*Colletotrichum* spp.) from flowering to harvest in mango orchards of the "Ataulfo" variety.

#### 2. MATERIALS AND METHODS

##### 2.1. Establishment of the experiment

Two experiments were established in commercial mango orchards of the "Ataulfo" variety in the last week of January and the first week of February 2022. One orchard was located in the Ejido (common) of Huaristemba, Municipality of San Blas, Nayarit, and the other in the Ejido (common) of El Corte, Municipality of Santiago Ixcuintla, Nayarit, in trees of 9 and 10 years of age, respectively. The selection of the aforementioned orchards was made based on the incidences of anthracnose that they had presented in the 2021 production cycle.

##### 2.2. Application of treatments

Biological agents (antagonistic bacteria and fungi of the genus *Trichoderma*) were used based on the results of the in vitro tests. Table 2 presents the treatments for each of the orchards. The applications were made every 15 days, with a total of 8 applications from flowering to harvest. The equipment used was Hyundai® Liquid/Powder HYD4514L engine sprayers.

##### 2.3. Evaluations and variables

The evaluations were carried out to determine the incidence of anthracnose in panicles and fruits. The variables to be evaluated were: a) Incidence of anthracnose in panicles; b) Incidence of anthracnose in fruits between 3 and 5 cm in length; c) Incidence of anthracnose in fruits of more than 5 cm in length; d) Incidence of anthracnose in fruits with physiological maturity ready for harvest; e) Severity of anthracnose in fruits; f) Effectiveness of control of biological agents. Four evaluations were carried out, one in flowering and the other three during the development of the fruit. To determine the incidence of anthracnose, the fruits that showed two or more lesions per fruit equal to or greater than 3 millimeters in diameter were considered.

❖ The incidence was expressed as a percentage, for which the following formula was used:  
Incidence of anthracnose

$$\text{Incidence of anthracnose (\%)} = \frac{\text{Fruits with anthracnose symptoms}}{\text{Total fruits}} \times 100 \dots$$

❖ The severity was determined by means of a diagrammatic logarithmic scale, and the scalar values were expressed in percentage of damaged tissue.

**Table 1. Scale to determine the severity of anthracnose in mango fruits in the "Ataulfo" variety.**

<b>Class</b>	<b>Disease severity (%)</b>
0	0
1	0-3
2	3-6
3	6-12
4	12-25
5	25-50
6	50-75
7	75-87
8	87-94
9	94-97
10	97-100
11	100

❖ Control Effectiveness was calculated using the following formula:

$$\text{EC (\%)} = \frac{\text{IAC} - \text{IAt}}{\text{IAC}} \times 100 \dots$$

Where:

EC = Control effectiveness of biological agents (%)

IAC = Incidence of anthracnose in the control

IAt = Incidence of anthracnose in treatment

**Table 2. Treatments with biological agents to control anthracnose in a commercial mango orchard of the "Ataulfo" variety, located in the Huaristemba, San Blas Municipality, Nayarit, and in the El Corte, Santiago Ixcuintla Municipality, Nayarit, in the 2022 production cycle.**

<b>NUMBER</b>	<b>TREATMENT</b>	<b>MICROORGANISMS</b>
T1	Tricho 6 hule	<i>Trichoderma</i> spp.
T2	BAC 5-Chi	<i>Bacillus subtilis</i> strain 2
T3	BAC 4	<i>B. subtilis</i>
T4	BAC 2-Chi	<i>Azotobacter chroococcum</i>
T5	BAC 6	<i>B. subtilis</i>
T6	BAC 3- Chi	<i>B. mucilaginosus</i>
T7	BAC 17	<i>B. subtilis</i>
T8	BAC 4-Chi	<i>Pseudomonas fluorescens</i>
T9	BAC 21	<i>B. subtilis</i>
T10	BAC 6- Chi	<i>B. amyloliquefasciens</i> strain 2
T11	BAC 23	<i>B. subtilis</i>
T12	BAC 7-Chi	<i>B. licheniformis</i>
T13	BAC 9-café	<i>B. subtilis</i>
T14	Tricho-Cesix	<i>Trichoderma</i> spp.
T15	BAC-10-café	<i>B. subtilis</i>
T16	BAC 8-Chi	<i>Streptomyces microflavus</i>
T17	BAC 13-café	<i>B. subtilis</i>
T18	Fungifree	<i>B. subtilis</i>
T19	Tricho pino	<i>Trichoderma</i> spp.

#### **2.4. Treatment design**

A completely randomized design with five replicates per treatment (five trees) was used. In each tree, 10 fully developed panicles (50 panicles per treatment) were selected and marked. In the case of anthracnose in fruits, all the fruits of each tree were taken as a sample, where the experimental unit was a fruit. An analysis of variance (ANOVA) and comparison of means (Tukey;  $p \leq 0.05$ ) were made using SAS (SAS Institute, Inc., 2010).

## 3. RESULTS

### 3.1. Incidence of anthracnose in fruits

#### 3.1.1. Orchard in Huaristemba

The incidence increased in all treatments as the evaluation times elapsed. The control fruits showed the highest incidence. They started with an incidence close to 18 % in the first evaluation. In the second evaluation it increased to 34 %, and for the third and last evaluation the incidence increased to 44 % (Figure 1). In the case of treatments with antagonistic bacteria and *Trichoderma*, the incidence in the first evaluation was less than 9 % in all treatments, while in the second evaluation it was less than 17 %. For the third evaluation, it did not increase significantly, and the incidence was below 18% (Figure 1). In the last evaluation, the fruits treated with the treatments T16 and T18 showed the lowest incidence with 11 %, followed by the treatments T1, T6, T8 and T12 with 12 %. On the other hand, the treatments T4, T9, T17 and T19 registered 13 % incidence. The rest of the treatments were in the range of 14 to 19 % incidence for their third evaluation (Figure 2). The average incidence of control fruits in the three evaluations was 32 %, while in the rest of the treatments it was less than 14 %, including the antagonistic bacteria, *Trichoderma* and the commercial product (Figure 3).

#### 3.1.2. Orchard in El Corte

As in the Huaristemba, the incidence increased in all treatments as the evaluations progressed. The control fruits showed the highest incidence. They started with an incidence close to 16 % in the first evaluation. In the second evaluation it increased to 37 %, and for the third and last evaluation the incidence increased to 43 % (Figure 4). In the case of treatments with antagonistic bacteria and *Trichoderma*, the incidence in the first evaluation was less than 15 % in all treatments, while in the second evaluation it increased to 18 %. For the third evaluation it increased significantly, and the incidence was below 28 % (Figure 4). In the last evaluation, the fruits treated with treatments T3 and T7 showed the lowest incidence with 15 %, followed by treatments T15, T16 and T17 with 16 %. The rest of the treatments were in the range of 18 to 29 % incidence for their third evaluation (Figure 5). The average incidence of control fruits in the three evaluations was 32 %, while in the rest of the treatments it was less than 1 %, including the antagonistic bacteria, *Trichoderma* and the commercial product (Figure 6).

