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Use of Species of Trichoderma sp. as an Alternative for Phytosanitary Control and Promotion of Plant Growth

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Abstract

Yield standards and diseases in horticultural crops are traditionally achieved with synthetic fungicide applications that increase production costs, however, this measure has shown negative effects. Therefore, the challenge of sustainable production imposes the transformation of conventional agricultural production systems to low-cost agroecological ones. Therefore, the objective was to determine the biocontrol capacity of native Trichoderma spp against Phytophthora cinnamomi and growth promotion in avocado and banana crops. Trichoderma spp were isolated by trap cultures in avocado crops and identified by molecular techniques. The antagonistic activity against P. cinnamomi and the growth promoting activity of Trichoderma was performed in vitro and in the greenhouse on avocado seedlings. Sequence analysis of the tef 1a gene of the 9 Trichoderma isolates identified them as T. viride and T. harzianum which showed inhibition against P. cinnamomi of 93.7% and 82.2%, respectively, while in the greenhouse the avocado plants showed no disease symptoms according to the severity scale (0). In vitro growth promotion showed that both species solubilized phosphate, produced siderophores and IAA and, in the greenhouse, increased all growth promotion variables in avocado and chili crops, compared to the control and the commercial product. In conclusion, T. viride and T. harzianum represent an important agroecological and biotechnological alternative that is low cost and environmentally friendly since they are native soil microorganisms.

Keywords: *Trichoderma, Biological Control, Phytophthora, Avocado.*

Introduction

The avocado crop in recent decades has gained demand in the national and international market given the high nutritional contribution and industrial applications of its fruit (Araújo et al., 2018). This has made countries such as Colombia, which has the appropriate agroclimatic conditions for its cultivation, arouse interest in it, managing to position itself as the fourth largest producer country worldwide (Arias and Moors, 2018), with the departments of Caldas, Antioquia, Bolívar, Cauca, Risaralda, Quindío and Tolima, being the ones with the highest production (MADR, 2019). However, the Montes de María in the department of Sucre, has been for many years the agricultural pantry of the country, with avocado cultivation as a flagship, but in recent decades the presence of crops and their yield, has decreased due to the impact of the internal armed conflict, which caused the displacement of thousands of farming families leading to the abandonment of crops, from which they earned their livelihood (Osorio et al., 2017). Recently, several crops have been introduced to generate income for families, such as chili peppers (Díaz, 2013; Osorio et al., 2017), which are consumed by more than a quarter of the inhabitants of the land every day (Halikowski, 2015).

However, these crops are attacked by several pathogens, among which are P. infestan, P. capsici, P. cinnamomi, C. gloeosporioides and Fusarium that are crucial problems every year, since they cause wilting of plantations generating important economic losses worldwide and in the Mountain of María (Hardham and Blackman, 2018; Blaya et al., 2015; Adlercreutz et al., 2014). These phytopathogens are transmitted by soil, water and air, penetrating the different plant tissues (roots, leaves and fruits) forming necrotic areas with rotting appearance, cause obstruction of vascular bundles, which reduces the transit of water and nutrients to the interior of the plant, causing wilting, stem drop, leaf blight and fruit rot, severely limiting the production of chili, once attacked the susceptible host rarely manage to recover, although it is often

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controlled by the application of fungicides, crop rotation, etc. (Rodriguez, 2015; Wu et al., 2013; Morrison et al., 2011).

Currently, agricultural production involves fertilization of soils with synthetic chemical agents to increase production yields (Bader et al., 2020). Nutrients such as phosphorus (P) are applied to crops to favor plant growth, although more than 80% of the P present in fertilizers is not available for plant uptake and remains retained in the soil (Bader et al., 2020; Silva et al., 2013; Kapri and Tewari, 2010). Probably, this ability to make insoluble nutrients available is made possible by the production of different organic acids and phosphatase enzymes secreted by beneficial fungi of the genus Trichoderma (Gravel et al., 2007).

