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ISOLATION, IDENTIFICATION AND *IN VITRO CHEMICAL CONTROL* OF PATHOGENS RELATED TO ROÑA SYNDROME IN AVOCADO

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Abstract: Mexico is the world leader in the avocado market. The country contributes 34% of the volume of the product worldwide, however, it is often affected by various diseases that cause economic losses and quality in the aesthetics of the fruit, among which scab stands out. Symptoms appear mainly on fruit, leaves and young branches. Scab is identified as any symptom of corchosis as a defense response of the plant to various biotic and abiotic factors. Therefore, the present work was carried out with the following objectives: isolate and identify the fungi associated with scab syndrome in avocado and evaluate *in vitro* the fungi related to this syndrome, using eight products: Bankit Gold ® (Azoxystrobin and fludioxonil) 1.125 mL, Bankit® (Azoxystrobin) 0.2 mL, Folpan ® (Folpet) 1.75 g, Tecto 60 ® (Thiabendazole) 0.3 g, Uniform ® (azoxystrobin +metalaxyl) 0.25 g, Headline ® (Piraclostrobin) 0.078 mL, Cabrio ® (Boscalid + Pyraclostrobin) 0.4g and Blue Shield ® (copper hydroxide) 1 g dose of the formulated product, and a control without fungicide. With a completely randomized design with three repetitions. The isolated fungi were *Colletotrichum* sp., *Alternaria* sp., *Pestalotiopsis* sp., Nigrospora sp. and *Curvularia* sp. Bankit Gold (Azoxystrobin + Fludioxonil) was statistically the product that exerted a better control to all the fungi evaluated. While Folpan ® (Folpet) and Blue Shield ® (copper hydroxide) were the least effective.
Keywords: corchosis, association, evaluation, efficacy.

INTRODUCTION

Mexico contributes three out of every 10 tons of avocado (*Persea americana,* Mill.) cultivar hass that are produced in the world; which places it as the number one fruit exporting country (SAGARPA, 2020).

The importance of avocado production derives from the benefit it generates, an economic benefit of more than 750 million pesos, the creation of 40,000 permanent jobs, 9 million wages a year, and 60,000 seasonal jobs linked to indirect activities (Echánove, 2008).

However, the avocado during its development stages is attacked by phytopathogens that cause various diseases, some of which are endemic. Of these phytosanitary problems, the diseases caused by fungi stand out due to their wide distribution and dissemination (Ochoa, 2011).

The importance of a phytopathogenic organism varies depending on the country, producing region and the type of market (national or international) and may be given by the distribution and severity of damage caused by pathogens or by their quarantine importance for an importing country (Téliz and Mora, 2007). In general, the establishment and spread of diseases in an avocado orchard is due to poor management of the crop (ICA, 2012).

Scab is one of the main diseases that limits avocado exports (Teliz *et al.*, 2000; Moroccan, 2000). This is presented in Mexico, USA, Argentina, Brazil, Haiti, Peru, Cuba, Jamaica, Puerto Rico and Africa. In Mexico it has been registered in the states of Michoacán, Guanajuato, Puebla, Querétaro, Morelos, Nayarit, Tamaulipas and Jalisco (Coria, 2009).

This disease is important due to its endemic nature, which implies that every year it is present in the orchards. When control is not carried out, the disease spreads rapidly, estimating incidences of 30 to 40% in some orchards and in extreme cases up to more than 70%. It attacks all varieties established in Mexico (Vidales, 1999). The direct damages of the scab are to the production and quality of the fruit. The production of quality fruit can be affected by up to 60%, which reduces the sale price between 27 and 53% (Vidales *et al.*, 1999).

The scab syndrome could be defined as any symptom of corchosis that results as a defense mechanism of the plant to isolate an infection. This mechanism can be activated in the presence of more than one causative agent, both biotic (high incidence of thrips and mites, pathogens such as S phaceloma *perseae, Colletotrichum* sp., *Alternaria sp., Pestalotiopsis* sp., *Nigrospora* sp. *and Curvularia* sp.) as abiotic (mechanical damage, damage from wind friction). (Cruz-Borruel *et al.*, 2006; Robles *et al.* (2014) Alfaro *et al.*, 2017; Martínez *et al.*, 2017; Becerra, 2019).

