Colegio de Postgraduados

Isolation and Characterization of fungal pathogens associated with *Carica papaya* L. and their biocontrol with *Trichoderma* sp.

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ABSTRACT

Objective: In this work, we undertook the task of isolating and identifying *Fusarium* obtained from papaya fruits grown in the Veracruz region and carried out antibiotic tests to find a beneficial fungus that could exert biological control.

Methodology: Juvenile fruits with necrosis and rot were collected from papaya plants of the Maradol variety, from here the pathogenic fungi were obtained, which were morphologically and molecularly characterized using the ITS gene. Subsequently, the isolated pathogenic fungi were confronted with the *Trichoderma* sp. fungus.

Results: Ten isolates were obtained, of which four were *Fusarium solani*. From the confrontations, a 66% to 100% percentage of inhibition in these pathogenic fungi was obtained.

Limitations of the study: It is proposed that future evaluations carry out long-term follow-ups to evaluate the persistence and effectiveness of biological control.

Findings/conclusions: Timely identification of pathogens could represent a biological control strategy in disease management programs.

Keywords: Antagonism, *Fusarium solani*, biological control.

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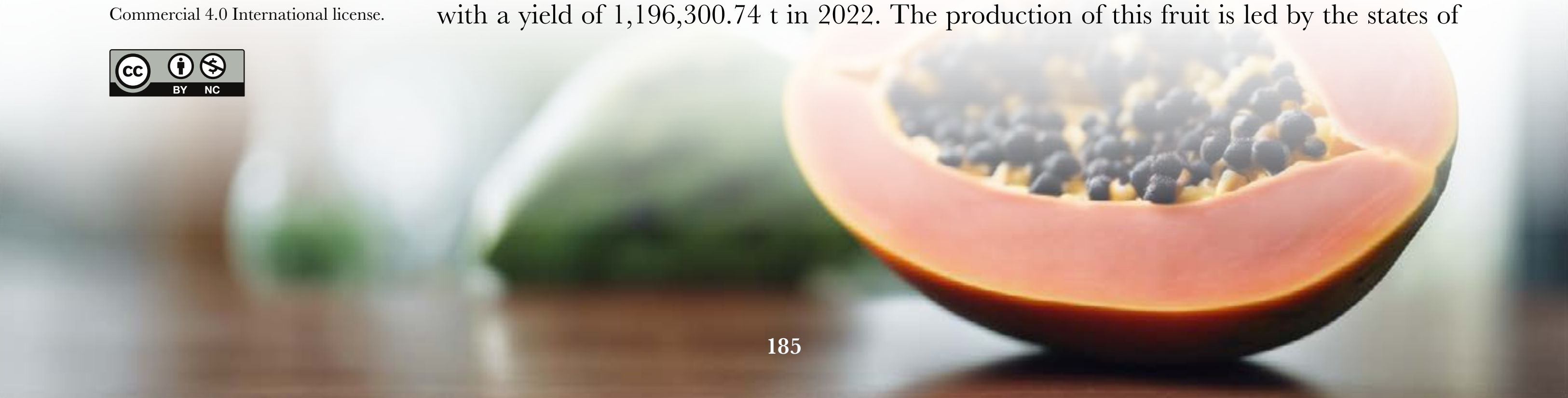
INTRODUCTION

Papaya (*Carica papaya* L.) is a perennial herbaceous plant belonging to the Caricaceae. It is among the most popular fruits in the world as it represents 15% of the total production of tropical fruits worldwide (Fatombi *et al.*, 2019). India is included in the five central papaya-producing countries, followed by Brazil and Mexico, in third place (Sharma *et al.*, 2016, FAOSTAT 2021). In Mexico, the cultivation of the Maradol variety predominates, with a yield of 1,196,300.74 t in 2022. The production of this fruit is led by the states of



Oaxaca, Colima, Chiapas, Veracruz and Michoacán, which together produce more than 80% of this fruit at the national level (Granados *et al.*, 2015; Miranda-Ramírez *et al.*, 2020; SIAP, 2023; Cisneros-Sanguilán *et al.*, 2023).