Trichoderma species have beneficial effects on plant growth and development by promoting high secondary root proliferation, leaf area, stem length, dry weight and crop yield (Hermosa et al., 2013; Mukherjee et al., 2013). It also favors the natural sustenance of agricultural yields (Lorito et al., 2010) thanks to the fact that together with other beneficial microbes it helps to maintain soil fertility and compost maturation for the production of natural fertilizers (Harman et al., 2004). Trichoderma spp. influence plant growth by producing siderophores, phosphate solubilization, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, indole acetic acid (IAA), phytase and acid phosphatase activity under biotic or abiotic stress (Babu et al., 2014). Additionally, species of this genus are producers of secondary metabolites of clinical importance and enzymes with wide industrial application (Mukherjee et al., 2013), such as Pyrones (Rubio et al., 2009), Butenolides (Cai et al., 2013), Peptaibols (Vizcaino et al., 2005) and terpenes such as Trichothecenes that are mycotoxins (Malmierca et al., 2013, 2012; Cardoza et al., 2011). In addition, they have biocontrol properties through the secretion of hydrolytic enzymes, such as glucanases, chitinases and aspartic proteases that degrade the cell wall of pathogens (Mandujano et al., 2016), a key step for mycoparasitism (Gruber and Seidl-Seiboth, 2012) since the hydrolytic enzyme proteases appears to regulate the action of other hydrolytic enzymes involved in mycoparasitism (Deng et al., 2018). They have also been used in consortium with arbuscular mycorrhizae for rust biocontrol in wheat, achieving significant disease reduction, since they induced the production of peroxidase and polyphenol oxidase enzymes, and increased total phenol content, which improved growth and yield parameters (El-Sharkawy et al., 2018). Certain Trichoderma strains are systemic endophytes (Druzhinina et al., 2011) and have growth-promoting activity through phytohormone production/stimulation (Contreras et al., 2013) and confer resistance against nematodes to tomato (Medeiros et al., 2017). A small secreted cysteine-rich protein called qid74 modifies root architecture to increase surface area (Samolski et al., 2012) and thereby increase nutrient uptake. Trichoderma spp. hyphae release numerous elicitors that induce different types of signals within the plant, e.g., by salicylic acid, jasmonic acid or ROS, triggering the expression of defense proteins, and as a result of this gene activation, the plant produces enzymes involved in direct pathogen suppression and enhancement of plant biochemical and structural barriers (Nawrocka and Malolepsza, 2013).

Trichoderma-induced resistance has been observed in plants inoculated with Rhizoctonia solani and P. capsici (Morán et al., 2009; Sriram et al., 2009). The result of this symbiotic relationship is an increase in plant growth and productivity (Hermosa et al., 2012) that can help to improve food security in agricultural areas of scarce economic resources such as the municipalities of Chalán and Ovejas, in the Montes de María, due to the reduced need to use pesticides and can provide an economic advantage for farmers (Harman et al., 2012).

Thanks to measures taken by the national government with the help of international entities, the country has established various strategies that seek to return families to their places of origin, which is why the Montes de María have been declared a post-conflict zone. One of the strategies is through agricultural strengthening, which seeks productive inclusion and the contribution of business and innovation skills to promote economic development in these national agricultural scenarios. Therefore, the objective of this research consisted in the search for Trichoderma strains native to avocado crops in Mountain of María with phytosanitary agricultural bioprospecting and as biofertilizers for avocado and chili bell pepper crops, the latter as an alternative for generating income in the short term.

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Materials and Methods

Study Area

The study area corresponds to the municipalities of Ovejas (9°31'33"N 75°13'38"W and altitude of 254 m asl) and Chalán (9°32'38"N 75°18'45"W and altitude of 270 m asl). They belong to the Montes de María subregion, department of Sucre, Colombia. It corresponds to a tropical dry forest zone and its characteristic landscape is mountainous. It has an average temperature of 27°C and rainfall that can vary between 1,000 and 1,200 milliliters per year (Martínez et al., 2008; Diaz, 2014).

Trap Cultures for the Capture of Trichoderma Spp

This methodology was proposed by the Microbiological Research Laboratory of the University of Sucre, which consists of placing traps with portions of pre-cooked and sterilized rice in 1,000 cm3 containers, covered by a muslin mesh. The traps were placed in the soil around the avocado plants with the highest vigor and fruit production. Ten traps were placed randomly throughout the crop lot, selecting those places with the highest humidity and presence of decomposing organic matter. In the samples taken during the dry season, the traps were left in the open, placing plant debris from the soil (pieces of decomposing leaves and stems) on top of the traps. After 14 days, the traps were dug up, sealed and processed. The Trichoderma specimens were isolated taking into account morphological characteristics of the mycelial colonies and consecutively replicated directly in Petri boxes with PDA culture media, and incubated at 28±2°C with photoperiods of 12 h of light and 12 h of darkness, monitored daily with the objective of eliminating possible contaminants, performing reisolation until pure cultures were obtained.

Isolation of Phytophthora Cinnamomi.

