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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 EurasiaandIt 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 andGuanajuato (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 (Vaccinum 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°, 15lb during15 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 bySonnenbichler 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 + Pyaclostrobin 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.

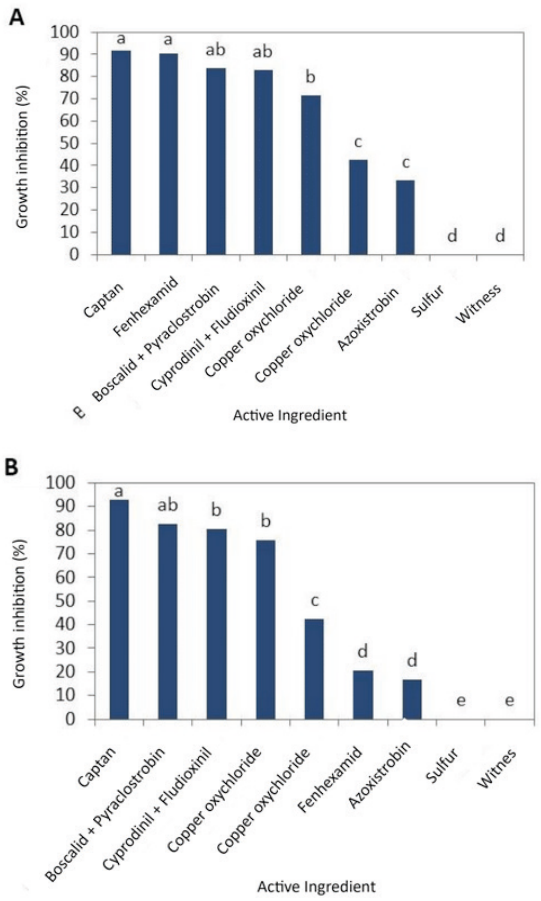
Experimental design:A randomized block experimental design was used, where six treatments with five repetitions were used.

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 chlamydospores, 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 byTerrones 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).



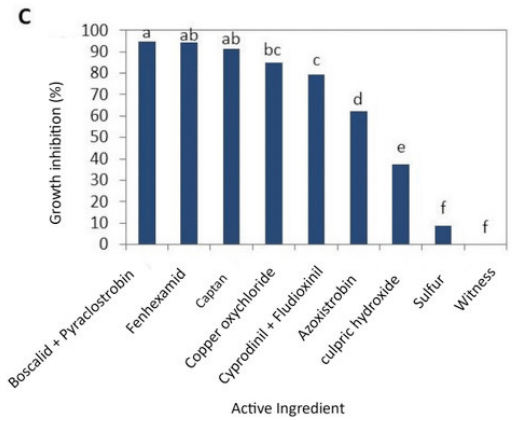


Figure 1. Percentage of inhibition of radial growth of isolates from the Municipalities of Ario de Rosales (A), Tacámbaro (B) and Ziracuaretiro (C) before the active ingredients.

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).

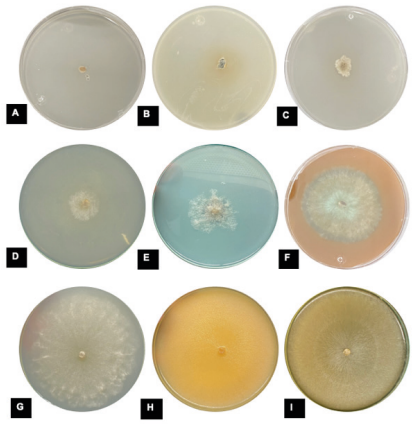


Figure 2: Active Ingredients: Captan (A), Fenhexamid (B), Cyprodinil + Fludioxinil (C), Boscalid + Pyraclostrobin (D), Copper oxychloride (E), Cupric hydroxide (F), Azoxystrobin (G), Sulfur (H) and Witness (I).

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%.

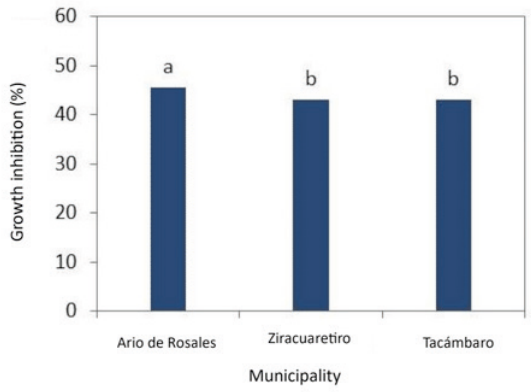


Figure 3: Percentage of radial inhibition of isolates of*Botrytis sp., from the municipalities of Ario de Rosales, Ziracuaretiro and Tacámbaro, Mexico., after 96 hours of confrontation with Trichoderma spp.*

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).

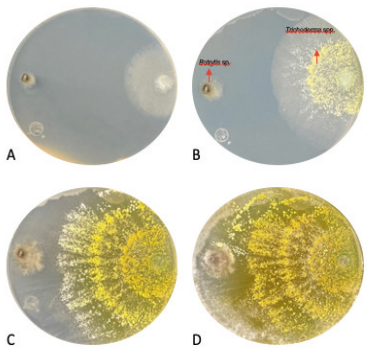


Figure 4. Confrontation of*Botrytis sp., Vs Trichoderma spp., 48 h (A), 72 h (B), 96 h (C), invasion of Trichoderma spp. against Botrytis sp. at 120 pm (D).*

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)

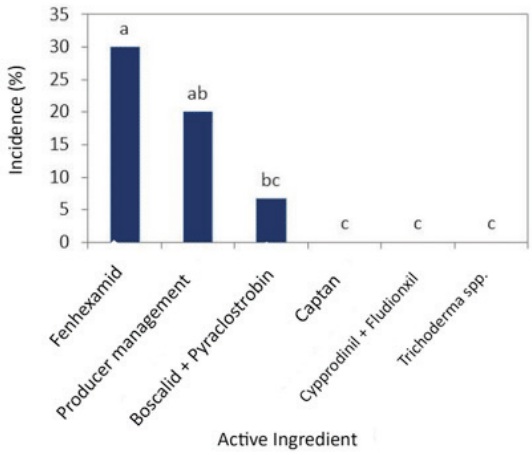


Figure 5. Percent incidence of damage caused by *Botrytis sp.* in each of the treatments evaluated.

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 structuresMacroscopic 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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