

## EFFICACY OF FUNGI TRICHO (*Trichoderma* spp.) AND CHEMICAL FUNGICIDES FOR THE CONTROL OF *Botrytis* sp., IN BLACKBERRY

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## INTRODUCTION

The blackberry (*Rubus* spp.) It is originally from Eurasia and It is consumed fresh, frozen, juices, pulps, extracts, which facilitates its marketing. Its importance as a crop worldwide is due to its flavor and versatility in the food industry, as well as its antioxidant properties (Ricárdez *et al.*, 2016).

In the last decade, blackberry production has had significant growth, becoming one of the most important crops in the Mexican agricultural sector, with a production of 215,923 tons. Mexico is the main producer of blackberries worldwide. It is grown in 12 states of the country, but Michoacán is the main producer with 97% of the total production of this strawberry. In the last record, it was determined that the main blackberry producing states are: Michoacán, Jalisco, Colima, Baja California and Guanajuato (SADER, 2022).

Among the main diseases that affect the blackberry crop, gray mold (*Botrytis cinerea*), orange rust (*Arthuriomyces peckianus*), leaf spot (*Cercospora* sp.), anthracnose (*Colletotrichum gloeosporioides*), mildew (*Peronospora sparsa*), and root rot (*Fusarium* sp.), (Contreras *et al.*, 2019).

One of the most relevant diseases is gray mold caused by *Botrytis cinerea*, which attacks hundreds of hosts, including other strawberries such as strawberries (*Fragaria mexicana*), raspberries (*Rubus idaeus*), blueberries (*Vaccinium corymbosum*) and grapes (*Vitis vinifera*). This fungus can live as a saprophyte (on dead tissue) in crop residues and other plants. In blackberry, *Botrytis* sp. It is always present and if conditions are favorable it causes infection in most cases without presenting symptoms until after harvest, so the producer and the technician must keep in mind the phenological stage of the crop and the environmental conditions for taking measurements decisions in the management

of the disease, because losses can be up to 100% in post-harvest if good management is not carried out in the field (Rebollar, 2011).

The infection by the fungus occurs in the field and remains quiescent, during the post-harvest the infection is activated and the disease develops during storage, transportation or even in the market, causing serious losses (Ippolito and Nigro, 2000).

Due to improper management and incorrect use of effective control methods, this disease has caused notable losses in blackberry production. Therefore, in the present investigation, the use of chemical fungicides and the product Fungi-Tricho, a biological fungicide, was proposed to control the damage caused by *Botrytis* sp. at laboratory and field level.

## MATERIALS AND METHODS

Blackberry producers were selected in the areas of Ario de Rosales, Tacámbaro and Ziracuaretiro, Michoacán, Mexico, whose crops in the last season had been most affected by fungus on the fruits. 30 orchards were selected, from which 10 fruits were collected per orchard.

**Characterization and morphological identification of the pathogen:** The collected fruits were transferred to the laboratory, According to the microscopic characteristics (type of mycelium, presence of structures, conidia, etc.) and macroscopic characteristics (shape, texture, color and growth rate), the pathogen was identified, taking as reference the descriptions of Rebollar, (2011) and the dichotomous keys of Barnett and Hunter (1998).

**Pathogen isolation:** According to what was described by Mata and Salmontes, (2021), sections of mycelium were taken from the fruits and subsequently placed in Petri dishes with PDA culture medium (Potato Dextrose Agar, BD Bioxon), added with V8 juice. Finally, the samples were incubated at 24 °C.

**Selection of fungicides:** The selection of fungicides was made based on the active ingredients, Captan, Boscalid + Pyraclostrobin, Fenhexamid, Cyprodinil + Fludioxinil, Copper oxychloride, Azoxystrobin, Sulfur, Cupric hydroxide. Due to the requirements for export to Europe, the Fungi-Tricho product based on strains of *Trichoderma* spp. A total of eight active ingredients were considered, plus the biological product, the doses used were those recommended by the manufacturer.

