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Control of avocado root rot caused by *Phytophthora cinnamomi* with different Trichoderma strains at Chavimochic Irrigation Project

Control de la pudrición de la raíz del palto causada por *Phytophthora cinnamomi* con diferentes cepas de Trichoderma en la Irrigación de Chavimochic

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Abstract

Avocado root rot caused by *Phytophthora cinnamomi* is one of the main problems affecting avocado (*Persea americana*) cultivation in Peru, especially at the Chavimochic Irrigation Project. The objective of this study was to evaluate the effect of different *Trichoderma* strains on the control of *Phytophthora cinnamomi* in Zutano rootstock under greenhouse conditions. Five isolates of Trichoderma were tested: *Trichoderma sp.* (Chav01); *Trichoderma harzianum* (Chavo2); *Trichoderma harzianum* (UNALM01); *Trichoderma viride* (UNALM02); and a commercial strain of *Trichoderma* sp. Evaluations were performed at 30, 45, and 60 days. All isolates colonized the rhizosphere of the avocado. No relation was found between the formation of more *Trichoderma* colonies and Phytophthora improved control. All strains controlled the root rot, but Chav01 and Chav02 showed the greatest diameter of stem, dry matter in the root, and percentage of healthy root in comparison with UNALM01, UNALM02, and the commercial strain. Thus, the native isolates of *Trichoderma* from the Chavimochic area can be added to the list of potential new *Trichoderma* species to control *Phytophthora cinnamomi*.

Keywords: *Trichoderma*, avocado, *Phytophthora cinnamomi*, biological control, root rot

Resumen

La pudrición de la raíz del palto causada por *Phytophthora cinnamomi* es uno de los principales problemas que afectan al cultivo de la palta (*Persea americana*) en el Perú, especialmente en el Proyecto de Irrigación Chavimochic. El objetivo de este estudio fue evaluar el efecto de diferentes cepas de Trichoderma en el control de *Phytophthora cinnamomi* en portainjertos de Zutano bajo condiciones de invernadero. Se probaron cinco aislamientos de Trichoderma: *Trichoderma* sp. (Chav01); *Trichoderma harzianum* (Chavo2); *Trichoderma harzianum* (UNALM01); *Trichoderma viride* (UNALM02); y una cepa comercial de *Trichoderma* sp. Las evaluaciones se realizaron a los 30, 45 y 60 días. Todos los aislados colonizaron la rizosfera del aguacate.

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No se encontró relación entre la formación de más colonias de Trichoderma y la mejora del control de Phytophthora. Todas las cepas controlaron la podredumbre de la raíz, pero Chav01 y Chav02 mostraron el mayor diámetro de tallo, materia seca en la raíz y porcentaje de raíz sana en comparación con UNALM01, UNALM02 y la cepa comercial. Por lo tanto, los aislados nativos de Trichoderma de la zona de Chavimochic pueden ser añadidos a la lista de nuevas especies potenciales de Trichoderma para controlar *Phytophthora cinnamomi*.

Palabras clave: *Trichoderma, aguacate, Phytophthora cinnamomi, control biológico, pudrición de la raíz*

Introduction

Root rot caused by *Phytophthora cinnamomi* is one of the most destructive diseases in global avocado cultivation. It attacks trees of all ages, including those cultivated under greenhouse conditions, and causes significant economic losses in avocado crops (Hardham et al., 2018; Coffey, 1987; Ploetz, 2013). *P. cinnamomi* was first described in 1922 by Rands as the causal agent of stem canker in cinnamon trees in Sumatra, and was first discovered on avocado trees (*Persea americana*) in Puerto Rico in 1929, where it caused severe root rot. Since then, *P. cinnamomi* has been reported in over 70 countries, with a wide host range (Zentmeyer, 1985).

The use of fungicides for the control of phytopathogenic fungi diseases has limitations, making biological control methods more appealing. The biological control of *P. cinnamomi* through the incorporation of different agents into the soil has been investigated by different authors, who have shown that bacteria present in the soil, such as *Pseudomonas* spp. and *Streptomyces* spp. inhibit the in vitro growth of *P. cinnamomi* (Mass & Kotzé, 1990; Finlay & McCracken, 1991; Stirling et al., 1992). Likewise, there are a large number of fungal species that show antagonistic effects toward *P. cinnamomi*, such as *Trichoderma* spp., *Myrothecium roridum, Aspergillus* spp., or *Paecilomyces* spp. (Reeves, 1975; Gees & Coffey, 1989; Casale, 1990; Finlay & McCracken, 1991; Duvenhage & Kotzé, 1993; McLeod et al., 1995). Soil suppressiveness is one of the main factors inhibiting the development of *Phytophthora cinnamomi*. This suppressiveness can be increased with the inoculation of *Trichoderma*, *Gliocladium*, *Bacillus*, and others (Erwin & Ribeiro, 1996).

