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# Antagonism of Native *Trichoderma* spp. from the Peruvian Central Rainforest on *Phytophthora capsici* and Effects on *Capsicum annuum*

## Antagonismo de *Trichoderma* spp. Nativos de la Selva Central Peruana frente a *Phytophthora capsici* y su Efecto en el Crecimiento de *Capsicum annuum*

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## Abstract

The genus *Trichoderma* is primarily recognized for its role as a biocontrol agent against various plant Among the actions exhibited by this genus is its activity against *Phytophthora capsici* (*P.c.*), the causal agent of crown rot, foliar blight, and fruit rot in pepper cultivation. This study evaluated the antagonistic activity of *T. koningiopsis* ( TKSAT001) and *T. lentiforme* (TLSAT002) against *P. capsici*. These species, isolated from the Peruvian central rainforest, were previously identified through morphological and molecular methods. The research was conducted in two phases: 1) In the *in vitro* phase, the treatments included isolates of *T. koningiopsis*, *T. lentiforme*, and *T. harzianum*, each tested both individually and against *P. capsici*, along with a positive control (+*P.c.*); 2) For the *in vivo* phase, a negative control (-*P.c.*) was included. The *in vitro* antagonism evaluation featured a dual plate confrontation assay between *Trichoderma* spp. and *P. capsici* on Potato Dextrose Agar (PDA) medium, wherein the percentage of radial growth inhibition (PRGI) and growth rate were assessed. In the greenhouse, bell pepper seedlings cv 'Piquillo' were inoculated with 20 mL of a *Trichoderma* spp. conidia suspension ($10^8$ spores/mL) at transplanting, 14, and 28 days after transplanting (dat). *P. capsici* was inoculated at 28 dat near the base of the plant using three grains (0.27 g) of previously colonized wheat. The experiments followed a completely randomized design (CRD) with Tukey's mean comparison test at a 0.05 significance level. The evaluated variables included plant height (cm), incidence (%), severity (%), fresh weight (g), and dry weight (g). The *in vitro* antagonism results showed that *T. koningiopsis* and *T. lentiforme* exhibited PRGI values of 38.08 % and 37.91 %, respectively, against *P. capsici*. In the greenhouse assay, the bell pepper plants co-inoculated with *T. lentiforme* and *P. capsici* exhibited higher values in plant height (21.11 cm), root length (11.02 cm), and fresh weight (1.4 g). Additionally, the degree of foliar and root severity in all treatments with *Trichoderma* spp. and *P. capsici* was lower (Grade 0 to Grade 2), unlike the treatment inoculated solely with the pathogen (Grade 3 to Grade 4). In conclusion, this study demonstrates the potential of *T. koningiopsis* and *T. lentiforme* as biocontrol agents against *P. capsici* in pepper crops.

***Keywords:*** *Trichoderma koningiopsis*, *Trichoderma lentiforme, Trichoderma harzianumCapsicum, PRGI.*

## Resumen

El género *Trichoderma* es reconocido principalmente por su papel como agente de biocontrol contra diversos patógenos vegetales. Entre las acciones exhibidas por este género se encuentra su actividad contra *Phytophthora capsici* (P.c.), el agente causal de la podredumbre de la corona, el tizón foliar y la podredumbre del fruto en el cultivo del pimiento. En este estudio se evaluó la actividad antagonista de *T. koningiopsis* y *T. lentiforme frente*

