Salud, Ciencia y Tecnología. 2025; 5:1616
doi: 10.56294/saludcyt20251616

ORIGINAL

Biocontrol potential of Trichoderma harzianum in fungal diseases associated with tomato crops (Solanum lycopersicum L.)

Potencial biocontrolador de Trichoderma harzianum en enfermedades fungosas asociadas al cultivo de tomate (Solanum lycopersicum L.)

Maryori C. Pineda Zambrano[1], Hebandreyna González García[2], Juan C. Escalante[3]

[1]Universidad Nacional Experimental Sur del Lago "Jesús María Semprúm" (UNESUR), Programa académico de Ingeniería de la Producción Agropecuaria, Laboratorio de Fitopatología. Santa Bárbara de Zulia, Venezuela.
[2]Corporación Universitaria del Meta (UNIMETA), Centro de Investigaciones Ambientales "José Antonio Cándamo". Villavicencio, Colombia.
[3]Tecnológico de Antioquia (TdeA), Facultad de Ciencias Administrativas y Económicas, Programa de Administración Financiera. Medellín, Colombia.

Cite as: Pineda Zambrano MC, González García H, Escalante JC. Biocontrol potential of Trichoderma harzianum in fungal diseases associated with tomato crops (Solanum lycopersicum L.). Salud, Ciencia y Tecnología. 2025; 5:1616. https://doi.org/10.56294/saludcyt20251616

Submitted: 14-09-2024 **Revised:** 01-01-2025 **Accepted:** 27-06-2025 **Published:** 28-06-2025

Editor: Prof. Dr. William Castillo-González

Corresponding author: Maryori C. Pineda Zambrano

ABSTRACT

Introduction: fungal diseases in tomato cultivation are the main phytosanitary factor that reduces yields. The objective of the investigation was to evaluate the potential biocontroller of *Trichoderma harzianum* in mushrooms associated with cultivation.
Method: the samples of leaves, roots and stems of diseased plants were collected and processed by the fungal isolation method in vegetable tissue and papa dextrose agar (PDA), the growths were purified, typified and tested for pathogenicity. The potential biocontroller was evaluated under in vitro conditions with the dual cultivation technique in PDA confronting the antagonist with each pathogen. The study employed an random design, with 7 treatments and 4 repetitions, conformed by: 3 dual cultures, 4 witnesses corresponding to each pathogen and the antagonist. The degree of mycoparasitism, the percentage of inhibition of the radial growth (PCR) of the antagonist and the presence of antibiosis between the antagonist and the pathogens were evaluated every 24 hours for 15 days.
Results: the fungi were identified: *Alternaria alternata*, *Fusarium oxysporum* and *Pythium* sp. The tests of confrontation evidenced the presence of the mechanisms of action characteristic of this species of biocontroller, although it varies according to the species. The antagonistic capacity for micoparasític action was grade 4 with *A. alternata* and *Pythium* and grade 3 with *F. oxysporum*, the strangulation and enzymatic lysis were observable at the microscopic level. The action of antibiosis was present with all pathogens, while the action of competition in growth was significant only with *A. alternata* and *F. oxysporum*. When evaluating the PICR it was found that the antagonist showed high and significant inhibition with *A. alternata* (55,43 %) and *F. oxysporum* (31,01 %), while no inhibition was found with *Pythium*, although when entering contact, the antagonist showed mycopasitic action stopping the growth and invading the pathogen until it sporulates on it.
Conclusions: that *T. harzianum* has the potential to be used as a biocontrol agent in fungal diseases associated with tomato cultivation.

Keywords: Antagonism; Alternaria Alternata; Biological Control; Phytopathogenic Fungi; Fusarium Oxysporum; Pythium Sp.

RESUMEN

Introducción: las enfermedades fúngicas en el cultivo tomate son el principal factor fitosanitario que reduce los rendimientos. El objetivo de la investigación fue evaluar el potencial biocontrolador de *Trichoderma harzianum* en hongos asociados al cultivo.
Método: las muestras de hojas, raiz y tallo de plantas enfermas fueron colectadas y procesadas por el método de aislamiento de hongos en tejido vegetal en agar papa dextrosa (PDA), los crecimientos fueron purificados, tipificados y comprobados su patogenicidad. El potencial biocontrolador se evaluó bajo condiciones in vitro con la técnica de cultivo dual en PDA confrontando el antagonista con cada patógeno. El estudio empleó un diseño al azar, con 7 tratamientos y 4 repeticiones, conformados por: 3 cultivos duales, 4 testigos correspondientes a cada patógeno y el antagonista. Se evaluó el grado de micoparasitismo, el índice porcentaje de inhibición del crecimiento radial (PCR) antagonista y la presencia de antibiosis entre el antagonista y los patógenos cada 24 horas por 15 días.
Resultados: se obtuvo la identificación de los hongos: *Alternaria alternata*, *Fusarium oxysporum* y *Pythium* sp. Las pruebas de confrontamiento evidenciaron la presencia de los mecanismos de acción característicos de esta especie de biocontrolador, aunque vario según la especie. La capacidad antagónica por acción micoparasítica fue en grado 4 con *A. alternata* y *Pythium* y grado 3 con *F. oxysporum*, el estrangulamiento y lisis enzimática fueron observables a nivel de microscopio. Las acciones de antibiosis fueron presentes con todos los patógenos, mientras que la acción de competencia en crecimiento fue significativa solo con *A. alternata* y *F. oxysporum*. Al evaluar el PICR se encontró que el antagonista mostró alta y significativa inhibición con *A. alternata* (55,43 %) y *F. oxysporum* (31,01 %), mientras que con *Pythium* no se encontró alguna inhibición, aunque al entrar en contacto el antagonista mostró acción micoparasítica deteniendo el crecimiento e invadiendo el patógeno hasta esporular sobre él.
Conclusiones: el *T. harzianum* tiene el potencial para ser empleado como agente de biocontrol en enfermedades fungosas asociadas al cultivo de tomate.

Palabras clave: Antagonismo; Alternaria Alternata; Control Biológico; Hongos Fitopatógenos; Fusarium Oxysporum; Pythium Sp.

INTRODUCTION

Tomato (Solanum lycopersicum L.) is one of the most essential horticultural species in the world, with a global production of 180 766 329 tons and an area of 5 030 545 hectares.[1] In Venezuela, this vegetable represents one of the high-value and in-demand items for the industrial and consumer sectors, being a sine qua non ingredient of the rich national culinary gastronomy, which has motivated the increase in the planted area of this crop in several regions of the country.[2] Its tolerance to different climates and agricultural technical investment has allowed it to grow heterogeneously over the years, and today it is cultivated in several productive zones, both in the field and under cover in semi-arid, Andean, and central-eastern regions.[3]

However, this crop is highly susceptible to a large number of phytopathogens, of which fungi are the leading group that stands out, agents such as: Alternaria, Botrytis, Fusarium, Laveillula, Phytopthora, Pythium and Rhizoctonia can reduce production by 60 % in association or individually, causing the development of different types of lesions in the plant that trigger the reduction of photosynthetic capacity, deterioration of the support and anchorage until its death.[4] The effects and consequences of these pathogens' actions on the crop primarily derive from the susceptibility of the species, the soil habitat of most fungi, predisposing favorable environmental conditions, and soil nutrient deficiencies.

