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In vitro biological and chemical control of fungi associated with gummosis in citrus fruits in Yucatan, Mexico

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ABSTRACT

Background/Objective. In all citrus-producing regions in the world, gummosis is a disease that has caused losses in citrus production. This disease is caused by several pathogens. The objectives were to identify the fungi associated with gummosis in citrus orchards of Plan Chac, Sacalum, Yucatan; and to evaluate chemical and biological alternatives for the control of fungi associated with gummosis.

Materials and Methods. From fragments of plant tissue and soil, the associated fungi were isolated. The isolates were identified morphologically in plant tissue as *Lasiodiplodia pseudotheobromae* and in soil as *Fusarium solani* and *Pestalotia* spp. The pathogenicity test determined that *L. pseudotheobromae* is an agent associated with this disease. The isolates were subjected to *in vitro* tests with chemical fungicides and antagonist agents.

Results. Thiabendazole showed effectiveness for *F. solani* with an effective concentration to inhibit 50 % of the population (EC50) of 0.0612 mg L^{-1}, with *Pestalotia* spp. inhibited growth at all concentrations evaluated and for *L. pseudotheobromae*, it showed an EC50 of 0.0049 mg L^{-1}. In the case of *Bacillus subtilis* strain QST 713, the growth of *F. solani* (EC50 0.0496 mg L^{-1}), *Pestalotia* spp. (EC50 0.0487 mg L^{-1}) and *L. pseudotheobromae* (EC50 0.0528 mg L^{-1}) decreased. On the other hand, *Trichoderma harzianum* showed a greater inhibition

against *F. solani*, *Pestalotia* spp. and *L. pseudotheobromae* of 61.08, 62.93 and 35.64 %, respectively.

Conclusion. In the management of gummosis in citrus fruits, the use of biological agents such as *Trichoderma* and *B. subtilis* can be efficiently included, offering alternatives with less impact on the environment.

Keywords: *Bacillus*, *Lasiodiplodia*, mycelial inhibition, Thiabendazole, *Trichoderma*.

Introduction

Different phytopathogenic agents have been associated to the gummosis of citrus fruits. Species of the *Phytophthora* genus are the main ones that have been reported (Bright *et al*., 2004; Brentu and Vicent, 2015; Graham and Feichtenberger, 2015). However, the members of the Botryosphaeriaceae family have displayed certain relevance due to their capacity to adapt in order to infect other crops near the original native host fields (Mondragon-Flores *et al*., 2021).

Gummosis is distributed in all the citrus-producing areas of the world. It has caused losses in Italy (Aloi *et al*., 2021), Algeria (Linaldeddu *et al*., 2015; Berraf-Tebbal *et al*., 2020), Iran (Abdollahzadeh *et al*., 2010) and the United States (Adesemoye *et al*., 2014), and in Mexico, it is distributed in the areas that produce sweet citrus fruits and Mexican and Persian limes in the states of Colima (Rocha-Peña *et al*., 2003), Morelos (Valle-de la Paz *et al*., 2019a), Puebla, Veracruz (Bautista-Cruz *et al*., 2019), Nuevo León and Tamaulipas (Polanco *et al*., 2019), with important data that range between an incidence of 2 and 14% (Medina-Urrutia *et al*., 2002).

Symptoms were observed in necrotic lesions in stems and branches, along with the appearance of a gummy exudate, followed by wilting, the yellowing of leaves, defoliation and finally, the partial or complete death of the tree (Bautista-Cruz *et al.,* 2019; Berraf-Tebbal *et al*., 2020; Aloi *et al*., 2021). The pathogen survives in the stubble of diseased plants, and is spread via trimming tools, but also via rainfall, irrigation, wind and insects (Moreira-Morillo *et al*., 2021). The disease prevails largely thanks to temperatures between 26 and 32 °C and a high relative humidity (80%) (Úrbez-Torres *et al.,* 2010; Picos-Muñoz *et al*., 2015).

The control of gummosis has become more efficient with the use of preventive chemical fungicides such as Carbendazim (Da Silva Pereira *et al.,* 2011; Valle-de la Paz *et al.,* 2019b), Thiabendazole (Da Silva Pereira *et al.,* 2011; Camacho-Tapia *et al.,* 2021), Benomyl and copper-based compounds (Everett and Timudo-Torrevilla,

2007; Sáenz *et al.,* 2019; Valle-de la Paz *et al.,* 2019). Antagonistic microorganisms have also been used, such as *Trichoderma* spp. and *Bacillus subtilis* (Bhuvaneswari and Rao, 2001; Rusin *et al.,* 2021). However, the use of chemical fungicides must be revised depending on the pathogen under study, as well as avoiding the generation of resistance. Obtaining biological alternatives for control is important for the conservation of biodiversity. This study has been developed with the goals of identifying the pathogens associated to gummosis in citrus fruit orchards in Plan Chac, Sacalum, Yucatan, as well as to evaluate the chemical and biological control alternatives that offer alternatives for the management of the disease.

Materials and Methods

Plant material and isolation of fungi. Plant tissue (bark) and soil samples were used, taken from a semi-commercial orchard with orange (*Citrus sinensis*), lemon (*C. latifolia*) and grapefruit trees (*C. paradisi*) with symptoms of exudate and necrosis. The orchard is located in the town of Plan Chac, Sacalum, Yucatan. The samples were extracted in July and October, 2023. For the isolations of the tissue, 5 mm^2 were cut, which were disinfected with 1 % sodium hypochlorite per minute and washed three times with sterile distilled water (Bautista-Cruz *et al.,* 2019). The tissues were left to dry in sterile paper towels and then planted in dishes with natural PDA media (200 g potato, 20 g agar-agar, 15 g dextrose in 1000 mL of water) and incubated at 24 °C for 24 h.