**Preparation and addition of the active ingredient in the PDA culture medium:** For the evaluation of the chemicals, the poisoned culture medium technique was used. The PDA culture medium was prepared and sterilized at 121°, 15 lb during 15 min. Subsequently, in a laminar flow hood, They added the active ingredients to the PDA culture medium. For this evaluation, five repetitions per treatment and one control were considered.

**Inoculation of the strains:** Once the active ingredient was added to the PDA culture medium, the strains isolated from the different areas were placed in the center of the Petri dish. In a laminar flow hood and with the help of a punch, a *Botrytis* mycelial disk of approximately 5 mm in diameter was placed in the center of each Petri dish. There were five repetitions per active ingredient plus an absolute control. Finally, the boxes were placed in the incubator at 24 °C.

**Antagonism test:** to determine the percentage of inhibition of the biological product based on *Trichoderma* spp. (Fungi-Tricho), *in vitro*, the dual culture technique used by Sonnenbichler *et al.* (1983), against *Botrytis* isolates from different areas. 5 repetitions were established for each of the isolated *Botrytis* strains with their respective controls.

**Establishment of the field experiment:** The experiment was carried out in the municipality of Ziracuaretiro, Mexico, in the town of Zirimicuaro, in the “El Perimal”

orchard at an altitude of 1300 meters above sea level.

**Field treatments:** The selection of treatments was based on the results obtained *in vitro*. Four active ingredients were selected: Fenhexamid, Captan, Boscalid + Pyraclostrobin and Cyprodinil + Fludioxinil, which had the greatest inhibition effect on growth. In addition, the product Fungi-Tricho (*Trichoderma* spp.) was included as another treatment and a conventional control, the latter consisting of the management that the technical advisor establishes in the orchard.

**Collection of fruit samples and preparation of humid chambers:** In order to evaluate the incidence of *Botrytis* sp., in each of the evaluated treatments, samples of ripe fruits were collected, a total of 30 fruits per treatment. Subsequently, the preparation of humid chambers was carried out.

**Experimental design:** A randomized block experimental design was used, where six treatments with five repetitions were used.

## RESULTS AND DISCUSSION

**Isolation and identification of the pathogen:** From the samples obtained in the 3 areas, *Botrytis* sp. was isolated. Colonies with pigmentation and segmented mycelium structures, as well as conidia characteristic of this pathogen, developed in the sowing. The PDA culture medium, added with V8 juice, allowed the growth of *Botrytis* sp. was faster and the morphology of the colony was observed more clearly. The ovoid, unicellular, colorless or gray conidia could be seen, the formation of chlamydo spores, thin, hyaline or pigmented conidiophores, branched in the form of a cluster, their particularity in the structures, the irregular black sclerotia often present flat, hard and black. According to the mentioned characteristics, they coincide with the aspects described by Terrones *et al.*, 2019.

**Sensitivity tests:** According to the results obtained, it was identified that in the Ario de Rosales area the treatments with Captan, Fenhexamid, Boscalid + Pyraclostrobin produced a statistically significant control; while, in the Tacámbaro area, it was found that the treatments with Captan, Boscalid + Pyraclostrobin and Ciprodinil + Fludioxinil obtained a statistically significant control and in the Ziracuaretiro area, the treatments Boscalid + Pyraclostrobin, Fenhexamid and Captan produced a significant control (Figure 1).