One of the leading causes that hinder papaya production is biotic stress, which includes attacks by bacteria, fungi, viruses, nematodes and insects. Among the primary diseases that attack papaya is the papaya ring spot virus; some others cause root and stem rot and post-harvest fruit rot, all caused by *Fusarium* spp. (Vega-Gutiérrez *et al.*, 2019; Correia *et al.*, 2013; Ivarez and Nishijima, 1987; Nery-Silva *et al.*, 2007; Nishijima, 1993; Margaret and Egwari, 2015). *Fusarium* control has led to excessive use of chemical pesticides. This has brought environmental damage, such as air pollution, groundwater tables, and decreased soil microbiota. Furthermore, the continuous use of pesticides exerts high selection pressure on pathogens, developing resistance to these chemical inputs (Gómez-Godínez *et al.*, 2019; Bhardwaj *et al.*, 2019; Kumar *et al.*, 2008; Rodríguez *et al.*, 2021). For this reason, it is essential to use alternative biological control strategies that have less impact on the environment and, at the same time, maintain crop yields. These defense and biological control mechanisms can be carried out by interacting with plants and microorganisms, among which we can find *Trichoderma* spp. (Oldroyd, 2013; Vinale *et al.*, 2008).

Fungi of the genus *Trichoderma* spp. with saprophytes and ubiquitous constituents of the soil microbiota are capable of colonizing roots and modulating plant-soil interactions (Vinale *et al.*, 2008; Ghorbanpour *et al.*, 2018; Tyśkiewicz *et al.*, 2022). *Trichoderma* can help plants cope with biotic and abiotic stress conditions, favors the absorption of nutrients by the plant and is known to have the ability to act as an antagonist against phytopathogenic microorganisms so that they do not affect the plant (Mendoza *et al.*, 2015; Stewart & Hill, 2014; Hermosa *et al.*, 2013). The fungi of *Trichoderma* spp. They are capable of exerting antagonism through various mechanisms, for example, the release of enzymes with the capacity to degrade polysaccharides, which can generate a systemic acquired response (SRA) and induced systemic resistance (ISR) in plants (Mathys *et al.*, 2012). Another mechanism is the production of suzukacillin and alamethicin (Ghisalberti & Sivasithamparam, 1991). Furthermore, several enzymes are secreted by *Trichoderma* spp., such as chitinase and protease, which are responsible for degrading the cell wall of some phytopathogenic fungi (Gajera & Vakharia, 2012).

The current work includes the isolation, morphological, and molecular characterization of pathogenic fungi present in papaya fruit and the analysis of the effect that a biological controller, such as *Trichoderma* sp., can exert against these pathogens.

MATERIALS AND METHODS

Isolation of pathogenic fungi from papaya fruit

In different municipalities of Veracruz, Mexico (Table 1), juvenile fruits with necrosis and rot and asymptomatic fruits of papaya plants of the Maradol variety were collected in January and February 2023. The plant material was washed with water, disinfested with sodium hypochlorite at 1.0% for 5 min, washed three times with sterile distilled water, dried with absorbent paper towels, sown in potato-dextrose-agar (PDA) culture medium (BD *et al.*) and incubated for 5 d at 25 °C with white light.

Table 1. Municipalities where infected papaya fruits were extracted were in the center of the state of Veracruz.