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sobre *P. capsici*. Estas especies, aisladas de la selva central peruana, fueron previamente identificadas mediante métodos morfológicos y moleculares. La investigación se realizó en dos fases: 1) En la fase in vitro, los tratamientos incluyeron aislados de *T. koningiopsis*, *T. lentiforme* y *T. harzianum*, cada uno probado individualmente y contra *P. capsici*, junto con un control positivo (+P.c.); 2) Para la fase in vivo se incluyó un control negativo (-P.c.). La evaluación del antagonismo in vitro consistió en un ensayo de confrontación en placa doble entre *Trichoderma* spp. y *P. capsici* en medio Agar Papa Dextrosa (PDA), en el que se evaluó el porcentaje de inhibición del crecimiento radial (PRGI) y la tasa de crecimiento. En el invernadero, las plántulas de pimiento cv 'Piquillo' se inocularon con 20 mL de una suspensión de conidios de *Trichoderma* spp. ($10_8$ esporas/mL) al trasplante, 14 y 28 días después del trasplante (dat). *P. capsici* se inoculó a los 28 dat cerca de la base de la planta utilizando tres granos (0.27 g) de trigo previamente colonizado. Los experimentos siguieron un diseño completamente aleatorizado (DCA) con la prueba de comparación de medias de Tukey a un nivel de significación de 0.05. Las variables evaluadas incluyeron altura de planta (cm), incidencia (%), severidad (%), peso fresco (g) y peso seco (g). Los resultados de antagonismo in vitro mostraron que *T. koningiopsis* y *T. lentiforme* exhibieron valores de PRGI de 38.08 % y 37.91 %, respectivamente, contra *P. capsici*. En el ensayo en invernadero, las plantas de pimiento co-inoculadas con *T. lentiforme* y *P. capsici* mostraron valores superiores en altura de planta (21.11 cm), longitud de raíz (11.02 cm) y peso fresco (1.4 g). Además, el grado de severidad foliar y radicular en todos los tratamientos con *Trichoderma* spp. y *P. capsici* fue menor (Grado 0 a Grado 2), a diferencia del tratamiento inoculado únicamente con el patógeno (Grado 3 a Grado 4). En conclusión, este estudio demuestra el potencial de *T. koningiopsis* y *T. lentiforme* como agentes de biocontrol contra *P. capsici* en cultivos de pimiento.

***Palabras clave:*** *Trichoderma koningiopsis*, *Trichoderma lentiforme, Trichoderma harzianum, Capsicum, PRGI.*

## Introduction

The *Capsicum* genus, belonging to the Solanaceae family, includes many important vegetables that are cultivated worldwide. Its importance lies in its applications in spices, medicine, ornamental purposes, and food preparation (Hulse et al., 2016; Quiñones et al., 2022). Over 350 varieties of this genus are cultivated in Peru, including crops such as paprika, yellow chili, rocoto, piquillo pepper, and bell pepper, with a combined national production totaling 184 000 tons (Ministerio de Desarrollo Agrario y Riego [MIDAGRI], 2021). Furthermore, in 2020, the top three exported species were paprika (28 %), piquillo pepper (22 %), and bell pepper (6 %) (Redagrícola, 2020). In 2022, the major export destinations were North America (63.2 %) and Europe (30.5 %) (Quintanilla, 2024).

Capsicum production is threatened by various soilborne pathogens, including those causing 'wilt' such as *Fusarium oxysporum*, *Phytophthora capsici*, and *Rhizoctonia solani* (Vallejo-Gutierrez et al., 2018). Among these soilborne pathogens, *P. capsici* is the causal agent of pepper wilt (Vélez-Olmedo et al., 2020). This pathogen is an oomycete, capable of infecting several plant families, such as cucurbits and solanaceous (Granke et al., 2012; Parada et al., 2021).

The symptoms caused by *P. capsici* vary depending on the plant host, the infected plant part, and environmental conditions (Lamour et al., 2012). In the *Capsicum* genus, it induces symptoms such as root rot, stem, and fruit rot, as well as leaf blight (Quispe et al., 2022; Vélez-Olmedo et al., 2020). Root infections can lead to damping off in seedlings, while in mature plants, delayed growth, wilting, and eventual death are commonly observed (Lamour et al., 2012).

The continuous search for new alternatives in biological disease control has led to an intensification in the detection of potential antagonistic microorganisms (Gonzáles et al., 1999). Among these microorganisms, those naturally present in the soil can serve as potential antagonists in controlling plant root pathogens (Volynchikova & Ki, 2022). The most commonly used genera include *Rhizobium*, *Pseudomonas*, *Bacillus*, *Streptomyces*, *Trichoderma*, and others (Ezziyyani et al., 2006).