Given the importance of this disease, the objectives of this research were to identify the pathogens associated with scab syndrome and to evaluate *in vitro* eight products for the control of the pathogens found.

MATERIALS AND METHODS

SAMPLE COLLECTION

A directed sampling was carried out collecting avocado fruits cv. Hass with different degrees of development of the scab syndrome in different orchards of the avocado producing areas in Michoacán, (Municipalities of: Nuevo San Juan Parangaricutiro, Tancítaro, Uruapan and Ario de Rosales). The collected samples were placed in plastic bags with their respective labels and transferred to the Phytopathology laboratory for analysis.

ISOLATION OF THE PATHOGEN

The fruits were washed with soap and water, fragments of approximately 5x5 mm were cut from the epicarp of the fruit with symptoms, trying to take from the diseased part and the healthy part. For disinfestation, 3% sodium hypochlorite was used for 30 seconds, then rinsed with sterile distilled water three times to eliminate the presence of chlorine in the tissues, they were placed on sterile paper towels to eliminate excess water, they were planted with equidistantly in Petri dishes with the PDA BD Bioxon M culture medium and incubated at 26°C. They were monitored daily to observe the growth of fungi.

PURIFICATION OF PATHOGENS

Once the growth of the different colonies was observed, a 1 cm diameter mycelium disc was taken from each of them to place them in a new Petri dish and grow individually without contaminants.

IDENTIFICATION OF THE PATHOGENS OBTAINED

In order to identify the causative agent, its macroscopic characteristics, color, appearance and shape of the colony and microscopic characteristics were taken into consideration, using the keys of Barnett and Hunter (1998).

IN VITRO CONTROL TESTS

Eight chemical treatments were used: Bankit Gold ® (Azoxystrobin and fludioxonil) 1.125 mL, Bankit ® (Azoxystrobin) 0.2 ml, Folpan ® (Folpet) 1.75 g, Tecto 60 ® (Thiabendazole) 0.3 g, Uniform ® (azoxystrobin + metalaxyl) 0.25 g, Headline ® (Piraclostrobin) 0.078 mL, Cabrio ® (Boscalid + Pyraclostrobin) 0.4g, Blue Shield ® (Copper hydroxide) 1 g and the control without fungicide, with three repetitions, at doses of the formulated product. An ANOVA statistical analysis and Tukey's mean tests ($P< 0.05$) were performed, the statistical analysis package was used in SAS® System for Windows v6.12.

To carry out the bioassays, filter paper discs were impregnated with each of the treatments and placed equidistantly at the four cardinal points of the Petri dish, a disc with mycelium of the pathogens found was placed in the center.

The boxes were sealed and incubated at 26°C. They were checked daily every 24 hours and mycelium growth was measured. Once

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the pathogen filled the control boxes or the fungus with the products touched the filter paper, all the boxes corresponding to that fungus stopped being measured.

RESULTS AND DISCUSSION

PATHOGEN IDENTIFICATION

Colletotrichum sp.

The symptoms of the fruits from which this fungus was isolated are rough, irregularly shaped brown coloration that covers a large part of the fruit, including the peduncle. The colony seen from the obverse is white to greyish in color with plush mycelium. On the reverse, the coloration is olive green with a white halo on the edge. The conidia are some elongated and others short with a cylindrical shape (Figure 1).

Alternaria sp.

In fruits with symptoms from which this pathogen was isolated irregularly shaped brown lesions can be seen. The colonies are fast growing, cottony and gray with white dots when viewed from the obverse. A darker color can be seen on the reverse, forming a halo of olive green color at the end. The conidia are characterized mainly because they are in the form of an ovoid mass with longitudinal and transverse septa, which can be solitary or in a chain. The mycelium is septate (Figure 2).

P stalotiopsis sp.

The symptoms seen in the fruit appear in a more united way with a brown coloration, forming large rough areas. Seen from the obverse, the colony is white and cottony with concentric rings, which when it has filled the box begins to present some small black dots on the middle, with a shiny appearance and a hard consistency that correspond to the pycnidia of the fungus. On the reverse you can see a salmon and white color with concentric rings. The conidia are short, generally presenting 3 to 4 transverse septa, each with three hyaline appendages on one of their apices and another on the opposite (Figure 3).

Nigrospora sp.