The management used with agrochemicals, although they act as a response to the problem, in the long term represents a loss of effectiveness because phytopathogens generate resistance to them, toxicological risks due to contamination of food and soil, reduction of self-regulating fungi of the ecosystem and persistence in water.[5] Therefore, the use of biocontrol agents in disease management represents an alternative to minimize damage caused by pathogens and reduce the use of chemical products. The species Trichoderma harzianum has been, until now, the most widely used antagonist fungus in the control of plant diseases, varying its response according to the pathogen with which it is confronted. Additionally, numerous biotypes are reported to be tolerant to fungicides.[6] Based on the above, this investigation proposes to evaluate the biocontrol potential of Trichoderma harzianum in fungal diseases associated with the tomato crop under in vitro conditions.

METHOD

Description of the study area

The research was conducted under in vitro conditions in the facilities of the Microbiology and Phytopathology

Laboratory at the Universidad Nacional Experimental Sur del Lago "Jesús María Semprum" (UNESUR), located in the municipality of Colón, Santa Bárbara parish, Zulia state, Venezuela. A controlled environment of temperature (29±1 ºC) and stable humidity (70 %) was used for the development of the microorganisms. A strain native to southern Lake Maracaibo, isolated, characterized, and identified as Th-BST.0108 (6), was used as an antagonist. The selected strain was previously activated in Papa Dextrose Agar (PDA) medium. The pathogenic fungi were isolated from a preliminary sampling in El Silencio, a farm with tomato crop production, located at 952 m above sea level (m.a.s.l.) in the El Peñon sector, Tovar parish, Tovar municipality, Mérida state, Venezuela.

Sampling of the affected tomato crop

For the collection of samples in the field, a permanent monitoring of the plants that had signs of the disease caused by the phytopathogen was carried out, the type of symptoms classified the lesions, the plant material was collected in different organs of the plants extracting sections of tissue (leaf, stem); later, it was stored individually in ziploc plastic bags, and transferred to the laboratory for their respective isolation and identification.

Isolation of samples

The plant material collected was isolated as follows: the tissue of interest to be processed was washed with running water; then, cuts were made from the edge of the infected lesion in the injured tissues of approximately 5 mm, under totally aseptic conditions; and under a laminar flow chamber the cuts were subjected to disinfection with hypochlorite solution for 1 minute with permanent agitation, after which the tissue was washed three times with sterile distilled water to remove excess disinfectant and then dried with sterile paper towel.[7] Subsequently, the sections were placed equidistantly in the Petri dishes containing Potato Dextrose Agar PDA medium, incubated at a temperature of 28 ºC±1 for 5 days until the development and subsequent purification of the colonies.

Identification of the genus of isolated fungi

Once the colonies were obtained in the previous procedure, the sample was purified to obtain a pure culture. The typing of the isolated genus was performed through the micromorphological observation of each fungus obtained, performing slide mounts with lactophenol blue staining, and with the help of the taxonomic keys, the structures of each isolate were characterized in optical microscopy at 40X and 100X up to the fungal taxon of the species.[8]

Pathogenicity tests

The pathogenicity tests were evaluated in an umbraculum, which consisted of selecting five healthy tomato plants in the nursery stage for each fungus group identified.[9] Subsequently, the inoculating suspension was made by pathogen, adding 5 ml of ADE on a fungal colony of 7 days in growth, and with the help of a sterile brush, the developed spores were swept, resulting in a suspension to which the amount of conidia/mL was quantified through its count in a Neubauer chamber, The content of the preparation to be applied to the ADE plants was then adjusted until the final concentration of 2,5 x 105 conidia/mL was obtained, and 3 mL of Tween 20 solution was added to disperse the aggregates of conidia.

The groups of healthy plants were inoculated with biological suspensions and subjected to a humid chamber for 3 days to induce the penetration and establishment of the fungi; subsequently, they were monitored daily until the first symptoms were recognized. Compliance with Koch's postulates was confirmed by reisolation and identification of pathogens.

In vitro confrontation

The in vitro confrontation tests between the antagonist and the identified pathogenic fungi consisted of placing a slide in the center of a Petri dish, on which PDA medium was poured under aseptic conditions. A 5 mm diameter disc with the mycelium of the fungus T. harzianum was then placed. The dish was incubated for 7 days under aseptic conditions. harzianum fungus in 7 days of active growth, and at the opposite end of the Petri dish, the 5 mm disc with mycelium of the phytopathogenic fungus, at a distance of approximately 5 cm between them and 2 cm from the edge of the capsule. Five replicates per isolate were prepared, and the plates were then incubated at 28 ± 1 °C, with their daily behavior measured for 10 days.[6]

The biocontrol potential of the fungus T. harzianum on pathogenic fungi associated with tomato crops was tested by evaluating the three mechanisms of action [10] related to this species. First, the antibiosis action of T. harzianum on each pathogen was measured. harzianum on each pathogen, which was evaluated qualitatively by the pathogen growth in the dual culture before the contact between both fungi (48 h), concerning its control, taking into account the antibiotic capacity of the antagonist, to promote the formation of an inhibition halo during the radial growth of the pathogenic fungus, generally witnessed at 10 days of growth.

Second: Evaluating competition for space and nutrients by estimating the percentage of radial growth inhibition (PGRI) in the dual culture, and measuring the radius of growth of the pathogen and antagonist with their respective controls. The PICR was calculated using the formula PICR [(R1-R2/R1) x 100]. Where R1 is the diameter of the control (average of the radial growth of the replicates of each pathogen), and R2 is the diameter of the pathogenic organism in the dual culture with the antagonist.

And third: Measuring the degree of mycoparasitism through the scale[10] (table 1); and corroborating these results through the direct interaction of the hyphae when in contact between both fungi, for which the slide was extracted from the center of the dual culture and observed under an optical microscope with a magnification of 40X.

Table 1. Scale for the evaluation of the antagonistic capacity of Trichoderma harzianum

Grade	Antagonistic capacity
0	No invasion of the surface of the pathogenic fungus colony.
1	Invasion of ¼ of the surface of the pathogenic fungal colony.
2	Invasion of ½ of the surface of the pathogenic fungus colony.
3	Total invasion of the surface of the pathogenic fungus colony.
4	Total invasion of the surface of the colony of the pathogenic fungus sporulating on it.

Statistical analysis

A completely randomized experimental design was employed, consisting of seven treatments and four replicates (one control for each treatment), where the experimental unit was each Petri dish. Dual cultures between T. harzianum and the respective pathogenic fungus formed the treatments. In contrast, the controls were only the antagonist and each pathogenic fungus in a Petri dish. Statistical processing was performed parametrically using an ANOVA. Multiple comparisons of means were performed using Tukey's test with a significance level of $\alpha = 0,05$. Data were statistically processed in the IBM SPSS Statistics version 25 statistical package.

RESULTS

Identification of the pathogen of the samples collected.

When isolations were made from plant tissue samples (stem, root and leaves) with disease symptoms, three isolates were identified: Alternaria alternata, Fusarium oxysporum and Pythium sp.,[8] The result of the pathogenicity tests proved that the fungi present pathogenic activity in the crop from the development of lesions in the tomato crop, with specific characteristics in each case, diagnosing diseases of fungal origin: early blight, root rot and damping off in tomato.

The characteristic foliar lesions of the tissues where Alternaria alternata was obtained presented concentric spots, round or oval in shape, brown on leaves, in addition to a faint yellow necrotic halo around them (figure 1a); while on the stem, petiole and peduncle, the slightly sunken necrotic formation characteristic of cankers was observed (figure 1b). Likewise, at the fungal colony level of A. alternata, a fast-growing culture was found, with aerial mycelia, cottony initial texture, and olive green coloration, which soon became velvety and dark green when sporulating (figure 2a), with the presence of aggregates of whitish wool-like conidia.