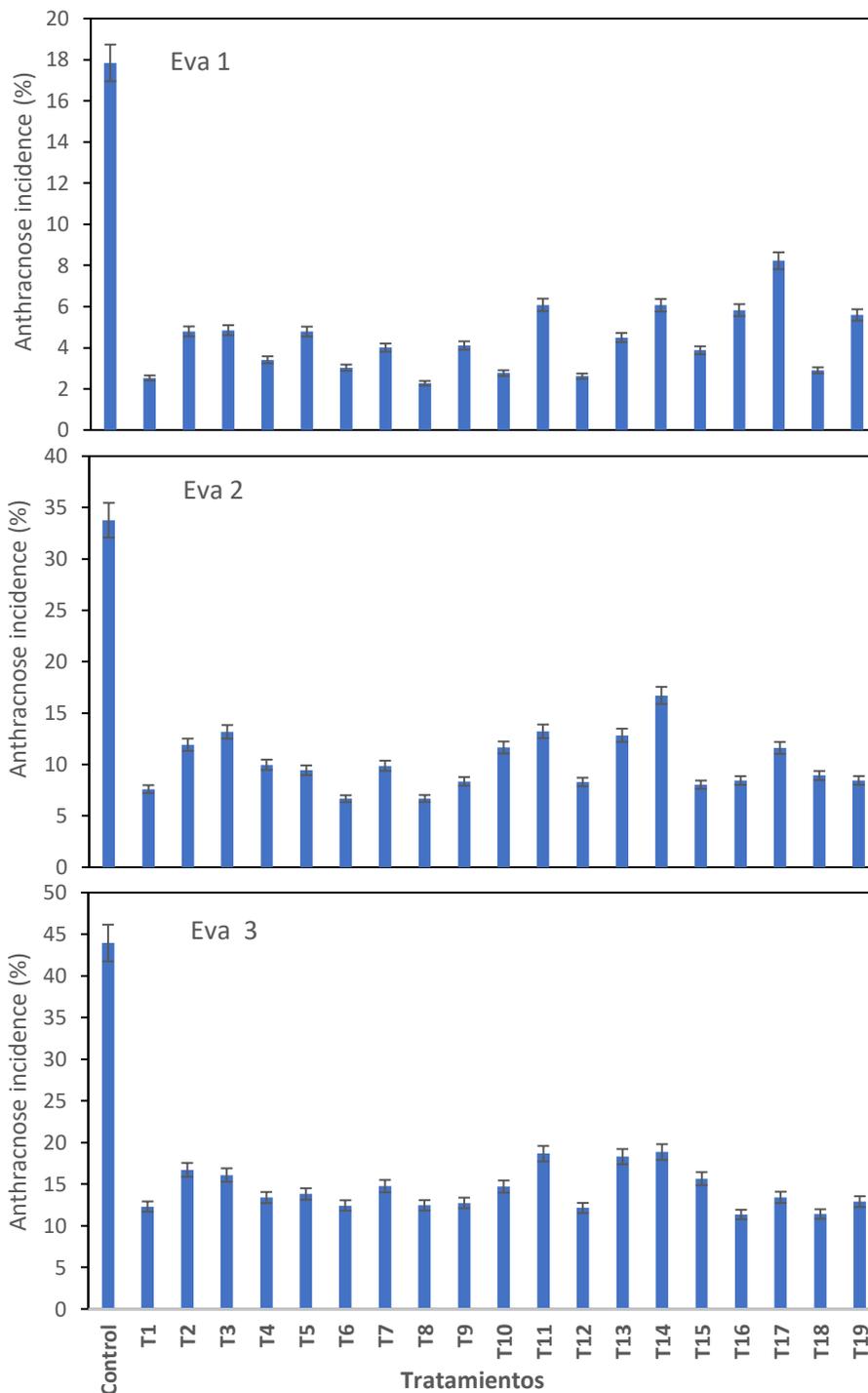


Figura 1. Incidencia de antracnosis en tres evaluaciones, en un huerto de mango “Ataulfo” en el ejido de Huaristemba, Municipio de San Blas, Nayarit. Tratamientos: Control; T1= Tricho-6 hule (*Trichoderma*); T2= Bac-5 Ch (*Bacillus subtilis*); T3= Bac-4 (*B. subtilis*); T4= Bac-2 Ch (*Azotobacter chroococcum*); T5= Bac-6 (*B. subtilis*); T6= Bac-3 Ch (*B. mucilaginosus*); T7= Bac-17 (*B. subtilis*); T8= Bac-7 Ch (*Pseudomonas fluorescens*); T9= Bac-21 (*B. subtilis*); T10= Bac-6 Ch (*B. amyloliquefasciens*); T11= Bac-23 (*B. subtilis*); T12= Bac-7 Ch (*B. licheniformis*); T13= Bac-9 café (*B. subtilis*); T14= Tricho-Cesix (*Trichoderma*); T15= Bac-10 Café (*B. subtilis*); T16= Bac-8 Ch (*Streptomyces microflavus*); T17= Bac-13 Café (*B. subtilis*); T18= Fungifree (*B. subtilis*); T19= Tricho-Pino (*Trichoderma*).

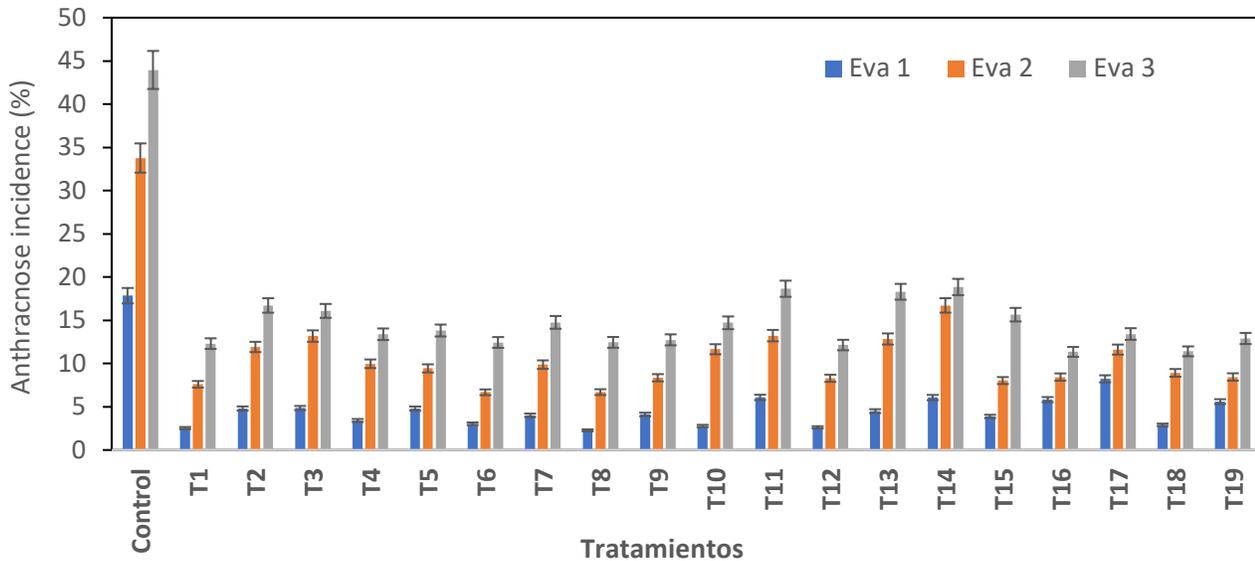


Figura 2. Comparación de la incidencia de antracnosis en tres evaluaciones, en un huerto de mango “Ataulfo” en el ejido de Huaristemba, Municipio de San Blas, Nayarit. Tratamientos: Control; T1= Tricho-6 hule (*Trichoderma*); T2= Bac-5 Ch (*Bacillus subtilis*); T3= Bac-4 (*B. subtilis*); T4= Bac-2 Ch (*Azotobacter chroococcum*); T5= Bac-6 (*B. subtilis*); T6= Bac-3 Ch (*B. mucilaginosus*); T7= Bac-17 (*B. subtilis*); T8= Bac-7 Ch (*Pseudomonas fluorescens*); T9= Bac-21 (*B. subtilis*); T10= Bac-6 Ch (*B. amyloliquefasciens*); T11= Bac-23 (*B. subtilis*); T12= Bac-7 Ch (*B. licheniformis*); T13= Bac-9 café (*B. subtilis*); T14= Tricho-Cesix (*Trichoderma*); T15= Bac-10 Café (*B. subtilis*) T16= Bac-8 Ch (*Streptomyces microflavus*); T17=Bac-13 Café (*B. subtilis*); T18= Fungifree (*B. subtilis*); T19= Tricho-Pino (*Trichoderma*).