The lesions on the fruits from which this fungus was isolated they are brown, corky, and united, covering much of the fruit. This fungus grows very quickly. The colony seen from the obverse is initially white, turning grayish and dark with time with white dots and growing irregularly. On the reverse the coloration goes from white to salmon. The conidia are smooth, solitary, completely black and spherical (Figure 4).

Curvularia sp.

It can be observed in the fruits from which this fungus was isolated, that the symptoms of scab are appreciated in approximately 90% covering the fruit. Colonies are cottony, white to gray in color on the obverse. On the reverse they are presented with a dark color forming a white halo on the edge. The conidia are mostly curved with three transverse septa (Figure 5).

The above coincides with Robles *et al.*, 2014; 2006; Alfaro *et al.*, 2017; Martinez *et al.* and Becerra, 2019, who, when isolating fruits with scab symptoms, found the following genera more frequently: *Alternaria, Colletotrichum, Nigrospora, Epicocum, Phomopsis, Botriosphaeria, Pestalotiopsis* and *Glomerella.*

This also coincides with Esquivel (2014), who mentions *Alternaria* sp. causing freckles on fruit and *Pestalotiopsis* sp. causing corky spots also on the fruit, but in this case in guava cultivation (*Psidium hahaha* L.).

IN VITRO CONTROL TESTS:

Colletotrichum sp.

The products with which mycelium growth was lower were Tecto 60 ® (Thiabendazole) and

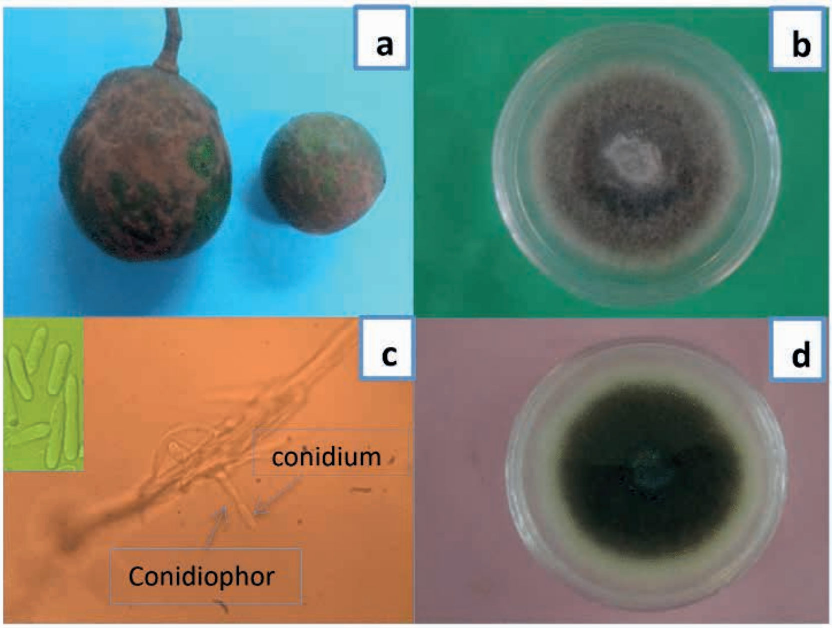
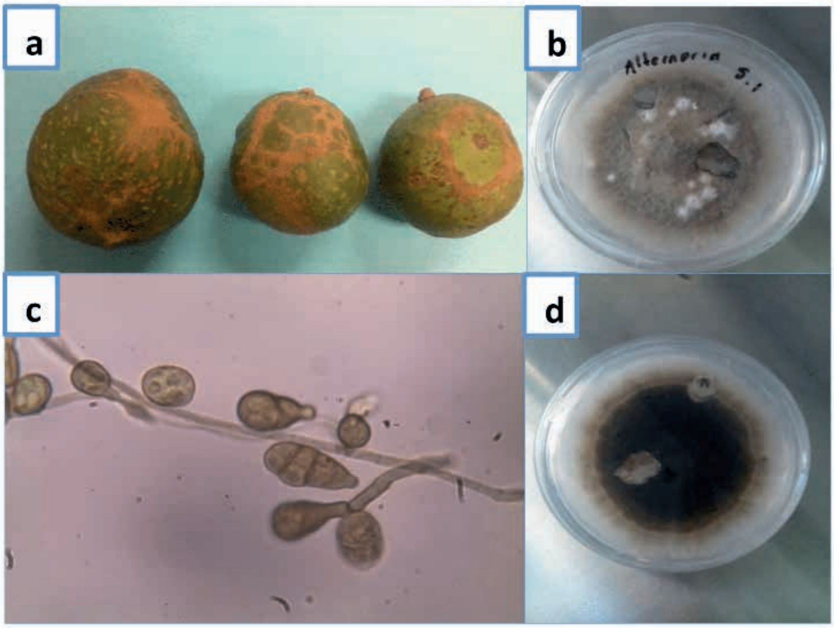