Microscopically, observations of A. alternata show it with macroconidia, fusiform, curved at the ends and septate, short erect conidiophores, forming chains of simple or branched conidia of dark color, measuring 12-20 X 120-296 µm (figure 2b and 2c). The conidia are large, dark with a mallet appearance and present longitudinal and transverse septa (figure 2c), they were observed after eight days.

The Fusarium oxysporum pathogen typing came from crop tissues that showed unilateral presence of yellowing in the basal leaves that gradually spread to the youngest ones, with brown wilting in the root system; necrosis initially lodged in the neck (figure 3a) and root of the plant progressively spread to the upper tissues, with the appearance of brown spots on the leaves, extending from the stem to the apex (figure 3b), and as the generalized wilting of the plant progressed, it led to the total death of the tissues (figure 3c).

The purified colony of Fusarium oxysporum was characterized by an initially sparse topography, and after 7 days semi-aerial, with filamentous texture, ivory-white mycelium (figure 4a), with aggregates of pink and light purple conidia that develop after 6 days; on the back of the Petri dish, a slightly purple rhizoid growth of the mycelium is observed. Micromorphologically, sect hyphae were observed, presence of large, elongated conidia of oblong shape (banana) with the presence of 3 to 4 septa inside, with the development of slightly ovoid and hyaline colored microconidia (figure 4b).

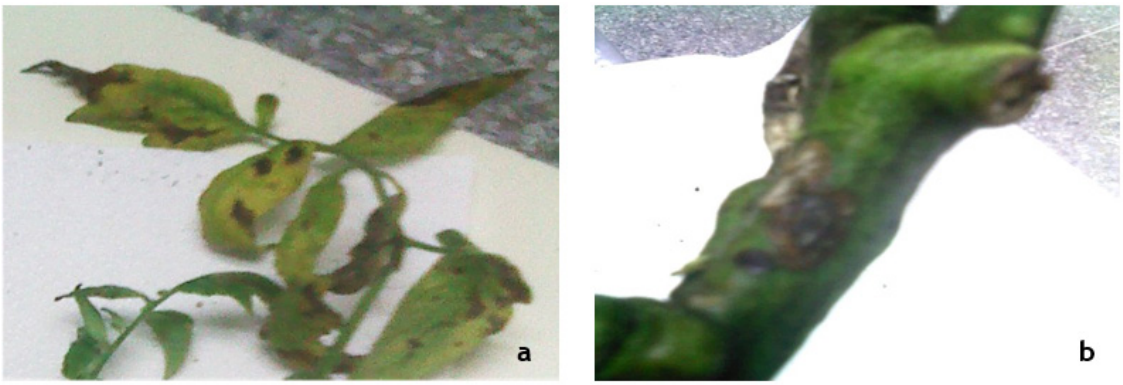

Figure 1. Necrotic lesions on tomato crop by A. alternata. 1a: Lesions on leaves, 1b: Lesions on stem

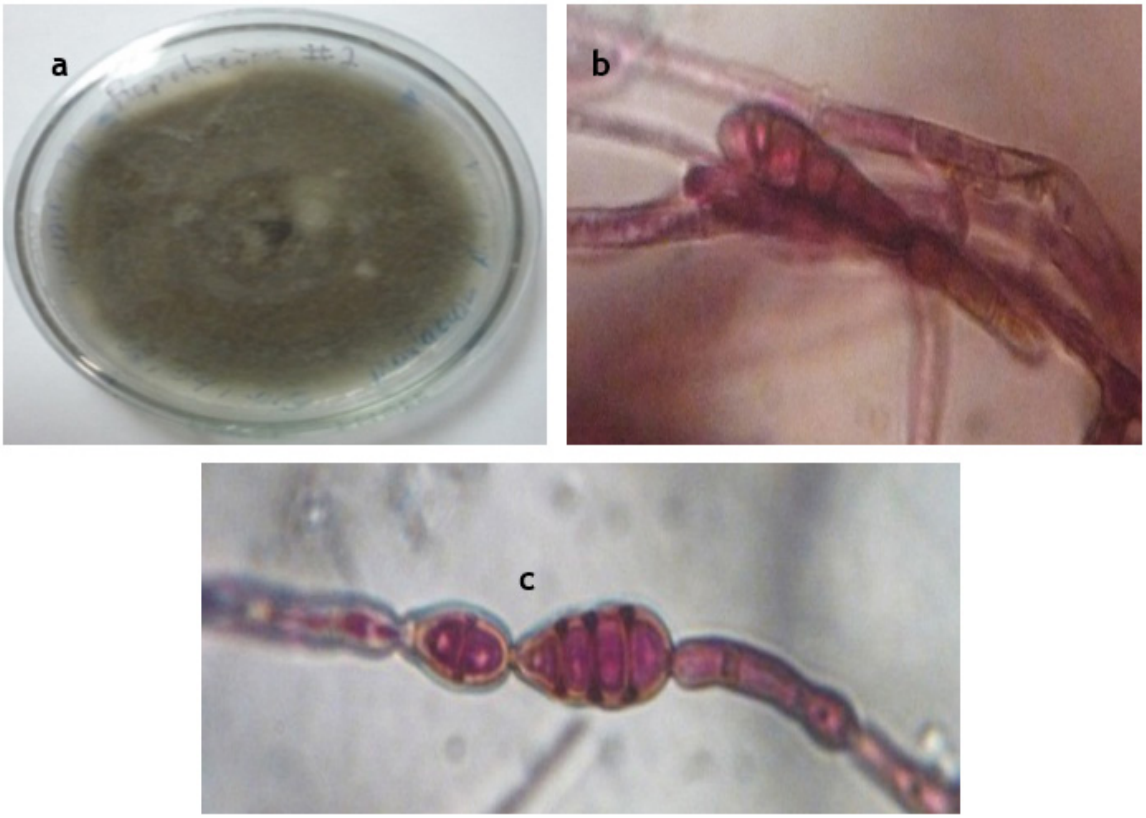

Figure 2. Cultural and morphological characteristics of A. alternata on tomato plants. 2a: Pure culture of A. solani; 2b and 2c: Conidia of the fungus

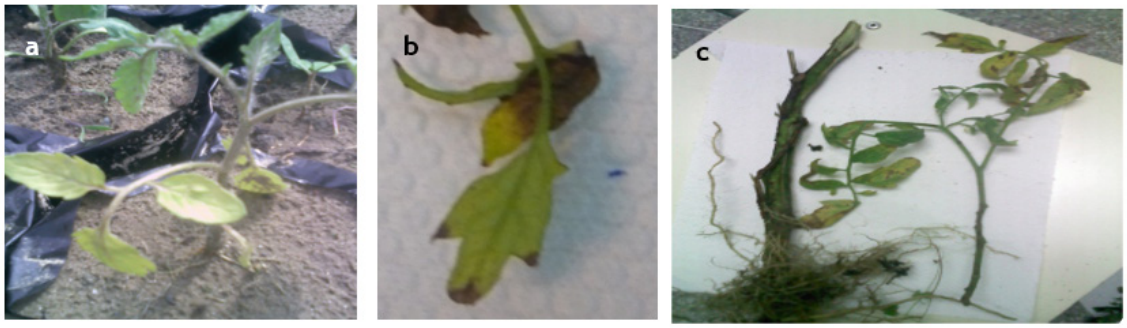

Figure 3. Necrotic lesions in tomato crop due to Fusarium oxysporum. 3a: Stem symptoms; 3b: Brown spots and yellowing on leaves; 3c: Complete section of the plant