Soil samples were taken around the trunks of trees with symptoms and at a depth of 30 cm. For the isolation of the soil fungi, the technique consisted in serial dilutions. Thus, in the first tube, 1 g of soil in 9 mL of sterile distilled water was added; later, 1 mL of that solution (soil-water) was transferred to a series of tubes with the same characteristics (Aziz and Zainol, 2018). Three dilutions were made of each sample (10^{-1} to 10^{-3}). Out of the final solution, 20 µL were transferred into dishes with the natural PDA medium, which were incubated at 24 °C for 24 h and observed under the microscope to detect fungal growth.

Purifying and identifying isolates. The isolates were purified using hyphal tip, planted in a PDA medium (BD BIOXON®, Cuautitlán Izcalli, Mexico) and kept for a 7 to 14-day day period at 24 °C in an incubator (BINDER, Model BD53-UL, Tuttlingen, Germany). The morphological characterization of the purified isolations was carried out with the following culture media: PDA with antibiotics (39 g, 200 mg of streptomycin and 1000 mL of water) and Clover Leaf Agar (CLA) (20 g agar, 1000 mL water, five pieces of carnation leaf per dish). In the PDA medium, the pigmentation and growth rates of the cultures were determined. To

study the morphology of the *Fusarium* genus, the CLA medium and moist chambers (Petri dish, wet paper and pieces of aluminum) which were evaluated after five days for the measurement of phyalids. In addition, macro and microconidia and chlamydospores were measured. For *Pestalotia*, conidia were measured, and for *Lasiodiplodia*, conidia, mature conidia and pycnidia were measured. After the incubation period, semipermanent glycerin preparations at 50% were prepared and examined with an optical microscope (Leica DM500, Heerbrugg, Germany) with a digital camera installed, with a 40x lens and the images were processed using the LAS EZ Software (version 3.4; Leica Microsystems, Germany).

Pathogenicity test. Based on the highest incidence found in the isolates, four *C. sinensis* plants were inoculated with *L. pseudotheobromae* in sour orange *C. aurantium*. The surface of the healthy plant tissue was disinfected with 70 % alcohol and a lesion was made in which a disk of active, 5-day old *L. pseudotheobomae* growth in PDA (5 mm) was placed (Bautista-Cruz *et al.,* 2019; Berraf-Tebbal *et al.,* 2020). The lesions were healed using wet cotton and Parafilm paper (Bemis, U.S.A.). The control plants received a sterile PDA disk. The plants were evaluated under microtunnel conditions for 21 days, with a mean temperature of 24 °C (max. 47 °C, man. 11 °C) and a relative humidity of 97 % (max. 100 %, min. 85 %). Once the period is finished, the presence of signs and symptoms of gummosis was found. The leaves and stems with lesions were used, and the fungi were reisolated in PDA medium. When the culture was developed, the morphological and cultural characterization was carried out to confirm the presence of structures that coincide with the original isolation.

***In vitro* evaluation of chemical fungicides and antagonists.** The technique consisted in the planting of the isolates in a PDA medium with fungicides at different concentrations (Dhingra and Sinclair, 1995). A 5 mm inoculant dick was extracted from the edge of the pure culture of the fungus in question. Four chemical fungicides were evaluated (Benomyl, Mancozeb, Carbendazim, Thiabendazole) and a biological product based on *B. subtilis*, strain QST 713. For the three isolations, seven concentrations were used with three repetitions, including the control (Table 1). The concentrations were calculated according to the volume of PDA medium and active ingredient. They were incubated at a temperature of 24 °C. The inhibition percentages of the mycelial growth (IPMG) and the effective concentration (EC) were obtained. Once the mycelial growth of the control occupied the total area of the Petri dish, the growth percentage of each one of the treatments, was calculated using the formula by Arce-Araya *et al.,* (2019): % fungal growth = [(Fungi diameter in PDA without fungicide - Fungi diameter in PDA with fungicide) / (Fungi diameter in PDA without fungicide) * 100]. The EC

Table 1. Treatments evaluated for the control of phytopathogenic fungi associated to the gummosis of citrus fruits in Plan Chac, Sacalum, Yucatan.

Fungus	Brand	Active ingredient (a.i.)	Concentrations evaluated (mg/L)
Fusarium solani	Carbendazim	Carbendazim a 500 g	0, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0
	Tecto	Thiabendazole a 600 g	0, 0.01, 0.1, 0.5, 1.0, 2.5, 5.0
	Benomilo	Benomyl a 500 g	0, 0.01, 1.0, 5.0, 10, 50, 100
	Serenade	*Bacillus subtilis* cepa QST 713 a 146 g	0, 0.005, 0.01, 0.05, 0.1, 0.5, 1.0
Pestalotia spp.	Benomilo	Benomyl a 500 g	0, 0.01, 0.1, 1.0, 5.0, 10.0, 100
	Mancozeb	Mancozeb a 800 g	0, 0.1, 1, 10, 50, 100, 500
	Tecto	Thiabendazole a 600g	0, 0.01, 0.1, 1.0, 5.0, 10.0, 100
	Serenade	*Bacillus subtilis* cepa QST 713 a 146 g	0, 0.005, 0.01, 0.05, 0.1, 0.5, 1.0
Lasiodiplodia pseudotheobromae	Mancozeb	Mancozeb a 800 g	0, 0.1, 1.0, 10, 50, 100, 500
	Benomilo	Benomyl a 500 g	0, 0.001, 0.01, 0.1, 1.0, 5.0, 10
	Tecto	Thiabendazole a 600 g	0, 0.001, 0.01, 0.1, 1.0, 5.0, 10
	Serenade	*Bacillus subtilis* cepa QST 713 a 146 g	0, 0.005, 0.01, 0.05, 0.1, 0.5, 1.0

that inhibits 50 % of the mycelial growth (EC_{50}) was calculated using the GraphPad Prism Software (version 8.0.1; GraphPad, San Diego, CA).