Avocado roots with characteristic signs of the disease caused by *P. cinnamomi* were collected. Once the roots were collected, 1.5 cm cuts were made to be subjected to a superficial disinfection process with ethanol (70%) for 30s, followed by washing in sterile water for 40s, then immersed in sodium hypochlorite (13%) and finally washed with distilled water for 30s. The disinfected samples were inoculated in V8 medium (Mamani, 2017). The inoculated Petri dishes were incubated at28°C for 8 days (Chañag et al., 2018).

Identification of the Microorganisms

The isolates that presented cultural characteristics of Trichoderma spp. were purified and inoculated in PDA culture medium for growth. Once the strains had presented optimal growth, a small paper tape was taken and placed on the surface of the Trichoderma spp. colony, then the tape was gently removed and placed on a slide containing a drop of lactophenol and observed under a 4X to 40X microscope. Structures such as conidia, conidiophores and phialides were observed from each strain. The taxonomic keys proposed by Barnett and Hunter (1998) were used. The keys proposed by Drenth and Sendall (2001) were used to identify the strains of P. cinnamomi.

Assay of inhibitory activity in vitro of Trichoderma spp. against Phytophthora cinnamomi.

The methodology used by Bell et al., (1982) was followed. For this, isolates of *Phytophthora cinnamomi* and Trichoderma spp. were taken, placed at the ends of the Petri dish and incubated at 28 ± 2°C. The radial growth of each organism was measured to determine the antagonistic activity using the scale of Ezziyyani et al. (2005) and the formula for obtaining the Percentage Inhibition of Radial Growth (PICR):

$$PICR = \frac{R_1 - R_2}{R_1} x\ 100$$

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Where R1 is the radial growth (mm) of the control phytopathogen and R2 is the radial growth of the phytopathogen exposed to the antagonist (Jaramillo, 2014).

Likewise, the antagonism scale proposed by Ezziyyani et al., (2005), was used in order to determine the degree of *in vitro* antagonistic activity of Trichoderma spp. against P. cinnamomi.

In vitro plant growth promotion activity of native strains of Trichoderma spp.

- *Phosphate Solubilization*

With the help of a punch, a 5 mm block of mycelium was taken and inoculated in SRS culture medium. The fungi were incubated for 7 days at 30°C. The color change from purple to yellow in the medium is considered positive for phosphate solubilization (Sundara & Sinha, 1963).

- *Siderophore Production*

Siderophore production was determined using the chromium azurol-S (CAS) medium proposed by Schwyn and Neilands (1987). A 5 mm block of mycelium was taken from each fungus and inoculated into the culture medium with the aid of a punch. These were incubated for 7 days at 30°C. The ability of the fungus to produce siderophores was evidenced by the formation of a transparent halo around it. Strains showing siderophore production and phosphate solubilization are candidates for in vivo plant growth promotion in avocado plants.

- *Molecular Identification of Trichoderma spp.*

Pure colonies of each strain of Trichoderma spp. were extracted from PDA medium and incubated at 25±2°C for three days with photoperiod (12 h light/dark). We weighed 100 mg of fresh mycelium which was macerated in liquid nitrogen with the aid of a mortar and pestle. DNA was extracted with the commercial DNeasy Plant Mini® kit following the manufacturer's protocol (Vázquez et al., 2015; Fernández-Gamarra et al., 2017). To verify the presence of DNA, 0.5% agarose gel electrophoresis in TBE buffer was performed; 3 µL of pure DNA was taken and mixed with 4 µL of Orange dye, a 100 bp molecular marker was used as a reference. For visualization with a run time of 2 hours at 70 volts using a Power PacTM Basic 300V/400mA/75W BIO-RAD power supply.

- *Amplification of the Translation Elongation Factor 1 Gene (Tef1)*

The extracted DNA was subjected to polymerase chain reaction (PCR) to amplify the tef1 gene region of rDNA. A final volume of 25µL of the reaction mixture, prepared as follows, was used in the PCR reaction: 2.5µL of buffer; 1.5µL of 25 mM MgCl2; 1.5µL of 10mM dNTP; 0.75µL of EF1-728F5'-CATCGAGAAGTTCGAGAAGAAGG-3'primer at 0.2mM and 0.75µL at 0.2mM Tef1-Llevrev 5'-AACTTGCAGGCAGGCAA-TGTGG-3'; and 0.5µL Taq DNA polymerase in 12.5µL of sterile ultrapure water (Druzhinina, 2009; Maniscalco and Dorta, 2015). Trichoderma harzianum was used as positive control and sterile water as negative control.

