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# BIOLOGICAL CONTROL in vitro of *Colletotrichum* sp. ISOLATED FROM ROÑA SYNDROME IN AVOCADO *Persea americana* Mill. IN MICHOACAN

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**Abstract:** Phytosanitary reviews in different avocado orchards in agroecological zones of Michoacán reveal diversity of symptoms attributed to *Colletotrichum* spp. The objective of this research was to isolate the pathogens associated with avocado scab syndrome, verify their pathogenesis, as well as carry out an in vitro biological control. Samples of avocado fruits with scab syndrome were collected in the municipalities ofSan Juan Nuevo, Los Reyes and Uruapan. The pathogen was isolated, pathogenicity tests were performed, and in vitro biological control was carried out based on four treatments:Baktillis (*Bacillus subtilis*) 1 mL/L, Natucontrol (*Trichoderma harzianum*), 0.3 g/L, Labrador (toxins of *T. harzianum*) 0.024 g/L and Bactiva (*Bacillus* spp.) 0.16 g/L, and an absolute Witness, with a completely random design. It was determined the presence of *Colletotrichum gloeosporioides*., based on morphological attributes. Corchosis symptoms were produced in the inoculated fruits, which were not similar to the symptoms presented by the fruits from which the pathogen was isolated. In which, the *Colletotrichum gloeosporioides*, it can also cause scab syndrome, but it is not responsible for the typical scab or purple spot on avocado. The product that had greater control was Natucontrol (*T. harzianum*).
**Keywords:** Biological products, Mushrooms, *Colletotrichum* spp.

## INTRODUCTION

There are more than 60 countries that produce avocado worldwide and there are more than 500 varieties; however, the Mexican countryside is the generator of three of the most important: Hass, Criollo and Fuerte. (APEAM, 2013)

In Mexico, avocado production has grown significantly in recent years, between 2002 and 2015 the average annual growth rate was 4.2%, while between 2011 and 2015 the average growth was 8.7% (SIAP, 2016).

The state of Michoacán is the main supplier of avocado, contributing in 2015 78.6% of the national production. (Fishing, 2016)

Pests and diseases in the avocado crop affect to a greater or lesser extent the yield per ha (Márquez, 2014)

One of the diseases that affects the quality of the fruit is avocado scab, which is the common name for the symptom of fungal origin that, according to Antonelli, 1980, is caused by the stimulation of structural defense mechanisms. induced in plants in the presence of an invading agent (biotic or abiotic), which involve histological changes. In this case, according to Ramírez-Gómez and Rodríguez, 2012, the plant produces layers of cork that prevent the advance of the fungal mycelium to healthy tissue.

The species that have been identified to date and that are involved in scab syndrome: *Sphaceloma perseae* (Everett *et al.*, 2011, Fan *et al., 2*017, López, 20021, Hinojosa, 2021) although *Colletotrichum gloeosporioides,* it has been reported as a secondary fungus (Crous et al., 2009), and other biotic factors such as a high incidence of Thrips and mites or abiotic mechanical damage damage from wind friction (López, 20021, Hinojosa, 2021).

Some of the common names of the disease caused by the genus Colletotrichum in avocado fruit are: chicken pox, smallpox, clove, anthracnose, purple spot or black spot. (Morales, 2013)

Regarding the genus Colletotrichum, a wide range of control measures for this fungus is mentioned (Pérez et al., 2013)

In this context, the present work evaluated the in vitro biological control of *Colletotrichum* sp. isolated from avocado scab syndrome *Persea americana with different fungus-based fungicides* (*Trichoderma* sp.) and bacteria (*Bacillus* spp.) under laboratory conditions.

## MATERIALS AND METHODS

### LOCATION AND LOCATION OF THE SAMPLING AREA

Samples of avocado fruits with scab syndrome were collected from different orchards located in **Uruapan:** 19º25' N, 102º03' O, at 1620 meters above sea level. Vegetable garden: San José -1, owner: Mr. José Manuel Méndez Padilla, location: Las Tejerías. **Los Reyes:** 19° 35' N and 102° 28' W, 1140-1536 m.s.n.m. Huerta: They attack. Owner: Senor: Carlos Méndez Ubicación. Atapan and **San Juan Nuevo:** 19º25' N and 102º08' O, 1880 m.s.n.m. The samples were processed under laboratory conditions at the Faculty of Agrobiology "presidente Juárez" belonging to the Michoacana University of San Nicolás de Hidalgo.