Dual *in vitro Trichoderma* test. In order to evaluate the antagonistic ability of *Trichoderma*, the dual culture technique, described by Morton and Stroube (1955), was used. The test was carried out with the phytopathogenic agents obtained, and with two antagonistic agents: *Trichoderma harzianum* and *T. viride*, which belong to the INIFAP collection. The test consisted in placing a mycelium disk of each fungus (5 mm) on an edge of the Petri dish and on the opposite edge, a disk of the antagonistic agent (5 mm) was placed, with a separation of 2 cm. For each treatment, three repetitions and one control were included. They were kept at a temperature of 24 °C. Upon the termination of the period, the % of radial inhibition (PRI) was calculated using the formula by Osorio *et al.* (2016): PRI= [(Fungi diameter without *Trichoderma*-Fungi diameter with *Trichoderma*) / (Fungi diameter without *Trichoderma*) * 100].

Statistical analysis. The experimental design used was completely randomized. An analysis of variance was carried out along with a comparison of means using Tukey's test ($P \leq 0.05$). The results were analyzed using the SAS statistical software (version 9.0; SAS Institute, Cary, N.C.).

Results and Discussion

Morphological characterization of fungus associated with gummosis. Cultures were obtained from the plant samples that correspond to only one fungus with the following characteristics: A whit, circular growth and a filamentous edge with a spongy texture that turned military green and finally black (Figure 1a). Picnidia, 133 µm wide x 212.69 µm long appeared, with conidia that measured (17.75-) 25.30

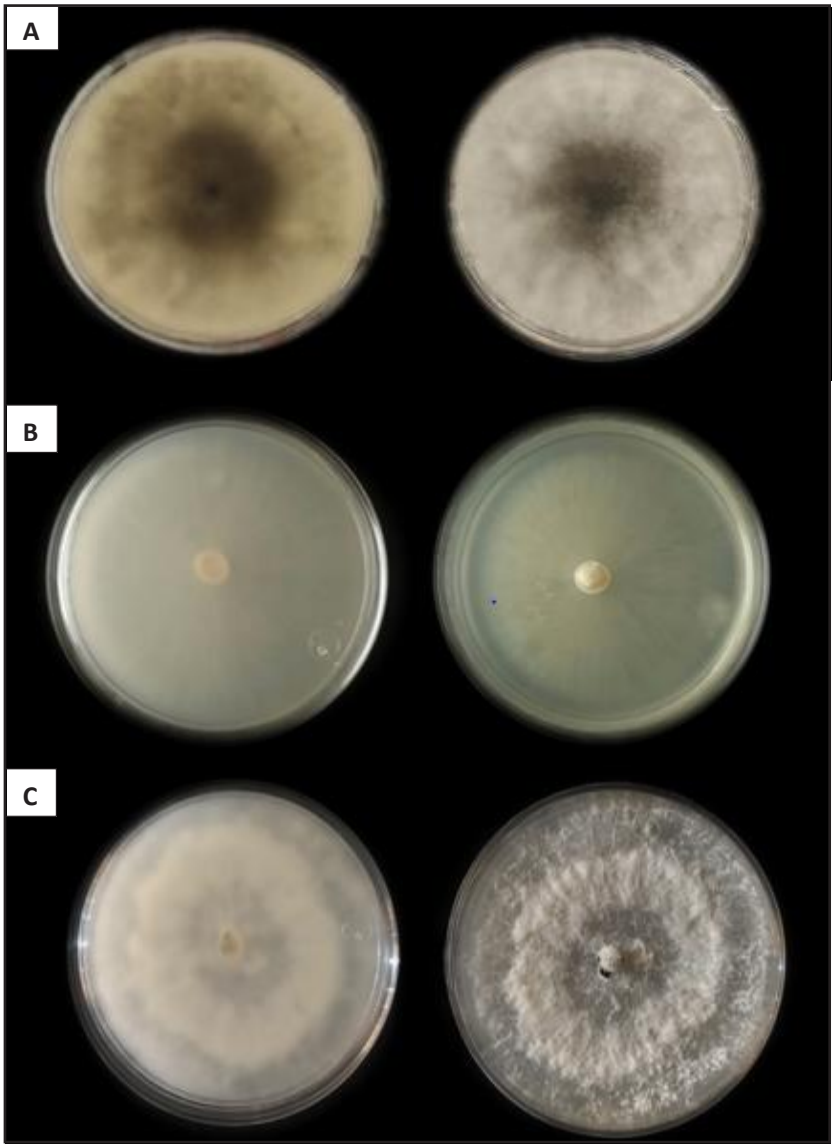

Figure 1. Front and back of isolates in PDA culture medium of fungi related to the gummosis of citrus fruits. **A)** Six-day old *Lasiodiplodia pseudotheobromae* culture, obtained from tissue from the cortex. **B)** Six-day old *Fusarium solani* culture. **C)** Six-day old *Pestalotia* spp. culture, both obtained from the soil.

