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Article

Antagonistic and Mycoparasitic Potential of *Trichoderma* spp. Against *Mycena citricolor*, a Major Coffee Pathogen in Southern Ecuador

Darío Cruz [1,3]*, *Débora Masache* [2], *Ricardo Albuja* [2]
[1] *MS2E, BIETROP research groups, Department of Biological and Agricultural Sciences, Universidad Técnica Particular de Loja, San Cayetano Alto s/n C.P. 11 01 608, Loja, Ecuador.*
[2] *Biology School, Universidad Técnica Particular de Loja, San Cayetano Alto s/n C.P. 11 01 608, Loja, Ecuador*
Email: drmasache@utpl.edu.ec.
Email: rmalbuja@utpl.edu.ec.
[3] *Instituto Nacional de Biodiversidad INABIO.Ecuador*
* *Correspondence: djcruz@utpl.edu.ec*

ABSTRACT

Trichoderma is a fungus with important applications in agriculture, as a biocontrol agent for pathogenic fungi or other pests that cause various diseases in crops. The pathogenic fungus *Mycena citricolor* causes "ojo de gallo" or "Rooster Eye" in coffee crops, mainly in areas above 1000 m.a.s.l. Therefore, its management of coffee crops, which is an economically important product for Ecuador, is vital. In this sense, this study sought to evaluate the percentage inhibition of mycelial growth using dual antagonist/pathogen in vitro cultures, to assess the mycoparasitic activity of Trichoderma spp., and to test its crude extract as a biocontroller of *Mycena citricolor*. The evaluated strains were molecularly characterized with the ITS-5.8S DNArn marker. The results determined four different *Trichoderma* species: *T. asperellum*, *T. harzianum*, *T.* sp1, and *T.* sp2. It was determined that three of the four *Trichoderma* species evaluated were effective, with inhibition values exceeding 40% and significantly different ($p < 0.05$) between treatments. These *Trichoderma* species show promise as mycoparasitic biocontrol agents against the pathogen *M. citricolor*. The crude extract from *T. harzianum* alone was ineffective in controlling the growth of M. citricolor, suggesting the need for further analysis of solvent-extracted extracts.

Keywords: ITS-5.8S, biocontroller, mycoparasitism, *Trichoderma asperellum*, *Trichoderma harzianum.*

INTRODUCTION

Fungi are diverse organisms that contribute significantly to natural ecosystems and agriculture through their roles in nutrient cycling and pest biocontrol [1]. However, several fungal species are considered devastating pathogens, destroying strategic crops such as coffee (*Coffea arabica* L.) [2]. In Ecuador, coffee is an agricultural product of national economic importance, with a reported production of 5,421 metric tons (mt) that contributes to global exports, generating between USD 120 and 150 million according to the Ministry of Agriculture and Livestock (2024) (accessed via the Agricultural Public Information System [SIPA] on January 9, 2026). Nevertheless, its productivity is severely threatened by the basidiomycete fungus *Mycena citricolor* (Berk. & M.A. Curtis) Sacc. (Mycenaceae; Index Fungorum, 2026), which causes one of the most critical diseases affecting coffee plantations [3,4].

While global reports estimate damage between 13% and 90%, local studies in Ecuadorian plantations have quantified significant yield impacts, including reduced cherry quality and premature defoliation, particularly in shaded, high-humidity microclimates typical of the Andean foothills [4]. This represents a direct economic loss that potentially affects international exports to countries such as Colombia, the United States, Germany,

France, Japan, and Russia [5,6]. In some cases, the losses caused by this agent are more severe than those triggered by coffee rust (*Hemileia vastatrix* Berk. & Broome) [2].

The disease determined by *Mycena citricolor* on coffee plants is commonly known as "ojo de gallo" (Rooster Eye). It predominantly affects plantations situated above 1,000 m.a.s.l, where fructifications appear on leaves and fruits under excessive shade and humidity [7,8]. Consequently, the agricultural sector is increasingly seeking environmentally friendly solutions, such as biological competitors that control pathogens while increasing production [9,10]. In countries like Costa Rica, where this pathogen is a major constraint, integrated management programs have successfully incorporated native *Trichoderma* strains. These isolates promote plant growth and exhibit potent antagonism against *M. citricolor*, effectively reducing disease incidence and severity while preventing plant mortality [11]. Despite these advances, identifying specific biological competitors remains a global priority.

Fungi such as Ascomycetes (e.g., *Trichoderma* spp.) can be pest biocontrol agents or competitors of agricultural pathogens, some of which are effective by directly attacking pests (e.g., fungi, bacteria, or nematodes) or by activating the plant immune system [12,13]. Species such as *Trichoderma asperellum and T. harzianum*, have been reported to have biocontrol potential, acting antagonistically against several pathogenic organisms: nematodes (e.g., *Meloidogyne* sp.), Oomycetes (e.g., *Pythium* spp., *Phytophthora* spp.), Ascomycetes (e.g., *Fusarium* spp. *Rhizoctonia* spp.), and Basidiomycetes fungi (e.g., *Mycena citricolor*, *Hemileia vastatrix*) [14,15,16].

Trichoderma species have also been documented to act indirectly by strengthening the immune systems of various plant hosts [17,18]. Consequently, *Trichoderma* extracts and formulations have been implemented as biological control agents worldwide, including several applications across Latin America [19]. To ensure efficacy, these promising species must be thoroughly characterized at multiple levels, particularly through molecular analysis using the ITS-5.8S rDNA marker, which is recognized as the universal fungal barcode [20].

In this context, *Trichoderma* spp. has emerged as a strategic candidate for the Ecuadorian coffee sector. The novelty of this study lies in leveraging local biodiversity to combat *Mycena citricolor* through a "green" strategy, thereby reducing reliance on synthetic fungicides and promoting environmental health. Given the aggressiveness of this pathogen in coffee plantations—particularly in the Ecuadorian landscape—it is imperative to evaluate the antagonistic effects of native Trichoderma species to develop effective, sustainable biocontrol agents.