In Peru, Chavimochic irrigation is one of the main avocado cultivation zones, with an area of 7500 ha. *P. cinnamomi,* is one of the main pathogens causing avocado root rot in this region. It is estimated that 10% of plantations are affected by this disease (Villavicencio, 2018). The main source of inoculum are the chlamydospores brought in by irrigation water from the Santa River (Ancash, Peru).

This situation lead us to investigate the effectiveness of *Trichoderma* strains by incorporating them into the soil for the control and isolation of *P. cinnamomi*, which was obtained from avocado plantations in the Chavimochic valley. The objectives of this research were: i) to determine the best *Trichoderma* strain for the control of *P. cinnamomi* in avocado plantations; and ii) to evaluate the effect of the different strains on the biometry of the avocado Zutano rootstock inoculated with *P. cinnamomi.*

Materials and methods

This experiment was performed in the greenhouse facilities at the Arato Perú S.A. company, in the province of Virú, department of La Libertad, Peru from September to December (2015) with temperatures fluctuating between 17 °C and 25 °C.

Plant material

Zutano variety avocado seeds were disinfected by immersion for 10 minutes in a 0.1% methyl thiophanate + Thiram (Homai WP) solution. The seeds were pregerminated. Once they presented a radicle of 3 cm, they were sown in 8 liter polypropylene bags containing a mixture of sterile sand plus earthworm humus in the ratio of 3:1. Once the plants had a growth of 60 cm and 8 formed leaves, they were inoculated with *P. cinnamomi*.

Inoculation method

Plant roots with wilt symptoms and regressive death were planted on a selective corn meal agar (NutriSelect® Basic, Merck) with the antibiotic Pimaricin Ampicillin Rifampicin Benomyl. Once a pure isolation of *P. cinnamomi* was obtained, it was increased on plates with Papa dextrose Agar-Difco medium. Slices of 3 cm in diameter with mycelial growth were then extracted from the medium and placed in 200 g bags with sterile wheat. These bags were incubated at 25°C for 21 days until *P. cinnamomi* completed growth throughout the bag.

For *Phytophthora* inoculation, 90-day-old avocado seedlings were placed on containers with water for 24 hours. Once the substrate was saturated, 35 g of wheat with mycelium of *P. cinnamomi* was placed around the neck of each plant and covered with the same saturated substrate.

Treatments with Trichoderma

The different *Trichoderma* treatments used are shown in Table 1. The *Trichoderma* isolates (Cha01 and Cha02) were obtained from soil collected from the rhizosphere of healthy avocado plants from the Chavimochic Irrigation Project. The isolate Chav01 was obtained in the Chao area from the rhizosphere soil of Hass avocado rootstock grafted on Zutano rootstock. The isolation of *T. harzianum* was isolated from the Virú area in the rhizosphere soil of Hass avocado rootstock grafted on Lula rootstock. The isolation was carried out in Papa Dextrose Agar Oxytetracycline (PDAO) medium through serial dilutions. The other isolates were provided by the institutions indicated in Table 1. Identification to genus and species level was carried out at the Phytopathology Diagnostic Clinic of the Universidad Nacional Agraria La Molina (Lima, Perú).

The inoculations with the antagonist *Trichoderma* was performed as follows. For the first inoculation, each *Trichoderma* isolate was inoculated in bags with sterile corn. In the sowing stage of the Zutano avocado seeds in substrate, 20 g of corn with each strain of *Trichoderma* growth were used per 8 liter substrate bag per plant. For the second inoculation, the *Trichoderma* strains were extracted from the bags of wheat with a *Trichoderma* suspension of 1×10^8 colony-forming units (cfu) per milliliter of sterile water solution. This was applied directly to the roots using 200 cm^3 of the solution per plant 30 days after the avocado seeds were sown in the substrate. For the third inoculation, the same procedure was carried out 30 days after the second *Trichoderma* inoculation.