(-34.97) µm long x (11.37-) 13.30 (-18.08) µm wide, sub-ovoidal to ellipsoidal in shape, with a truncated apex and base (Figure 2). The conidia are initially hyaline and aspetated, and then brown and septated with a longitudinal striated. In addition, other structures such as conidiogenous cells and paraphyses appeared. These characteristics correspond to those described by Phillips *et al.* (2013) and Liang *et al.* (2020) for *L. pseudotheobromae.*

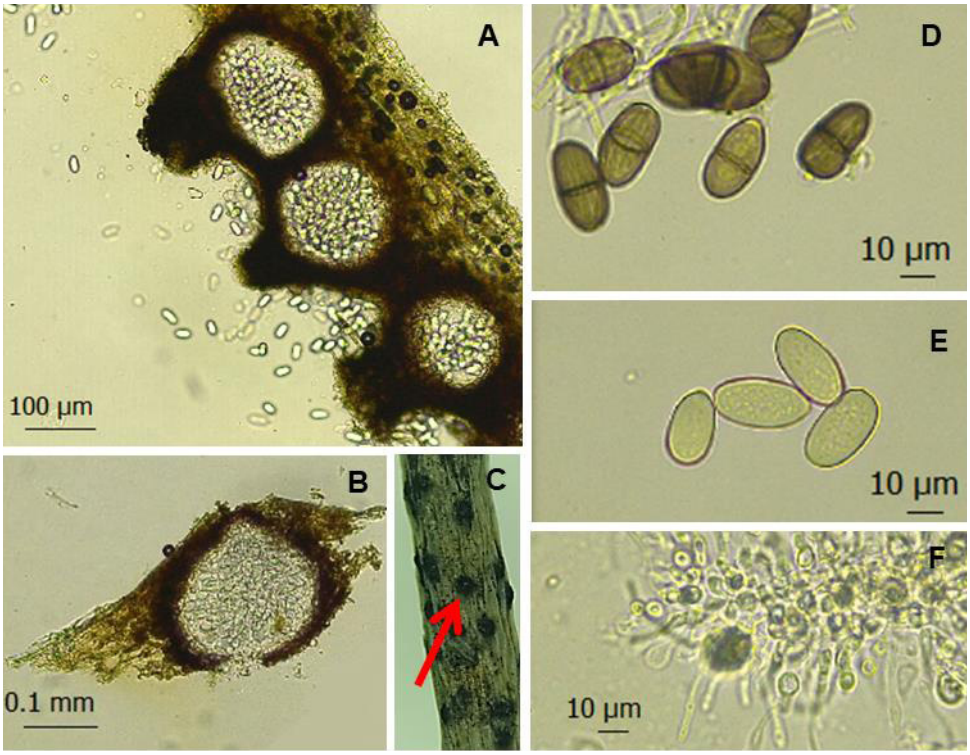

Figure 2. Microscopic morphology of fruiting *Lasiodiplodia pseudotheobromae* bodies. **A, B** and **C)** Globose pycnidial conidiomata, **D)** mature septate conidia with longitudinal striations, **E)** conidiogenous cells and paraphyses and **F)** immature aseptate conidia.

From the soil samples, cultures were obtained with a circular, cream-colored growth, filamentous edge and flat texture that turned light brown (Figure 1b). The slightly curved, triseptate macroconidia that measured (28.99-) 35.02 (-42.76) µm in length by (4.18-) 5.18 (-6.99) µm in width. The microconidia with or without a septum, (6.86-) 10.02 (-14.08) µm in length by (5.24-) 3.75 (-2.41) µm in width. The long and cylindrical phialides measuring 97.30 µm, and double-wall chlamidospores, terminal or intercalary measuring 8.69 µm in diameter (Figure 3). The above description coincides with *F. solani* (Leslie and Summerell, 2006). In addition, a third, white culture with circular rings was formed, cottonlike in texture

Figure 3. Microscopic morphology of *Fusarium solani* associated to the gummosis in citrus fruits. **A)** Phialides and conidia grouped into flower heads, **B)** Mature septated conidia, **C)** Mature microconidia.

and filamentous edges (Figure 1c). Presence of acervuli measuring 506 µm and curved conidia measuring (13.19-) 21.61 (-28.07) µm in length (5.93-) 8.01 (-9.97) µm in width, with five cells of hyaline ends (terminal and apical). The terminal end presented three appendages that measured 4.96-30.3 µm, 5.33-31.06 µm and 5.28-29.81 µm. On the other hand, the apical end of just one short, 2.86-7.83 µm long appendage. This description coincides with the *Pestalotia* genus, with no possible distinction of a species, according to Guba (1961) and Barnett and Hunter (1998).

Isolate pathogenicity test. On day 21, necrotized lesions were observed with amber-colored exudates in stems, as well as the presence of pycnidia in stems and leaves (Figure 4). Similar to findings by Bautista-Cruz *et al.* (2019) and Berraf-Tebbal *et al.* (2020). The causal agent of the gummosis disease was confirmed to correspond to *L. pseudotheobromae*, using Koch's postulates.