MATERIAL AND METHODS

Strains applied in the study: Trichoderma spp. and the pathogen

The strains morphologically assigned to *Trichoderma* spp. isolated from soil (codes F11, F11#2, J1, and CEFI) (Figure 1) were obtained from the HUTPL mycotheque. The pathogen *Mycena citricolor* was collected from a coffee plantation located in the El Cristal farm (4°07'14.4" S, 79°11'56.6" W; 1,800 m.a.s.l.) in southern Ecuador. This region is characterized by a cloud forest microclimate, with an average annual humidity of 82% to 85% and a mean annual temperature of 12°C to 15°C (with fluctuations from 6°C to 22°C depending on altitude). Furthermore, the area receives annual precipitation of 1,500-2,000 mm [21]. Fructifications of *M. citricolor* were extracted from parts of the plant affected by Rooster Eye, such as leaves, fruits, and coffee leaf litter (Figure 2). *Mycena citricolor* was isolated directly on Potato Dextrose Agar (PDA) supplemented with 0.01% chloramphenicol [22].

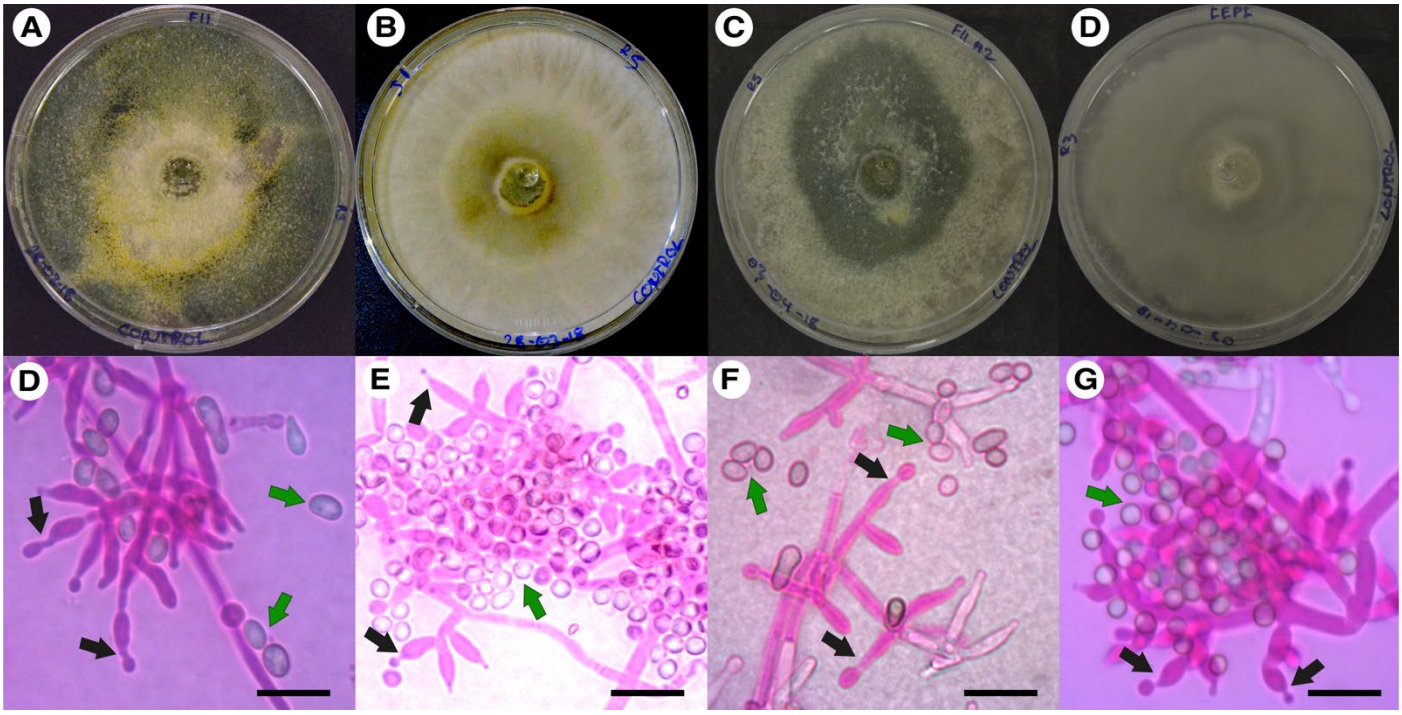

Figure 1. ***Trichoderma*** **mycelial growth in a Petri dish with PDA. Each strain corresponds to the codes: A) = F11; B) = J1; C) = F11#2; D) = CEFI. Structures such as hyphae and conidiophores (bottom, black arrows) and conidia (bottom, green arrows) typical for** ***Trichoderma*** **spp. according to each strain, respectively. Scale Bars = 20µm in 100X magnification.**