Orchard location	Geographical coordinates	Isolation name
1. Santa Teresa, Cotaxtla, Ver.	18° 47' 57" 96° 21' 53"	T14, V2
2. Las Minas, Cotaxtla, Ver.	18° 55' 17" 96° 12' 41"	V4
5. Campo Cotaxtla, Medellín, Ver.	18° 56' 03" 96° 11' 23"	V8
6. Casa Blanca, Tlalixcoyan,Ver.	18° 43' 48" 96° 24' 51"	T7, T19
7. Mata Guitara, Tlalilxoyan, Ver.	18° 43' 07" 96° 21' 00"	T24

Monoconidial cultures purified the fungal colonies formed

The pathogenicity of the fungi isolated from diseased tissues was verified in asymptomatic fruits in February 2023. Each isolation was considered a treatment, and the experimental unit was a fruit. Each fungus was inoculated into 5 asymptomatic juvenile fruits; 5 healthy fruits were used as controls (sprayed with sterile distilled water). The inoculum was prepared by culturing the fungi in PDA at 25 °C with white light for 15 days, and a solution with 1×10^3 conidia mL^{-1} was prepared from each one. Before inoculation, the experimental units were disinfested with 1.0% sodium hypochlorite for 2 min and rinsed with sterile distilled water. Each was sprayed with 3 mL of the inoculant solution and covered for the first 3 days with a disinfested plastic bag. With alcohol.

Morphological and molecular characterization of pathogenic fungi associated with papaya

The fungi from papaya were isolated and cultured in a PDA medium and incubated in the dark for 3 days at 28±2 °C. The isolated strains were preserved in 30% glycerol and were stored in deep freezing at −80 °C. Colony morphologies such as growth, colony color, texture, and pigmentation were described. The conidiophores' morphology and conidia's characteristics were described using the LEICA compound microscope with magnifications of 10X and 40X. DNA was isolated with the Zymo kit, following the manufacturer's instructions. The DNA obtained was used as template DNA for the polymerase chain reaction (PCR), where the ITS region was amplified using the universal primers ITS1 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5' TCCTCCGCTTATTGATATGC 3'). (White *et al.*, 1990). Amplifications were carried out in a thermocycler (ThermoFisher), using an initial denaturation of 94 °C for 5 min, followed by 30 cycles of initial denaturation of 94 °C for 1 min to 94 °C, annealing for 1:20 min at 57 °C and extension for 1 min at 72 °C and a final extension of 5 min at 72 °C. The PCR products were verified on 1% agarose gels and sent for macrogen sequencing. The sequences obtained were aligned and compared in the GenBank National Center for Biotechnology Information (NCBI) database using the elemental local alignment search tool BLAST (Johnson *et al.*, 2008).

RESULTS AND DISCUSSION

Isolation of fungi associated with papaya

It is known that fruit rot in papaya is a post-harvest disease that causes significant losses. It is generally caused by improper handling, storage and transportation after harvest.

Fusarium spp. is a phytopathogenic fungus associated with papaya fruit rot (Coates *et al*., 1997). This phytopathogen infects through cuts or abrasions created during harvesting and handling or through injuries caused by insects. Symptoms caused by *Fusarium* on papaya can be observed as rounded areas that later become small depressions. As these lesions develop, rot and mycelia appear on the surface (Nishijima, 1993; Rahman *et al*., 2018). Diseased papayas were identified at the sites to isolate the fungi present (Figure 1) subsequently.

A total of ten fungi were isolated from the papaya samples, which were named as follows: T7.2, T14, V8, 7.1, V4, V2, T31, T24, T7.3 and T19.

Morphological identification of pathogenic fungi isolated from papaya

In the Potato Dextrose Agar (PDA) culture media, most pathogenic fungi showed moderate mycelial growth except isolate 7.1. The isolates T7.2, T24, and T7.3 presented purple tones, and the isolates V8, T31, and T19 presented orange tones (Figure 2). Under the microscope, hyaline and septate vegetative hyphae with a smooth wall were identified. Microconidia were observed as abundant, short, non-septate and elliptical structures. They were abundant and elongated, cylindrical, slightly curved, fusiform and with septate structures (Figure 2).

Molecular identification of pathogenic fungi isolated from papaya

The ITS regions were successfully amplified by PCR, obtaining a product of around 650 bp. BLASTn (Nucleotide BLAST)-based sequence identification which confirms the strains as different Fusarium species (Table 2).