Evaluation

Three evaluations were performed at 30, 45, and 60 days after inoculation. For each evaluation there were 10 replicates in a completely randomized design. For the evaluation, the plants were extracted from the bags and the

Table 1. Trichoderma strains used for the control of *P. cinnamomi* in avocado under greenhouse conditions. Chavimochic - Trujillo**.**

Trial	Strains of Trichoderma	Code	Place of origin
T1	*Trichoderma* sp.	Chav01	Chavimochic Irrigation Project
T2	*Trichoderma harzianum*	Chav02	Chavimochic Irrigation Project
T3	*Trichoderma harzianum*	UNALM01	Universidad Nacional Agraria La Molina[(a)]
T4	*Trichoderma viride*	UNALM02	Universidad Nacional Agraria La Molina[(a)]
T5	*Trichoderma* sp.	Trichomax Sol	Solagro S.A.C.[(b)]
T6	Inoculated control *(Phytophthora cinnamomi)*		Chavimochic Irrigation Project
T7	Absolute control		

[(a)] Isolate from fungi collection of Phytopathology department of Universidad Nacional Agraria La Molina.
[(b)] Isolate from Solagro SAC company, marketed under the name of Trichomax

root systems were washed, then the biometric parameters were evaluated, including stem diameter, dry weight, total root length, and the percentage of healthy root.

Stem diameter was measured using a Vernier by measuring the neck of the plant 2 cm from the end of the seed. The dry weight of each root was determined by allowing the fresh roots to air dry for 3 days, then drying the roots in a paper bag in the oven for another 3–4 days at an average temperature of 70 °C. The total root length was measured by photographing each experimental unit, then processing the photos with the ASSES 2.0 program (Lamari, 2008). The percentage of healthy root was determined visually by visual estimation using the graphic scale shown in Fig. 1 after the fresh roots were washed. This scale had 11 classes ranging from 0 % to 100 % healthy root.

Trichoderma colony-forming units

Rhizosphere samples were taken from each evaluation unit 45 and 60 days after inoculation with *P. cinnamomi*. These samples were diluted in distilled water, then sown in PDAO culture medium to quantify the *Trichoderma* colonies.

Results and discussion

Biometric parameters

The results are shown in Table 2 and Fig. 2. After 60 days, the stem diameters in Chav01 and Chav02 were statistically different than the inoculated control. The stem diameter of the rest of the treatments did not differ from the control. The dry weight of the roots from Chav01 and Chav02 also showed differences to the *P. cinnamomi*-inoculated control, but Chav01 did not show any differences to the control that was not inoculated. No differences were observed among the different treatments in total root length, but statistical differences were observed between the treated samples and both controls.

Of these parameters, dry weight best differentiates and evaluates the effects of the different *Trichoderma* strains on *P. cinnamomi* due to the fact that roots affected by *P. cinnamomi* normally undergo a process of root necrosis and tissue death that significantly reduces their weight. Sid Ahmed et al. (1999) found that the control of *Phytophthora capsica* in peppers using *Trichoderma harzianum* was best indicated by the dry matter weight results.