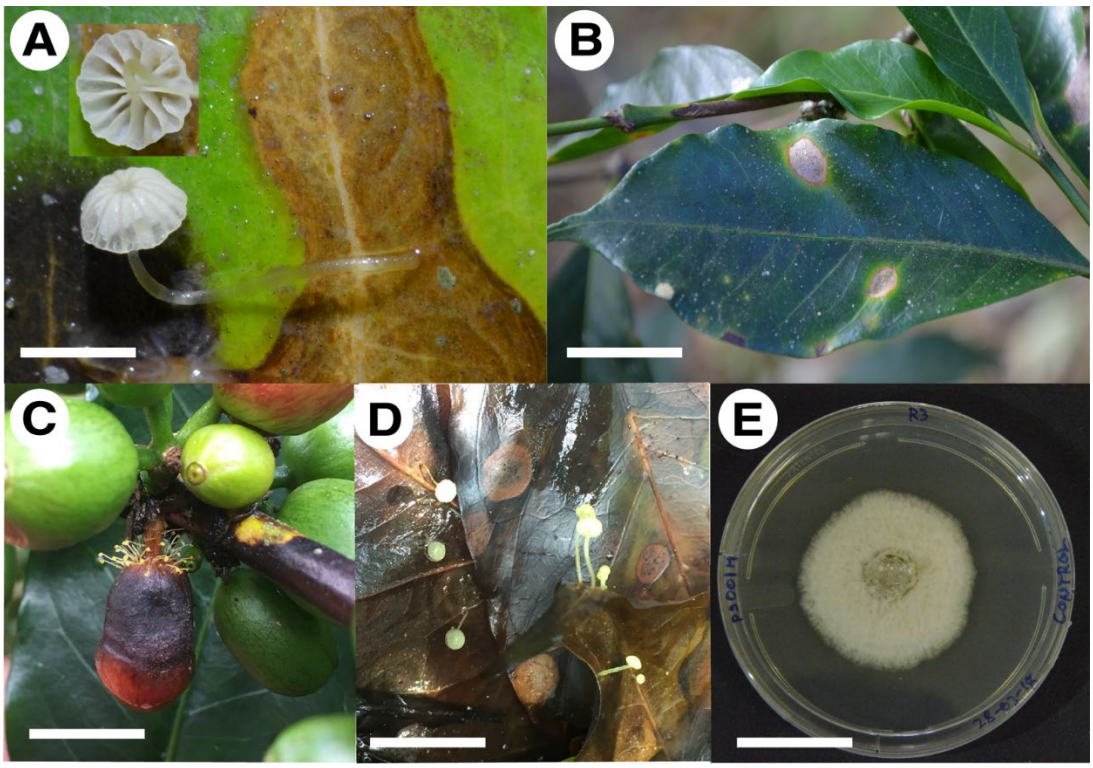

Figure 2. Cultivation of ***Mycena citricolor*** **and damage to** ***Coffea arabica*** **coffee. A) Basidiocarp of** ***M. citricolor.*** **B) leaf with circular damage caused by** ***M. citricolor.*** **C) healthy coffee fruit (green color) and fruit showing infection with Rooster Eye (red color). D) leaf litter on soil with fruiting bodies of** ***M. citricolor.*** **E) Growth of** ***M. citricolor*** **(P500H) on PDA culture medium. Scale bars A) = 3mm. B, C, D, E) = 2cm.**

Molecular analysis

All strains used in the study were genetically identified using the following steps: DNA was extracted with Invitrogen® commercial kit (Purelink DNA plant), amplified by PCR applying the universal primers ITS1 5'-TCC GTA GGT GAA CCT GCG G-3' and NL4 5'-GGT CCG TGT TTC AAG ACGG-3' [23] with PCR

conditions: initial denaturation at 94°C for 3 minutes, 35 cycles, each consisting of a denaturation step at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 2 minutes, and a final extension at 72°C for 10 minutes. The PCR reaction volume was 20 µL: 18 µL of Platinum ® PCR Supermix from Invitrogen, 0.2 µL of each primer, 0.4 µL of 10% BSA (bovine serum albumin), and 1.5 µL of DNA. PCR products were verified by electrophoresis on 1% agarose gel plus 1X Gel Red solution (Biotium) [24]. Amplified PCR products were purified with the PureLink PCR Purification Kit (Invitrogen) and sequenced at Macrogen Seoul, Korea.

Dual antagonism tests

Dual antagonism tests were carried out in 9-cm-diameter Petri dishes containing PDA medium. At a distance of 2 cm from the border of the dish, a 5 mm diameter agar disc with mycelium of the pathogen (*M. citricolor*) was placed, and at the opposite end, a 5 mm diameter disc colonized with mycelium of the antagonist *Trichoderma* spp. was placed. The spacing between the discs was approximately 4 cm, as recommended by Howell [25] (Figure 3A). Positive growth controls with similar diameters were planted in Petri dishes designated for antagonists and pathogens, as detailed in the design (Table 1). All cultures were incubated at 27°C for 5 days, with 70% relative humidity and pH 5.5 as optimal growth conditions for fungi [25]. Radial measurements of the mycelium were taken every 24h.

Treatments (replicates n = 5)	Antagonists evaluated
T1	*Trichoderma* sp. (F11) vs. *M. citricolor* (P500H*)
T2	*Trichoderma* sp. (J1) vs. *M. citricolor* (P500H*)
T3	*Trichoderma* sp. (CEFI) vs. *M. citricolor* (P500H*)
T4	*Trichoderma* sp. (F11#2) vs. *M. citricolor* (P500H*)
C1	*Trichoderma* sp. F11
C2	*Trichoderma* sp. J1
C3	*Trichoderma* sp. CEFI
C4	*Trichoderma* sp. F11#2
C5*	*M. citricolor* P500H*

Table 1. Treatments (T) and controls (C) were evaluated in the dual antagonism tests. * Pathogenic fungus.

For each replicate, we measured the Antagonistic growth radius (AGR) and Pathogenic growth radius (PGR) to observe nutrient and space competition according to growth rate. In addition, the percentage inhibition of radial growth (PIRG) was calculated to verify the efficacy of the treatments using the formula PIRG = (R1-R2)/(R1) × 100 [25], where R1 represents the radial growth of the pathogen in the control treatment and R2 the radial growth of the pathogen in confrontation.

Mycoparasitism (MICMO) was assessed by macroscopic observation of dual cultures (antagonist effect on the pathogen via invasion of the growth space) using the scale in Table 2.

Rating	Antagonistic capacity	Biocontrol potential
0	No surface invasion of the pathogenic strain	Very bad
1	¼ surface invasion of the pathogenic strain	Bad
2	½ surface invasion of the pathogenic strain	Deficient
3	Partial surface invasion of the pathogenic strain	Good
4	Total invasion of the surface of the pathogenic strain, sporulation on it	Very good

Table 2. Antagonistic capacity assessment scale [25].

Antagonist/pathogen crude extract test

The *Trichoderma* isolate exhibiting the highest radial growth inhibition percentage (PIRG) was selected for crude extract evaluation. To recover the enzymatic complex, the isolate was inoculated into Yeast Extract Peptone Dextrose (YPD) liquid medium and incubated under constant agitation for seven days. The secretome, containing potential lytic enzymes such as chitinases and β-1,3-glucanases, was harvested by filtration through a 0.22 µm membrane to remove mycelial fragments. The resulting filtrate was freeze-dried for 48 hours to preserve the integrity of the synergistic enzymatic complex or preserve other thermolabile compounds [26]. Finally, the lyophilizate was reconstituted in sterile distilled water to a final concentration of 160 mg/mL for subsequent antagonistic assays. Minimum inhibitory concentration (MIC) tests were performed by inoculating 25 µL at three concentrations (8 mg/mL, 4 mg/mL, and 2.5 mg/mL) onto PDA medium with a *Mycena citricolor* (0.2mm diameter disc). Positive control (only inoculated with *M. citricolor*) and negative control (without inoculation) were applied (Figure 3B). Pathogen growth on cell culture plates was monitored for five days.