Effectiveness of fungicides. Our results helped identify the fungicide with the highest effectiveness in the reduction of the mycelial growth of *F. solani*, *Pestalotia* spp. and *L. pseudotheobromae.* For *F. solani,* the analysis of variance displayed significant differences between Carbendazim, Thiabendazole and Serenade ($P \leq 0.05$). The Thiabendazole was the most effective chamical fungicide, since minimum doses were able to inhibit *F. solani* (Table 2, Figure 5). The benzimidazoles such as Carbendazim and Thiabendazole have been effective to control the *Fusarium* genus (Agrios, 2005). Zárate-Ramos *et al.* (2022) determined that Thiabendazole

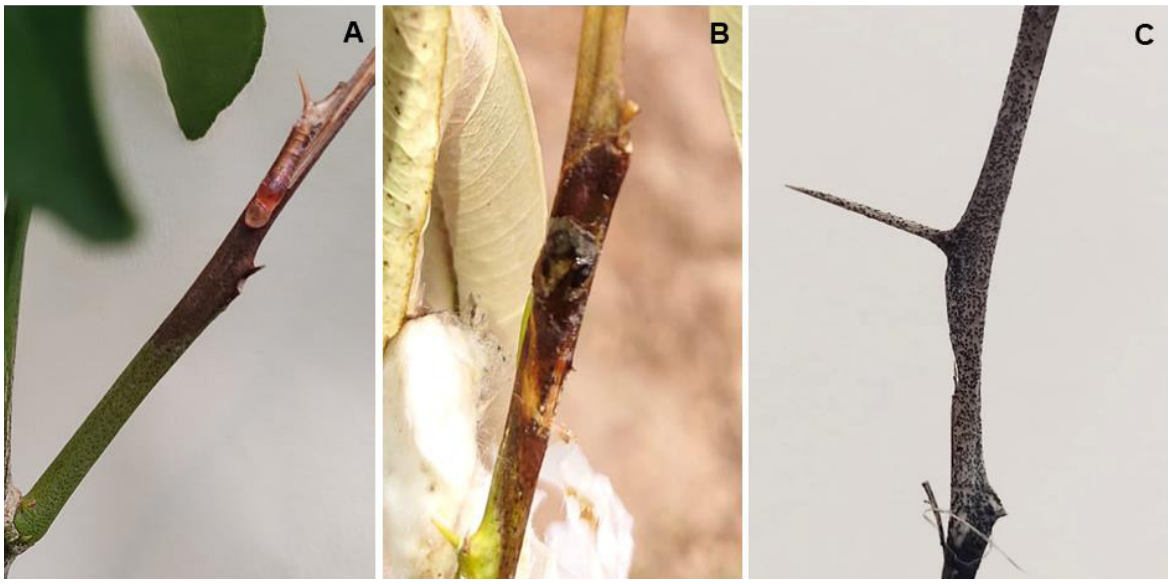

Figure 4. Symptoms and signs caused by *Lasiodiplodia pseudotheobromae* on citrus fruits, A) stem necrosis, B) presence of pycnidia on stems, and C) production of exudate or gum.

Table 2. Mean effective concentration (EC_{50}) (mg L^{-1}) of each fungicide tested *in vitro* for the inhibition of the mycelial growth of *Fusarium solani* obtained from citrus orchards in Plan Chac, Sacalum, Mexico.

Fungicides	Mean EC_{50}	Optimal value EC_{50}	Confidence interval at 95%
Benomyl	0.4807 a[z]	0.4150	0.2687 a 0.6068
Carbendazim	0.4274 ab	0.4243	0. 3477 a 0.5037
Thiabendazole	0.0612 b	0.0610	0.05179 a **
Serenade	0.04970 b	0.0496	0.04613 a 0.05303

[z]Means with the same letter in the column are statistically equal, according to Tukey's test ($P \leq 0.05$). NA= Not applicable. ** = Undefined. Value of $P = 0.0121$.

was effective with an EC_{50} of 14.50 mg L^{-1} against *F. incarnatum*, while Medina-Osti *et al.* (2022) reported that Thiabendazole was effective at an EC_{50} of 7.2 mg L^{-1} against *F. sacchari*. In addition, the, biological product Serenade was effective (Table 2, Figure 5). The bacteria of the *Bacillus* genus have been reported to produce antimicrobial compounds against *Fusarium* (Mardanova *et al.,* 2017). In the scae of *F. solani,* to inhibit more than 50 % of the fungus, a concentration of 0.0497 mg L^{-1} was needed (Table 2). Carbendazim proved to be efficient to inhibit