### ISOLATION OF THE PATHOGEN

The collected fruits with scab syndrome were washed with running water and soap (gently rubbing). They were rinsed and allowed to air dry. 2-5 mm2 fragment of the damaged tissue areas were cut with the aid of a scalpel. They were disinfected with 3% sodium hypochlorite for 30 seconds, rinsed three times with sterile distilled water and placed on sterile paper towels to remove excess water. Five fragments were then placed per Petri dish with Potato-Dextrose-Agar (PDA) culture medium distributed equidistantly, the Petri dishes were sealed with a kleen pack and placed in the incubation chamber at a temperature of 26 ºC, monitored. every 24 hours for 5 days until observing development of mycelium of the fungus.

### IDENTIFICATION OF THE ISOLATED PATHOGEN

To identify the pathogen, preparations were made on slides, a drop of lactophenol was added, then fragments of mycelium were placed with a dissection needle and a coverslip was placed, later they were observed under a microscope (40 X). The identification was made based on the shape of the mycelium, its coloration and the morphology of the conidia based on what was described in the Barnett (1998) keys and what was described by Sutton (1992).

### STRAIN PURIFICATION

This was carried out using the technique of successive sowing of hyphal tips in PDA culture medium, this technique was applied until completely pure strains of the pathogen were obtained, the boxes were placed at a temperature of 24 to 26 ºC for their development.

### PATHOGENICITY TESTS

Pathogenicity tests were performed with each of the strains isolated from the municipalities.

All the collected strains were adjusted to a concentration of 1.25 x108 conidia per milliliter. The inoculations were carried out in nursery avocado plants cultivar Méndez with healthy fruits which were in an area conditioned as a greenhouse at the Faculty of Agrobiology U.M.S.N.H.

For the inoculation, wounds were made on the fruits of the plants, the conidia suspension was sprayed, bags were placed as a humid chamber for three days, they were labeled and they were monitored daily until the appearance of symptoms.

### *IN VITRO* BIOASSAYS

Four Products were used to carry out the bioessays: Baktillis (*Bacillus subtilis*). Natucontrol (*T. harzianum*), Bactiva (*Bacillus* spp.) and Labrador (toxins of *T. harzianum*). (Table 1).

PDA culture medium was used, in which a disc with mycelium of 1 cm in diameter of the pure fungus was placed in the center of the Petri dish. Filter paper discs of approximately 1 cm in diameter were impregnated with each of the Biological Products. With forceps, 4 disks were placed in each Petri dish equidistantly. They were monitored at 26ºC, measuring the growth of the fungus every 24 hours until the Witness filled the box.

## STATISTIC ANALYSIS

A completely randomized experimental design was used with 4 treatments, a Witness and 4 repetitions. With the data obtained, an analysis of variance and the Tukey test ($P<0.05$) were performed for the comparison of means between treatments. Statistical analysis was performed with the SAS program. version 9.0.

# RESULTS

## IDENTIFICATION OF THE CAUSAL AGENT

It was identifed: *Colletotrichum gloeosporioides*., based on the keys of Barnett (1998) and what was described by Sutton, (1992). Due to the shape and color of the colony, which varied from light gray to dark gray, it presented aerial mycelium and the colony was fast growing, unicellular, hyaline conidia of 12 to 17 x 3.5 to 6 µm were observed, which coincides with what was observed. reported by Morales, (2000) (Figure 1).

## PATHOGENICITY TESTS

The first symptoms were observed 14 days after the inoculations on the surface of the fruit, the symptoms were not similar to those presented by the fruits from which it was isolated. *Colletotrichum* sp., agreeing with López 2021 and Hinojosa 2021, who found that when inoculating avocado fruits with *Colletotrichum* sp. these showed symptoms of corchosis, but were not similar to corchosis caused by *Sphaceloma perseae*, nor to the typical scab (Figure 2).

And it also agrees with Crous et al., 2009 who mentions that *Coletotrichum* sp. is a secondary fungus in scab syndrome.