- *Sequencing of PCR Products*

The amplified products were sent for sequencing to Macrogen (Seoul, South Korea) in an automatic sequencer with a 3730XL capillary. The nucleotide sequence entities obtained were compared with those stored in databases of the National Center for Biotechnology Information (NCBI). Base alignment was performed by cluster W, phylogenetic inferences were obtained by Neighbor-Joining method based on the kimura-2-parameter model with bootstrap test 1,000 replicates with the MEGA X program.

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Antagonistic activity of Trichoderma spp. in vivo against Phytophthora cinnamomi in avocado seedlings var. Lorena and chili bell pepper

- *Disinfection of Avocado and Chili Bell Pepper Seeds*

The methodology proposed by Alvarado (2017) was followed with modifications in the disinfection process, which consisted of extracting the seed of the avocado variety Lorena when the fruit was in a state of commercial maturity. Then they were washed and dried for 30 minutes, subsequently the seeds that presented better quality and were free of pests or diseases were selected. The tegument covering the seed surface was removed and a 2 to 3 mm cut was made at the apex to promote germination. Finally, they were placed in a benomyl solution for 20 minutes to carry out the disinfection process. The previously disinfected seeds were sown superficially with the vertical apex upwards in the previously sterilized soil contained in polyethylene bags with a capacity of 4 kg.

On the other hand, chili bell pepper seeds were surface sterilized in agitation at 180 rpm for 10 min in 70% ethyl alcohol and 2% sodium hypochlorite and subsequently washed with sterile distilled water four times (Rubio et al., 2017) and dried in the air of the laminar flow cabinet. Subsequently, they were inoculated in sterile soil until germination.

- *Preparation of Trichoderma Spp. and Phytophthora Cinnamomi Inoculums*

Soil-native Trichoderma spp. strains with the best *in vitro* antagonistic activity were bioaugmented on PDA agar at 28°C, with 12 h light and 12 h dark intervals. A stock solution of PDA broth was prepared and inoculated with 5mL Trichoderma spp. After 10 days, 1 mL of the sample was taken and added in the neubauer chamber and with the help of a microscope the number of spores/mL was determined and by means of dilutions were adjusted to different concentrations for avocado (1x107, 1x108, 1x109 spores/mL) and for chili (0.5x106, 1.5x106, 3.0x106 and 4.5x106 spores/mL) (Cardenas et al., 2005; Hoyos-Carvajal et al., 2008; Leal et al., 2014); while for P. cinnamomi it was bioaugmented on V8 agar and adjusted to a concentration of 1x105 sporangia/mL (Leal et al., 2014). For the case of commercial Trichoderma, it was left at the same concentration given by the commercial house 1x108 sporangia/mL.

Inoculation was carried out 20 days after seed germination, 200 mL of solution was added to each seedling at the concentration determined for each treatment. A total of 3 applications were made every 30 days. After inoculation with the pathogen, the severity of wilt was evaluated twice a week, using a scale of 0 to 2 (0 = healthy plant; 1 = wilted plant; 2 = dead plant) (Guigón, 2004). A 200 mL of agriphos was applied and added for a period of 2 months when the plant showed symptoms of the disease.

- *In Vivo Growth Promotion in Avocado Seedlings Var. Lorena and Chili Bell Pepper.*

Avocado and chili bell pepper seeds, previously sterilized, were inoculated at different concentrations as described above. For each seedling, 250 mL of each concentration were added and three applications were made, at 30 days, 60 days and 90 days for avocado; while for chili bell pepper two applications were made at 30 days and 45 days. According to agronomic recommendations.

Results and Discussion

Molecular Identification of the Strains of Trichoderma Spp.

Amplification of genomic DNA extracted from Trichoderma spp strains using primers EF1-728F and Tef1-Llevrev resulted in an expected product of 1200 bp, whose sequence analysis allowed identification of strains C1OVLIMB, C3OVLIMB, C4OVLIMB, C2CHLIMB, C3CHLIMB as Trichoderma harzianum, while C2OVLIMB, C4OVLIMB, C1CHLIMB, C4CHLIMB, C5CHLIMB were identified as Trichoderma viride (Table 1).

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In Vitro Growth Promotion Assays

The results of the qualitative *in vitro* growth promotion activity assay show that all the isolated Trichoderma spp strains produced, in different proportions, siderophores, IAA and solubilized phosphate (Table 1), which was evidenced, when the strains presented a transparent halo in the CAS medium indicating siderophore production, while phosphate solubilization generated a color change in the SRS culture medium from purple to yellow and the production of IAA changed from transparent medium from pink to brick color.