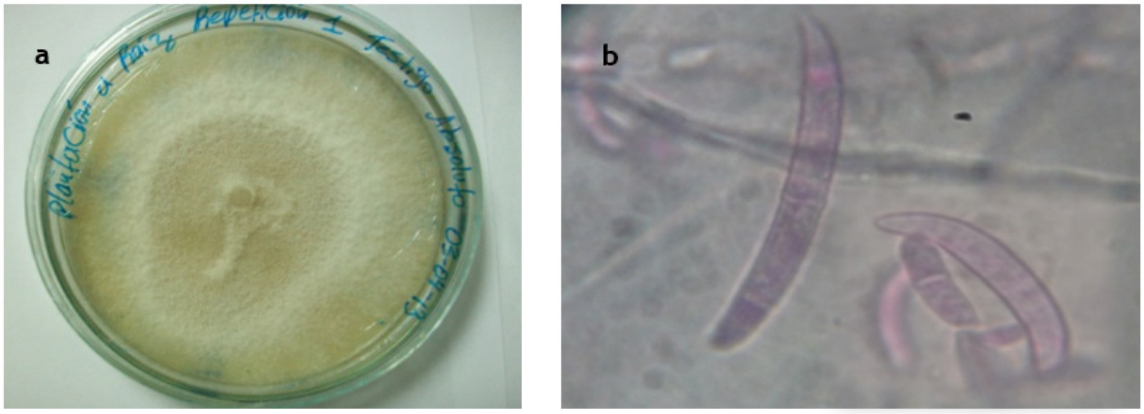

Figure 4. Cultural and morphological characteristics of Fusarium oxysporum on tomato plants. 4a: Pure culture; 4b: Conidia of the fungus

Finally, the presence of the genus Pythium sp. was confirmed by the symptoms of plant neck drowning. Although initially the symptomatological manifestation of wilting and loss of turgor in the aerial organs of the plants can be confused with water deficit, as the pathogen infection evolves in the tissues, small lesions are observed in the stem that darken, indicating the death of the tissue, preventing the mobilization of nutrients throughout the plant. These lesions cause systemic yellowing, stunting, and wilting throughout the plant (figure 5a).

Pure cultures of Pythium reveal it as a fast-growing fungus that rapidly covers the Petri dish, characterized by whitish mycelia with aerial topography, a cottony and mucilaginous texture (figure 5b). Microscopically, it presents coenocytic hyphae with ramifications from which circular vesicles called sporangia and oogonia are formed (figure 5c), inside which the flagellated spores known as zoospores are formed.

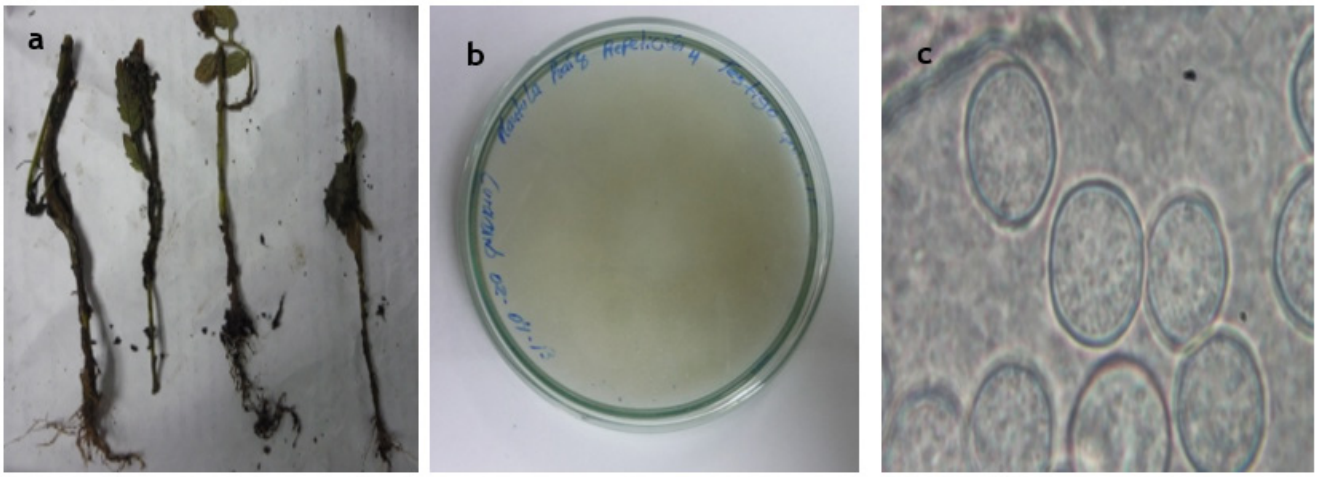

Figure 5. Symptomatology and cultural and morphological characteristics of Pythium sp. 5a: Lesions on tomato plants associated with Pythium sp infections; 5b: Pure culture of Pythium. 5c: Pythium oogonia

Evaluation of the in vitro biocontrol potential of Trichoderma harzianum on fungi associated to diseases in tomato crop.

The biocontrol potential is associated with the capacity and aggressiveness of the strain of the antagonist used. In this study, the mechanisms of action of T. harzianum on A. alternata, F. oxysporum, and Pythium associated with tomato crop diseases were evaluated. It was found that the aggressiveness of the action employed by T. harzianum responds according to the pathogen being confronted. At the same time, the mechanisms associated with the species act synergistically among them as a response to the antagonistic evolution of T. harzianum.

Antibiosis action

As a result of observing the dual cultures, it was found that T. harzianum generated a positive response to the antibiosis action against the studied pathogens through both direct (non-volatile) and volatile secretion of

organic compounds. Two repetitive behaviors were observed in each of the described fungi. In the first case, it was observed that a borderline band formed without coloration of the medium, in the border zone between the horizontal growth of the hyphae of the antagonist and those of the three pathogens under study. This visually appreciable formation was manifested at 98 hours, indicating a positive result for the antibiosis action of T. harzianum against Alternaria alternata, Fusarium oxysporum, and Pythium. The diffusion of the antagonist's possible non-volatile organic compounds in the medium towards the pathogens resulted in a fungistatic effect, inhibiting growth and altering the colony characteristics as the mycelia acquired a curved or straight pattern, depending on the direction of T. harzianum (figure 6a1, 6b, and 6c).

The direct chemical response of T. harzianum is complex. In dual cultures, the antagonist exhibits the development of modified somatic structures, which enable it to reach adjacent areas with the pathogen, thereby limiting its expansion and forming the described borderline band. These observations were particularly seen in all isolates with A. alternata, where the growth rate of the antagonist was initially constant, until reaching the border zone, where the mycelium showed increased growth rate, changes in color of the soma formed (from green to light soft), texture (from aerial cottony to sparse) and reduction of hyphal thickness, allowing it to reach the pathogen (figure 6a2).