*Trichoderma* provides many benefits in crops through multiple mechanisms of action, such as nutrient supply, phytohormone production, and antagonism against phytohormone production, and antagonism against phytopathogens (Valdés, 2014). In Mexico, Andrade-Hoyos et al. (2019)

indicated that different species of *Trichoderma* where effective against *Phytophthora capsici, Fusarium oxysporum,* and *Rhizoctonia solani.* Additionally, a study conducted in the Dominican Republic found that *Trichoderma harzianum* and *Trichoderma asperllum* exhibited a biocontrol effect against *Fusarium, Phytophthota, Rhizoctonia,* and *Pythium* in chili cultivatium (Moya, 2022). In our region, Huallanca & Cadenas (2014) evaluated *T. viride* (49.38 %) and *T. harzianum* (41.15 %), finding an inhibitory effect on the mycelial development of *P. capsici* at the in vitro level; however, in vivo tests did not manage to inhibit the development of the disease. Additionally, Romero et al. (2022) tested *T. viride* as a biocontrol agent against *P. capsici* in three planting methods (direct sowing, seedling, and bare root), where the highest control efficacy was observed in seedling and bare root planting with 10 % and 32 % effectiveness, respectively. These results present a promising outlook for biocontrol using microorganisms that demostrate outstanding perfomance without causing environmental toxicity (Volynchikova & Ki, 2022).

In agriculture, the ongoing search for antagonistic microorganisms with a high potential for effective biological control is vital to mitigate the overapplication of active ingredients to the soil. Currently, the limited alternatives available in the market for controlling *P. capsici* position biological control as an attractive option. Additionally, the diversity of soils in Peru presents a challenge for the adaptation of antagonistic microorganisms across different agroecosystems. Therefore, it is important to evaluate potential isolates of antagonistic fungi, including those of the genus *Trichoderma*, extracted from various regions and adapted to different conditions.

Therefore, this study aimed to evaluate the effectiveness of *T. koningiopsis* and *T. lentiformis*, both isolated from the Peruvian central rainforest, along with a commercial strain of *T. harzianum*. The scope of the study had two objectives: i) to evaluate the efficacy of *Trichoderma* isolates under in vitro conditions by determining the percentage of radial growth inhibition (PRGI) and ii) to evaluate their efficacy under in vivo conditions by measuring biometric parameters in capsicum plants. The present study will contribute to the analysis of these isolates as alternatives for the management of *P. capsici*, promoting sustainable management.

## Materials and methods

### Location

The experiment was conducted at the laboratory level (*in vitro*) and in a greenhouse (*in vivo*) (12.0799° S, 76.9510° W). Both phases were carried out at the Department of Plant Pathology's facilities at Universidad Nacional Agraria La Molina (UNALM) in 2022.

### *Trichoderma* isolates obtained from Peruvian rain forest

Each isolate of *T. koningiopsis* (TKSAT001) and *T. lentiforme* (TLSAT002) was acquired from the culture collection of The Plant Disease Clinic at UNALM. These isolates, previously morphologically and molecularly identified, were obtained from soil in "El Sinaín Farm" (11° 24' 15.9" S, 74° 21' 31.1" W), where cocoa (*Theobroma cacao*) was cultivated. The farm is located in San Martín de Pangoa district, Satipo Province, Junín region (Central rainforest of Peru). The isolates were preserved in 1.5 mL cryovials filled with distilled water, once filled they were double-sterilized. To activate the isolates, a portion of the mycelium was extracted from the cryovial using a sterilized wood stick in a laminar flow hood. This extracted mycelium was then placed on a Potato Dextrose Agar(PDA) medium. The Petri dishes were incubated at 25 °C for 3 d in dark conditions. Then, mycelial plugs, measuring 0.5 cm in diameter, were taken from a region of fungal growth and transferred onto PDA. These Petri dishes were sealed with parafilm, incubated at 25 °C, and used for *in vitro* and *in vivo* experiments.