Siderophores are compounds synthesized by many organisms including Trichoderma, when exposed to Fe deficient medium, binding to insoluble ferric ion (Fe3+) to convert it into ferrous ion (Fe2+), which is soluble for plants (Ghosh et al., 2020a), being one of the mechanisms that induces the promotion of plant growth and defense against pathogens (Ghosh et al., 2017b). Different Trichoderma species can produce hydroxamate and carboxylate type siderophores, for example T. harzianum produces them in 85.0%, T. viride in 65.0%, T. asperellum in 60.3% and T. longibrachiatum in 45.5% (Ghosh et al., 2017). But it has also been reported that T. virens can produce ferricrocin-type siderophores (Mukherjee et al., 2018).

Table 1. Results of in Vitro Growth Promoting Activity of the Identified Native Trichoderma Strains

Isolate code	Origin	*Best BLAST match*	Growth promotion *In vitro*		
			Phosphate solubilization	Siderophores production	IAA production
C1OVLINM	Ovejas	*Trichoderma harzianum* (99%)	+++	+++	+++
C2OVLINM	Ovejas	*Trichoderma viride* (98%)	++	+	++
C3OVLINM	Ovejas	*Trichoderma harzianum* (99%)	++	++	+++
C4OVLINM	Ovejas	*Trichoderma harzianum* (99%)	+++	+	++
C1CHLINM	Chalán	*Trichoderma viride* (98%)	+	+	++
C2CHLINM	Chalán	*Trichoderma harzianum* (99%)	++	++	++
C3CHLINM	Chalán	*Trichoderma harzianum* (99%)	+++	++	+
C4CHLINM	Chalán	*Trichoderma viride* (98%)	++	+	+++
C5CHLINM	Chalán	*Trichoderma viride* (98%)	+++	+++	+++
Comercial product			+	+	-

Additionally, T. koningiopsis solubilizes phosphates through different mechanisms, such as the production of organic acids like citric acid and glucanic acid or by accumulation of polyphosphates and synthesis of phosphatase enzymes (Tandon et al., 2019), which is corroborated by Busato et al. (2020) who determined that T. asperellum and T. virens increased soluble phosphate content by releasing citric acid in vermicompost and Babu et al. (2014) found that Trichoderma positively stimulates plant growth through the production of siderophores, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, indole acetic acid (IAA), phosphate solubilization, phytase and acid phosphatase activity.

In Vivo Growth Promotion Assays

The results of the plant growth promotion assays indicate significant differences between T. harzianum and T. viride species with the commercial product for each of the fruit and vegetable crops (avocado and chili)

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evaluated. viride showed the best values of growth promotion in the variables analyzed in the cultivation of avocado (Table 2) and chili pepper plants (Table 3), however, for some variables both strains of Trichoderma native to the Montes de María, did not present significant difference between them, although they evidenced an increase in the variables of growth promotion when compared with the commercial Trichoderma. In the avocado crop, T. viride increased the diameter of the stem in 141.2% with respect to the control and 68.6% with respect to the commercial product, while the length of the stem increased it in 291% with respect to the control and 154.2% with respect to the commercial product and finally, the dry weight of the root increased it in 236.8% with respect to the control and 81.5% with respect to the commercial product (Table 2). In addition, T. viride on the chili bell pepper crop doubled all the variables with respect to the control (Table 3).

The results of this research are consistent with those reported by Bader et al. (2020) who argue that Trichoderma fungi can be considered as biofertilizers, since they increase the efficiency of nutrient capture for plants, given that it interacts with the roots producing secondary metabolites such as phytohormones that stimulate and regulate plant metabolism, resulting in better growth and yield (Padmavathi et al., 2015). In addition, it can interact with other rhizosphere microorganisms improving growth variables, production and biological control of phytopathogens (Bader et al., 2020). Complementarily, Nykiel et al. (2020) state that the application of spores of T. koningii, T. citrinoviride, T. harzianum, T. viride, and T. viriens promote plant growth through siderophore production, phosphate solubilization, and 1-aminocyclopropane-1-carboxylate deaminase (ACCD) activity.

Table 2. Results of in Vivo Growth Promoting Activity of Native Trichoderma Strains on Avocado Plants.