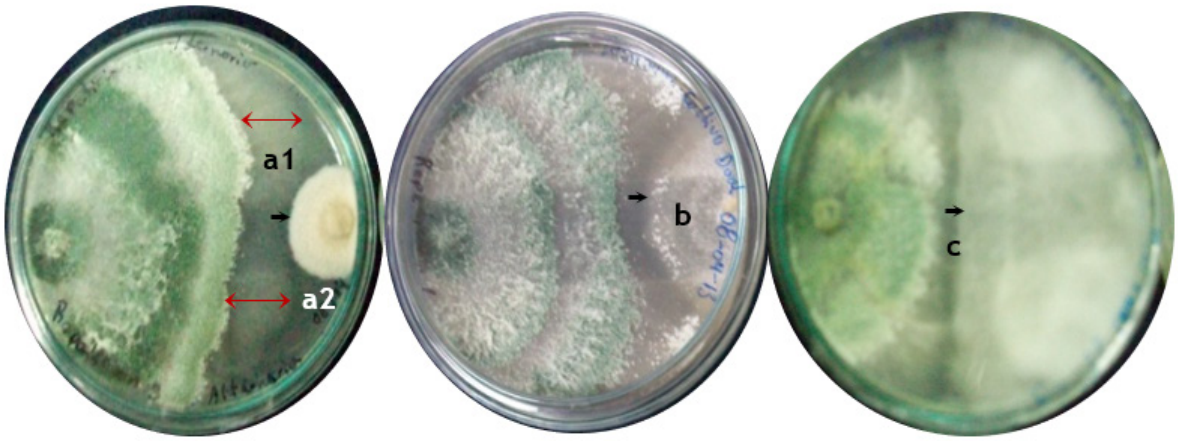

Figure 6. Antibiosis action of T. harzianum in dual cultures. Black arrow: Possible fungistasis. Red arrow: Somatic cells modified for antibiosis action

In the second case, the antibiosis response of T. harzianum by possible production of volatile substances, is particularly stimulated by the type of fungus with which it is confronted, for which, the phenomenon is shown by reducing at a distance the growth radius of the pathogens, being observed only with A. alternata and F. oxysporum, that is, it prevents the growth of the opposing fungus even though the microorganism is not yet at the point of confrontation.

It is possible to infer the presence of volatile compounds due to the fungistatic effect that limited the normal development of the pathogens in the dual cultures, allowing T. harzianum to develop a greater radius of growth until reaching the growth point of the colony of Alternaria and Fusarium. With Pythium, this phenomenon was not observed. The pathogen in the dual culture (4,93) increased its growth rate (4,25 cm)(figure 7) up to the confrontation point, and then exhibited the fungistatic action described in the first case (figure 6c).

When comparing the growth radii of the three pathogens in the dual culture, significant differences were found ($p<0,05$) in the inhibition among them, being A. alternata (1,24 cm) and F. oxysporum (2,04 cm) the ones that showed fungistatic response at a distance by presenting reduction from the beginning of the evaluation; while in their controls the growth was 3,21 cm and 3,25 cm, respectively. The antibiosis mechanism shown was statistically significant ($p < 0,05$). Although it turned out to be positive in the strain of T. harzianum used, this action is not maintained in time, because the antagonist, gains area of occupation in the medium, the competitiveness for space is manifested on this in the hours, the bordering band of antibiosis fades as a consequence of the expansion of the antagonist towards that direction.

Columns accompanied by different letters indicate statistical differences ($p<0,05$) between the different growth radii of pathogens in dual crops and the control lot. Each bar represents the mean mycelial growth radius obtained by each pathogen in the dual crops and the control group.

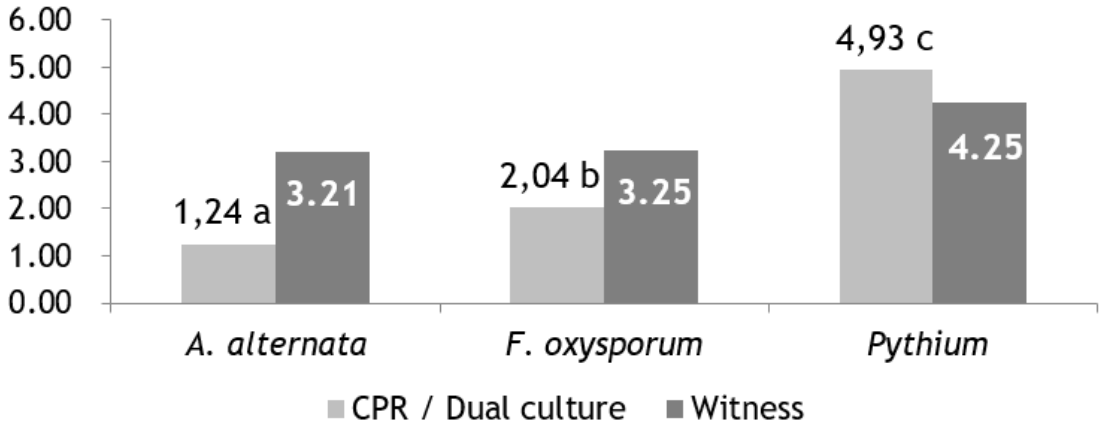

Figure 7. Comparison of growth radius of pathogenic fungi associated with tomato crop

Competition action

Evaluating the growth radius of the antagonist in dual crops highlights the ability to compete in a habitat limited by space, nutrients, and water. Although this evolutionary action differs from antibiosis, it demonstrates a supportive relationship between the two mechanisms. Because the effect of inhibiting the growth of the pathogen by chemical action allows T. harzianum to have more area for mycelial occupation, having more of the available nutrients compared to the contrasting fungus, or weakening the pathogen so that the antagonist can even advance to grow on top of it. When comparing the radius of growth of the antagonist in the control plates and in those of the confrontation with all the pathogens studied (figure 8), it was found that there was a significant difference ($p<0,05$), being greater in the dual cultures than in the control so that this mechanism may be activated by the chemical response of chemotrophic recommitment in the medium.

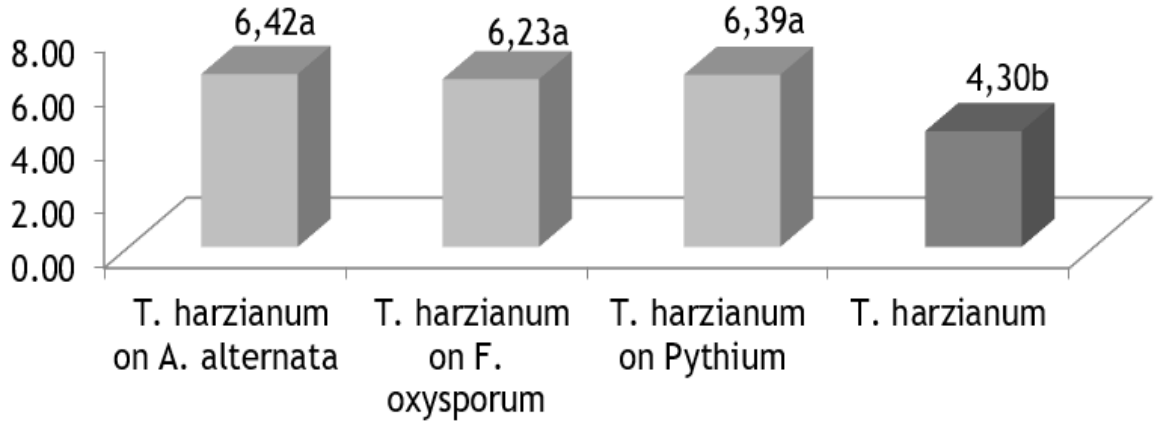

Figure 8. Trichoderma harzianum growth radius in dual crops whit pathogenic fungi associated whit tomato cultivatiom and witness group

The competitive action of the antagonist, expressed by the percentage of radial growth inhibition (PICR) in the dual cultures, shows that Trichoderma harzianum exerted a significant effect ($p<0,05$) on the fungi studied. The PICR in Alternaria alternata (56,64 %) and Fusarium oxysporum (31,78 %) indicate the competitive capacity of the antagonist compared to these two genera of pathogens; while with Pythium, they did not present significant differences in the percentage of inhibition, the PICR was negative (-12,14 %) which indicates that the pathogen presented greater radius of growth in the dual culture than in the control plates.