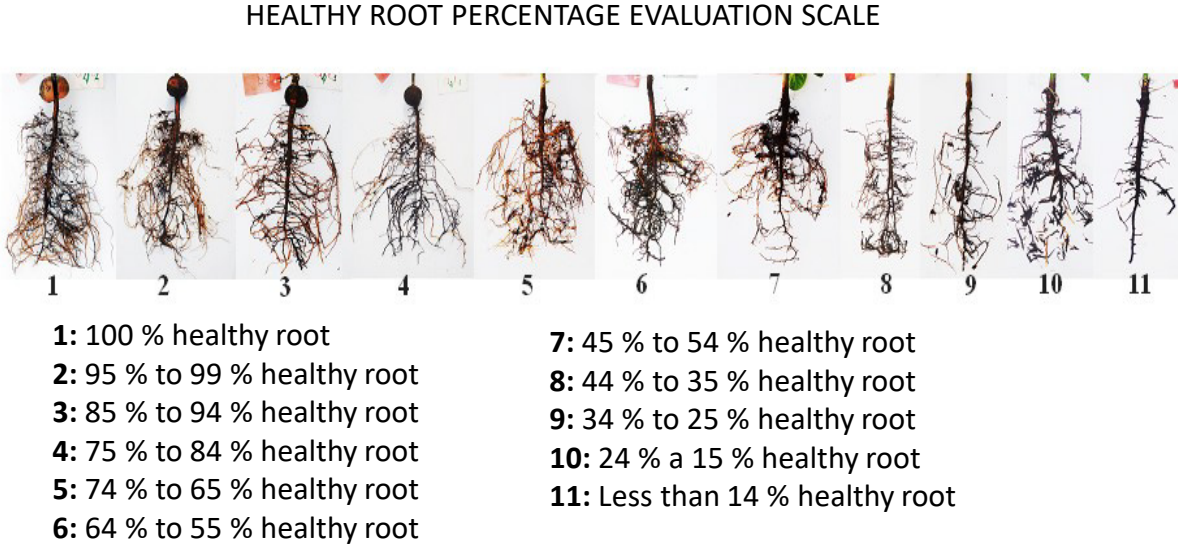

Fig. 1. Pictographic scale used to evaluate the percentage of healthy avocado roots inoculated with *Phytophthora cinnamomi.*