## BIOASSAYS

### Strain collected in the Municipality of Uruapan

The best treatment for in vitro control of the strain of *Colletotrichum gloeosporioides* isolated from Mpio. from Uruapan was Natucontrol (T. *harzianum*) since it presented the lowest growth 36 %, followed by Bactiva (*Bacillus* spp.) which showed 46% growth: Baktillis (*B. subtilis*) con 78 %, and Labrador (toxins of *T. harzianum* with 89 % growth, all treatments were statistically superior to the Witness treatment which presented 100 % growth (Table 2 and Figure 3).

### Strain collected in the Municipality of Los Reyes

The best treatment for in vitro control of the strain of Colletotrichum sp. isolated from los Reyes was Natucontrol (*T. harzianum*), since it presented the lowest growth 47%, followed by Bactiva (*Bacillus* spp.) which showed 60% growth, Baktillis (*B. subtilis*) with 86 %, and Labrador (toxins of *T. harzianum*) (toxins of *T. harzianum*) were statistically superior to the control treatment which presented 100 % growth (Table 3 and Figure 4).

### Strain collected in San Juan Nuevo

The best treatment for in vitro control of the strain of *Colletotrichum* sp. isolated from San Juan was Natucontrol (*T. harzianum*), since it presented the lowest growth 38%, followed by Bactiva (*Bacillus* spp.) which showed 48% growth, Baktillis (*B. subtilis*) with 78%, and Labrador (toxins from *T. harzianum*) with 98% growth, all treatments were statistically superior, which was equal to the control treatment which presented 100% growth (Table 4, Figure 5).

| Trade name | Active ingredient | Average Dose | Dose/L of water |
|---|---|---|---|
| Baktillis | *Bacillus subtilis* | 1.5 L/ha | 0.075 mL |
| Natucontrol | *Trichoderma harzianum* | 600 g/ha | 0.3g |
| Labrador | Toxins of *Trichoderma harzianum* | 480 g/ha | 0.024g |
| Bactiva | *Bacillus polymyxa, B. subtilis, B. megaterium, T. harzianum T. reesei, T. viride.* | 325 g/ha | 0.16g |
| Witness | *Without fungicide* | 1 L | 1 L |

Table 1: Products and doses used in the experiment.

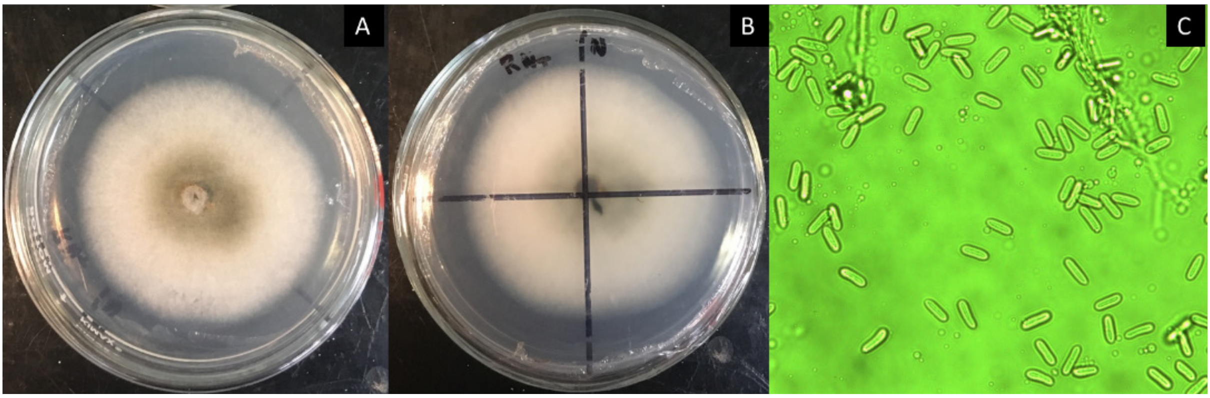

Figure 1: Strain of *Colletotrichum gloeosporioides*. A) Shape and color of the vine. B) Growth of the strain. C) Shape and size of the conidia.