Columns accompanied by different letters indicate statistical differences ($p<0,05$) between the percentages of PICR radial growth inhibition in the dual cultures. Each bar represents the mean PICR obtained by pathogenic fungi in the dual cultures.

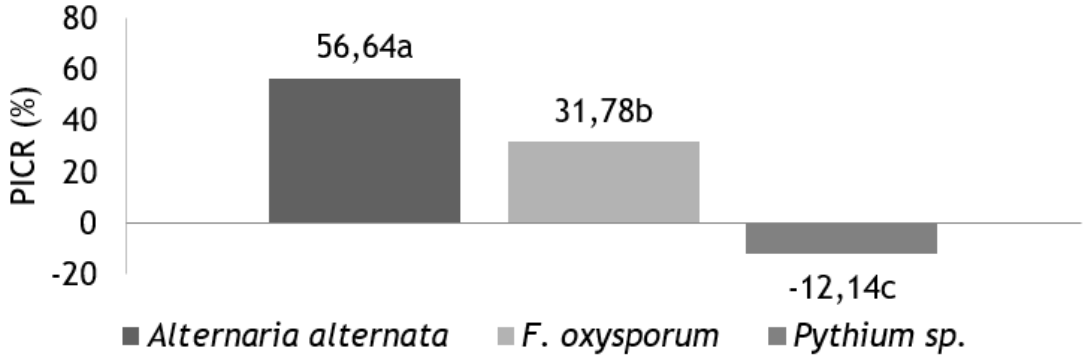

Figure 9. Percentage inhibition of radial growth in dual cultures of T. harzianum with A. alternata, F. oxysporum and Pythium

Mycoparasitic action

The in vitro confrontation in the dual culture showed the antagonistic ability of Trichoderma harzianum to parasitize the pathogens under study. In this case, its capacity to parasitize the biomass of the pathogens was proved, presenting intense green sporulations on them. That is, the mycoparasitic action on Alternaria alternata, Fusarium oxysporum, and Pythium was of grade 4 according to the proposed scale,[10] which allowed for total invasion of the pathogen's surface and sporulation on it (figure 10).

The mycoparasitism effect was evidenced in the microscopic observations, where it was found that the antagonist was able to penetrate the hyphae of the pathogens, exerting coiling, strangulation and penetration (figure 11a), enzymatic lysis with distortion (figure 11b), degradation of the pathogen hyphae (figure 11c) and deformation of the Pythium sporangia.

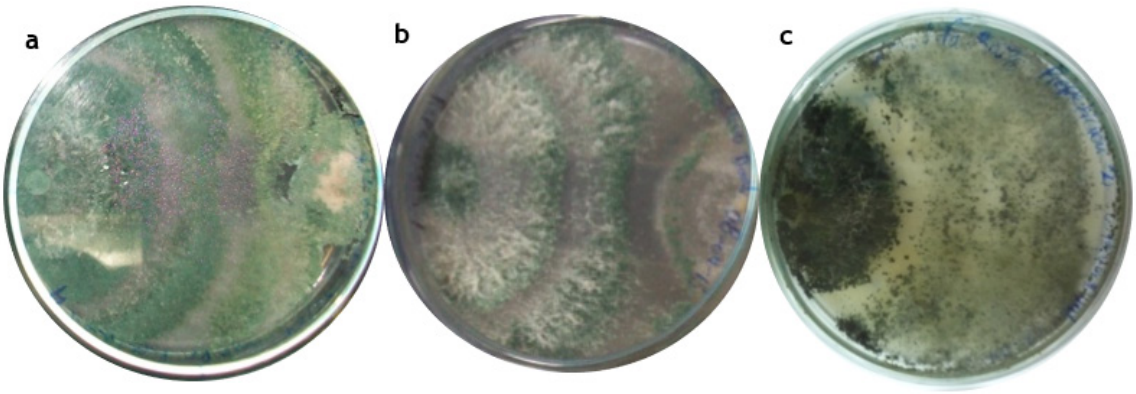

Figure 10. Mycoparasitism action of T. harzianum on disease-associated fungi in tomato crop. Distinct letters indicate dual cultures with pathogens a: A. alternata. b: F. oxysporum c: Pythium.

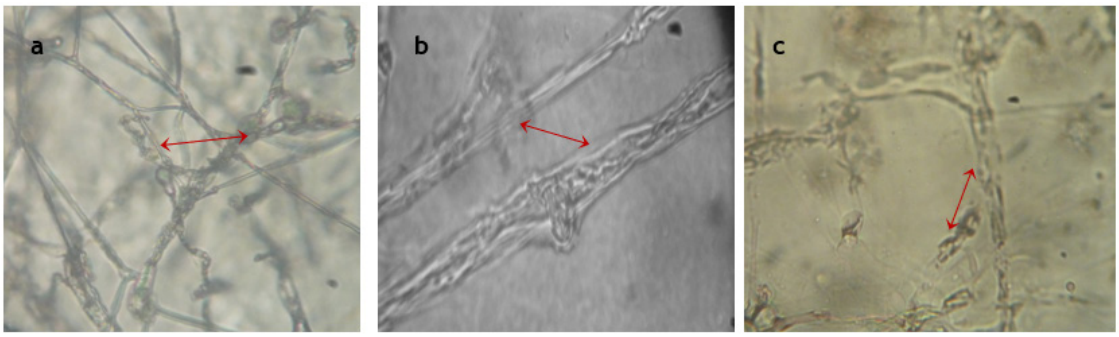

Figure 11. Microscopic observations of the mycoparasitism action of T. harzianum on fungi associated with diseases in tomato crops. a: Coiling of hyphae in A. alternata. b: Lysis of hyphae in F. oxysporum. c: Lysis of hyphae in Pythium.

DISCUSSION

The results obtained in the diagnosis of Alternaria and Fusarium in tomato crops, which coincide with the symptomatology described, align with those indicating that these two genera are pathogens associated with commercial tomato plantations, and their lesions and damage are considerable. Therefore, the authors are evaluating biotechnological mechanisms to combat them.[11]

For their part,[12,13] describe that the Fusarium genus causes stem and root rot, since its mechanism of action is to colonize the roots in a pathogenic way, since it hinders the absorption of water and nutrients, altering the metabolism of the plant. The authors describe how phytofungi hijack the secondary metabolic pathways of the host for better establishment, through the production of toxins such as fusarins, fusaric acid, and moniliformin, which are characteristic of F. oxysporum. These toxins can cause harmful effects to humans and animals when toxicity levels increase.

While [14] point out that after this pathogen spreads through the vascular system to inhabit the xylem vessels of the plant, it causes wilting and rapid death of the plants, which is generally known as vascular wilt, which occurs when the plants show yellowing, These symptoms described by the authors are consistent with the pathogenesis observed in the tissues of the crop.

When evaluating the biocontrol potential of Trichoderma, it was found that this control agent responds differently according to the fungal pathogen it confronts. At the same time, the aggressiveness of the mechanism employed is associated with the susceptibility of the pathogen. The observations of antibiosis and competitive action indicate the chemical and biological responsiveness of the antagonist. The direct fungistatic action from the formation of an antibiosis band that limits the growth of the exposed pathogen, indicate the production of organic substances produced by this strain, similar behavior reported [15] in studies of T. harzianum where the authors noted the ability to inhibit the growth of phytopathogens because it produces numerous antibiotics such as tricodermin, suzukacillin, alamethicin, dermadin, penicillin, trichothecenes, trichorzianins, among others.