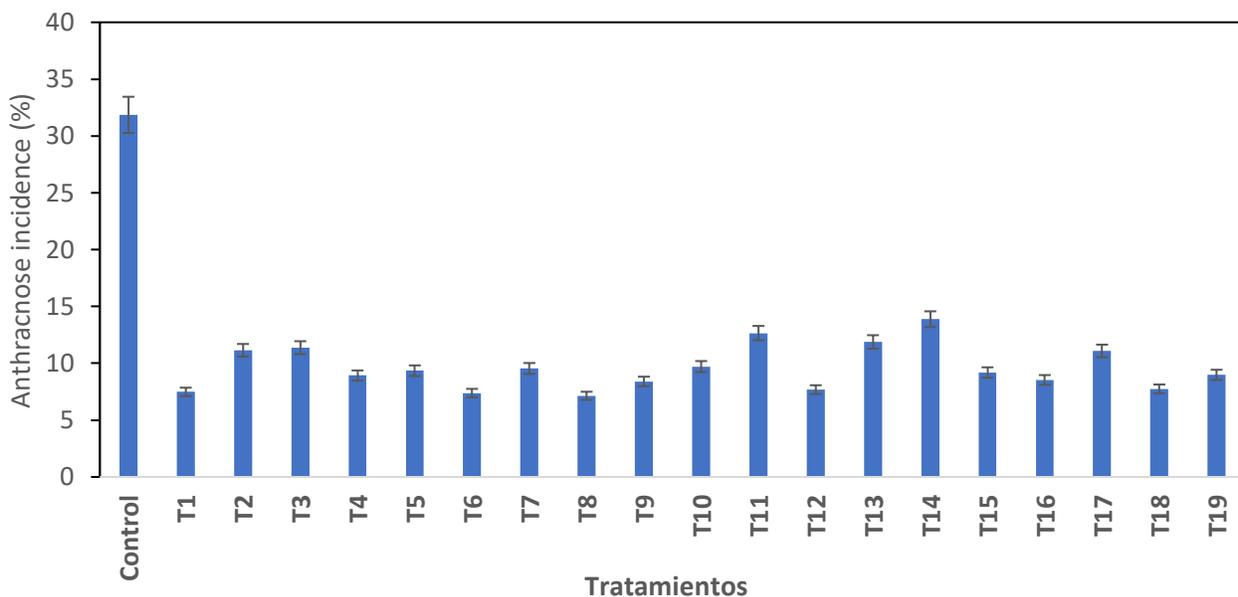


Figura 3. Incidencia promedio de antracnosis en tres evaluaciones, en un huerto de mango “Ataulfo” en el ejido de Huaristemba, Municipio de San Blas, Nayarit. Tratamientos: Control; T1= Tricho-6 hule (*Trichoderma*); T2= Bac-5 Ch (*Bacillus subtilis*); T3= Bac-4 (*B. subtilis*); T4= Bac-2 Ch (*Azotobacter chroococcum*); T5= Bac-6 (*B. subtilis*); T6= Bac-3 Ch (*B. mucilaginosus*); T7= Bac-17 (*B. subtilis*); T8= Bac-7 Ch (*Pseudomonas fluorescens*); T9= Bac-21 (*B. subtilis*); T10= Bac-6 Ch (*B. amyloliquefasciens*); T11= Bac-23 (*B. subtilis*); T12= Bac-7 Ch (*B. licheniformis*); T13= Bac-9 café (*B. subtilis*); T14= Tricho-Cesix (*Trichoderma*); T15= Bac-10 Café (*B. subtilis*) T16= Bac-8 Ch (*Streptomyces microflavus*); T17=Bac-13 Café (*B. subtilis*); T18= Fungifree (*B. subtilis*); T19= Tricho-Pino (*Trichoderma*).