Figure 1. Strain of *Colletotrichum* sp. a) Symptoms on fruit. b) Colony seen from the obverse. c) Conidium attached to the conidiophore and mature conidia d) Colony seen from the reverse.



Alternaria *sp.* a) Symptoms in avocado fruits. b) Colony seen from the obverse. d) Mature conidia and mycelium d) Colony seen from the back.

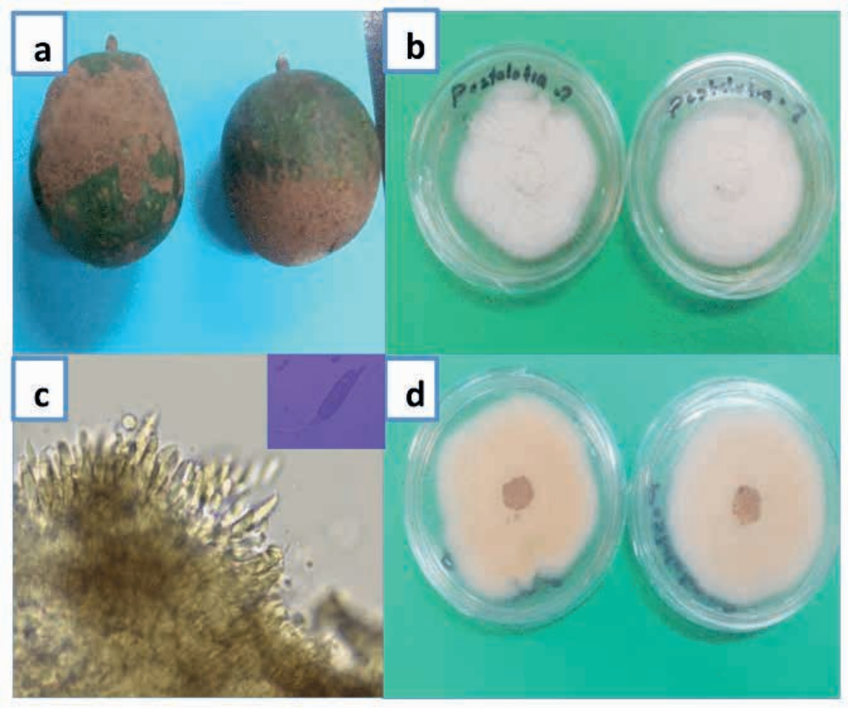


Figure 3. Strain of *Pestalotiopsis* sp. a) Symptoms on avocado fruits b) Colony seen from the obverse c) Typical conidia of *Pestalotiopsis* sp. d) Colony seen from the back.