In this regard, they indicated the presence of tetramic acid in strains of Trichoderma harzianum, demonstrating remarkable biological properties, including plant growth promotion, antimicrobial activity against different pathogens such as Pythium irregulare, Sclerotinia sclerotiorum, and Rhizoctonia solani, as well as the ability to chelate soil iron (Fe3+), thus facilitating its absorption by the plant.[16] This type of compound may have been present during the confrontation with the pathogen Pythium, which resulted in fungistasis of the fungal growth upon encountering the antibiosis band developed by Trichoderma harzianum.

The two types of phytochemical recognition of Trichoderma observed in the pathogenic species in the medium enabled it to produce volatile substances, exerting a distant action that limited and deformed the development of the pathogen's somatic structures. The authors [17] described that the volatile secondary metabolites secreted by T. viride can make the mycelium of Phytophthora nicotianae grow irregularly, break, or even dissolve, evidencing the capacity to produce secondary metabolites that give Trichoderma selective advantages in processes such as competition, symbiosis, metal transport, signaling, and mycoparasitic activity. In the same way, they pointed out the capacity of some species of Trichoderma to produce volatile metabolites that generate fungistasis,[18] which means they inhibit the germination of spores of their competitors, giving them an advantage when competing for space and nutrients.

Trichoderma exhibits strong adaptability to its environment.[19] By growing rapidly in dual crops, the antagonist demonstrated its ability to compete for niches and nutrients. When measuring growth rates, the rapid colonization of the medium by the antagonist against A. alternata and Fusarium oxysporum was found. In their studies,[20] they explained this effective phenomenon of increased growth rate of Trichoderma against phytopathogenic fungi. However, in the investigation it was found that when confronting the antagonist with Pythium the pathogen initially developed greater radius of mycelial growth compared to Trichoderma, on this occurrence they had already manifested that the speed of development of the antagonist does not determine the effective colonization of the niches, but the uniform application of the same one in all the substrate, and at the end of the days of evaluation this effect was observed.[21]

In this sense,[22] express that several species of Trichoderma have effective strategies of colonization of plants to be abundant in a niche where occurs the competition with other fungi considering it aggressive competitors; therefore, this behavior gives it biological advantage in the medium when it is shared with fungi of lower rates of daily growth. On the other hand,[23] explains that T. harzianum species rapidly colonize various substrates and eliminate slower-growing pathogens. The competitiveness of the T. harzianum species expressed in the study may be attributed to the aggressiveness of the competitive mechanism employed by the strain, combined with the high availability of dextrose in the synthetic medium. Thus,[24] associated the growth of the Trichoderma fungus in different media, finding particularly high growth rates in the presence of glucose and dextrose.

The radial growth inhibition behavior of PICR has been reported in several Trichoderma species, highlighting it as an effective biocontrol agent of several crop pathogens. In this regard,[25] reported 53 % of PICR of

T. harzianum with Fusarium solani and 55 % with the same pathogen against T. koningiopsis. Although the results were significant and practical, the studies carried out with 13 species of Trichoderma against Fusarium oxysporum showed greater inhibition of the pathogen, with a PICR of 84,4 to 90,5 %, emphasizing the species T. erinaceum.[26]

The mycoparasitism is one of the essential mechanisms in the biological control of Trichoderma, in the study this indicator was present in grade 4 with all the pathogens, with action of strangulation and lysis of the hyphae of the pathogens by the species T. harzianum at microscopic level, which indicates the aggressiveness of the strain towards the biocontrol of the fungi evaluated, coinciding with the results described by [27,25] where when comparing the effect of species such as T. harzianum and T. koningiopsis on Fusarium solani they obtained response and changes only with the species T. harzianum due to the production of chitinases that allow dissolving the cell wall of the hyphae of the pathogens.

The enzymatic activity of the mycoparasitism appreciated in the microscopic observations indicate that in the process of mycoparasitism, Trichoderma secretes enzymes [28] that hydrolyze the cell wall of the fungi that parasitize, being the most known: the proteases, chitinases and glucanases; which causes the retraction of the plasmatic membrane and the disorganization of the cytoplasm. In this sense,[29] indicates that this action on the part of Trichoderma inhibits the germination of spores and the elongation of the germinative tube of the pathogens, resulting from the degradation of their enzymes, which allows the antagonist to feed on them.

CONCLUSIONS

The microorganisms Alternaria alternata, Fusarium oxysporum, and Pythium sp. were identified as the causal agents of fungal diseases in the lesions of the sampled tomato plants.

As the in vitro action responses of the antagonist in the studied phytofungi were positive, it is concluded that the fungus Trichoderma harzianum presents the potential to be an effective biocontrol in fungal diseases associated with tomato crop, since the fungus responds to the presence of pathogens in its shared habitat, jointly activating the mechanisms attributed to it: colonization, antibiosis and mycoparasitism.

REFERENCES

1. Organización de las Naciones Unidas para la Alimentación y la Agricultura. «Food Agricultural Organization of the United Nations», 2019. https://www.fao.org/statistics/es.

2. Doaz, R. «Situación actual y potencial de las principales hortalizas que se siembran en Venezuela». Revista FONIAP Divulga, 1993.

3. Marín, D. «Rendimiento y Producción agrícola vegetal: Un análisis del entorno mundial (1997-1999) y de Venezuela (1988 - 2001).» Revista Agroalimentaria, 2002.

4. Salas, A., Osorio, E., Espinoza, C., Rodríguez, R., Segura, M., Ramírez, E., y Estrada, B. «Principales enfermedades del cultivo de tomate (Solanum lycopersicum L.) en condiciones de campo.» Revista Científica Multidisciplinar, 2022.

5. Sánchez, F. «La resistencia de las plagas y enfermedades ante el control convencional y la búsqueda de alternativas de biocontrol.» Tierra Infinita, 2019.

6. Pineda, Z, M., Pineda, R, D., Labarca, M, J., y González, G. H. «Caracterización y comportamiento biológico de una cepa nativa de Trichoderma harzianum del sur del lago de Maracaibo – Venezuela». Revista Ciencia y Tecnología Agropecuaria 5, nº 1 (2020): 9-15.

7. Agrios, G. Plant Pathology. 5ta ed. London, UK: Editorial Elsevier Academic Press, 2005.

8. Pitt, J., y Hocking, A. Fungi and Food Spoilage. 1era ed. London.: Blackie Academic & Professional., 1997.

9. Pineda, Z, M., y González, G, H. «Bioactividad fungistática del extracto acuoso artesanal de Azadirachta indica en hongos fitopatógenos asociados al cultivo de guanábana». Revista Ambiental Aire y Agua 13, nº 2 (2022): 65-77.

10. Ezziyyani, M., Pérez, C., Sid, A., Requena, M., y Candela, M. «Trichoderma harzianum como biofungicida para el biocontrol de Phytopthora capsici en plantas de pimiento (Capsicum annum L.).» Revista Anuales de Biología 26 (2004): 35-45.

11. Martínez, F., Andrade, G., Aispuro, E., Hernández –Montiel, L., Holguin, R., y Rueda-Puente, E. «Antisuero

vs hongos fitopatógenos en el cultivo de tomate en Sonora, México». Revista Mexicana de Ciencia Agrícolas 10, nº 4 (2020): 873-84. https://doi.org/10.29312/remexca.v10i4.1706.