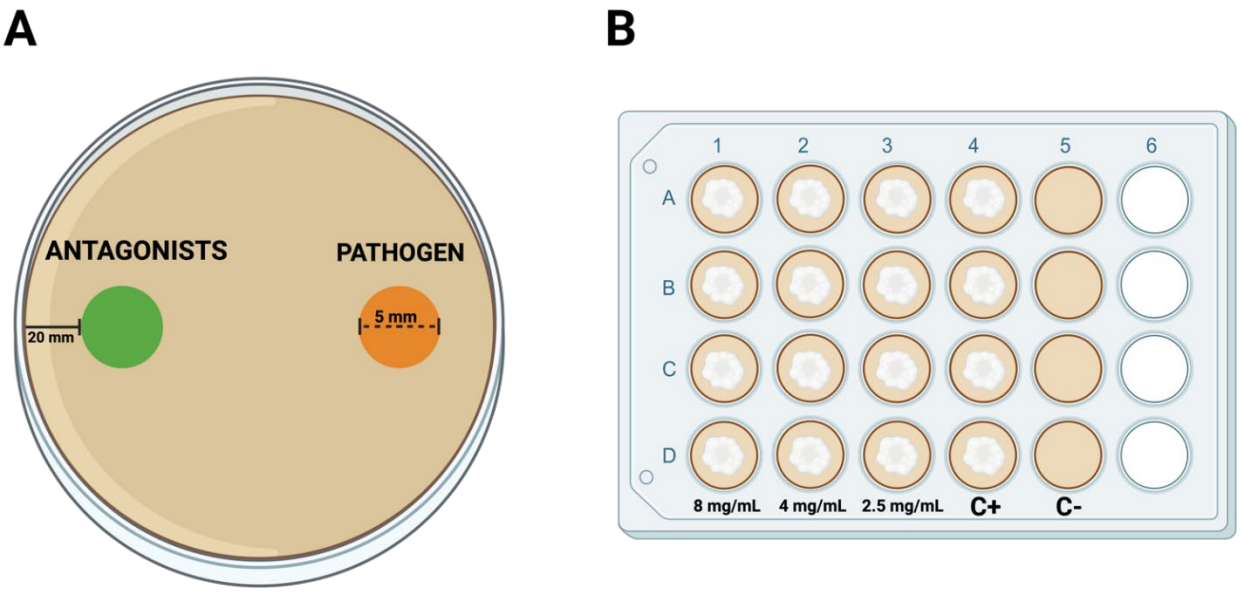

Figure 3. A) Scheme of dual antagonist/pathogen antagonism test adapted from [19]. B) Design for inhibitory action on plate at different concentrations of fungal extract (*Trichoderma* spp.) against the pathogen *Mycena citricolor* (white circle) and C+ and C- (respective controls). Image created with BioRender (https://biorender.com/).

Statistical analysis

Differences in AGR, PGR, and PIRG among treatments were assessed using ANOVA, followed by Tukey post hoc tests. Before the analyses, normality was tested using the Shapiro-Wilk test. All analyses were performed in the statistical program R [27], with a significance level of 0.05.

RESULTS

Specimens analyzed

The morphotypes belonging to *Trichoderma* and *Mycena citricolor* strains were molecularly corroborated, revealing four distinct genotypes for Trichoderma spp.: two with species identity and two currently undescribed (Table 3). The pathogen was identified as *Mycena citricolor* (Table 3).

Strain, code, and treatment	Accession and % similarity (GenBank)	Genotypes
***Trichoderma* sp. (F11) = T1**	MH455287 / 99%	*Trichoderma harzianum*
***Trichoderma* sp. (J1) = T2**	MH455289 / 90%	*Trichoderma* sp. 1
***Trichoderma* sp. (CEFI) = T3**	MH455290 / 90 %	*Trichoderma* sp. 2
***Trichoderma* sp. (F11#2) = T4**	MH455285 / 94%	*Trichoderma asperellum*
***Mycena citricolor* P500H**	MF417760 / 98.15%	*Mycena citricolor*

Table 3. Similarity comparison in the NCBI GenBank database for sequences from the study strains.

AGR and PGR inhibition tests

The *Trichoderma* species in treatments T1 (*T. harzianum* vs *M. citricolor*), T2 (*T. sp. 1* vs *M. citricolor*), and T4 (*T. asperellum* vs *M. citricolor*) showed high antagonist radial growth (AGR), with mean values ranging between 2.6 and 2.8 cm (Figure 4). In these treatments, the pathogen *Mycena citricolor* exhibited markedly reduced radial growth (PGR), with values ranging from 0.6 to 0.8 cm, indicating a strong inhibitory effect. In contrast, treatment T3 (*T.* sp. 2 vs *M. citricolor*) showed a lower AGR value (approximately 1.8–1.9 cm), which was still higher than the corresponding PGR of *M. citricolor* (approximately 1.0–1.1 cm), suggesting a weaker antagonistic effect compared to the other Trichoderma treatments (Figure 4).

The ANOVA indicates a significant difference between treatments ($p < 0.0012e^{-16}$). The post-hoc tests indicated that treatments T1, T2, and T4, and their respective controls (C1, C2, and C4), were not significantly different, except for C5 corresponding to M. citricolor, which showed significance (p-value <). On the other hand, the treatment T3 and its control C3 are significantly different from each other (p value ≤0.05), but not significantly different from the pathogen control M. citricolor (C5) (p value >0.05). Most treatments show a significant growth difference against the pathogen (Figure 4).