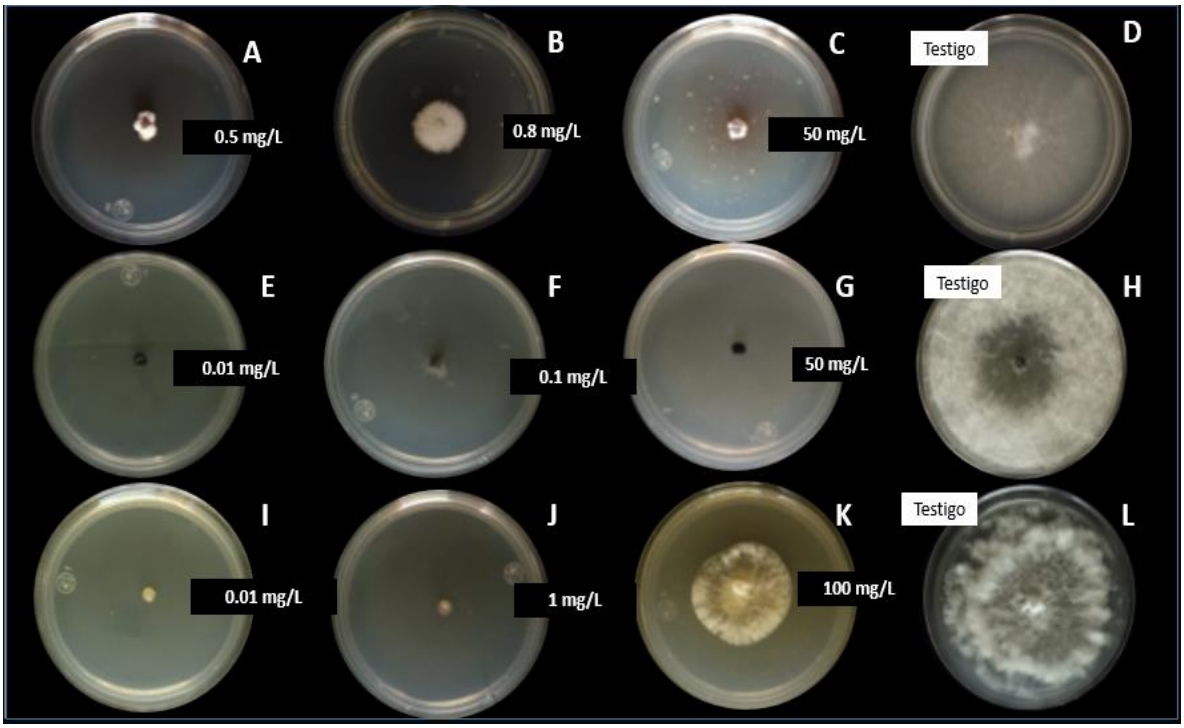

Figure 5. *In vitro* effect of fungicides against fungi associated to the gummosis of citrus fruits in five days. Growth of *Fusarium solani*: **A)** Medium with Thiabendazole at 0.5 mg L^{-1}. **B)** Medium with Carbendazim at 0.8 mg L^{-1}. **C)** Medium with Benomyl at 50 mg L^{-1}. **D)** Control. Growth of *Lasiodiplodia pseudotheobromae*: **E)** Medium with Thiabendazole at 0.01 mg L^{-1}. **F)** Medium with Benomyl at 0.1 mg L^{-1}. **G)** Medium with Mancozeb at 50 mg L^{-1}. **H)** Control. Growth of *Pestalotia* spp.: **I)** Medium with Thiabendazole at 0.01 mg L^{-1}. **J)** Medium with Benomyl al 1 mg L^{-1}. **K)** Medium with Mancozeb at 100 mg L^{-1}. **L)** Control.

its mycelial growth (Table 2, Figure 5). In general, Carbendazim has displayed good results against *F. oxysporum* in tomato (Jahanshir and Dzhalilov, 2010) and against *F. solani* in chili pepper (Madhavi and Bhattiprolu, 2011). González-Oviedo *et al*. (2022) reported that strains of *F. oxysporum*, from vanilla, were sensitive to Benomyl and Carbendazim. However, Benomyl was less effective, since the highest percentage of inhibition was displayed at high elevations (Table 2, Figure 5). Applying Benomyl on the soil restricted the colonization of *F. oxysporum* in melon (Maraite and Meyer, 1971). Benomyl has been shown to reduce the germination rate of the *Fusarium* conidia (Decallonne and Meyer, 1972). Romero-Velázquez *et al.* (2015) determined that an EC_{50} of 0.01 mg L^{-1} was presented by *B. subtilis* against *Fusarium* in chayote. On the other hand, Zarate-Ramos *et al.* (2022) indicated that *B. subtilis* was efficient with an EC_{50} 0.00014 mg L^{-1}, and completely inhibited *Fusarium* at 0.01, 0.05 and 1 mg L^{-1},

On the other hand, the analysis of variance ($P \leq 0.05$) for *Pestalotia* spp. proved that there are significant differences between Benomyl and Mancozeb. Thiabendazole proved to be the most efficient in all the concentrations evaluated (Table 3). Hernández-Ceja *et al.* (2021) reported that Thiabendazole, starting at 5 mg mL^{-1} inhibited 100% of the fungi associated with the regressive death of the cranberry: *Pestalotiopsis clavispora*, *Colletotrichum gloeosporioides* and *L. pseudotheobromae*. With Serenade, an optimum value was obtained of EC_{50} 0.04870 mg L^{-1}. Monroy and Lizarazo (2010) found no antagonistic properties against *Pestalotia* spp., with the cultivation of the fungus with *Pseudomonas fluorescens* and *B. subtilis* bacteria at a concentration of 10^6 UFC/mL^8 in PDA. Mancozeb was the least effective (Table 3). The 100 % inhibition of the fungus was achieved with the use of the highest dose evaluated. In strawberry, Ara *et al.* (2017) and Rajnish & Gauta, (2022) managed to completely inhibit *Pestalotia* spp. at concentrations of 250, 500 and 1000 mg L^{-1}. On the other hand, Carbendazim and Mancozeb were the ones to control *P. anacardii* in mango (Patil *et al.*, 2019).

Table 3. Mean effective concentration (EC_{50}) (mg L^{-1}) of each fungicide tested *in vitro* for the inhibition of the mycelial growth of *Pestalotia* spp. obtained from citrus orchards in Plan Chac, Sacalum, Yucatan.

Fungicides	Mean EC_{50}	Optimal value EC_{50}	Confidence interval at 95 %
Mancozeb	58.73 a[z]	58.09	45.44 a 73.40
Benomyl	0.0819 b	0.06934	0.03579 a **
Serenade	0.0481 b	0.04870	0.04547 a 0.05117
Thiabendazole	NA	NA	NA

[z]Means with the same letter in the column are statistically equal, according to Tukey's test ($P \leq 0.05$). NA= Not applicable. ** = Undefined. Value of $P = 0.0001$.