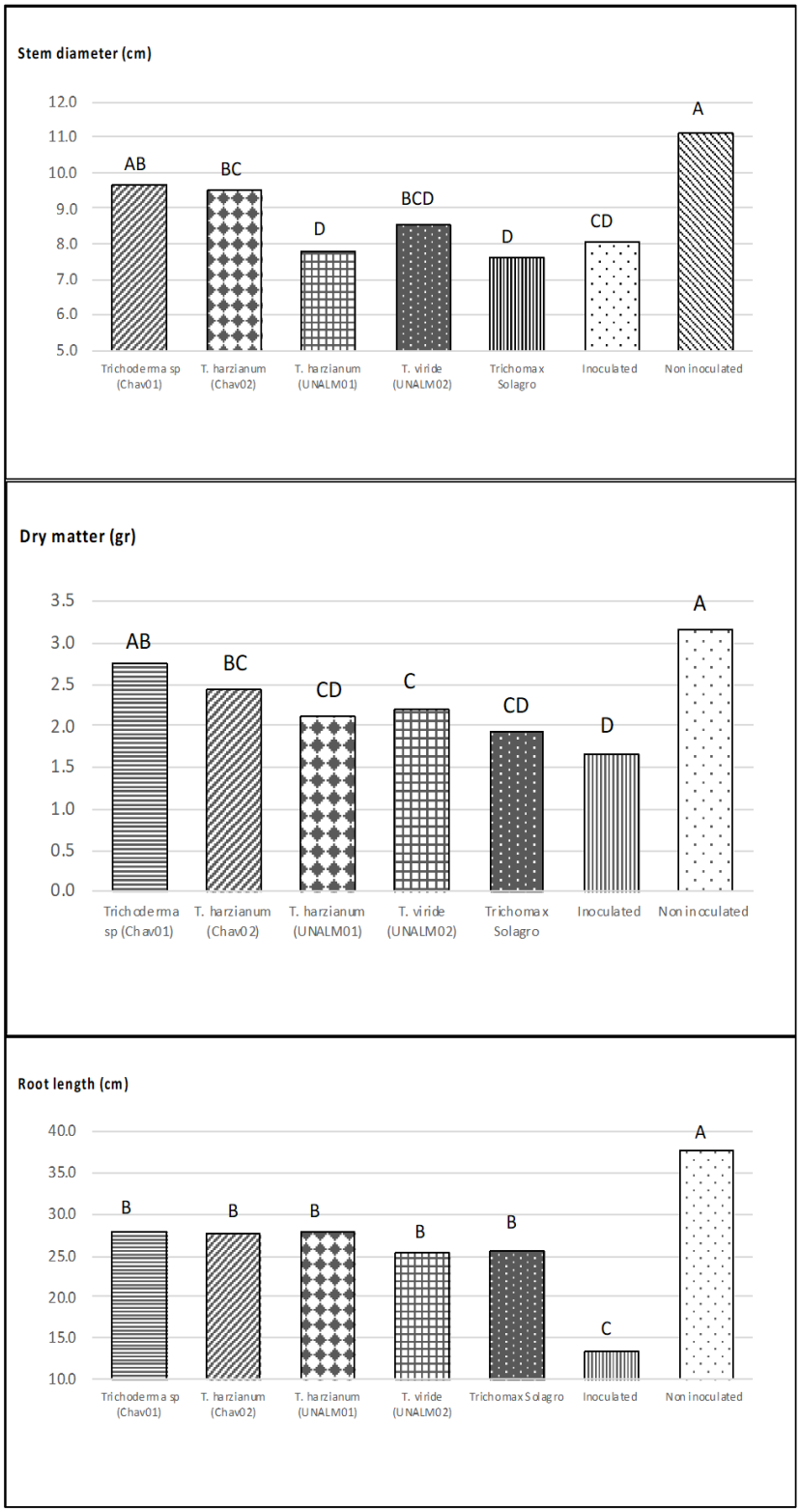

Fig. 2. Stem diameter (cm), root dry weight (g), and root length (cm) of avocado seedlings inoculated with *Phytophthora cinnamomi* and treated with different Trichoderma isolates for 60 days

Table 2. Biometric parameters: Stem diameter (cm), root dry weight (g) and root length (cm) in avocado seedlings inoculated with *Phytophthora cinnamomi* treated with different Trichoderma strains

		Stem diameter (cm)[a]						Root dry weight (g) [a]						Root length (cm) [a]					
Treat.	Antagonist Strain	1° Eval.		2° Eval.		3° Eval.		1° Eval.		2° Eval.		3° Eval.		1° Eval.		2° Eval.		3° Eval.	
1	*Trichoderma sp.* (Chav01)	7.01	AB	8.04	BC	9.67	AB	1.56	B	2.17	BC	2.75	AB	15.4	B	25.4	ABC	28.0	B
2	*T. harzianum* (Chav02)	6.76	B	8.62	AB	9.48	BC	1.51	B	2.37	AB	2.43	BC	14.6	B	27.4	AB	27.6	B
3	*T. harzianum* (UNALM01)	7.18	AB	6.38	D	7.80	D	1.57	B	1.98	BC	2.11	CD	16.0	B	17.8	CD	27.8	B
4	*T. viride* (UNALM01)	6.81	B	8.04	BC	8.56	BCD	1.52	B	2.12	BC	2.19	C	15.9	B	25.2	AB	25.4	B
5	*Trichomax Solagro*	7.38	AB	7.04	CD	7.61	D	1.54	B	1.72	C	1.94	CD	17.6	B	22.0	BCD	25.6	B
6	Inoculated control	6.56	B	7.19	BCD	8.07	CD	1.45	B	1.70	C	1.65	D	17.7	B	16.8	D	13.5	C
7	Control no inoculated	8.25	A	9.59	A	11.12	A	2.13	A	2.79	A	3.17	A	25.6	A	31.8	A	37.8	A
	Variability coefficient (%)	13.6%		14.9%		12.98%		11.4%		17.2%		17.0%		18.7%		24.7%		27.8%	
	P Value	0.05		0.05		0.05		0.05		0.05		0.05		0.05		0.05		0.05	

[a] Tukey with alfa = 0.05. Equal letter has no statistical differences

Table 3. Percentage of healthy roots in avocado seedlings inoculated with *Phytophthora cinnamomi* treated with different *Trichoderma* strains

Treat.	Antagonist strain	Percentage of healthy Root (%)[a] 1° Eval. (30 days)		2° Eval. (45 days)		3° Eval. (60 days)	
1	*Trichoderma* sp (Chav01)	14	B	68	B	82	B
2	*T. harzianum* (Chav02)	16	B	50	C	64	C
3	*T. harzianum* (UNALM01)	14	B	28	E	50	CD
4	*T. viride* (UNALM02)	14	B	40	D	60	C
5	Trichomax Solagro	12	B	20	F	42	D
6	Inoculated control	14	B	16	F	20	E
7	Control no Inoculated	98	A	100	A	100	A
	Variability coefficient (%)	22.5%		13.2%		18.1%	
	Value P > alfa	0.05		0.05		0.05	

[a]Tukey with alfa = 0.05. Equal letter has no statistical differences.