### *Phytophthora capsici* isolate

*P. capsici* was isolated from "Katya" tomato roots exhibiting necrosis symptoms in the province of Mala, Lima. Corn meal agar (CMA) medium supplemented with Piramycin, Ampicillin, Rifampicin, and Benomyl (PAR-B) was used to isolate the pathogen. This isolate was identified

morphologically by the UNALM Plant Disease Clinic by microscopic observations of its structures and characteristics of the colonies following the 'Synoptic Keys of Phytophthora Species' by Ho (1981). Pathogenicity tests were carried out on "Ktya" tomato seedlings. Preservation and activation procedures were carried out following the protocol described previously for *Trichoderma* spp. After ten days of growth of *P. capsici* in PAR-B medium, 0.5 cm diameter plugs were cut from the actively growing mycelium. These plugs were then transferred to PDA and colony growth was monitored and used for in vitro and in vivo assays.

## *In vitro* phase

### Antagonistic activity of *Trichoderma* spp. against *P. capsici*

Dual confrontation assays were conducted between *Trichoderma* spp. and *P. capsici* isolates, following the methodology described by Bell et al. (1982) with modifications. For the experiment, 9 cm diameter Petri dishes and PDA media were used. Initially, a 5 mm diameter mycelial plug of *P. capsici* was placed 0.5 cm from the edge of the Petri dish. Five days later, a 5 mm diameter mycelial plug of *Trichoderma* spp. was strategically placed 6 cm away from the phytopathogen and 0.5 cm from the edge of the Petri dish. Seven treatments were considered for this test. Each treatment had five replicates and was tested twice (Table 1).

The Petri dishes were incubated at 25 °C in darkness. Evaluations were conducted every twenty-four hours for six days. The mycelial growth of the pathogen and antagonists was measured to determine the percentage of radial growth inhibition (PRGI). This parameter was calculated using the formula proposed by Ezziyyani et al. (2004): PRGI = $(r_1 - r_2) / r_1 \times 100$, where $r_1$ and $r_2$ represent the radius of the pathogen's mycelial growth in the control treatment and the confrontation test, respectively.

Additionally, the level of antagonism was evaluated by observing the growth of the pathogen's mycelium. The scale proposed by Bell et al. (1982) was used: Grade 1, where *Trichoderma* completely colonizes the pathogen and covers the entire surface of the medium; Grade 2, *Trichoderma* colonizes two-thirds of the surface of the medium; Grade 3, both *Trichoderma* and the pathogen colonize approximately half of the surface, with no organism dominating the other; Grade 4, shows the pathogen colonizing two-thirds of the medium's surface and appearing to resist invasion by *Trichoderma*; and Grade 5, where the pathogen completely colonizes *Trichoderma* and covers the entire surface of the medium.

## *In vivo* phase

### Preparation of *Trichoderma* inoculum

The protocol proposed by Hernández (2022) was followed with some modifications. Isolates were cultivated on Petri dishes with a PDA medium. The isolates were incubated in dark conditions at 25 °C ± 2 °C for three days to stimulate mycelial growth and fungal sporulation. This was followed by exposure to constant fluorescent light for an additional three days at room temperature (Lewis & Papavizas, 1985).

**Table 1.** Treatments of the dual confrontation between *Trichoderma* spp. and *P. capsici*, under *in vitro* and *in vivo* conditions

| Treatment | Experiment condition | |
|---|---|---|
| | *in vitro* | *in vivo* |
| T1 | *T. harzianum* + *P. capsici* | *T. harzianum* + *P. capsici* |
| T2 | *T. koningiopsis* + *P. capsici* | *T. koningiopsis* + *P. capsici* |
| T3 | *T. lentiforme* + *P. capsici* | *T. lentiforme* + *P. capsici* |
| T4 | *T. harzianum* | *T. Harzianum* |
| T5 | *T. koningiopsis* | *T. koningiopsis* |
| T6 | *T. lentiforme* | *T. lentiforme* |
| T7 | *P. capsici* | Positive Control (+*P.c.*) |
| T8 | - | Negative Control (-*P.c.*) |