The accession IDs of the ten strains fell under different NCBI GenBank numbers (Table 2). Several species of *Fusarium* are associated with diseases in papaya; for example, *F. solani* has been reported in different countries such as Hawaii, India, the Philippines, Malaysia and Brazil, causing diseases such as stem rot (Nishijima *et al*., 1993; Rahman *et al*., 2018

Figure 1. Identification of areas with diseased fruits, A) Area/field where isolation was carried out. B) Isolation of the diseased fruit in the laboratory. C) Infection of papaya fruits with fungi isolated from diseased papaya fruits.

Figure 2. Morphological characteristics of pathogenic fungi associated with papaya in PDA medium and microscopic characteristics under a 40X optical microscope.

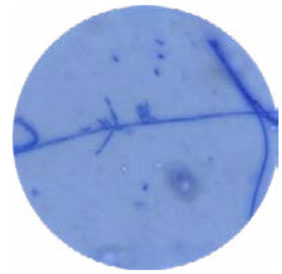
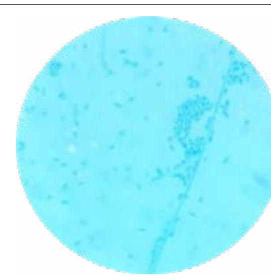
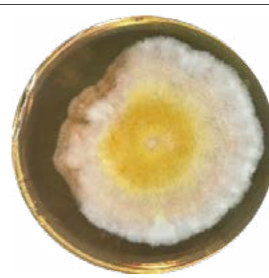
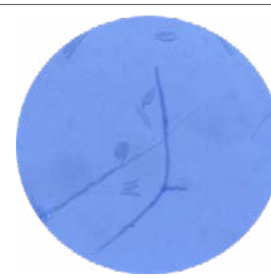
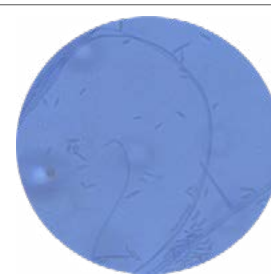
Name isolate	Description	Macroscopic image	Microscopic image
T7.2	White mycelium with purple tones in the center, filamentous aerial mycelium without elevation and regular edge.		
T14	White mycelium, filamentous aerial mycelium without elevation and regular edge.		
V8	White mycelium with orange tones of filamentous type, without elevation and regular edge.		
7.1	White mycelium with a yellow center ring, cottony aerial mycelium without elevation and irregular edge.		
V4	White mycelium, filamentous aerial mycelium without elevation and regular edge.		

Figure 2. Continues...

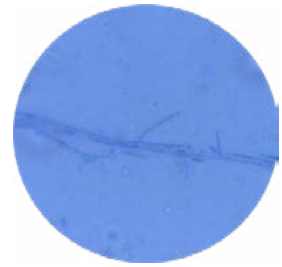
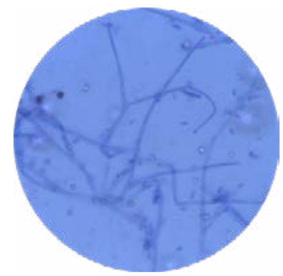
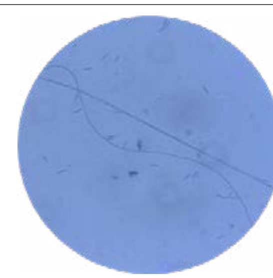
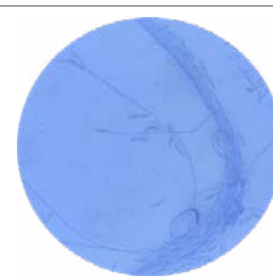
Name isolate	Description	Macroscopic image	Microscopic image
V2	White mycelium with purple tones in the center, cottony aerial mycelium, convex elevation and regular edge.		
T31	Whitish mycelium with orange tones, filamentous aerial mycelium with umbonate elevation and regular edge.		
T24	Whitish mycelium with purple tones, cottony aerial mycelium with ring formation, umbonate elevation and regular edge.		
T7.3	White mycelium with purple tones in the center, cottony aerial mycelium without elevation and regular edge.		
T19	White mycelium with orange tones, filamentous aerial mycelium without elevation and regular edge.		