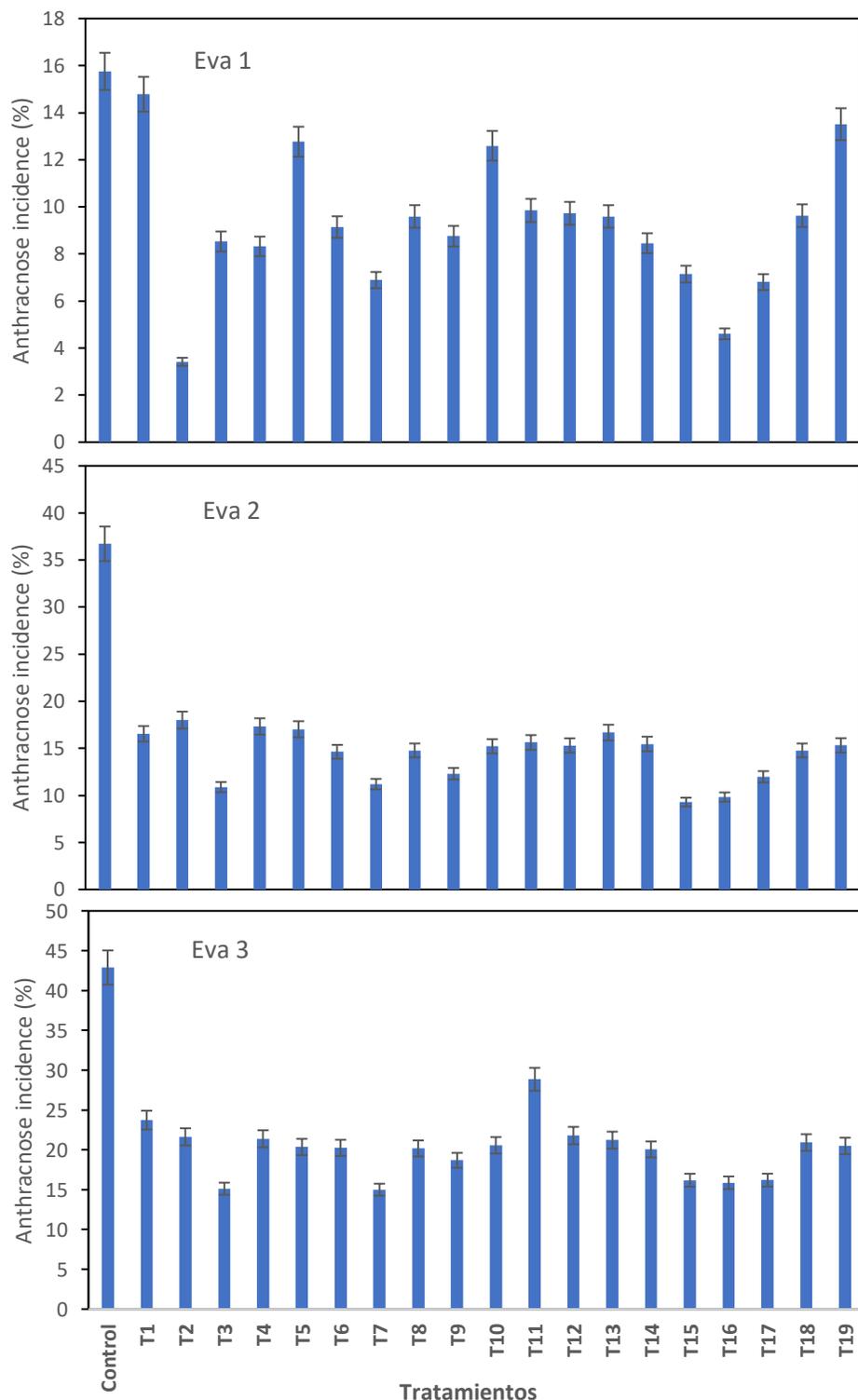


Figura 4. Incidencia de antracnosis en tres evaluaciones, en un huerto de mango “Ataulfo” en el ejido de El Corte, Municipio de Santiago Ixcuintla, Nayarit. Tratamientos: Control; T1= Tricho-6 hule (*Trichoderma*); T2= Bac-5 Ch (*Bacillus subtilis*); T3= Bac-4 (*B. subtilis*); T4= Bac-2 Ch (*Azotobacter chroococcum*); T5= Bac-6 (*B. subtilis*); T6= Bac-3 Ch (*B. mucilaginosus*); T7= Bac-17 (*B. subtilis*); T8= Bac-7 Ch (*Pseudomonas fluorescens*); T9= Bac-21 (*B. subtilis*); T10= Bac-6 Ch (*B. amyloliquefasciens*); T11= Bac-23 (*B. subtilis*); T12= Bac-7 Ch (*B. licheniformis*); T13= Bac-9 café (*B. subtilis*); T14= Tricho-Cesix (*Trichoderma*); T15= Bac-10 Café (*B. subtilis*) T16= Bac-8 Ch (*Streptomyces microflavus*); T17=Bac-13 Café (*B. subtilis*); T18= Fungifree (*B. subtilis*); T19= Tricho-Pino (*Trichoderma*).

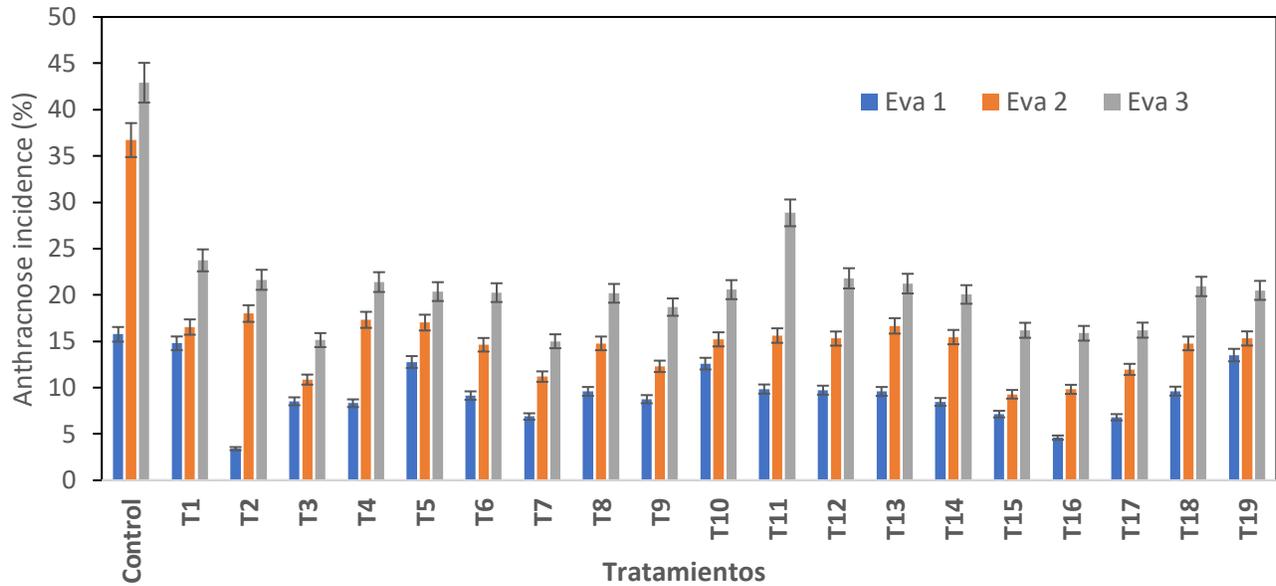


Figura 5. Comparación de la incidencia de antracnosis en tres evaluaciones, en un huerto de mango “Ataulfo” en el ejido de El Corte, Municipio de Santiago Ixcuintla, Nayarit. Tratamientos: Control; T1= Tricho-6 hule (*Trichoderma*); T2= Bac-5 Ch (*Bacillus subtilis*); T3= Bac-4 (*B. subtilis*); T4= Bac-2 Ch (*Azotobacter chroococcum*); T5= Bac-6 (*B. subtilis*); T6= Bac-3 Ch (*B. mucilaginosus*); T7= Bac-17 (*B. subtilis*); T8= Bac-7 Ch (*Pseudomonas fluorescens*); T9= Bac-21 (*B. subtilis*); T10= Bac-6 Ch (*B. amyloliquefasciens*); T11= Bac-23 (*B. subtilis*); T12= Bac-7 Ch (*B. licheniformis*); T13= Bac-9 café (*B. subtilis*); T14= Tricho-Cesix (*Trichoderma*); T15= Bac-10 Café (*B. subtilis*); T16= Bac-8 Ch (*Streptomyces microflavus*); T17= Bac-13 Café (*B. subtilis*); T18= Fungifree (*B. subtilis*); T19= Tricho-Pino (*Trichoderma*).