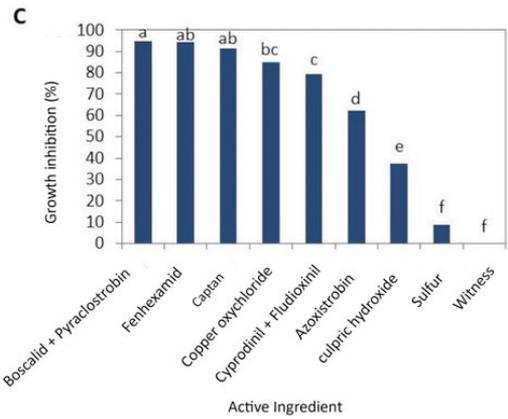
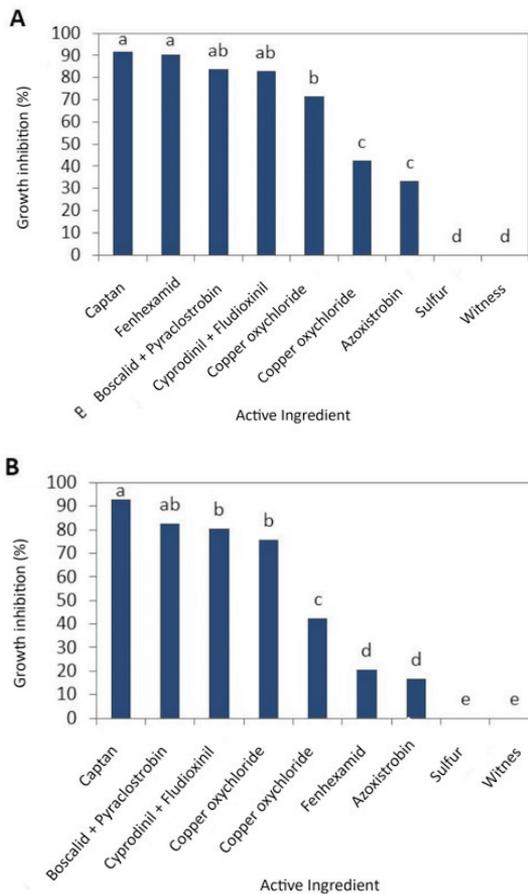


Figure 1. Percentage of inhibition of radial growth of isolates from the Municipalities of Ario de Rosales (A), Tacámbaro (B) and Ziracuaretiro (C) before the active ingredients.

**Effect of active ingredient Vs pathogen:** visual behavior and its effect on the growth inhibition of *Botrytis* sp., in each of the active ingredients evaluated (Figure 2).

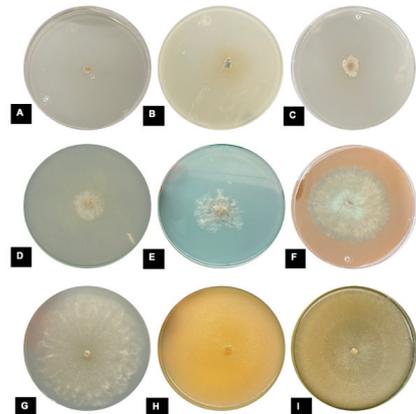


Figure 2: Active Ingredients: Captan (A), Fenhexamid (B), Cyprodinil + Fludioxinil (C), Boscalid + Pyraclostrobin (D), Copper oxychloride (E), Cupric hydroxide (F), Azoxystrobin (G), Sulfur (H) and Witness (I).

**Dual culture antagonism assay:** The percentage of inhibition of the growth of *Botrytis* sp. was obtained. against *Trichoderma* spp. in the different areas evaluated (Figure 3). Growth inhibition was observed after 96 hours, *Trichoderma* spp. inhibited the growth of *Botrytis* sp by 60%.

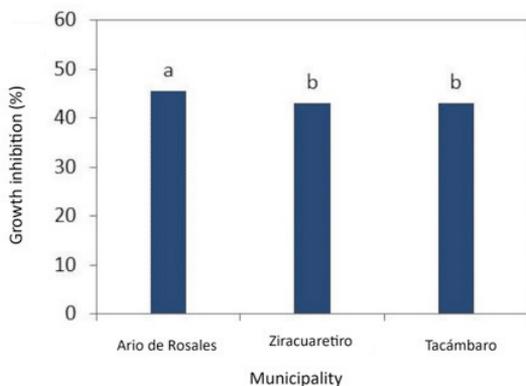


Figure 3: Percentage of radial inhibition of isolates of *Botrytis* sp., from the municipalities of Ario de Rosales, Ziracuaretiro and Tacámbaro, Mexico., after 96 hours of confrontation with *Trichoderma* spp.