After the Petri dishes with *Trichoderma* had sporulated, they were flooded with 6 mL of sterile distilled water. The Petri dishes were then scraped using a Drigalski spatula to release conidia. The resulting suspension was filtered through four layers of sterile gauze into a 1 L beaker to discard any mycelial remnants. Subsequently, 10 µL of the conidia suspension was extracted using a micropipette to determine the concentration using a Neubauer Chamber. Finally, the dilution was adjusted to a concentration of $10^8$ conidia/mL.

### Preparation and increase of *P. capsici* inoculum

This procedure was based on Romero et al. (2022) with some modifications as it will be described. For the increase in pathogen inoculum, precooked wheat grains were prepared and placed in polypropylene bags, which were then sterilized in an autoclave (15 min at 121 °C and 101.3 kPa).

In a laminar flow chamber, mycelial plugs of *P. capsici* growing in a PDA medium were obtained. These 5 mm plugs were extracted from the active region of mycelial growth. Subsequently, these plugs were placed inside polypropylene bags containing sterilized wheat grains. The bags with the inoculum were incubated at 25 °C and monitored until the mycelium completely invaded the substrate.

### Greenhouse antagonism test

Bell pepper cv. Piquillo seeds were sown in germination trays with a sterile substrate of sphagnum moss and vermiculite (Sunshine). One seed was placed per cell and kept in darkness for approximately five days until germination started. Subsequently, they were transferred to the greenhouse. After 45 d of growth, the seedlings were transplanted into 2 kg capacity pots containing agricultural soil, sand, and compost mixed at a ratio of 1:2:1, previously sterilized in the autoclave for 30 min at 121 °C and 101.3 kPa. The temperature was recorded daily during the experiment with an average of 22.5° C with a range of 15 °C, and 30 °C. This process was repeated twice.

The inoculation technique was adapted fron Eraso et al. (2014) for this study. Three inoculations were conducted with each Trichoderma isolate according to the described treatments (table 1). Initially, inoculation occurred during transplanting utilizing a drench method with 20 mL of a suspension containing 10 conidia/mL per seedling. Subsequent inoculations were conducted at 14 and 21 days after transplanting (dat), employing the same procedure as mentioned earlier. Pathogen inoculation was carried out at 21 dat, 30 min after transplanting (dat), employing the same procedure as mentioned earlier. Patogen inoculation was carried out at 21 dat, 30 mint after the third inoculation of Trichoderma isolates. The inoculation involved adding three grains of wheat (0.27 g) colonized by the pahtogen's hyphae at the root level of each seedling. Each experimental unit was considered for one plant. After inoculation, the pots were consistently watered every three days.

Seven days after the inoculation with *P. capsici* the assessment of foliar severity was conducted by observing secondary symptoms and classified according to the scale described by Romero et al. (2022). Furthermore, at the end of the experiment, root severity was assessed using the scale proposed by Kim & Hwang (1992) (Table

**Table 2.** Severity scale of leaf and root in the confrontation assay of *Trichoderma* spp. and *P. capsici* in pepper plants

| Grade | Foliar [a] | Root [b] | |
|---|---|---|---|
| | Description | Description | % Severity |
| 0 | Healthy root, healthy vigorous plant | Healthy plant | 0 |
| 1 | Dark brown color starting to appear on the stem or brown lesions on the leaves | Leaf epinasty | >0-30 |
| 2 | Dark roots with slightly very small lesions on the stem | Pronounced epinasty | > 30-50 |
| 3 | Dark root, large lesions on the stem | Epinasty plus early leaf fall | > 50-70 |
| 4 | Dark root, large lesions on the stem, entire plant wilted and growth halted | Severe leaf loss | > 70-90 |
| 5 | Dead plant | Dead plant | > 90-100 |

[a] Adapted from Romero et al. (2022); [b] Adapted from Kim & Hwang (1992).