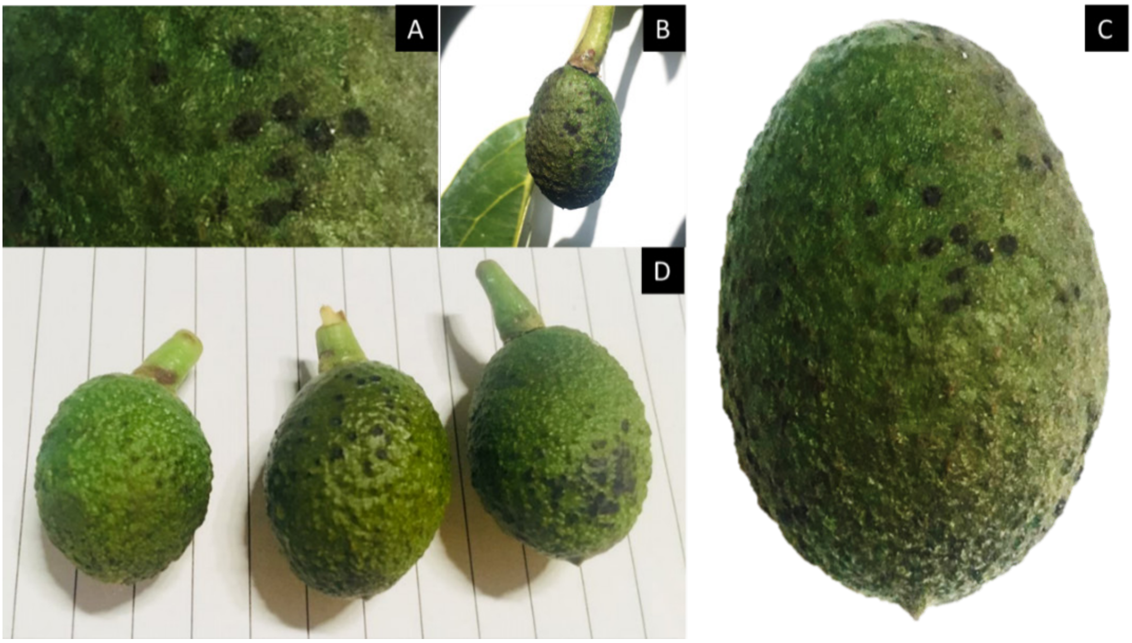

Figure 2: Pathogenicity tests. A) Purple to brown spots. B), C) Fruit with purple to brown spots. D) Fruits of the three municipalities inoculated with the fungus: *Colletotrichum gloeosporioides.*

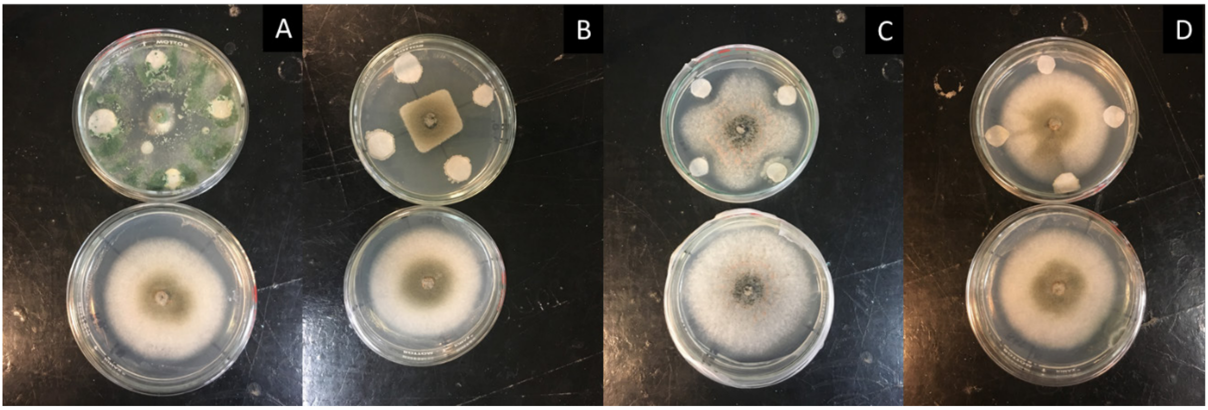


Figure 3: Treatments of the strain of *Colletotrichum gloeosporioides*. Collected from Uruapan A) Natucontrol A) Natucontrol (*T. harzianum*). B) Bactiva (*Bacillus* spp.). C) Baktillis (*B. subtilis*). D) Labrador (toxins of *T. harzianum*). In the top image is the treatment against the bottom image which is the Witness treatment.