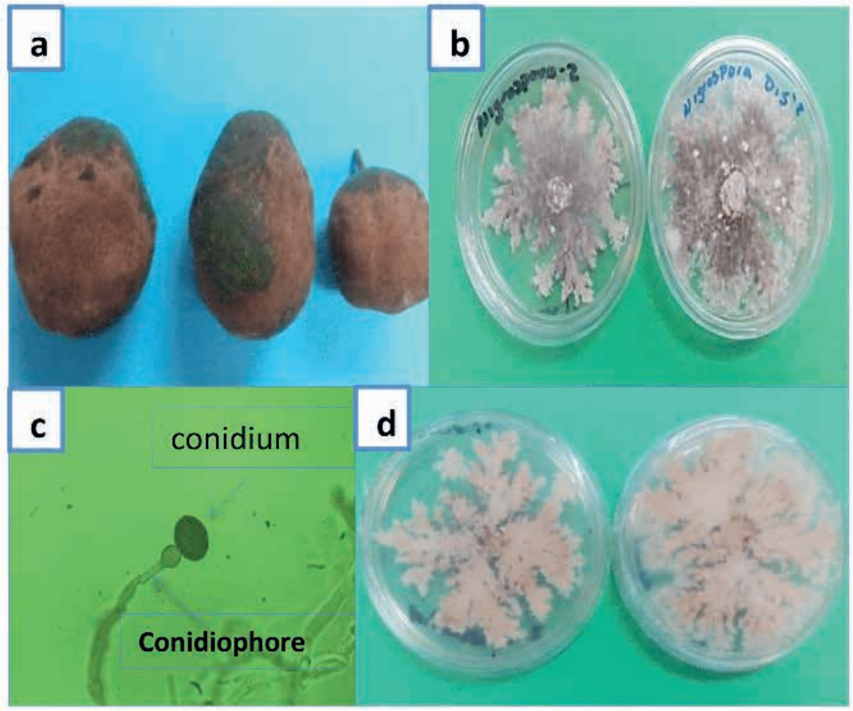


Figure 4. Strain of *Nigrospora* sp. a) Symptoms in avocado fruits. b) Colony seen from the obverse. c) Conidia attached to the conidiophore. d) Colony seen from the back.

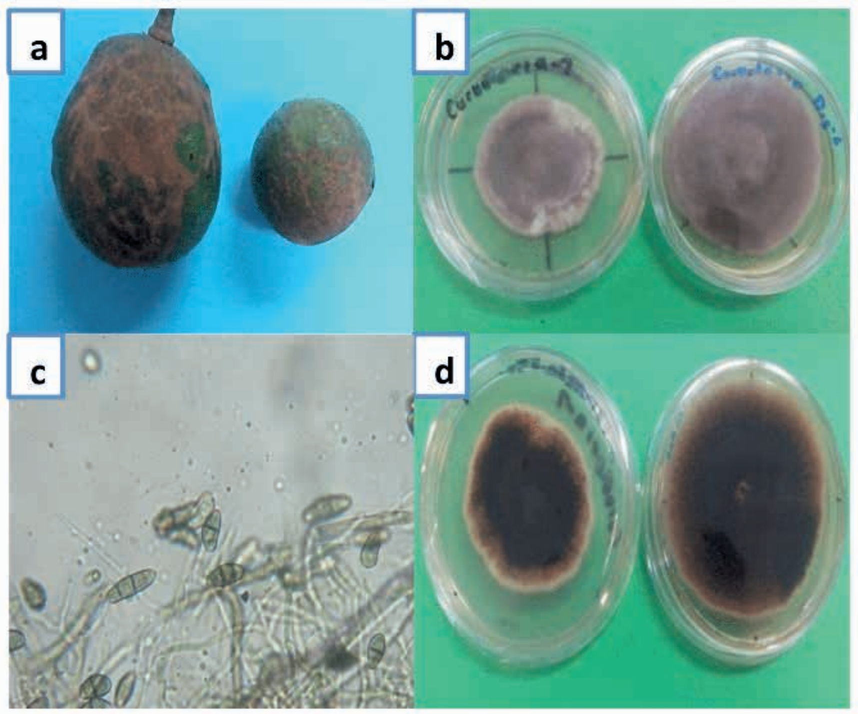
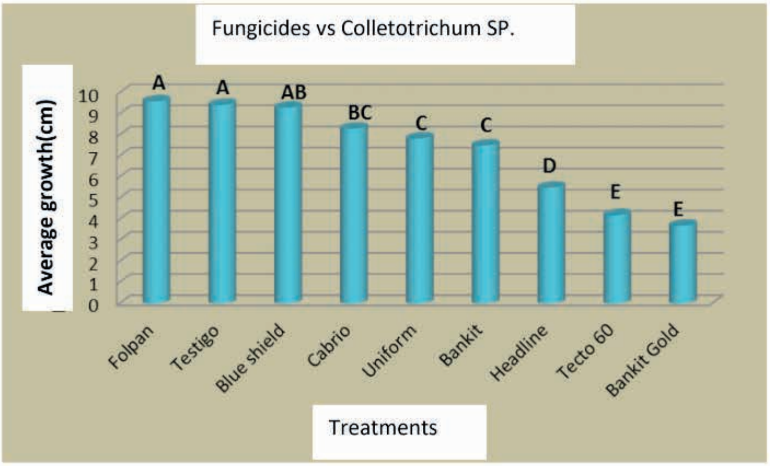


Figure 5. Curvularia *sp*. a) Symptoms in avocado fruits. b) Colony seen from the obverse ***c)*** *mature* conidia ***d)*** Colony seen from the reverse.