2). Other variables were also recorded, such as plant height, number of leaves, root length, as well as total fresh and dry weight.

### Statistical analysis

For both *in vitro* and *in vivo* experiments, a Completely Randomized Design (CRD) was applied, and Tukey's mean comparison test with a significance level of 0.05 was conducted using the statistical software InfoStat. The *in vitro* assay comprised seven treatments, each with five replications (i.e., five Petri dishes), while the *in vivo* phase involved eight treatments, also with five replications (i.e., five pots).

## Results and discussions

### Percentage of radial growth inhibition (PRGI)

All *Trichoderma* species demonstrated PRGI values ranging from 36 % to 38 % and 50 % to 51 % against *P. capsici* three and seven days after the assay started, respectively. *T. harzianum* exhibited the lowest PRGI values against *P. capsici*, obtaining 36.05 % ± 1.06 % and 50.02 % ± 0.83 % on the third and seventh days of pathogen colony growth, respectively. In both *T. koningiopsis* (KSAT001) and *T. lentiforme* (TLSAT002) against *P. capsici*, the antagonist exhibited higher PRGI values compared to other treatments, reaching 38.08 % ± 0.73 % and 37.91 % ± 0.53 % at three days, and 51.60 % ± 0.57 % and 51.47 % ± 0.44 % on the seventh day, respectively. PRGI values obtained with *T. koningiopsis* and *T. lentiforme* showed no statistical differences, but both showed differences with *T. harzianum*. Regarding antagonistic activity, significant differences were observed among thetreatments. *T. lentiforme* exhibited 100 % in its antagonistic capacity (grade 1), showing significant differences with *T. koningiopsis* (66.67 %) and *T. harzianum* 59.96 %, which obtained a grade 2 (Table 3).

These results differ from those found by Mousumi (2019), where, four days after the confrontation between *P. capsici* and *T. harzianum*, PRGI values of 70.9 % ± 0.3 % and 100 % were obtained when applying 0.1 mg/µL and 0.25 mg/µL of the antagonist, respectively. Likewise, total inhibition was observed with *T. asperellum* strain AFP, and a value of 59.0 % ± 0.4 % was obtained with *T. brevicompactum* using 0.25 mg/µL. Additionally, Andrade-Hoyos et al. (2019) obtained PRGI values of 88.25 %, 87.22 %, and 87.8 % with *T. asperellum*, *T. viride*, and *T. harzianum*, respectively, against *P. capsici*, showing no significant differences among them.

The inhibition of *Trichoderma* species evaluated on *P. capsici* can be explained by Romo & Ávila (2000) as cited by Eraso et al. (2014), who mentioned that this genus presents antifungal and antibacterial properties, as well as extracellular enzymes that degrade the host's cell walls, facilitating their penetration. However, the antagonistic action depends on the production of extracellular enzymes corresponding to the composition and structure of the cell walls of the parasitized fungi (Infante & Martínez, 2019). Although our study did not include microscopic observations to understand the mechanism of the antagonist on the pathogen, we observed *Trichoderma koningiopsis* and *T. lentiforme* hyphal growth in all treatments on the *P. capsici* mycelium at a macroscopic level. Notably, *T. koningiopsis* and *T. lentiforme* exhibited sporulation, with the latter being particularly abundant, potentially supporting its hyperparasitic ability (Sandoval & López, 2001; Swapan et al., 2022).