Isolate code	Origin	Identification	Concentration (Spores/mL)	Growth promotion on avocado (Greenhouse)		
				Stem thickness (Cm)	Plant height (Cm)	Dry root biomass (g)
C1OVLINM	Ovejas	*T. harzianum*	$1x10^7$	$0,65\pm0,06^{bcd}$	$39,0\pm2,6^{cd}$	$3,42\pm0,15^{c}$
			$1x10^8$	$0,69\pm0,15^{bcd}$	$46,3\pm5,5^{def}$	$4,53\pm0,03^{e}$
			$1x10^9$	$0,79\pm0,18^{de}$	$61,6\pm1,5^{g}$	$5,51\pm0,05^{g}$
C5CHLINM	Chalán	*T. viride*	$1x10^7$	$0,61\pm0,26^{bc}$	$48,8\pm4,3^{ef}$	$3,96\pm0,29^{d}$
			$1x10^8$	$0,86\pm0,08^{e}$	$50,3\pm8,0^{f}$	$4,94\pm0,25^{f}$
			$1x10^9$	$0,99\pm0,12^{e}$	$71,0\pm8,7^{h}$	$5,85\pm0,30^{h}$
Comercial product			$1x10^7$	$0,48\pm0,08^{ab}$	$26,3\pm1,5^{b}$	$2,63\pm0,07^{b}$
			$1x10^8$	$0,56\pm0,02^{bc}$	$35,0\pm2,1^{c}$	$3,72\pm0,14^{c}$
			$1x10^9$	$0,72\pm0,06^{cd}$	$41,7\pm1,2^{cde}$	$4,83\pm0,12^{e}$
Control			N/A	$0,34\pm0,01^{a}$	$14,5\pm3,2^{a}$	$1,46\pm0,05^{a}$

Tabla 3. Results of in Vivo Growth Promoting Activity of Native Trichoderma Strains on Chili Pepper Plants.

Variables GP	Control	*T. harzianum* (C1OVLINM)	*T. viride* (C5CHLINM)
Number of fruits	$0,40\pm0,02^{a}$	$1,15\pm0,17^{a}$	$2,05\pm0,22^{b}$
Fruit Diameter (mm)	$16,15\pm0,51^{a}$	$30,65\pm1,45^{b}$	$39,49\pm1,15^{c}$
Fruit Length (mm)	$25,44\pm1,03^{a}$	$115,91\pm1,89^{b}$	$161,87\pm1,31^{c}$
Dry weight fruit (g)	$1,62\pm0,11^{a}$	$2,21\pm0,09^{b}$	$2,84\pm0,07^{c}$
Plant Length (cm)	$63,28\pm1,72^{a}$	$86,12\pm1,05^{b}$	$97,47\pm1,25^{c}$
Dry weight of plants (g)	$14,52\pm0,85^{a}$	$29,84\pm1,51^{b}$	$42,95\pm1,44^{c}$
Stem thickness (mm)	$6,90\pm0,66^{a}$	$8,22\pm0,84^{b}$	$9,98\pm0,72^{c}$
Root Length (mm)	$14,34\pm0,97^{a}$	$36,71\pm1,52^{b}$	$49,97\pm0,92^{c}$
Dry weight root (g)	$1,44\pm0,37^{a}$	$3,42\pm0,23^{b}$	$4,95\pm0,38^{c}$

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Rubio et al. (2017) and Carillo et al. (2020) also claim that the application of T. harzianum on tomato plants improved production yields by 40%, finding higher number of fruits per plant and higher fruit weight, similar results were found in this research (Table 3). In addition, Candelero et al. (2015) reported increases of 55.57% and 47.62% in plant height, 41.6% in length and 55.0% in root volume when C. chinense plants were inoculated with T. harzianum, consistent with this study.

In Vitro Inhibitory Activity of Trichoderma Spp. Against P. Cinnamomi

Trichoderma species are characterized by presenting different inhibition mechanisms such as production of volatile secondary metabolites, mycoparasitism, competition for space and nutrients. According to the *in vitro* results, T. harzianum and T. viride presented inhibition by mycoparasitism, where T. harzianum presented an antagonistic activity grade 5, occupying completely the surface of the medium and sporulating on the pathogen, inhibiting it in 82.24±0.17%; while T. viride was grade 3, occupying 50% of the surface of the culture medium and inhibiting the phytopathogen in 93.7±0.16% (Table 4).