| **Products** | **Tukey 0.05** | | | **Growth averages(cm)** | **Growth rate (%)** |
|---|---|---|---|---|---|
| Natucontrol (*T. harzianum)* | | | C | 1.0000 | 36 |
| Bactiva (*Bacillus spp)* | | | C | 1.2750 | 46 |
| Baktillis *(Bacillus subtilis)* | | B | | 2.1750 | 78 |
| Labrador (toxins of *T. harzianum)* | A | B | | 2.5000 | 89 |
| Witness | A | | | 2.8000 | 100 |

Table 2. Comparison of means (Tukey 0.05) and Growth rate of the strain of *Colletotrichum gloeosporioides*. Collected from Uruapan with the application of fungicides.

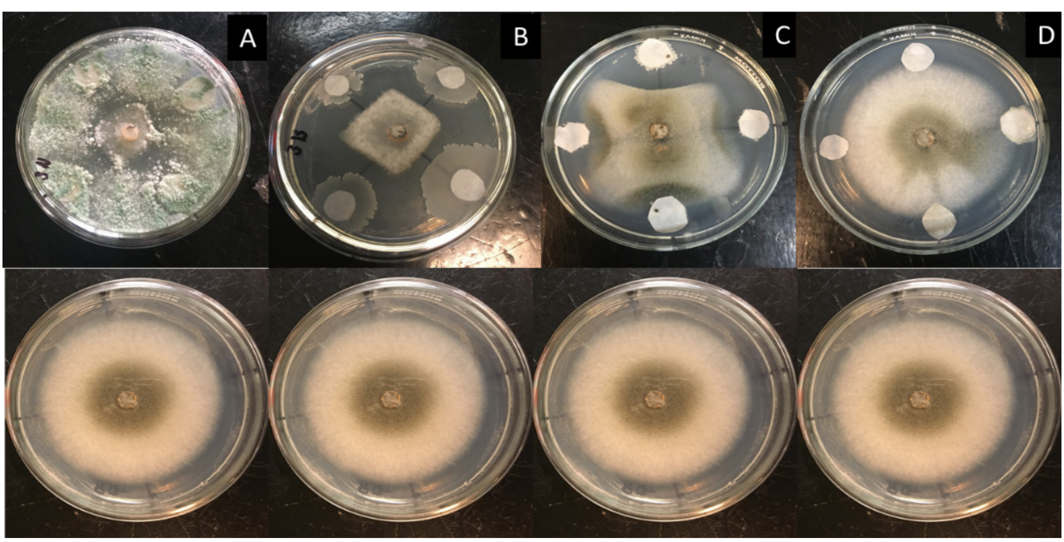


Figure 4: Treatment of the Colletotrichum sp. Collected from the Municipality of Los Reyes. A) Natucontrol (*T. harzianum*). B) Bactiva (*Bacillus* spp.). C) Baktillis (*B. subtilis*). D) Labrador (toxins of *T. harzianum*). In the upper image is the treatment against the lower image which is the control treatment.

| Products | Tukey 0.05 | | | Growth averages(cm) | Growth rate (%) |
|---|---|---|---|---|---|
| Natucontrol (*T. harzianum)* | | | C | 1,1250 | 47 |
| Bactiva (*Bacillus spp)* | | B | C | 1,4500 | 60 |
| Baktillis *(Bacillus subtilis)* | A | B | | 2,0750 | 86 |
| Labrador (toxins of *T. harzianum)* | A | | | 2,4000 | 100 |
| Witness | A | | | 2,4000 | 100 |

Table 3. Comparison of means (Tukey 0.05) and Growth rate of the Colletotrichum sp. collected in the Municipality of Los Reyes with the application of fungicides.