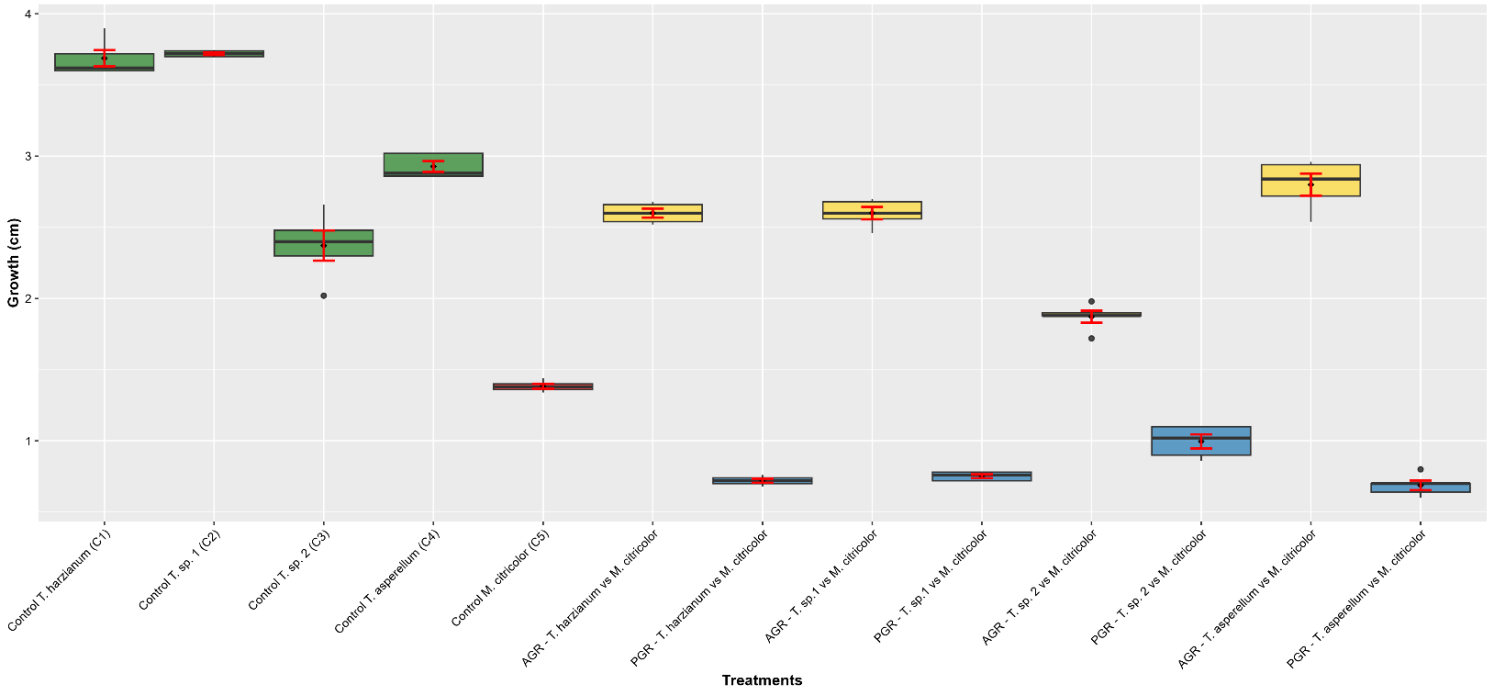

Figure 4. Radial growth of *Trichoderma* spp. (AGR) and *Mycena citricolor* (PGR) in dual antagonism assays. Growth controls for each species are shown in green (antagonist) and red (pathogen) plots. Bars represent mean values ± standard error. Detailed data for all treatments are provided in Table 1.

Radial growth inhibition (PIRG)

The percentages of radial growth inhibition (PIRG) were higher in treatments T1 (*T. harzianum* vs *M. citricolor*), T2 (*T.* sp. 1 vs *M. citricolor*), and T4 (*T. asperellum* vs *M. citricolor*), with values ranging from 47% to 55%, in contrast to T3, which had a low PIRG of 28%.

Statistically, the ANOVA indicates a significant difference between treatments ($p < 0.001$). The Tukey test indicates that only treatment T3 showed significant differences in PIRG compared to the other treatments ($p < 0.05$) (Figure 5).

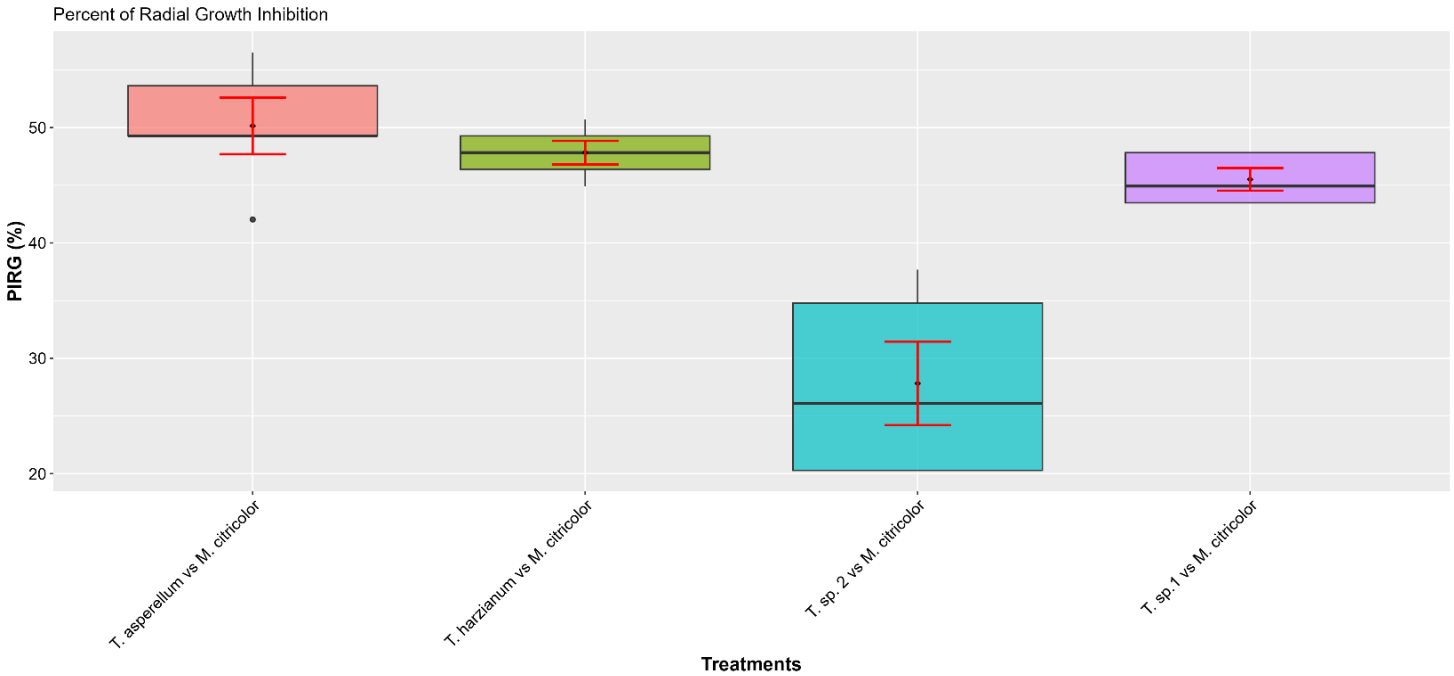

Figure 5. Percentage of radial growth inhibition (PIRG) after five days for the different antagonism treatments (*Trichoderma* spp. vs. *Mycena citricolor*) (see Table 1). Red symbols represent mean values ± standard error.

Evaluation of mycoparasitism (MICMO)

Macroscopic observation of the treatments showed that the species used in treatments T1, T2, and T4 had an efficient inhibitory effect on *M. citricolor* (Figure 6), with a score of 4 (see Table 2). On the other hand, the *Trichoderma* species used in T3 was evaluated as a poor biocontrol agent of *M. citricolor* (Figure 6), with a score of 2 (see Table 2).