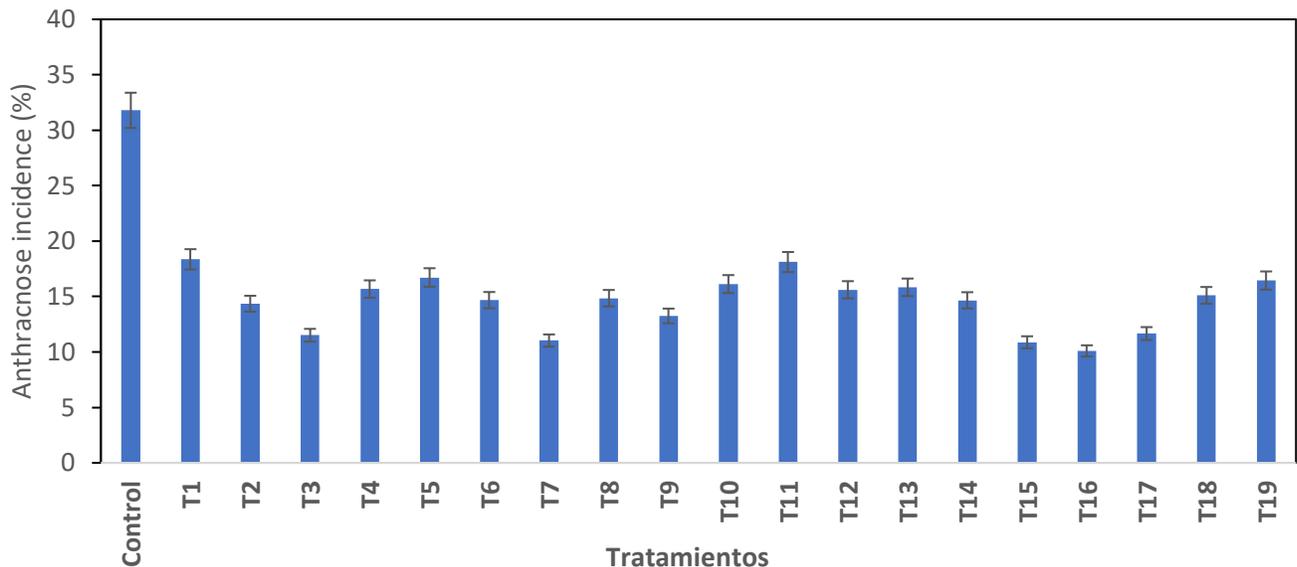


Figura 6. Incidencia promedio de antracnosis en tres evaluaciones, en un huerto de mango “Ataulfo” en el ejido de El Corte, Municipio de Santiago Ixcuintla, Nayarit. Tratamientos: Control; T1= Tricho-6 hule (*Trichoderma*); T2= Bac-5 Ch (*Bacillus subtilis*); T3= Bac-4 (*B. subtilis*); T4= Bac-2 Ch (*Azotobacter chroococcum*); T5= Bac-6 (*B. subtilis*); T6= Bac-3 Ch (*B. mucilaginosus*); T7= Bac-17 (*B. subtilis*); T8= Bac-7 Ch (*Pseudomonas fluorescens*); T9= Bac-21 (*B. subtilis*); T10= Bac-6 Ch (*B. amyloliquefasciens*); T11= Bac-23 (*B. subtilis*); T12= Bac-7 Ch (*B. licheniformis*); T13= Bac-9 café (*B. subtilis*); T14= Tricho-Cesix (*Trichoderma*); T15= Bac-10 Café (*B. subtilis*); T16= Bac-8 Ch (*Streptomyces microflavus*); T17= Bac-13 Café (*B. subtilis*); T18= Fungifree (*B. subtilis*); T19= Tricho-Pino (*Trichoderma*).

## **3.2. Severity of anthracnose in fruits**

### **3.2.1. Orchard in Huaristemba**

The severity increased in all treatments as the evaluation times elapsed. The control fruits showed the highest severity in the last evaluation. In the first evaluation, the control fruits registered severities close to 8 %, however, in the second evaluation this increased significantly up to 24 % (Figure 7). In the first evaluation, most of the treatments showed severities below 10 %, however, only treatments T2, T6, T10 and T14 presented severities above 10 %. For the second evaluation, the fruits of the treatments T1, T4 and T15 obtained the lowest severities with values close to 8 %, followed by the treatments T18 (9 %), T8 and T17 (10 %), T19 (11%), T3 (12%), T11 and T9 (13%). The rest of the treatments had severities between 14 and 18 % (Figure 7). In the average severity of the two evaluations, the treatments T15, T4, T8, T17, T1, T18 and T19 showed the lowest severities in a range of 5 to 7 %. The rest of the treatments had severities above 9 %. The control fruits presented an average severity of 16 % (Figure 8).

### **3.2.2. Orchard in El Corte**

The severity increased in all treatments as the evaluation times elapsed. The fruits of the T5 and T10 treatments showed the highest severity with 21 and 20 % respectively, while the control fruits presented severities of 18 %. In the first evaluation, the control fruits registered severities close to 6 %, however, in the second evaluation this increased significantly up to 18 % (Figure 9). In the first evaluation, more than half of the treatments showed severities below 10 %, however, the treatments T2, T6, T8, T9, T10, T11, T17 and T19 presented severities above 10 %. For the second evaluation, the fruits that showed lower severities were T15 (8 %), T3 and T7 (10 %), T13 and T14 (11 %) and T9 (12 %). The rest of the treatments had severities between 14 and 21 % (Figure 9). In the average severity of the two evaluations, the treatments T3, T7, T13, T14, T15 and T17 showed the lowest severities in a range of 6 to 7 %. The rest of the treatments had severities above 9 %. The control fruits presented an average severity of 12 % (Figure 10).

## **3.3. Effectiveness of control of anthracnose in fruits**

In the Huaristemba orchard, it was determined that the T16 treatment showed control effectiveness of 74 %. Other treatments that had effectiveness equal to or greater than 70 % were T1, T6 and T8 (72%), T9 (71%), T4, T17 and T19 (70 %). While the fruits treated with Fungifree™ (T18) presented an efficacy of 74 % (Figure 11). On the other hand, in the El Corte orchard it was observed that the control effectiveness in all treatments were less than 70 %. The treatments that presented greater effectiveness were T3 and T7 (65 %), T16 (63 %), T15 and T17 (62 %), and T9 (57 %). The commercial product Fungifree™ (T18) showed an efficacy of 51 % (Figure 12).