12. Retana, K., Ramírez-Coché, J. A., Castro, O., y Blanco-Meneses, M. «Caracterización morfológica y molecular de Fusarium oxysporum f. sp. apii asociado a la marchitez del apio en Costa Rica.» Agronomía Costarricense 42, nº 1 (2018): 115-26. http://dx.doi.org/10.15517/rac.v42i1.32199.

13. Perincherry, L., Lalak-Kańczugowska, J., y Stępień, L. «Fusarium-Produced Mycotoxins in Plant-Pathogen Interactions». Toxins 11 (2019): 1-22. https://doi.org/10.3390/toxins11110664.

14. Albarracin, L., Hortua, S., y Acero, J. «Efecto inhibitorio del aceite esencial de Lippia graveolens sobre Fusarium oxysporum en la familia Solanaceae. Una revisión». Revista Tecnología en Marcha 36, nº 1 (2023): 54-65. http://dx.doi.org/10.18845/tm.v36i1.5877.

15. Andrade-Hoyos, P., Luna-Cruz, A., Osorio-Hernández, E., Molina-Gayosso, E., Landero-Valenzuela, N y Barrales-Cureño, H. «Antagonismo de Trichoderma spp. vs hongos asociados a la marchitez de chile.» Revista mexicana de ciencias agrícolas 10, nº 6 (2020): 1259-72. https://doi.org/10.29312/remexca.v10i6.1326.

16. Vinale, F., Sivasithamparam, K., Ghisalberti, E., Marra, R., Barbetti, M., y Li, H. «A novel role for Trichoderma secondary metabolites in the interactions with plants.» Physiology and Molecular Plant Pathology 72, nº 1-3 (2008): 80-86. https://doi.org/10.1016/j.pmpp.2008.05.005.

17. Manganiello, G., Sacco, A., Ercolano, MR., Vinale, F., Lanzuise, S., Pascale, A., Napolitano, M., Lombardi, N., Lorito, M., y Woo, SL. «Modulation of tomato responses to Rhizoctonia solani by Trichoderma harzianum and its secondary metabolite harzianic acid». Frontiers in Microbiology 9 (2018): 1-19. https://doi.org/10.3389/fmicb.2018.01966.

18. El-Sharkawy, H., Rashad, Y., y Ibrahim, S. «Biocontrol of stem rust disease of wheat using arbuscular mycorrhizal fungi and Trichoderma spp.» Physiological and Molecular Plant Pathology 103 (2018): 84-91. https://doi.org/10.1016/j.pmpp.2018.05.002.

19. Köhl, J., Kolnaar, R., y Ravensberg, W. «Modo de acción de los agentes de control biológico microbiano contra las enfermedades de las plantas: relevancia más allá de la eficacia.» Frontiers in Plant Science 10 (2019): 1-19. https://doi.org/10.3389/fpls.2019.00845.

20. Mohiddin, F., Padder, S., Bhat, A., Ahanger, M., Shikari, A., y Wani, S. «Filogenia y optimización de Trichoderma harzianum para la producción de quitinasa: evaluación de su comportamiento antifúngico frente a los prominentes fitopatógenos transmitidos por el suelo de la India templada». Microorganismos 9, nº 9 (2021). https://doi.org/10.3390/microorganismos9091962.

21. Infante, D., Martínez, B., González, N., y Reyes, Y. «Mecanismos de acción de Trichoderma frente a hongos fitopatógenos.» Revista Protección Vegetal, 2009.

22. Ghorbanpour, M., Omidvari, M., Abbaszadeh-Dahaji, P., Omidvar, R., y Kariman, K. «Mecanismos subyacentes a los efectos protectores de los hongos beneficiosos contra las enfermedades de las plantas». Control biológico 117 (2018): 147-57. https://doi.org/10.1016/j.biocontrol.2017.11.006.

23. Oszust, K., Cybulska, J., y Frac, M. «How Do Trichoderma Genus Fungi Win a Nutritional Competition Battle against Soft Fruit Pathogens? A Report on Niche Overlap Nutritional Potentiates». International Journal of Molecular Sciences 21, nº 12 (2020): 1-19. https://doi.org/10.3390/ijms21124235.

24. Jaroszuk-Scise, J., Tyśkiewicz, R., Nowak, A., Ozimek, E., Majewska, M., Hanaka, A., Tyskiewicz, K., Pawlik, A., y Janusz, G. «Fitohormonas (auxina, giberelina) y ACC desaminasa sintetizadas in vitro por la cepa micoparásita Trichoderma DEMTkZ3A0 y cambios en el nivel de Marcadores de auxinas y resistencia de plantas en plántulas de trigo inoculadas con conidios de esta cepa.» International Journal of Molecular Sciences 20, nº 19 (2019): 1-35. https://doi.org/10.3390/ijms20194923.

25. Ferrer, M., Romero, L., Andrade, O., Sánchez, P., Rivera, JA., y Hernández, S. «Actividad antifúngica de Trichoderma harzianum y T. koningiopsis contra Fusarium solani asociado en la germinación y vigor de plántulas

de chile Miahuateco.» Revista Mexicana de fitopatología 39, nº 2 (2021): 228-47. https://doi.org/10.18781/r.mex.fit.2101-5.

26. Martínez, O., Cristóbal, J., Tun, J., y Reyes, A. «Detección de genes Epl1 y Sm1 en Trichoderma spp. antagonistas contra hongos fitopatógenos.» Ecosistemas y recursos agropecuarios 8, n.º 2 (2021): 1-8. https://doi.org/10.19136/era.a8n2.2791.

27. Tian, Y., Tan, Y., Yan, Z., Liao, Y., Chen, J., y De Boevre M. «Antagonistic and Detoxification Potentials of Trichoderma Isolates for Control of Zearalenone (ZEN) Producing Fusarium graminearum». Frontiers Microbiology 8 (2018): 1-11. https://doi.org/10.3389/fmicb.2017.02710.

28. García, C., Mamani, M., Chávez, G., y Álvarez, M. «Evaluación de la actividad enzimática del Trichoderma inhamatum (BOL-12 QD) como posible biocontrolador». Journal of the Selva Andina Research Society, 7, nº 1 (2016): 20-32.

29. Romero, T., López, P., Ramírez, M., y Cuervo, J. «Modelado cinético del micoparasitismo por Trichoderma harzianum contra Cladosporium cladosporioides aislado de frutos de cacao (Theobroma cacao).» Chilean Journal of Agricultural and Animal Sciences 32, nº 1 (2016): 32-45. http://dx.doi.org/10.4067/S0719-38902016000100004.

FUNDING

The authors would like to thank INBIA MP 2023 C. A, for the logistical support, which allowed the realization and completion of this research that contributes to the strengthening of the research line in the biotechnological study and production of sustainable inputs for agricultura.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORSHIP CONTRIBUTION

Conceptualization: Maryori Carolina Pineda Zambrano.
Data Curation: Maryori Carolina Pineda Zambrano.
Formal analysis: Maryori Carolina Pineda Zambrano, Hebandreyna González García.
Research: Maryori Carolina Carolina Pineda Zambrano, Hebandreyna González García.
Methodology: Maryori Carolina Pineda Zambrano, Hebandreyna Gonzalez Garcia.
Project administration: Juan Carlos Escalante.
Resources: Maryori Carolina Pineda Zambrano.
Software: Juan Carlos Escalante.
Supervision: Hebandreyna González García.
Validation: Maryori Carolina Pineda Zambrano.
Visualization: Maryori Carolina Pineda Zambrano, Hebandreyna González García, Juan Carlos Escalante.
Writing - original draft: Maryori Carolina Pineda Zambrano.
Writing - proofreading and editing: Hebandreyna González García.