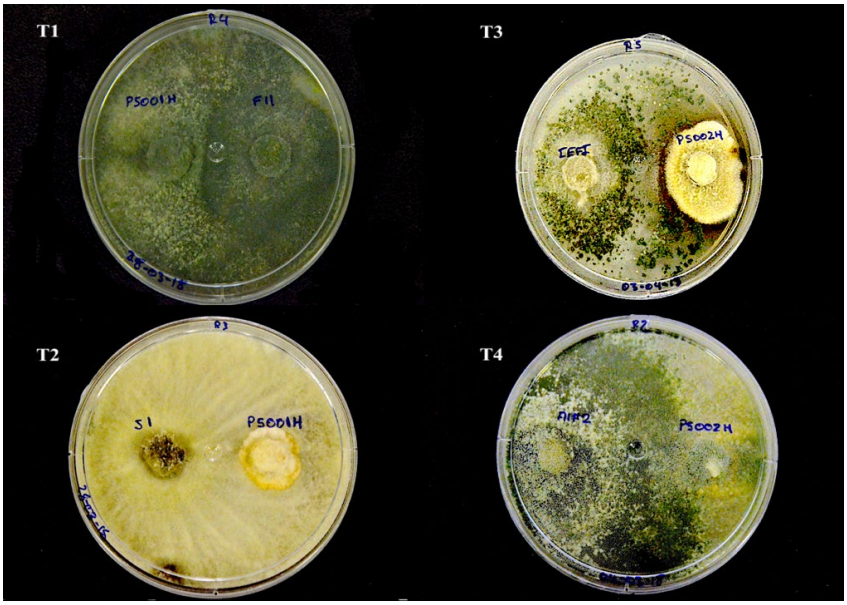

Figure 6. Macroscopic evaluation of mycoparasitism (MICMO) action, five days after growth of the different treatments (see Table 1). T1, T2, and T4 were evaluated as efficient biocontrol agents against the pathogen, whereas T3 showed a poor inhibitory effect (see Table 2). Petri dishes 90mm.

Fungal extract inhibition test

The crude extract from *T. harzianum* (T1) did not inhibit the growth of *M. citricolor*; the positive control and the different concentrations tested showed similar growth (Figure 7).

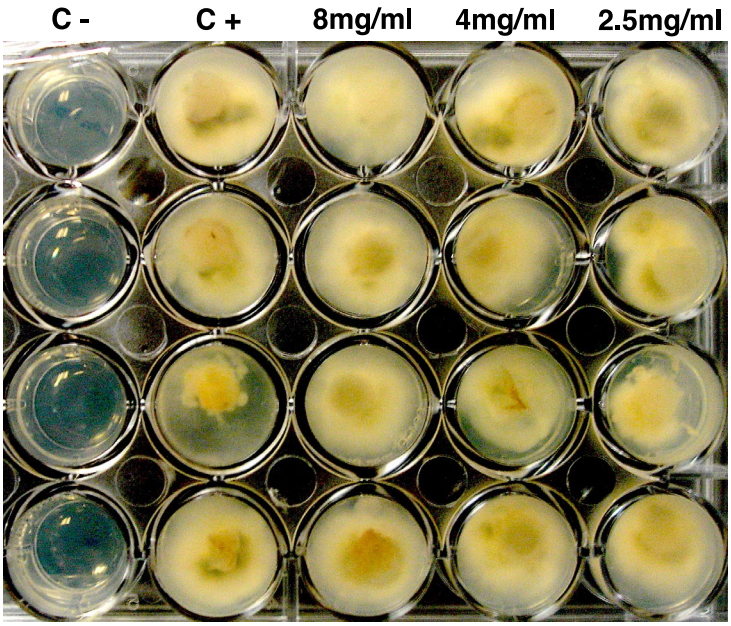

Figure 7. Inhibition test for the fungal extract from *T. harzianum* (three different concentrations), evaluated after five days.

DISCUSSION

Our results indicated the presence of four *Trichoderma* genotypes (*T. harzianum* = T1, T. sp1. = T2, T. sp2. = T3, and *T. asperellum* = T4), as well as *Mycena citricolor*, emphasizing the effectiveness of the DNArn molecular marker ITS-5.8S as a universal barcode [20]. However, we recommend that a multigene analysis (RPB1, RPB2, tub2/BenA, calmodulin, LSU, and SSU) be carried out in the future to provide a correct description of the *Trichoderma* species, given their genetic variability [28].

Three of the evaluated *Trichoderma* species (*T. asperellum*, *T. harzianum*, and *T.* sp.1) exhibited a very good inhibitory action (47% to 55% of PIRG) on the growth of the pathogen *M. citricolor,* with no significant differences between them. *Trichoderma* spp. has already been reported as effective against other fungi, such as Rhizoctonia solani J. G. Kühn, with radial inhibition percentages (PIRG) of 60-90% 29, although other studies found it to be antagonistically effective at 38-46% [13].

This suggests that the efficacy of these *Trichoderma* species varies depending on the pathogen's cell wall composition (30). Nevertheless, they function as effective biocontrol agents by exhibiting accelerated growth and early colonization of the substrate. This competition for nutrients and the resulting restriction of pathogen growth are consistent with previous reports [19,30]. *T. harzianum* is a more efficient competitor for nutrients compared to the pathogen [16], probably due to the production of inhibitory compounds, such as extracellular enzymes (peptidases, cutinases, and chitinases) [31,] which can alter the cell structure of pathogens such as *M. citricolor* [32].

The metabolic characteristics of *Trichoderma* spp. probably affect their mycoparasitism potential (MICMO), so that not all *Trichoderma* strains or species produce the same defense mechanisms, metabolites, or other effective compounds against pathogens [31,33]. This explains why one of the species tested in our experiments (*T.* sp2, treatment T3) generated a much less efficient control against *M. citricolor* compared to the species used in the other three treatments, which were capable of a total invasion on the surface of the pathogenic strain, with a rating of 4, similar to that reported in other studies [34].