Treatments with the same letter are statistically equal according to Tukey's test (a=0.05).

Bankit Gold ® (Azoxystrobin and fludioxonil) while Folpan ® (Folpet) and Blue Shield ® (copper hydroxide) had the least effect on the growth of the mycelium (Figure 6.)

Growth response of the fungus *Colletotrichum* sp. with respect to the tested fungicides.

Gutierrez *et al.* (2003) in their research work on the control of anthracnose in guava fruit caused by *Colletotrichum* sp. found that two of their isolates (Co-1, Co-5) presented 100 % inhibition of mycelium growth *in vitro* at 5 and 10 ppm and two other isolates (Co-2, Co-3) at 50 ppm, respectively using azoxystrobin.

Santamaria *et al.* (2011) report in their research that azoxystrobin showed sensitivity to *Colletotrichum* sp. Which inhibited its development *in vitro,* this in anthracnose in papaya fruits of maradol.

Alternaria sp.

Control of mycelial growth was more effective with Cabrio® (Boscalid + Pyraclostrobin), while with Bankit ® (Azoxystrobin) it was less effective (Figure 7).

Villanueva *et al.* (2005) isolated *Alternaria chrysanthemi* from chrysanthemum plants. The incidence of the disease was considered as a parameter to estimate the intensity of the disease through the characterization of epidemics and partial curves, adjusting to the Weibull description model. With this model, azoxystrobin, Folpet, Chlorothalonil, and captan allowed the lowest apparent infection rates, and at the end of the experiment, only azoxystrobin showed the least disease intensity. Different from what was found in this work, that *in vitro* azoxystrobin by itself did not control the growth of *Alternaria* sp.

P stalotiopsis sp.

The product that had a greater effectiveness in the inhibition of *Pestalotiopsis* sp. it was Bankit Gold ® (Azoxystrobin and fludioxonil), otherwise it happened with Folpan ® (Folpet) and Blue Shield ® (copper hydroxide), which behaved in the same way as the control, showing no effectiveness in inhibiting growth of the fungus (Figure 8).

Growth response of the fungus *Pestalotiopsis sp.* with respect to the tested fungicides.

In agreement with the work carried out by Martínez *et al.* 2017 who, when performing *in vitro* chemical treatment of *Pestalotiopsis* sp. with different chemical products found that growth was controlled with Bankit Gold ® (Azoxystrobin and fludioxonil).

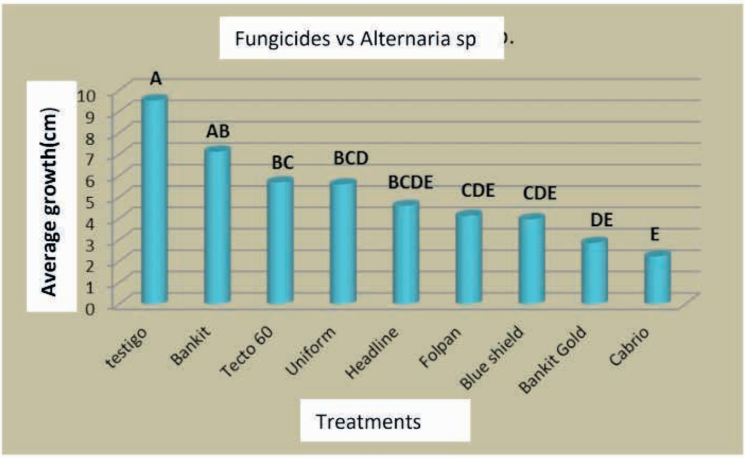
Nigrospora sp.

For *Nigrospora* sp. the product that was most effective in inhibiting this was Bankit Gold ® (Azoxystrobin and fludioxonil), while Folpan ® (Folpet) and Blue Shield ® (copper hydroxide) showed the least effectiveness in controlling the fungus (Figure 9).