In *L. pseudotheobromae*, the analysis of variance indicates that there were no significant differences on the EC_{50} between Benomyl, Serenade and Thiabendazole, although they do differ statistically with Mancozeb ($P \leq 0.05$). Thiabendazole was effective, since it displayed a lower value for EC_{50} (Table 4, Figure 5) and it displayed a greater inhibition, starting at 0.01 mg×L^{-1} (98.55%). Benzimidazoles have been effective against *L. theobromae*. Out of 120 *L. theobromae* isolates, 91.6% were reported to be sensitive to benzimidazoles with EC_{50} values ranging from 0.36 to 1.27 µg mL^{-1} for Thiabendazole (Da Silva *et al.,* 2012). Under field conditions, Camacho-Tapia *et al.* (2021) showed that the use of Thiabendazole provided good control over gummosis in lemon trees. Control with Benomyl was

Table 4. Mean effective concentration (EC_{50}) (mg L^{-1}) of each fungicide tested *in vitro* for the inhibition of the mycelial growth of *Lasiodiplodia pseudotheobromae* obtained from citrus orchards in Plan Chac, Sacalum, Yucatan.

Fungicides	Mean EC_{50}	Optimal value EC_{50}	Confidence interval at 95 %
Mancozeb	20.96 a[z]	25.59	-
Benomyl	0.0638 b	0.0638	0.04696 a **
Serenade	0.0528 b	0.0528	0.05052 a 0.05537
Thiabendazole	0.0049 b	0.0056	**

[z]Means with the same letter in the column are statistically equal, according to Tukey's test ($P \leq 0.05$). ** = Undefined (GraphPad Prism does not show a complete trust interval). Value of $P = 0.0004$.

achieved at low concentrations (Table 4, Figure 5); of *Lasiodiplodia* was achieved with an application of 0.1 mg L^{-1} (86.58 %) and complete inhibition, at 10 mg×L^{-1} (99.56 %). This is similar to results by Da Silva Pereira *et al*. (2021), in which the EC_{50} of Benomyl for *L. theobromae* was between 0.002 and 1.75 µg mL^{-1}. In addition, some species of *Botryosphaeriaceae* were controlled with Benomyl (EC_{50} ranging from 0.36 to 0.55 µg mL^{-1}) (Bester *et al.,* 2007). The application of Benomyl and copper oxychloride-based compounds against *L. theobromae* is effective in different phenological stages of the crop (Sáenz *et al.,* 2019). Serenade was the least effective, with an EC_{50} value of 0.0528 mg L^{-1}. Mancozeb managed to control *L. pseudotheobromae* at high concentrations of 50, 100, 500 mg L^{-1}, compared to the evaluated fungicides. This was confirmed by Dianda *et al.* (2020) and Sultana and Ghaffar (2010), since *Lasiodiplodia* was completely inhibited at 100 and 500 mg L^{-1}. Mancozeb, along with Carbendazim, has allowed for broader control (Jadeja and Bhatt, 2010; Valle- de la Paz *et al.,* 2019b). On the other hand, Sultana and Ghaffar (2010) obtained good results with the application of *B. subtilis* in pre- and post-emergence control of *L. theobromae.*

Effect of *Trichoderma*. Both *T. harzianum* and *T. viride* inhibited the development of fungi related to the gummosis of citrus fruits; however, according to the ANOVA, *T. harzianum* caused the greatest inhibition. Its effectiveness as a biological agent control against fungi, nematodes and insects has been reported (Ferreira and Musumeci, 2021). In *F. solani*, the ANOVA indicates that *T. harzianum* (61.08%) displayed significant differences in comparison with *T. viride* (22.17%) ($P \leq 0.05$) (Table 5, Figure 6). Likewise, Fernández and Suárez (2009) reported that applying *T. harzianum* inhibited over 50 % of *Fusarium* in passionfruit. In eggplants, Ganesh and Dwivedi (2019) reported that around 20 % of *Fusarium* was inhibited

Cuadro 5. Percentage of mycelial growth inhibition by the effect of *Trichoderma* against *F. solani*, *Pestalotia* spp. and *L. pseudotheobromae*.

Pathogen	Treatments	Mean
Fusarium solani	*T. harzianum*	61.08 a[z]
	T. viride	22.17 b
	P value	0.0001
	DMS	5.549
Pestalotia spp.	*T. harzianum*	62.93 a[z]
	T. viride	53.78 a
	P value	0.0001
	DMS	14.76
Lasiodiplodia pseudotheobromae	*T. harzianum*	35.64 a[z]
	T. viride	25.45 a
	P value	0.0001
	DMS	4.856

[z]Means with the same letter in the column do not differ statistically according to Tukey's test ($P \leq 0.05$). LSD; Least significant difference.

with *T. viride*. Madhavi and Bhattiprolu (2011) indicate that the integration of different treatments such as soaking seedlings with Carbendazim, the addition of vermicompost, soaking with fungicide and the application of *T. viride* is efficient for the control of the wilting disease by *Fusarium* in chili pepper. For *Pestalotia*, the analysis of variance ($P \leq 0.05$) indicates that *T. harzianum* (62.93%) displayed significant differences in comparison with *T. viride* (53.78%) (Table 5, Figure 6). In mango, the fungus was inhibited with the application of *Trichoderma* (72.88%) (Bhuvaneswari and Rao, 2001). In the case of *Lasiodiplodia*, the ANOVA ($P < 0.05$) indicates that *T. harzianum* (35.64%) did not display significant differences in comparison with *T. viride* (25.45%) (Table 5, Figure 6). Boat *et al.* (2022) determined that *T. harzianum* reduced *L. theobromae* by 64.1%, whereas Bhuvaneswari and Rao (2001) reported that 62.41% of the fungal mycelial growth was reduced. Likewise, Da Silva *et al.* (2022) showed that *Trichoderma* reduced the growth of *F. solani* (34%) and *L. theobromae* (89%) related to *Nopalea cochinillifera*. In Morelos, *L. citricola* was found to be sensitive to the evaluated doses of *T. harzianum* (0.55, 0.39 and 0.19 g/100 mL) (Valle-de la Paz, 2019a).