The crude fungal extract obtained from *T. harzianum* (T1) did not inhibit the growth of *M. citricolor.* This negative result is attributed to the extraction method, which yielded low metabolite concentrations. Higher concentrations could be obtained by applying solvents such as alcohols or acetone during the extraction and recovery of metabolites [26, 34]. Another factor that may have resulted in a lower concentration of active compounds is the culture medium, as *T. harzianum* was grown on it before the extraction; studies have shown that the expression of defense and other metabolites can be enhanced when the antagonistic fungus is grown in the presence of the pathogenic fungus [10, 33]. As a result, we suggest that *Trichoderma* spp. extracts obtained using a higher metabolite recovery methodology should be reevaluated in the future to assess their efficacy against *M. citricolor*. This is especially important since this pathogen attacks at the foliar and fruit levels (Figure 2 A, B, C), and not only through competition between antagonist/pathogen species that would work efficiently on *M. citricolor* fructifications in coffee leaf litter (Figure 2 D).

Currently, the search for biotechnological alternatives and the application of ecological compounds are research priorities 35 for controlling fungal diseases that affect economically important crops. Our study reports three *Trichoderma* species with mycoparasitic biocontrol capabilities against M. citricolor, the pathogen causing the "Rooster Eye" disease in coffee crops. As a next step, we recommend field-testing these Trichoderma species to assess their usefulness as effective, environmentally friendly alternatives for disease management, as already suggested by other plant pathogen biocontrol agents [36,37,38].

CONCLUSIONS

This study provides one of the first integrated, locally grounded evaluations of native *Trichoderma* isolates as biocontrol candidates against *Mycena citricolor* (coffee "Rooster Eye") in southern Ecuador, combining ITS-5.8S rDNA molecular characterization with dual-culture antagonism and mycoparasitism (MICMO) scoring. Molecular results resolved four distinct *Trichoderma* genotypes, including one isolate closely matching *T. harzianum* (99% similarity) and another related to *T. asperellum* (94%). At the same time, two isolates showed only ~90% similarity to their closest GenBank matches, suggesting the presence of potentially divergent or

under-characterized native lineages (here treated as Trichoderma sp. 1 and sp. 2 pending multilocus confirmation).

Functionally, three isolates—*T. harzianum*, *Trichoderma* sp.1, and *T. asperellum*—showed consistent and statistically significant antagonism against *M. citricolor*, with PIRG values of ~47–55% and high mycoparasitic performance (MICMO = 4), signifying strong promise as "green" biocontrol agents for coffee systems. In contrast, *Trichoderma* sp. 2 exhibited markedly weaker activity (PIRG ~28%; MICMO = 2), underscoring the importance of strain-level selection rather than assuming uniform potency across the genus.

Importantly, the lyophilized secretome/crude aqueous extract from *T. harzianum* did not inhibit *M. citricolor* at the tested concentrations. This result is novel and practically informative: under the evaluated conditions, inhibition appears to depend more on direct interaction methods (competition and mycoparasitism) than on water-reconstituted extracellular products alone. Subsequent work should therefore prioritize field validation, multilocus identification, and optimization of metabolite recovery (e.g., solvent-based extracts and/or induction conditions) to translate these native isolates into efficient biocontrol tools for Ecuadorian coffee production.

Patents: Not applicable.

Supplementary Materials: Not applicable.

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REFERENCES

1. Piepenbring M, López F, Cáceres O. La importancia de los hongos en los ecosistemas. *Puente Biológico*. 2016;8:57–91.
2. Hindorf H, Chrispine O, Omondi O. A review of three major fungal diseases of *Coffea arabica* L. in the rainforests of Ethiopia and progress in breeding for resistance in Kenya. *J Adv Res*. 2011;2(2):109–120. doi:10.1016/j.jare.2010.08.006.

3. Leyva Mir G. Principales enfermedades del café (*Coffea arabica*). *Rev Agroproductividad* [Internet]. 2018;3(32):12–23. Available from: https://revista-agroproductividad.org/index.php/agroproductividad/article/view/590
4. Pilozo Mantuano W, Indacochea Ganchozo B, Castro Landín A, Vera Tumbaco M, Gabriel Ortega J. Principales enfermedades causantes de la pérdida de rendimientos de los cultivos de café (*Coffea arabica* L.) en la zona sur de Manabí, Ecuador. *UNESUM Ciencias*. 2022;6(2):117–134. doi:10.47230/unesum-ciencias.v6.n2.2022.632.
5. Jiménez Cercado ME, Rangel Saltos JE, Lavayen Yávar HF, Cavagnaro Romero PC. Sustainability in Ecuadorian exports as part of the 4.0 revolution in the coffee sector. *J Bus Entrep Stud*. 2025;9(4):57–76. doi:10.37956/jbes.v9i4.405.
6. Venegas S, Orellana D, Pérez P. La realidad ecuatoriana en la producción de café. *Recimundo*. 2018;2(2):24–44. doi:10.26820/recimundo/2.(2).2018.24-44.
7. Allinne C, Savary S, Avelino J. Delicate balance between pest and disease injuries, yield performance, and other ecosystem services in complex coffee-based systems of Costa Rica. *Agric Ecosyst Environ*. 2016;222:1–12. doi:10.1016/j.agee.2016.02.001.
8. Avelino J, Allinne C, Cerda R, Willocquet L, Savary S. Multiple-disease system in coffee: from crop loss assessment to sustainable management. *Annu Rev Phytopathol*. 2018;56:611–635. doi:10.1146/annurev-phyto-080417-050117.
9. Leite SA, Santos MP, Resende-Silva GA, Costa DR, Moreira AA, Lemos OL, et al. Area-wide survey of chlorantraniliprole resistance and control failure likelihood of the Neotropical coffee leaf miner *Leucoptera coffeella* (Lepidoptera: Lyonetiidae). *J Econ Entomol*. 2020;113:1399–1410. doi:10.1093/jee/toaa017.
10. Venzon M. Agro-ecological management of coffee pests in Brazil. *Front Sustain Food Syst*. 2021;5:721117. doi:10.3389/fsufs.2021.721117.
11. Escudero-Leyva E, Granados-Montero MDM, Orozco-Ortiz C, Araya-Valverde E, Alvarado-Picado E, Chaves-Fallas JM, et al. The endophytobiome of wild Rubiaceae as a source of antagonistic fungi against the American leaf spot of coffee (*Mycena citricolor*). *J Appl Microbiol*. 2023;134(5):lxad090. doi:10.1093/jambio/lxad090.
12. Ezziyyani M, Sánchez CP, Ahmed AS, Requena ME, Castillo MEC. *Trichoderma harzianum* como biofungicida para el biocontrol de *Phytophthora capsici* en plantas de pimiento (*Capsicum annuum* L.). *An Biol*. 2004;26:35–45.
13. Infante D, Martínez B, González N, Reyes Y. Mecanismos de acción de *Trichoderma* frente a hongos fitopatógenos. *Rev Prot Veg*. 2009;24(1):14–21.
14. Gallagher, N. Effects of *Trichoderma harzianum* perimeter applications on preventing *Mycena citricolor* in Coffea arabica plots. *CIEE Fall Tropical Ecology and Conservation.* 2009.
15. Bastakoti S, Belbase S, Manandhar S, Arjyal C. *Trichoderma* species as biocontrol agents against soil-borne fungal pathogens. *Nepal Journal Biotechnol*. 2017;5:39–45.
16. Al-Saeedi SS, Al-Ani BM. Study of antagonistic capability of *Trichoderma harzianum* isolates against some pathogenic soil-borne fungi. *Agriculture and Biology Journal of North America*. 2014;5(1):15–23.
17. Astorga-Quirós K, Meneses-Montero K, Zúñiga-Vega C, Brenes-Madriz J, Rivera-Méndez W. Evaluación del antagonismo de *Trichoderma* sp. y *Bacillus subtilis* contra tres patógenos del ajo. *Rev Tecnol Marcha*. 2014;27(2):82. doi:10.18845/tm.v27i2.1929.
18. Stocco M, Consolo F, Monaco C, Kripelz N, Salerno G, Cordo C. Control biológico de la mancha de la hoja del trigo con especies del género *Trichoderma*. 2012;8.
19. Howell CR. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts. *Plant Dis*. 2003;87(1):4–10.
20. Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, et al. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. *Proc Natl Acad Sci U S A*. 2012;109:1–6.
21. Samaniego Burneo JP. Diseño arquitectónico de bajo impacto ambiental de un centro de investigación científica en la reserva "El Cristal" del cantón y provincia de Loja [thesis]. Loja: Universidad Internacional del Ecuador (UIDE), Facultad de Arquitectura; 2020. 139 p.
22. Cruz D, Suárez JP, Kottke I, Piepenbring M, Oberwinkler F. Defining species in *Tulasnella* by correlating morphology and nrDNA ITS-5.8S sequence data of basidiomata from a tropical Andean forest. *Mycol Prog*. 2011;10:229–238.