Table 2. Molecular identification of the ITS marker of pathogenic fungi isolated from papaya.

Isolate ID	Identified species	Identity percentage
T7.2	*Fusarium annulatum*	99.81
T14	*Fusarium solani*	99.81
V8	*Fusarium solani*	99.63
7.1	*Fusarium incarnatum*	100
V4	*Fusarium solani*	99.62
V2	*Fusarium solani*	99.75
T31	*Fusarium solani*	99.82
T24	*Fusarium solani*	99.63
T7.3	*Fusarium oxysporum*	99.70
T19	*Fusarium solani*	99.82

and Correira *et al*., 2019). The dry rot of papaya fruits is also known to be caused by *F. solani* (Alvarez & Nishijima, 1987). This report identified the isolates T14, V8, V4, T31, and T24 as *F. solani*.

Other *Fusarium* species are implicated in cases of papaya fruit rot, including *F. acuminatum*, *F. equiseti*, *F. nivale*, *F. oxysporum*, *F. thapsinum* and *F. chlamydosporum* (Margaret *et al*., 2015); Pathak *et al*., 1976; Gupta *et al*., 1990; Helal *et al*., 2018; Oke *et al*., 1991). In this report, it was also possible to identify *F. annulatum*, which is known to be a species that is also known as *F. proliferatum*, which is known to be pathogenic in up to 200 different plants (Yilmaz *et al*., 2021). This *Fusarium* species has been previously reported to cause decline in the grapevine plant (Úrbez-Torres *et al*., 2017; Bustamante *et al*., 2022).

Antibiosis tests: Inhibition of the growth of pathogenic fungi isolated from papaya

The ten isolates of pathogenic fungi were inhibited by the beneficial fungus *Trichoderma* CC1261, in different percentages, for example, the isolate T14, V4, T24, T7.2, T31, V8 and 7.1 were inhibited by 100%, the isolate T19 and V2 were inhibited by 68% (Figure 3). On the other hand, the *Trichoderma* CC647 strain inhibited isolate 7.1, V8, T7.3, by 100%, isolate V4, was inhibited by 75%, isolates T19, T7.2, were inhibited by 66% (Figure 3).

The two *Trichoderma* tested in this work inhibited papaya pathogenic *Fusarium* in different percentages and exhibited profuse sporulation, which indicates that they are highly competitive for space and nutrients. The application of beneficial antagonists to control pathogens is a sustainable and environmentally friendly strategy. It is known that *Trichoderma* are fungi antagonistic to many pathogens, capable of controlling phytopathogens through multiple mechanisms such as the production of enzymes, antibiotics, volatiles, among others (Tian *et al*., 2020). The selection and characterization of microorganisms highly targeted to a specific pathogen such as Fusarium could be concluded in disease management programs.

Figure 3. Antagonistic effects of *Trichoderma* C647 and C1261 against phytopathogens isolated from papaya.

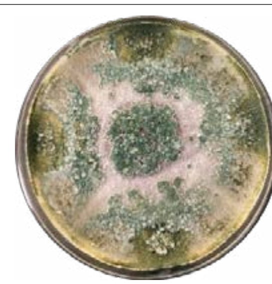
Strain *Trichoderma* sp.	**Strain *Fusarium* sp.**	***Fusarium* without treatment**	**Confrontation**	**PICR**
C647	T19			68.82 %
C647	7.1			100 %
C647	T7.2			68.03 %
C647	V2			53.72 %
C647	V8			100 %

Figure 3. Continues...