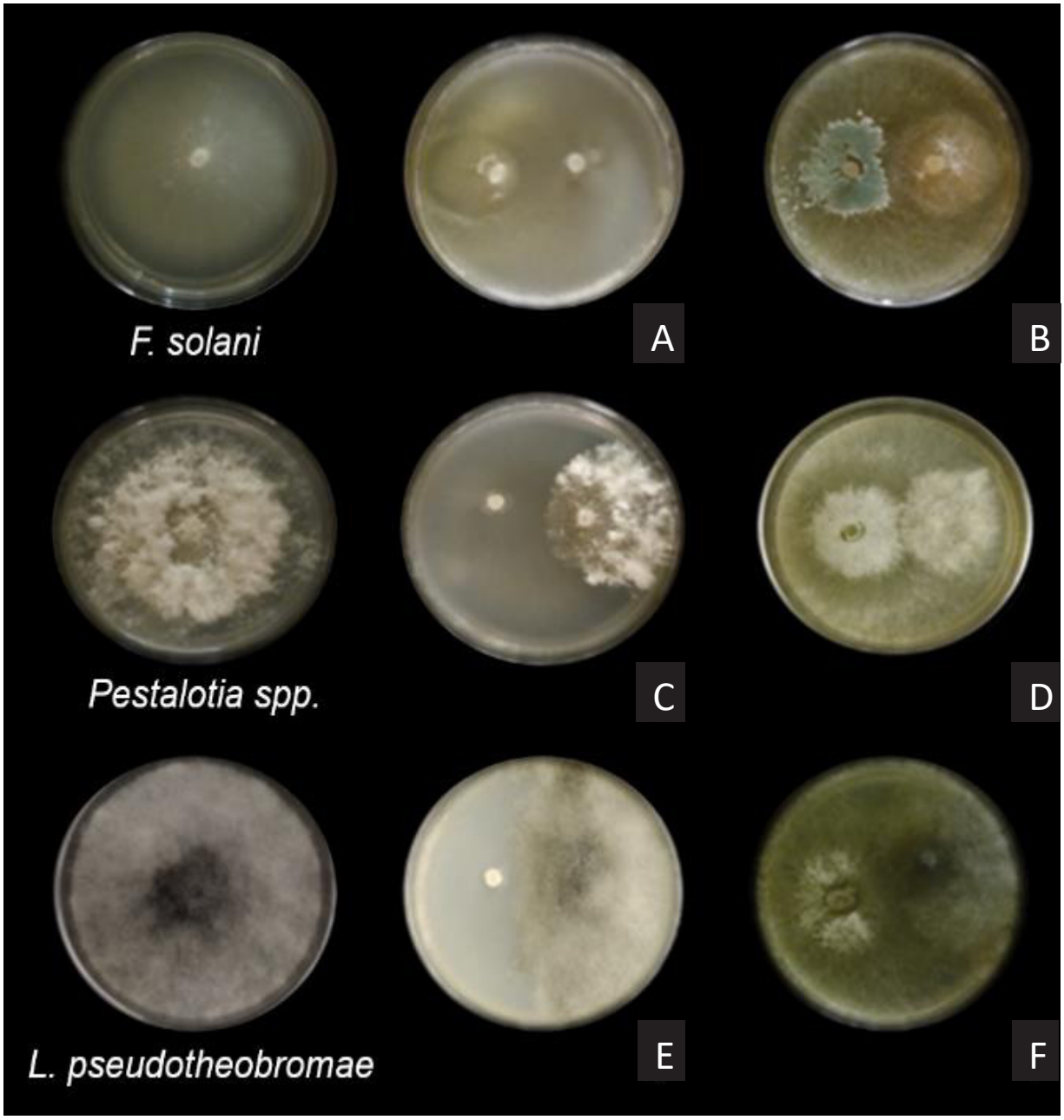

Figure 6. Effect of *Trichoderma* spp. In the development of fungi associated to the gummosis of citrus fruits. **A)** *Fusarium solani vs T. harzianum.* **B)** *F. solani* vs *T. viride.* **C)** *Pestalotia* spp vs *T. harzianum*. **D)** *Pestalotia* vs *T. viride.* **E)** *Lasiodiplodia pseudotheobromae* vs *T. harzianum.* **F)** *L. pseudotheobromae* vs *T. viride*.

Conclusions

The morphological identification and pathogenicity tests showed that the disease of gummosis in citrus fruits from the town of Plan Chac in Sacalum, Yucatan, is caused by *L. pseudotheobromae*. In addition, other phytopathogenic fungi were identified: *Pestalotia* spp. and *F. solani*. The treatment with Thiabendazole, *B. subtilis* strain QST 713 and *T. harzianum* control *F. solani*, L. *pseudotheobromae* and *Pestalotia spp*. efficiently. The management of gummosis fruits can be carried out in combination with the use of biological agents, thus reducing the use of chemical control and avoiding a possible long resistance with its long-term use.

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