Studies carried out by Larios *et al.* (2020) verified that the antagonistic agent *T. harzianum* inhibited the growth of the mycelium of *B. cinerea* by 36%; Although, the growth speed of *T. harzianum* was lower than that of the pathogen in the control treatment. *Trichoderma* spp. managed to stop the development of *Botrytis* sp., upon contact, inhibiting its mycelial growth (Figure 4).

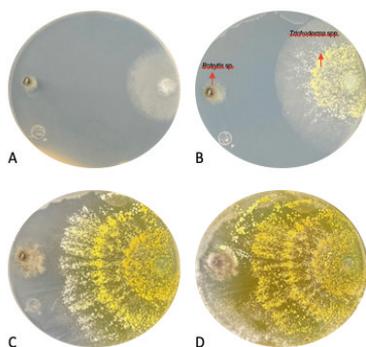


Figure 4. Confrontation of *Botrytis* sp., Vs *Trichoderma* spp., 48 h (A), 72 h (B), 96 h (C), invasion of *Trichoderma* spp. against *Botrytis* sp. at 120 pm (D).

#### Incidence of *Botrytis* sp. in wet chamber:

The incidence of *Botrytis* sp. was determined. In a humid chamber, eight days after the treatments, a decrease in the incidence of

*Botrytis* sp. was observed. with the Captan, Ciprodinil + Fludioxinil and *Trichoderma* spp. treatments, having 100% effective control; while, the treatments Fenhexamid, producer management and Boscalid + Pyraclostrobin had an incidence of *Botrytis* sp. below 35%, but greater than 5% (Figure 5)

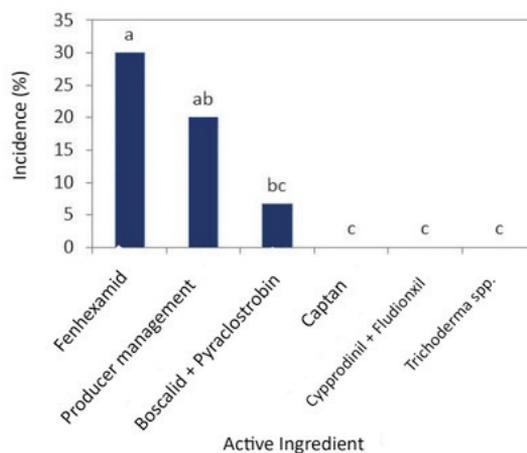


Figure 5. Percent incidence of damage caused by *Botrytis* sp. in each of the treatments evaluated.

## CONCLUSIONS

The symptoms of *Botrytis* sp., in fruits, are the presence of soft rot, grayish mycelium with structures that correspond to the characteristic conidia of the fungus and the structures Macroscopic and microscopic correspond to the fungus *Botrytis* sp. Treatments with Captan, Fenhexamid, Ciprodinil + Fludioxinil and Boscalid + Pyraclostrobin were significantly better in the in vitro control of *Botrytis* sp. In the dual mycoparasitism assay, *Trichoderma* spp. inhibited the growth of *Botrytis* sp by 60%. Under field conditions, a decrease in the incidence of *Botrytis* sp. was observed. with Captan, Ciprodinil + Fludioxinil and *Trichoderma* spp., having 100% effective control; while, Fenhexamid, Producer Management and Boscalid + Pyraclostrobin led to an incidence of *Botrytis* sp. below 35%.

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Secretaría de Agricultura y Desarrollo Rural (SADER) 2022. Consultado en línea el 31/10/22 en: <https://www.gob.mx/agricultura>

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