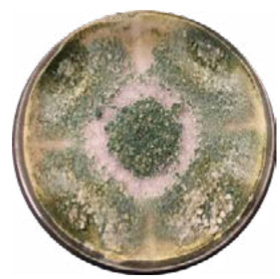
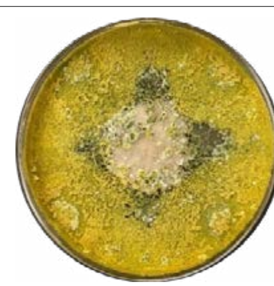
Strain *Trichoderma* sp.	Strain *Fusarium* sp.	*Fusarium* without treatment	Confrontation	PICR
C647	T7.3			100 %
C647	T31			100 %
C647	T24			100 %
C647	T14			100 %
C647	V4			75 %

Figure 3. Continues...

Strain *Trichoderma* sp.	**Strain *Fusarium* sp.**	***Fusarium* without treatment**	**Confrontation**	**PICR**
C1261	T7.3			71.37 %
C1261	T14			100 %
C1261	T19			68.82 %
C1261	V4			100 %
C1261	V2			68.43 %

Figure 3. Continues...

Strain *Trichoderma* sp.	Strain *Fusarium* sp.	*Fusarium* without treatment	Confrontation	PICR
C1261	T24			100 %
C1261	T7.2			100 %
C1261	T31			100 %
C1261	V8			100 %
C1261	7.1			100 %

CONCLUSION

Tropical fruit crops can be infected by one or more diseases caused by *Fusarium*. These diseases can represent limitations in the sustainable production of tropical fruit crops since they weaken crop production. Postharvest diseases affect the marketing of fruits since the product is not attractive to consumers. Identifying the fungi that cause postharvest diseases is crucial to search for biological control alternatives, such as *Trichoderma*, which was evaluated in this report, showing inhibition capabilities in the growth of the pathogenic Fusarium characterized in this report.

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REFERENCES

Alvarez, A.M.; Nishijima, W.T. Postharvest diseases of papaya. *Plant Dis.* 1987, 71, 681–686. DOI: 10.1094/PD-71-0681.

Bustamante, M. I., Elfar, K., Smith, R. J., Bettiga, L. J., Tian, T., Torres, G. A., & Eskalen, A. (2022). First report of *Fusarium annulatum* associated with young vine decline in California. *Plant Disease, 106*(10), 2752. https://doi.org/10.1094/PDIS-12-21-2790-PDN

Cisneros-Saguilán, P., Velasco-Galeana, B. R., Hernández-Bernardino, A. A., Antonio-Méndez, I., & Vázquez-Alfaro, M. (2023). Social and Economic Analysis of the Production of Maradol Papaya (*Carica papaya* L.): Case study in the coast of Oaxaca, Mexico. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i4.2473

Coates, L.; Johnson, G. Chapter 33: Postharvest diseases of fruit and vegetables. In Plant Pathogens and Plant Diseases; Brown, J., Ogle, H., Eds.; Rockvale Publications: Cambridge, UK, 1997; pp. 533–547.

Correia, K. C., Souza, B. O., Câmara, M. P. S., & Michereff, S. J. (2013). First report of stem rot of papaya caused by *Fusarium solani* species complex in Brazil. *Plant Disease, 97*(1), 140-140. https://doi.org/10.1094/PDIS-06-12-0519-PDN

Fatombi JK, Osseni SA, Idohou EA, Agani I, Neumeyer D, Verelst M, Mauricot R, Aminou T (2019) Characterization and application of alkali-soluble polysaccharide of *Carica papaya* seeds for removal of indigo carmine and Congo red dyes from single and binary solutions. *J Environ Chem Eng* 7:103343. https://doi.org/10.1016/j.jece.2019.103343

Gajera, H. P., & Vakharia, D. N. (2012). Production of lytic enzymes by *Trichoderma* isolates during *in vitro* antagonism with *Aspergillus niger*, the causal agent of collar rot of peanut. *Brazilian Journal of Microbiology*, 43, 43-52. https://doi.org/10.1590/S1517-83822012000100005

Ghisalberti, E. L., & Sivasithamparam, K. (1991). Antifungal antibiotics produced by *Trichoderma* spp. *Soil biology and Biochemistry, 23*(11), 1011-1020. https://doi.org/10.1016/0038-0717(91)90036-JGet rights and content