Table 4. Results of in Vivo and in Vitro Inhibitory Activity of Native Trichoderma Strains

Isolate code	Origin	Identification	Biocontrol against *P. cinnamomi*			
			Greenhouse (Severity)		*In vitro* (%AI)	
			Chili	Avocado	PIRG	GCA
C1OVLINM	Ovejas	*T. harzianum*	0	0	82,24±0,17%[a]	5
C5CHLINM	Chalán	*T. viride*	0	0	93,7±0,16%[b]	3
Comercial product			0	0	N/A	N/A
Control			0	0	N/A	N/A
P. cinnamomi (1x10_5 Sporangia/mL)			1	1	N/A	N/A
Agrochemical (Agrifos 100mL/L)			1	1	81,7±1,12[a]	N/A

Mycoparasitism of Trichoderma Against Phytophthora Cinnamomi

After the tests of antagonism *in vitro* we proceeded to make assemblies for observation in microscope of the capacity of the strains of Trichoderma to exert mycoparasitism on P. cinnamomi, results that are evidenced in figure 1. Where it is observed that the hyphae of Trichoderma envelop the hyphae of P. cinnamomi causing lesions in the form of pores that generate that all the intracellular material of the phytopathogen is dispersed in the medium and with it the death of P. cinnamomi (Figure 1 C and D).

The results of antagonistic activity of Trichoderma strains against Phytophthora are in agreement with reports that T. harzianum species are highly aggressive against a wide range of phytopathogens, including F. oxysporum with inhibition around 80% and Ganoderma boninense with 72% antifungal activity, suggesting a high potential as a biocontrol agent, either through mycoparasitism, antibiotic production and induction of systemic resistance in plants (Bader et al., 2020; Singh et al., 2019; Nowara et al., 2017; Siddiquee et al., 2009).

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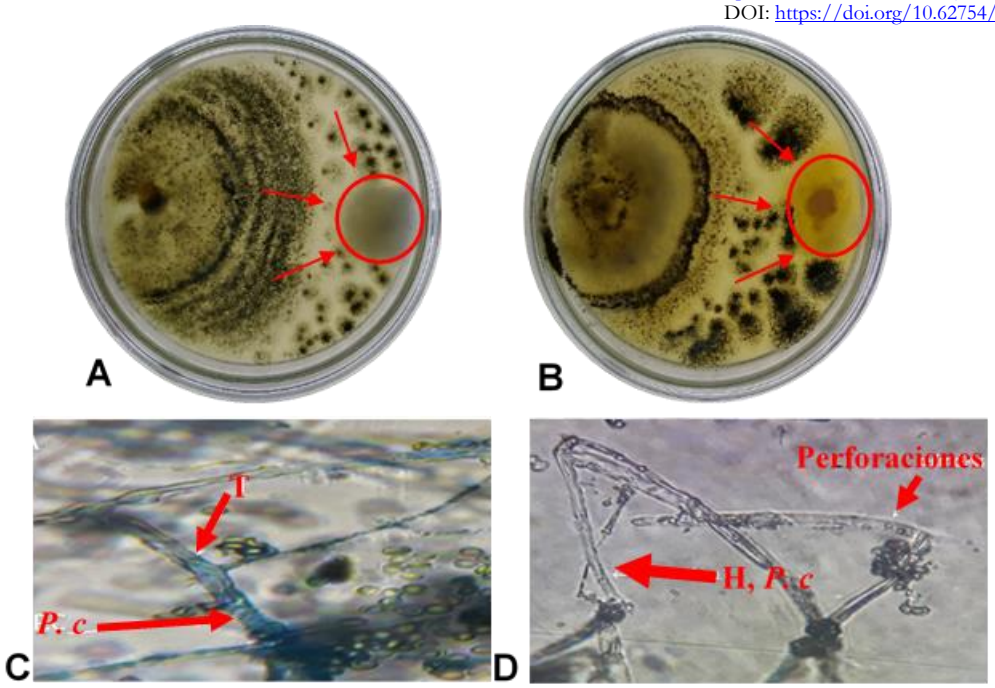

Figure 1. Results of *in vitro* antagonistic activity of T. harzianum (A and C) and T. viride (B and D) against P. cinnamomi. Source: Microbiological Research Laboratory of the University of Sucre.

These results are very similar to the different investigations that demonstrate the inhibition of T. harzianum and T. viride against pathogen species. Studies by Troian et al. (2014) demonstrated that Trichoderma harzianum inhibits the growth of Sclerotinia sclerotiorum by mycoparasitism in which the fungus releases enzymes such as glucanases and peptidases capable of degrading the cell wall of the pathogen. Once the cell wall was degraded, the fungus multiplied forming a dense mycelium that penetrated the hyphae of the pathogen. Likewise, Sanchez et al. (2019) demonstrated that Trichoderma spp. presented *in vitro* inhibition against Phytophthora rosacearum, P. inundata, P. lacustris, P. cactorum. In all cases, the coiling of Trichoderma spp. on the hyphae of the above-mentioned pathogens was observed under electron microscopy.