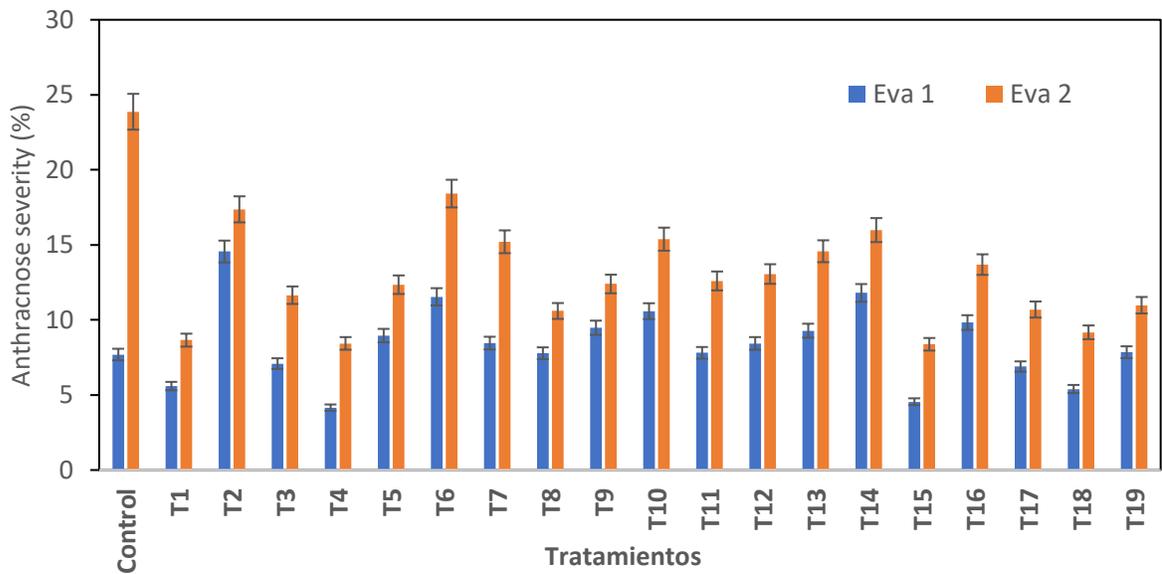


Figure 7. Severity of anthracnose in two evaluations, in an "Ataulfo" mango orchard in the common of Huaristemba, Municipality of San Blas, Nayarit. Treatments:Control; T1= Tricho-6 hule (*Trichoderma*); T2= Bac-5 Ch (*Bacillus subtilis*); T3= Bac-4 (*B. subtilis*); T4= Bac-2 Ch (*Azotobacter chroococcum*); T5= Bac-6 (*B. subtilis*); T6= Bac-3 Ch (*B. mucilaginosus*); T7= Bac-17 (*B. subtilis*); T8= Bac-7 Ch (*Pseudomonas fluorescens*); T9= Bac-21 (*B. subtilis*); T10= Bac-6 Ch (*B. amyloliquefasciens*); T11= Bac-23 (*B. subtilis*); T12= Bac-7 Ch (*B. licheniformis*); T13= Bac-9 café (*B. subtilis*); T14= Tricho-Cesix (*Trichoderma*); T15= Bac-10 Café (*B. subtilis*) T16= Bac-8 Ch (*Streptomyces microflavus*); T17=Bac-13 Café (*B. subtilis*); T18= Fungifree (*B. subtilis*); T19= Tricho-Pino (*Trichoderma*).

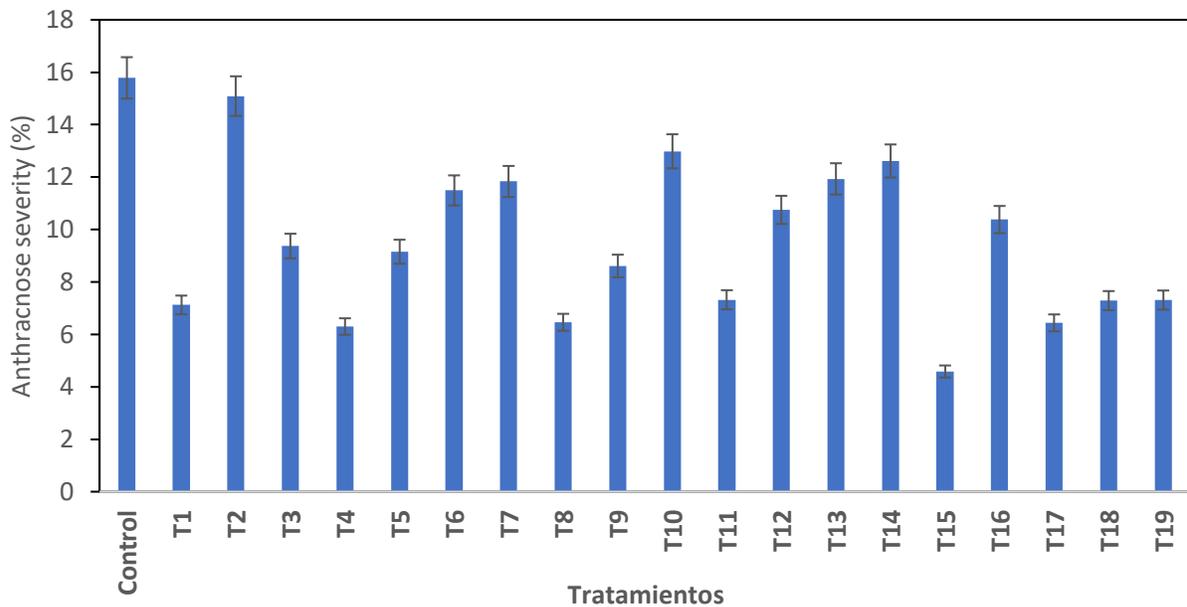


Figure 8. Average severity of anthracnose in two evaluations, in an "Ataulfo" mango orchard in the common of Huaristemba, Municipality of San Blas, Nayarit. Treatments:Control; T1= Tricho-6 hule (*Trichoderma*); T2= Bac-5 Ch (*Bacillus subtilis*); T3= Bac-4 (*B. subtilis*); T4= Bac-2 Ch (*Azotobacter chroococcum*); T5= Bac-6 (*B. subtilis*); T6= Bac-3 Ch (*B. mucilaginosus*); T7= Bac-17 (*B. subtilis*); T8= Bac-7 Ch (*Pseudomonas fluorescens*); T9= Bac-21 (*B. subtilis*); T10= Bac-6 Ch (*B. amyloliquefasciens*); T11= Bac-23 (*B. subtilis*); T12= Bac-7 Ch (*B. licheniformis*); T13= Bac-9 café (*B. subtilis*); T14= Tricho-Cesix (*Trichoderma*); T15= Bac-10 Café (*B. subtilis*) T16= Bac-8 Ch (*Streptomyces microflavus*); T17=Bac-13 Café (*B. subtilis*); T18= Fungifree (*B. subtilis*); T19= Tricho-Pino (*Trichoderma*).