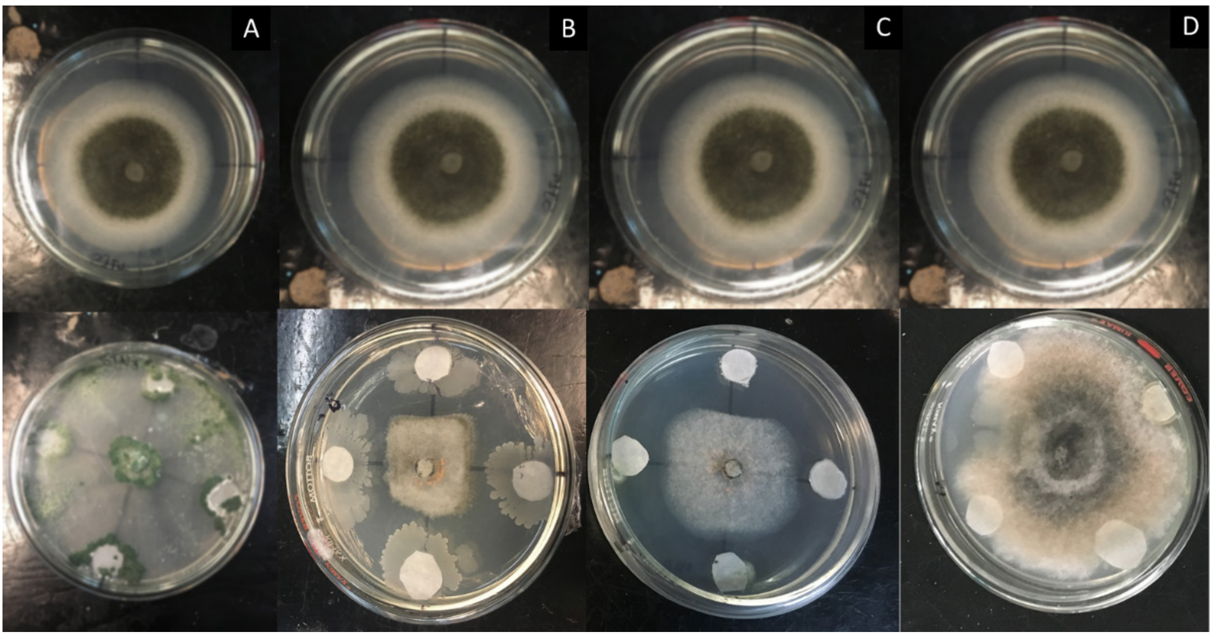


Figure 7: Treatment of the strain collected in the Municipality of San Juan Nuevo. A) Natucontrol (*T. harzianum*). B) Bactiva (*Bacillus* spp.). C) Baktillis (*B. subtilis*). D) Labrador (v*T. harzianum*). Table 4. Comparison of means (Tukey 0.05) and percentage of growth.

| Products | Tukey 0.05 | | Growth averages (cm) | Growth rate (%) |
|---|---|---|---|---|
| Natucontrol (*Trichoderma harzianum)* | | B | 1.0000 | 38 |
| Bactiva (*Bacillus polymyxa, B. subtilis, B. megaterium)* | | B | 1.2750 | 48 |
| Baktillis *(Bacillus subtilis)* | A | | 2.0750 | 78 |
| Labrador (toxins from *Trichoderma harzianum)* | A | | 2.6000 | 98 |
| Witness | A | | 2.6500 | 100 |

Table 4. Comparison of means (Tukey 0.05) and percentage of growth of the strain of *Colletotrichum* spp. collected in San Juan Nuevo with the application of fungicides.

*Trichoderma* sp. is considered the most studied antagonist for the control of phytopathogens. Infante et al. (2009) studied and made a summary about the modes of action. In the integration of the results, they mentioned that the mechanisms of action that allow the control of pathogens by *Trichoderma* sp., there is competition for the substrate, antibiosis parasitism, deactivation of pathogen enzymes, penetration and induced resistance, mainly.

## CONCLUSIONS

1. *Colletotrichum gloeosporioides* causes scab syndrome in avocado fruits that is not the same as that caused by Sphaceloma perseae or typical scab.

3. The product that best controlled *Colletotrichum gloeosporioides*, was Natucontrol (*Trichoderma harzianum*).

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