23. White TJ. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. *PCR protocols: a guide to methods and applications*. San Diego: Academic Press; 1990. p. 315–322.
24. Cruz D, Suárez JP, Kottke I, Piepenbring M. Cryptic species revealed by molecular phylogenetic analysis of sequences obtained from basidiomata of *Tulasnella*. *Mycologia*. 2014;106(4):708–722. doi:10.3852/12-386.
25. Fernández RJ, Suárez CL. Antagonismo in vitro de *Trichoderma harzianum* Rifai sobre *Fusarium oxysporum* Schlecht f. sp. *passiflorae* en maracuyá (*Passiflora edulis* Sims var. *flavicarpa*) del municipio zona bananera colombiana. *Rev Fac Nac Agron Medellín*. 2009;62(1):4743–4748.
26. Celis Á, Mendoza C, Pachón M, Cardona J, Delgado W, Cuca LE. Extractos vegetales utilizados como biocontroladores con énfasis en la familia Piperaceae: una revisión. *Agron Colomb*. 2008;26(1):97–106.
27. R Core Team. *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing; 2021. Available from: https://www.R-project.org/
28. Raja HA, Miller AN, Pearce CJ, Oberlies NH. Fungal identification using molecular tools: a primer for the natural product research community. *J Nat Prod***.** 2017;80(3):756–770. doi:10.1021/acs.jnatprod.6b01085.
29. Veitía MM, García V, Izquierdo D, Porras Á, Wong W. Control de *Rhizoctonia* sp. en albahaca blanca (*Ocimum basilicum* L.) con *Trichoderma harzianum* cepa 34. *Fitosanidad*. 2000;4(1–2):67–70.
30. Benítez T, Rincón AM, Limón MC, Codón AC. Biocontrol mechanisms of *Trichoderma* strains. Int Microbiol. 2004;7(4):249–260.
31. Durán E, Robles F, Martínez J, Brito M. *Trichoderma*, un hongo combatiente de patógenos. *Revista Técnico Ambiental Teorema Ambiental*. 2003;(92):20–27.
32. Contreras-Cornejo HA, Macías-Rodríguez L, Ek del-Val J, Larsen J. Ecological functions of *Trichoderma* spp. and their secondary metabolites in the rhizosphere: interactions with plants. *FEMS Microbiology Ecology* 2016;92(4):fiw036. doi:10.1093/femsec/fiw036.
33. Suárez Meza Carol Libeth, Fernández Barbosa Reinel José, Valero Nelson Osvaldo, Gámez Carrillo Rocío Margarita, Páez Redondo Alberto Rafael. Antagonismo in vitro de Trichoderma harzianum Rifai sobre Fusarium solani (Mart.) Sacc., asociado a la marchitez en maracuyá. Rev. colomb. biotecnol [Internet]. 2008 July; 10(2): 35-43. Available from: http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S0123-34752008000200005&lng=en
34. Tahía B, Rincón AM, Limón MC, Codón AC. Biocontrol mechanisms of *Trichoderma* strains. Int Microbiol. 2004;7(4):249–260.
35. López P. Aislamiento, purificación y caracterización estructural de nuevos principios bioactivos a partir de extractos fúngicos [dissertation]. Valencia: Universitat Politècnica de València; 2008. Available from: https://doi.org/10.4995/Thesis/10251/1823
36. Umaru FF, Simarani K. Evaluation of the potential of fungal biopesticides for the biological control of the seed bug, *Elasmolomus pallens* (Dallas) (Hemiptera: Rhyparochromidae). Insects. 2020;11(5):277. doi:10.3390/insects11050277.
37. Schilly A, Chaves N, Guzmán M, Sandoval J, Staver C, Dita M. Exploring root-associated endophyte microorganisms from *Musa* spp. for enhancing plant health. In: Proceedings of the 29th International Horticultural Congress; 2014 Aug 17–22; Brisbane, Australia.
38. Samuelian S. Potential of *Trichoderma harzianum* for control of banana leaf fungal pathogens when applied with a food source and an organic adjuvant. 3 Biotech. 2016;6(1):8. doi:10.1007/s13205-015-0327-0.

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Correspondence should be addressed to: djcruz@utpl.edu.ec;

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