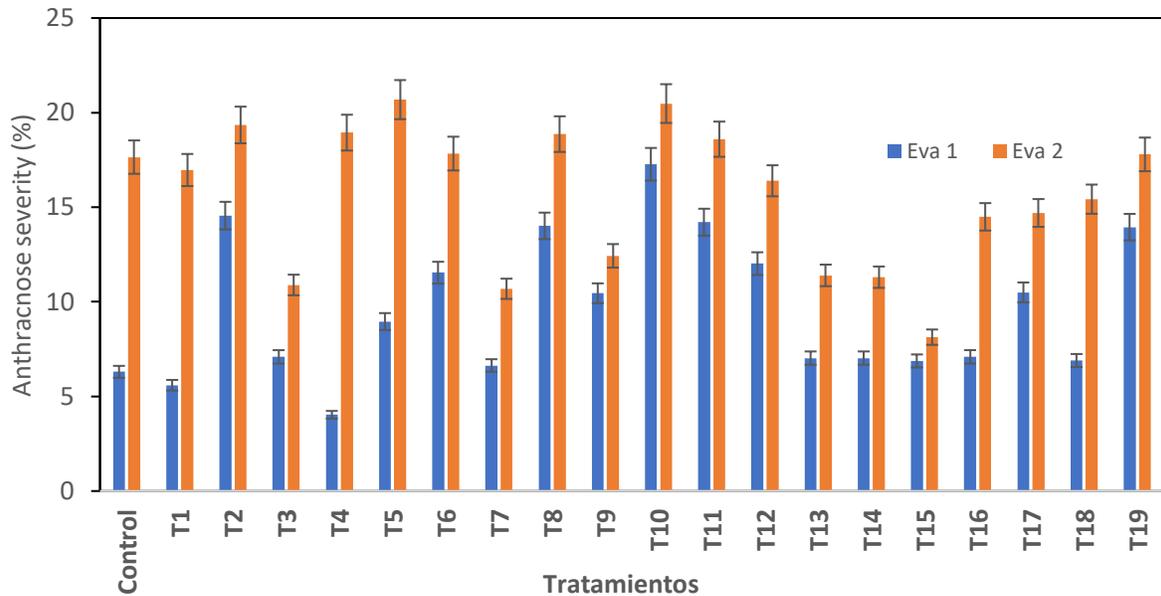


Figure 9. Severity of anthracnose in two evaluations, in an "Ataulfo" mango orchard in the common of El Corte, Municipality of Santiago Ixcuintla, Nayarit. Treatments: Control; T1= Tricho-6 hule (*Trichoderma*); T2= Bac-5 Ch (*Bacillus subtilis*); T3= Bac-4 (*B. subtilis*); T4= Bac-2 Ch (*Azotobacter chroococcum*); T5= Bac-6 (*B. subtilis*); T6= Bac-3 Ch (*B. mucilaginosus*); T7= Bac-17 (*B. subtilis*); T8= Bac-7 Ch (*Pseudomonas fluorescens*); T9= Bac-21 (*B. subtilis*); T10= Bac-6 Ch (*B. amyloliquefasciens*); T11= Bac-23 (*B. subtilis*); T12= Bac-7 Ch (*B. licheniformis*); T13= Bac-9 café (*B. subtilis*); T14= Tricho-Cesix (*Trichoderma*); T15= Bac-10 Café (*B. subtilis*) T16= Bac-8 Ch (*Streptomyces microflavus*); T17=Bac-13 Café (*B. subtilis*); T18= Fungifree (*B. subtilis*); T19= Tricho-Pino (*Trichoderma*).

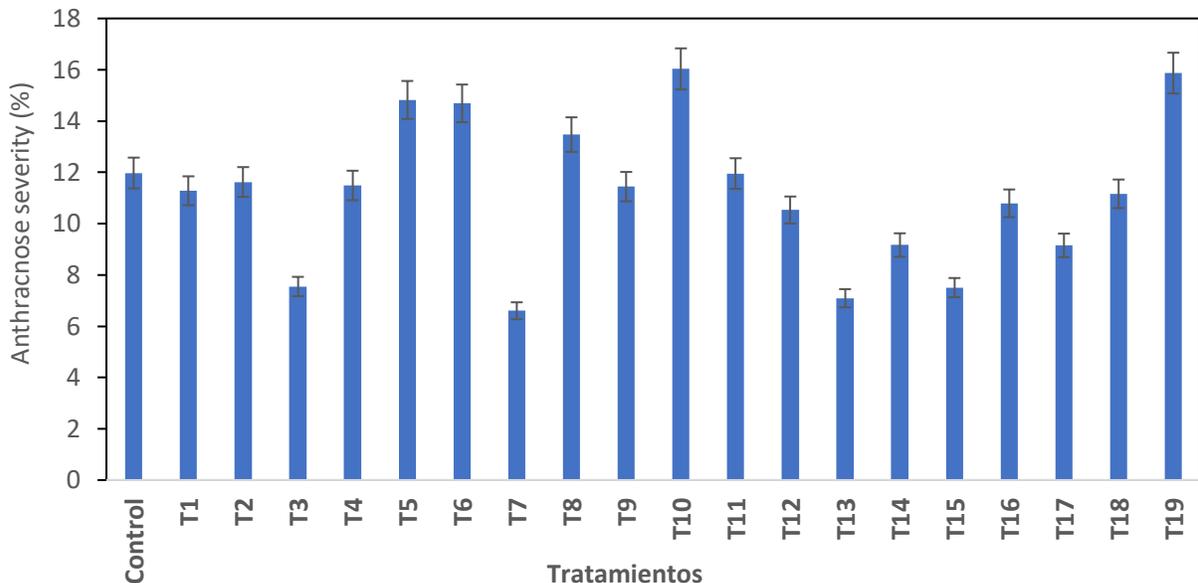


Figure 10. Average severity of anthracnose in two evaluations, in an "Ataulfo" mango orchard in the common of El Corte, Municipality of Santiago Ixcuintla, Nayarit. Treatments: Control; T1= Tricho-6 hule (*Trichoderma*); T2= Bac-5 Ch (*Bacillus subtilis*); T3= Bac-4 (*B. subtilis*); T4= Bac-2 Ch (*Azotobacter chroococcum*); T5= Bac-6 (*B. subtilis*); T6= Bac-3 Ch (*B. mucilaginosus*); T7= Bac-17 (*B. subtilis*); T8= Bac-7 Ch (*Pseudomonas fluorescens*); T9= Bac-21 (*B. subtilis*); T10= Bac-6 Ch (*B. amyloliquefasciens*); T11= Bac-23 (*B. subtilis*); T12= Bac-7 Ch (*B. licheniformis*); T13= Bac-9 café (*B. subtilis*); T14= Tricho-Cesix (*Trichoderma*); T15= Bac-10 Café (*B. subtilis*) T16= Bac-8 Ch (*Streptomyces microflavus*); T17=Bac-13 Café (*B. subtilis*); T18= Fungifree (*B. subtilis*); T19= Tricho-Pino (*Trichoderma*).

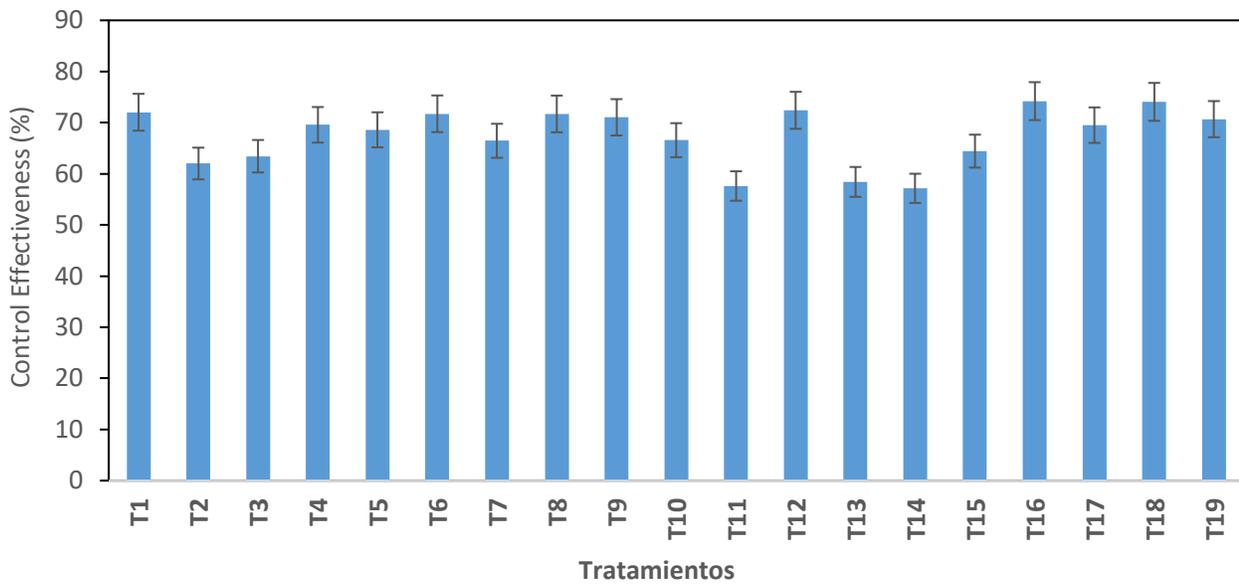


Figure 11. Effectiveness of control of anthracnose in fruits, in an "Ataulfo" mango orchard in the common of Huaristemba, Municipality of San Blas, Nayarit. Treatments: Control; T1= Tricho-6 hule (*Trichoderma*); T2= Bac-5 Ch (*Bacillus subtilis*); T3= Bac-4 (*B. subtilis*); T4= Bac-2 Ch (*Azotobacter chroococcum*); T5= Bac-6 (*B. subtilis*); T6= Bac-3 Ch (*B. mucilaginosus*); T7= Bac-17 (*B. subtilis*); T8= Bac-7 Ch (*Pseudomonas fluorescens*); T9= Bac-21 (*B. subtilis*); T10= Bac-6 Ch (*B. amyloliquefasciens*); T11= Bac-23 (*B. subtilis*); T12= Bac-7 Ch (*B. licheniformis*); T13= Bac-9 café (*B. subtilis*); T14= Tricho-Cesix (*Trichoderma*); T15= Bac-10 Café (*B. subtilis*); T16= Bac-8 Ch (*Streptomyces microflavus*); T17= Bac-13 Café (*B. subtilis*); T18= Fungifree (*B. subtilis*); T19= Tricho-Pino (*Trichoderma*).