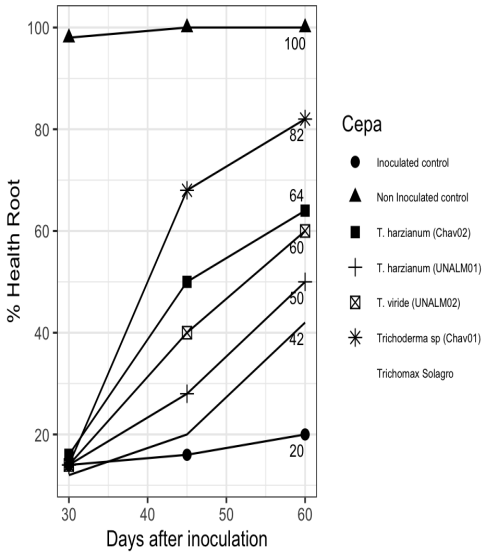

Fig. 3. Percentage of healthy roots treated with different *Trichoderma* strains in avocado seedlings inoculated with *Phytophthora cinnamomi*

Healthy root percentage

The healthy root percentage results are shown in Table 3, and Figs. 3 and 4. The healthy root percentage and incidence of symptoms in the aerial part of the plants made the differences between the different *Trichoderma* isolates clearer. It was observed that the best treatments were Chav01 and Chav02, which both showed statistical differences to the inoculated control and the rest of the treatments. These two isolates were obtained from the rhizospheres of healthy plants from the Chavimochic Irrigation Project, so their adaptations to the soil conditions and the irrigation environment was better than that of the isolates from the Universidad Nacional Agraria La Molina, which were obtained from capsicum and tomato plants. Differences between strains is a characteristic of *Trichoderma* as antagonists (Bae et al., 2011). The commercial strain Trichomax generally has a lower control efficiency than the