Das et al. (2019) isolated Trichoderma spp. strains from the rhizosphere of ginger cultivars grown in different regions of Palakkad and Idukki districts, India. They identified 4 isolates by biochemical and molecular characterization. The isolates were identified as Trichoderma asperellum, strain AFP, T. asperellum, strain MC1, T. brevicompactum MF1 and T. harzianum, strain CH1. subsequently, they were studied to evaluate them *in vitro* antifungal activity against Fusarium oxysporum, Phytophthora capsici, Rhizoctonia solani. The results of antagonistic activity showed that all four isolates of Trichoderma spp. had significant antagonistic activity against the above mentioned plant pathogens, indicating that Trichoderma spp. isolates can be used as effective microbial biological control agents. The above studies demonstrate the potential of Trichoderma spp. against phytopathogens that cause damage to economically important crops.

Nidhina et al. (2016) evaluated Trichoderma spp. strains for growth promoting ability and antagonistic activity. Molecular identification was performed by PCR amplifying internal transcribed spacer regions. The strains were identified as T. Asperellum, T. Harzianum and T. Longibrachiatum. *In vitro* test on dual medium against plant pathogens such as Phytophthora meadii, P. heveae, P. citrophthora, P. capsici and P. palmivora showed that all Trichoderma isolates grew rapidly and inhibited the growth of the pathogens by the third day. Growth-promoting activity was tested on green grain (Vigna radiate) seeds under greenhouse conditions; seed germination percentage, root length, sprout and vigor index were measured. All Trichoderma isolates showed a significant vigor index compared to the control, suggesting that these isolates can be used as biofertilizers.

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In addition, a research done by El-Sharkawy et al., (2018) performed a combination of arbuscular mycorrhizae with Trichoderma harzianum and Trichoderma viride in order to control stem rust disease in wheat plants caused by Puccinia graminis. The results obtained showed that under greenhouse conditions the combination of arbuscular mycorrhizae with the two Trichoderma species reduced the disease, induced the production of peroxidase and polyphenol oxidase enzymes, increased the total phenol content and improved growth parameters.

In Vivo Inhibitory Activity of Trichoderma Spp. Against P. Cinnamomi in Chili Bell Pepper and Avocado Seedlings.

In addition, a research by El-Sharkawy et al., (2018) conducted a combination of arbuscular mycorrhizae with Trichoderma harzianum and Trichoderma viride to control stem rust disease in wheat plants caused by Puccinia graminis. The results obtained showed that, under greenhouse conditions, the combination of arbuscular mycorrhizae with the two Trichoderma species reduced the disease, induced the production of peroxidase and polyphenoloxidase enzymes, increased total phenol content and improved growth parameters.

The genus Trichoderma has good qualities for the control of plant diseases caused by soil fungal pathogens, mainly of the genera Phytophthora sp., Rhizoctonia sp., Sclerotium sp., Pythium sp. and Fusarium sp, Pythium sp. and Fusarium sp. Trichoderma species act as competitive hyperparasites that produce antifungal metabolites and hydrolytic enzymes to which structural changes at the cellular level of the pathogen are attributed (Ezziyyani et al., 2005; Saravanakumar & Wang, 2020; Zhang et al., 2020).

Trichoderma spp. have the ability to synthesize a great diversity of molecules with biocontrol activity against phytopathogens and induce in the plant molecular mechanisms such as systemic acquired resistance (SAR) and induced systemic resistance (ISR) (Baiyee et al., 2019; Saravanakumar & Wang, 2020). They have proven to be a biotechnological tool because they present great benefits for agriculture, including plant growth promoting activity, production of enzymes and metabolites that help inhibit disease-causing pathogens in plants (Babu et al., 2014; Kidwai & Nehra, 2017; Saravanakumar & Wang, 2020). For example, in addition to the pathogen biocontrol effect, inoculation of T. harzianum and T. viride has been shown to provide other benefits to plants; through the decomposition of organic matter, it releases nutrients in forms available to the plant (Godes, 2007), and presents phosphate solubilizing activity (Sánchez et al., 2005), which is why it is frequently used as a biofertilizing organism in different commercial products (Moreno-Sarmiento et al., 2007).

Conclusion

The implementation of native Trichoderma strains is proposed as a phytosanitary and agronomic alternative to the conventional management of avocado and chili bell pepper crops, since it allows reducing production costs and increasing yields, an opportune condition in the agricultural scenarios of developing countries with vulnerable populations of scarce economic resources, such as the Montes de Maria in the Colombian Caribbean.

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Conflict of Interest. All the authors of the manuscript declare that they have no conflict of interest.

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