The above agrees with Gutiérrez *et al.* (2003) who, when testing three different fungicides on rice grain spotting, among which was *Nigrospora* sp. obtained as results that the fungicide Amistar could reduce the symptoms of the disease which has Azoxystrobin and Difenoconazole as active ingredients in comparison with the present work where the product that worked best was Bankit Gold which also contains Azoxystrobin but combined with Fludioxonil.

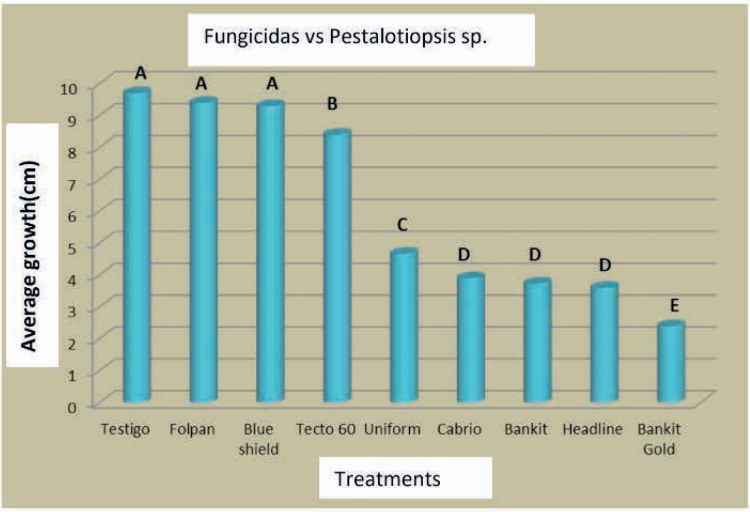
Curvularia sp.

For *Curvularia* sp. there was no statistical difference during the 8 days in which measurements were taken, the treatments behaved the same, due to the slow growth of the fungus with respect to the other species throughout the experiment, for which the mean significance test was not performed.

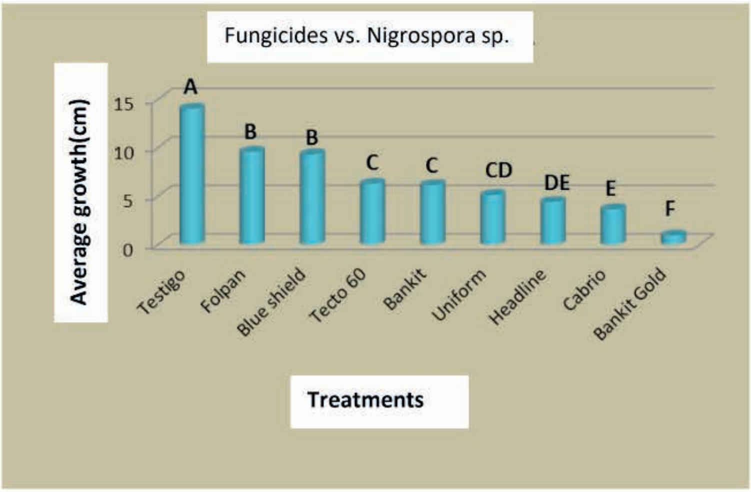


Treatments with the same letter are statistically equal according to Tukey's test (a=0.05).

Figure 7. Growth response of the fungus *Alternaria* sp. with respect to the tested fungicides.



Treatments with the same letter are statistically equal according to Tukey's test (a=0.05).



Treatments with the same letter are statistically equal according to Tukey's test (a=0.05).

Figure 9. Growth response of the fungus *Nigrospora* sp. with respect to the tested fungicides.

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CONCLUSIONS

1. An association of several phytopathogenic fungi was found in the fruits sampled with scab syndrome in avocado.

2. The pathogens associated with the scab symptom evaluated were *Colletotrichum* sp., *Alternaria* sp., *Pestalotiopsis* sp., *Nigrospora* sp. and *Curvularia* sp.

3. *Sphaceloma perseae* was not found to be associated with this evaluated scab symptom.

4. In general, it is observed that the product that had the greatest efficacy in inhibiting the fungi found was Bankit Gold (Azoxystrobin + Fludioxonil) and the least effective were Folpan® (Folpet) and Blue Shield (Cupric Hydroxide).

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