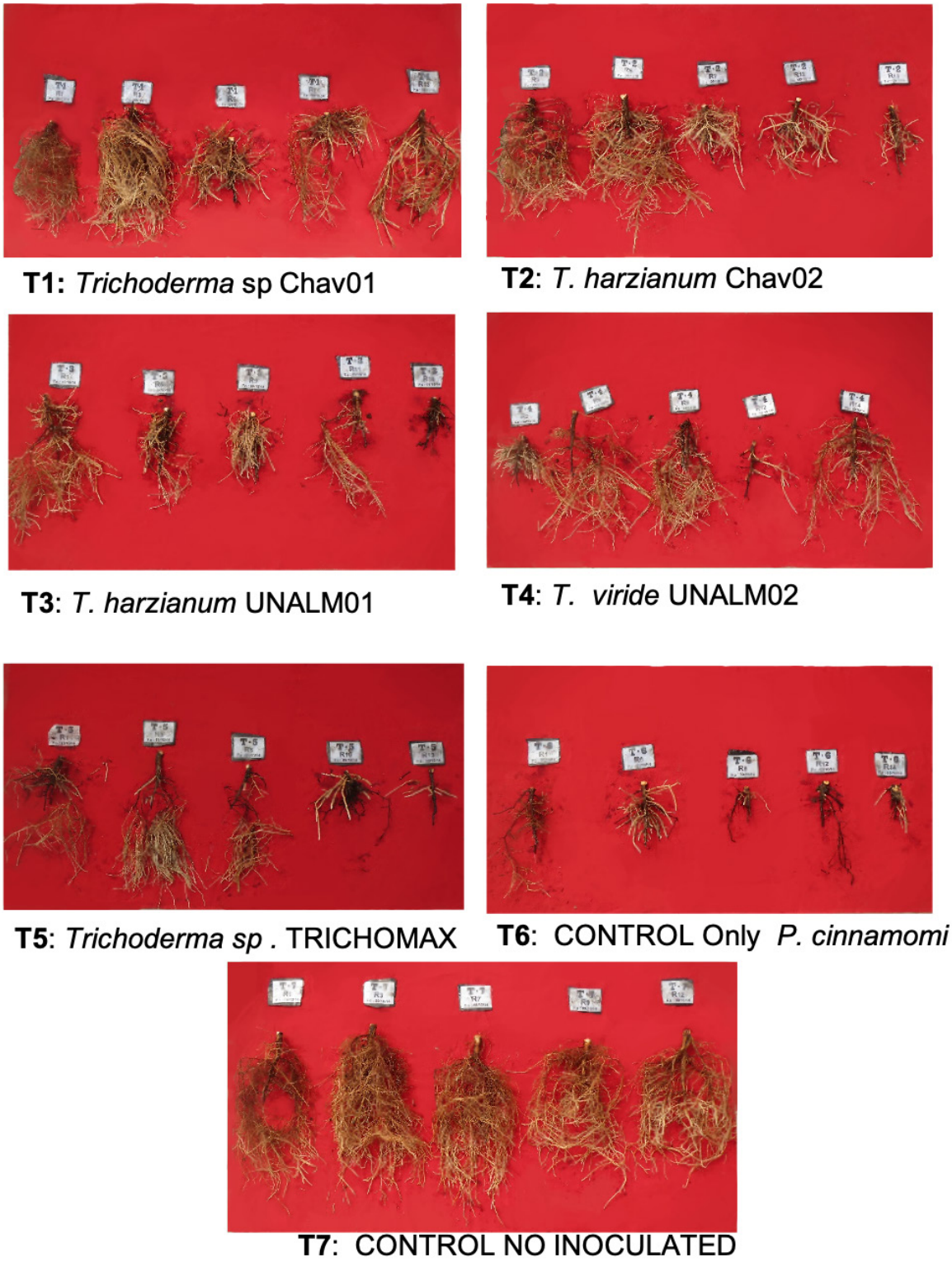

Fig. 4. Roots of Zutano avocado inoculated with *Phytophthora cinnamomi* and treated with different Trichoderma strains

Table 4. Colony-forming units of different *Trichoderma* strains in roots of Zutano avocado inoculated with *Phytophthora cinnamomi*

Treat.	Antagonist Strain	1st Sample (a)(b) (45 days) (UFC × g soil)		2nd Sample (a)(b) (60 days) (UFC × g soil)	
1	*Trichoderma* sp (Chav01)	2.0×10^5	BC	1.2×10^3	B
2	*T. harzianum* (Chav02)	2.0×10^5	BC	2.2×10^2	C
3	*T. harzianum* (UNALM01)	3.7×10^5	A	2.0×10^3	B
4	*T. viride* (UNALM02)	2.7×10^5	AB	3.3×10^3	A
5	Trichomax Solagro	1.0×10^5	C	2.2×10^3	AB
6	inoculated Control	0	D	0	D
7	Control non inoculated	0	D	0	D
	Variability coefficient (%)	27.14%		25.22%	

(a) Transformed data to root of X.

(b) Tukey with alfa = 0.05. Equal letter has no statistical differences

rest of the isolates. These results corroborate findings from other authors that antagonist adaptation to the agroecosystem conditions is a determining factor in their biological control of root pathogens (Benítez et al., 2004; Samuels, 2006). Pathogen control mechanisms occur mainly via antibiosis through metabolites, which inhibits pathogen development (Vinale, 2008; Bae et al., 2016).

Colony-forming units of Trichoderma

When the colony-forming units (cfu) of all the isolates were quantified, it was observed that the values 45 days after inoculation with *P. cinnamomi* were higher compared to after 60 days (Table 4). This is due to the fact that *Trichoderma* populations generally tend to decrease with time after being applied to the soil (Finlay & McCracken, 1991). In fact, *Trichoderma* was found in the rhizosphere zone, which is where the samples were extracted, indicating that all the isolates were able to colonize the rhizosphere of the avocado. No relationship was found between a greater quantity of *Trichoderma* cfu and better control. This can be explained by the fact that the presence of *Trichoderma* in the root already exerts control via antibiosis and competition, which is probably the most important characteristic with respect to its control. The population dynamics of *Trichoderma* are highly variable in the soil due to several factors, including temperature, humidity, roots, and the presence of organic matter, among others. In our study, *Trichoderma* colonies were detected in all soil treatments applied around the roots of the avocado seedlings.

Conclusions

All *Trichoderma* treatments controlled the avocado root rot caused by *P. cinnamomi*. Significant statistical differences with respect to the inoculated control were found for the percentage of healthy roots 60 days post inoculation. Chav01 best controlled the avocado root rot. Chav02 displayed the best performance for the percentage of healthy root and the incidence of plants with aerial symptoms. Overall, the native isolates from the Chavimochic region better controlled *Phytophthora cinnamomi* than the isolates from UNALM and the commercial strain Trichomax. No direct relationship was found between the number of colonies forming units of the different *Trichoderma* strains and the control effect on *Phytophthora cinnamomi.* All *Trichoderma* isolates were able to colonize the rhizosphere of the avocado.

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