Ghorbanpour, M., Omidvari, M., Abbaszadeh-Dahaji, P., Omidvar, R., Kariman, K. (2018). Mechanisms underlying the protective effects of beneficial fungi against plant diseases. *Biol. Control* 117, 147–157. doi: 10.1016/j.biocontrol.2017.11.006

Granados, R., Salceda, R., & Longar, M. P. (2015). Situación actual y perspectivas tecnológicas para la papaya (*Carica papaya* L.) en el distrito de Veracruz, Veracruz. *Revista Mexicana de Ciencias Agrícolas, 6*(4), 749-761

Gupta, A.K.; Pathak, V.N. Epidemiology and management of papaya fruit rots. *Summa Phytopathol.* 1990, 16, 92–105

Helal, R.B.; Hosen, S.; Shamsi, S. Mycoflora associated with post-harvest disease of papaya (*Carica papaya* L.) and their pathogenic potentiality. *Bangladesh J. Bot.* 2018, 47, 389–395.

Hermosa, R., Rubio, M. B., Cardoza, R. E., Nicolás, C., Monte, E., & Gutiérrez, S. (2013). The contribution of *Trichoderma* to balancing the costs of plant growth and defense. *Int. Microbiol, 16*(2), 69-80.

Johnson, M., Zaretskaya, I., Raytselis, Y., Merezhuk, Y., McGinnis, S., Madden, T. L. (2008). NCBI BLAST: a better web interface. *Nucleic Acids Res. 36* (suppl_2), W5–W9. https://doi.org/10.1093/nar/gkn201

Margaret, O.; Egwari, L. Fruit, Leaf and Stem Diseases of *Carica papaya*. *J. Int. Sci. Pub*. 2015, 3, 398–407.

Mathys, J., De Cremer, K., Timmermans, P., Van Kerckhove, S., Lievens, B., Vanhaecke, M., & De Coninck, B. (2012). Genome-wide characterization of ISR induced in *Arabidopsis thaliana* by *Trichoderma hamatum* T382 against *Botrytis cinerea* infection. *Frontiers in plant science*, 3, 108. https://doi.org/10.3389/fpls.2012.00108

Mendoza, J. L. H., Pérez, M. I. S., Prieto, J. M. G., Velásquez, J. D. Q., Olivares, J. G. G., & Langarica, H. R. G. (2015). Antibiosis of *Trichoderma* spp strains native to northeastern Mexico against the pathogenic fungus *Macrophomina phaseolina*. *Brazilian Journal of Microbiology,* 46, 1093-1101. https://doi.org/10.1590/S1517-838246420120177

Miranda-Ramírez, J. M., Aguilar-García, O., & Miranda-Medina, D. (2020). Comparación de la productividad agrícola-económica sustentable y convencional de papaya en Michoacán, México. *Agronomía Mesoamericana, 31*(2), 385-403. doi: 10.15517/am.v31i2.38615

Nery-Silva, F.A.; Machado, J.D.; Resende, M.L.; Lima, L.C. Inoculation methodology of papaya fruits with fungi causing stem-end rot. *Ciênc. Agrotec*. 2007, 31, 1374–1379. https://doi.org/10.1590/S1413-70542007000500015

Nishijima, W. *Fusarium solani*: Fruit and Seedling Rot of Papaya. Crop Knowl. Master.

Oke, O.A.; Banjoko, K.M. The effects of *Penicillium digitatum* and *Fusarium oxysporum* rots on nutritional content of pawpaw (*Carica papaya* L.). *Mycopathologia* 1991, 116, 199–202. https://doi.org/10.1007/BF00436835

Oldroyd, G. E. (2013). Speak., friend., and enter: signalling systems that promote beneficial symbiotic associations in plants. *Nat. Rev. Microbiol. 11*(4), 252–263. https://doi.org/10.1038/nrmicro2990

Pathak, V.N.; Goyal, J.P.; Bhatanagar, L.G. Effect of chemical and hot water treatment on *Fusarium* and *Rhizopus* rots of papaya. *Indian Phytopathol*. 1976, 29, 210–211.