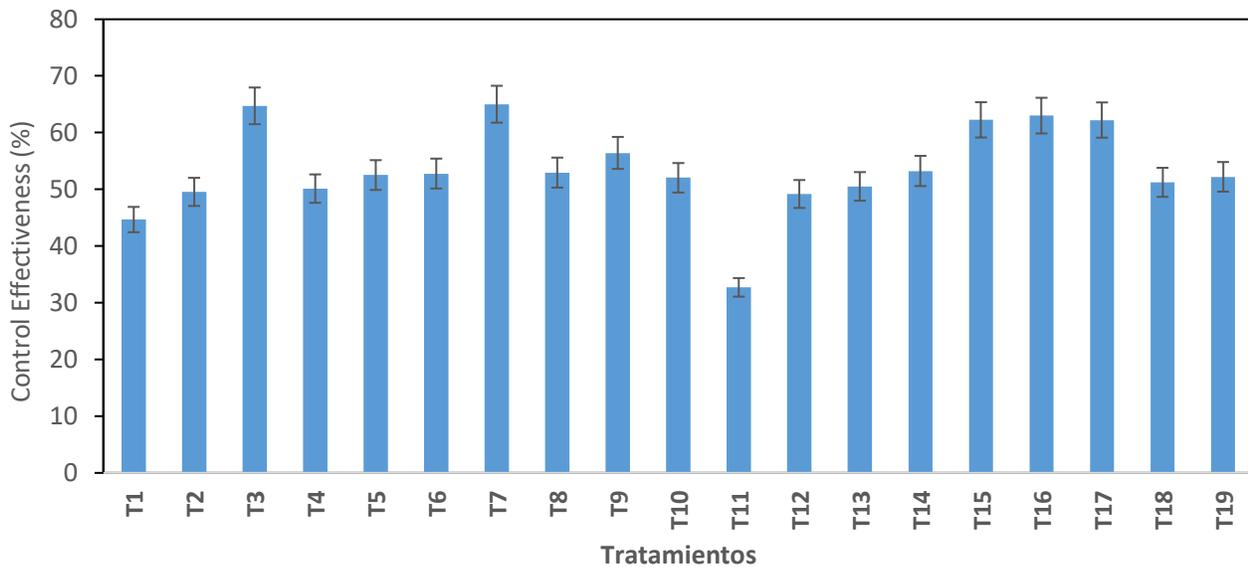


Figure 12. Effectiveness of control of anthracnose in fruits, in an "Ataulfo" mango orchard in the common of El Corte, Municipality of Santiago Ixcuintla, Nayarit. Treatments: Control; T1= Tricho-6 hule (*Trichoderma*); T2= Bac-5 Ch (*Bacillus subtilis*); T3= Bac-4 (*B. subtilis*); T4= Bac-2 Ch (*Azotobacter chroococcum*); T5= Bac-6 (*B. subtilis*); T6= Bac-3 Ch (*B. mucilaginosus*); T7= Bac-17 (*B. subtilis*); T8= Bac-7 Ch (*Pseudomonas fluorescens*); T9= Bac-21 (*B. subtilis*); T10= Bac-6 Ch (*B. amyloliquefasciens*); T11= Bac-23 (*B. subtilis*); T12= Bac-7 Ch (*B. licheniformis*); T13= Bac-9 café (*B. subtilis*); T14= Tricho-Cesix (*Trichoderma*); T15= Bac-10 Café (*B. subtilis*); T16= Bac-8 Ch (*Streptomyces microflavus*); T17= Bac-13 Café (*B. subtilis*); T18= Fungifree (*B. subtilis*); T19= Tricho-Pino (*Trichoderma*).

## 4. CONCLUSIONS

1. No presence of anthracnose was found in the inflorescences of treatment trees, including control trees.
2. It was determined that the control fruits showed the highest incidence and severity of anthracnose in the two orchards. In orchard one located in Huaristemba, the incidence was 44 % of diseased fruits with anthracnose. In the case of orchard two (El Corte), the incidence of anthracnose was 43 %. Regarding the severity of anthracnose in fruits, orchard one had a severity of 24 %. That is, of the total area of the fruit, 24 % presented anthracnose. On the other hand, orchard two showed a severity of 18%.
3. In the incidence of anthracnose in fruits treated with biological agents (bacteria and fungi) and the commercial product Fungifree™ (*Bacillus subtilis*), it was observed that there were differences between treatments. The treatments that showed lower incidences in the two orchards (Huaristemba and El Corte) were the treatments T15, T16 and T17 with incidences of 11 to 16 %, followed by T3 and T7 with an incidence of 15 % in both orchards. Fungifree™ showed an incidence of 11% in orchard one, and 20% in orchard two.
4. The treatments that showed less severity in the two orchards (one and two) were T15 (4.5-7.5 %), T17 (6.4-7.5 %), T4 (6-11 %), T1 (7-11 %), and T16. (10-11 %). The severity in orchard one was in the range of 8 to 24 %, while in orchard two it was from 8 to 21 %. Fungifree™ showed a severity of 7 % in orchard one, and 11 % in orchard two.
5. In the control effectiveness, orchard one was in the range of 57 and 72 % effectiveness, while in orchard two it was from 32 to 65 %. The treatments that showed better average effectiveness in the two orchards were T16 (69 %), T7 and T17 (66 %), T3 and T9 (64 %) and T15 (63 %). In the case of Fungifree™, the average effectiveness of both orchards was 62 %.
6. It was determined that the control fruits showed the highest incidence and severity of anthracnose. It was observed that some treatments with biological agents showed better effectiveness in controlling anthracnose than the commercial product Fungifree™. However, none of the treatments showed incidences less than 5% or control efficiencies above 85%.

## 5. PHOTOGRAPHIC APPENDIX



Figure 13. Application of antagonistic bacteria and fungi of the genus *Trichoderma* to control anthracnose in fruits, in "Ataulfo" mango orchards in the commons of Huaristemba, Municipality of San Blas, Nayarit, and El Corte, Municipality of Santiago Ixcuintla, Nayarit.



Figure 14. "Ataulfo" mango fruits with symptoms associated with anthracnose, in commercial orchards in the ejidos of Huaristemba, Municipality of San Blas, Nayarit, and El Corte, Municipality of Santiago Ixcuintla, Nayarit.