Rahman, M.A.; Mahmud, T.M.M.; Kadir, J.; Abdul, R.R.; Begum, M.M. Major postharvest fungal diseases of Papaya cv. 'Sekaki' in Selangor, Malaysia. Pertanika *J. Tropi. Agric. Sci*. 2008, 31, 27–34.

Rodríguez E., J. G.; Rodríguez F., R. y Rodríguez Q., C. G.; 2021. *Colletotrichum* spp. procedente de frutos de papaya, del centro de Veracruz, su control con fungicidas y efecto del ph del agua. *Brazilian Journal of Animal and Enviromental Research. 4*(3):4512-4523 DOI: 10.34188/bjaerv4n3-133

Servicio de Información Agroalimentaria y Pesquera (SIAP). (2022). Anuario Estadístico de la Producción Agrícola. https://nube.siap.gob.mx/cierreagricola/

Sharma, S.K.; Mitra, S.K.; Saran, S. Papaya production in India—History, present status and future prospects. *Acta Hort*. 2016, 1111, 87–94. 10.17660/ActaHortic.2016.1111.13

Stewart, A., & Hill, R. (2014). Applications of *Trichoderma* in plant growth promotion. In Biotechnology and biology of *Trichoderma* (pp. 415-428). Elsevier. https://doi.org/10.1016/B978-0-444-59576-8.00031-X

Tian, Y., Yu, D., Liu, N., Tang, Y., Yan, Z., & Wu, A. (2020). Confrontation assays and mycotoxin treatment reveal antagonistic activities of *Trichoderma* and the fate of *Fusarium* mycotoxins in microbial interaction. *Environmental Pollution*, 267, 115559. https://doi.org/10.1016/j.envpol.2020.115559

Tyśkiewicz, R., Nowak, A., Ozimek, E., Jaroszuk-Ściseł, J. (2022). *Trichoderma*: The current status of its application in agriculture for the biocontrol of fungal phytopathogens and stimulation of plant growth. *Int. J. Mol. Sci. 23*(4), 2329. doi: 10.3390/ijms23042329

Úrbez-Torres, J. R., J. Boule, and D. T. O'Gorman. «Investigating the role of *Fusarium* spp. in the young vine decline complex." *Phytopathologia Mediterranea 56*(2017): 527-528.

Vega-Gutiérrez, T.A.; Tirado-Ramírez, M.A.; López-Urquídez, G.A.; Angulo-Castro, A.; Martínez-Gallardo, J.A.; López-Orona, C.A. *Fusarium falciforme* (FSSC 3 + 4) causing root and stem rot in papaya (*Carica papaya*) in Mexico. *Plant Dis*. 2019, 103, 2681. https://doi.org/10.1094/PDIS-05-19-0917-PDN

Vinale, F., Sivasithamparam, K., Ghisalberti, E. L., Marra, R., Woo, S. L., & Lorito, M. (2008). *Trichoderma*–plant–pathogen interactions. *Soil Biology and Biochemistry, 40*(1), 1-10. https://doi.org/10.1016/j.soilbio.2007.07.002

White, T. J., Bruns, T., Lee, S., Taylor, J., Innis, M. A., Gelfand, D. H., et al. (1990). PCR protocols: a guide to methods and applications.

Yilmaz, N., Sandoval-Denis, M., Lombard, L., Visagie, C. M., Wingfield, B. D., & Crous, P. W. (2021). Redefining species limits in the *Fusarium fujikuroi* species complex. *Persoonia-Molecular Phylogeny and Evolution of Fungi, 46*(1), 129-162. https://doi.org/10.3767/persoonia.2021.46.05

Zhao, X., Hou, D., Xu, J., Wang, K., & Hu, Z. (2022). Antagonistic activity of fungal strains against *Fusarium* crown rot. *Plants, 11*(3), 255. https://doi.org/10.3390/plants11030255