### Effect on morphological variables

### Plant height and number of leaves

The evaluation was conducted at 45 dat. *T. lentiforme* vs. *P. capsici* exhibited the greatest height at 21.11 cm ± 4.30 cm, followed by *T. koningiopsis* vs. *P. capsici* with 18.24 cm ± 3.19 cm. Both treatments showed significant differences compared to each other, as well as

**Table 3.** Antagonistic capacity of *Trichoderma* spp. against *P. capsici* in dual confrontation tests.

| Treatment | % PRGI[a] | | | | ACG[b] |
|---|---|---|---|---|---|
| | 3 dai[c] | ds | 7 dai | ds | |
| *T. harzianum v.s P. capsici* | 36,05 b | 1.06 | 50.02 b | 0.83 | 2 b |
| *T. koningiopsis v.s P. capsici* | 38,08 a | 0.73 | 51.60 a | 0.57 | 2 b |
| *T. lentiforme v.s P. capsici* | 37,91 a | 0.56 | 51.47 a | 0.44 | 1 a |

[a] % PRGI: Percentage Relative Growth Inhibition; [b] ACG: Antagonistic Capacity Grades
[c] dai: days after inoculation

the negative control (-P.c.) and positive con control (+*P.c.*). The treatment *Trichoderma* ssp. vs *P. capsici* resulted in greater plant heights compared to treatments in which only *Trichoderma*, whitout the presence of *P. capsici*,where aplied. Among the aplications where only the antagonists were used, *T. lentiforme* and *T. koningiopsis* showed the highest (15.31 cm ± 1.21 cm) and lowest (10.71 cm ± 1.54 cm) values, respectively. *T. koningiopsis* did not exhibit statical differences compared to the negative control (-*P.c.*). Regarding the number of leaves, *Trichoderma* spp. vs. *P. capsici* did not show statical differences among treatments. However, they showed significant differences compared to the positive control (+ *P.c.*), which only had two leaves (Table 4).

The effect of *Trichoderma* spp. on plant height could be explained by Olmedo & Casas (2014) and Harman et al. (2004). who mentioned that this genus induces the production of growth hormones in plants, which results in better vegetal development. The results of our study are similar to those found by Candelero et al. (2015), who observed in *Capsicum chinense* that some isolates of *Trichoderma* spp. showed a significant effect on plant growth. Additionally, Danger et al. (2000), as cited by Candelero et al. (2015), reported increases in the height of *Solanum lycopersicum* seedlings from 9 % to 11 % compared to the negative control through the application of *T. harzianum*. Similarly, Cruz &Cisternas (1998) mentioned that *T. harzianum* increased plant height compared to the positive control (+*P.c.*) in pepper cultivation under greenhouse conditions.

In terms of leaf count, other studies have reported that maize plants inoculated with strains of *T. spirale* and *T. melanomagnum* exhibited a higher number of leaves (Cuenca et al., 2022). Similar findings were reported by Cruz & Cisternas (1998). They concluded that *T. harzianum* not only contributes to an increase in the number of leaves but also enhances the length and dry weight of roots and leaves. Furthermore, it increases the leaf area, stem diameter, and the number of flowers.

**Root length**

In the in vivo assay, it was observed that the root legths of plants inoculated with *T. lentiforme* and *T. kiningiopsis* against *P. capsici* were the highest, showing an average of 11.02 cm ± 1.39 cm and 9.52 cm ± 2.79 cm, respectively. Additionally, these treatments exhibited statistically significant differences compared to the others (Table 4). On the other hand, the root lengths of plants inoculated solely with *Trichoderma* spp. did not exhibit statistically significant differences among themselves or compared to the negative control (-*P.c.*). These findigs contrast with those reported by Nawaz et. (2018), who investigated nine *Trichoderma* species and observed greater root lengths compared to the negative control (-*P.c.*).
Furthermore, a study conducted by Rodríguez & Vargas (2022) demonstrated that plants inoculated with *T. asperelloides* displayed significantly longer root lengths compared to commercially available strains of *T. guizhouense* and *T. asperellum*, with differences of 11 cm and 24 cm, respectively. These results suggest that certain *Trichoderma* isolates may exhibit greater efficacy in promoting plant root growth under greenhouse conditions compared to commercial isolates, a phenomenon that was only observed in our investigation when the plants were inoculated with the antagonist and the pathogen.

| Treatment | Plant height | ds | N° of leaves | ds | Root length | ds |
|---|---|---|---|---|---|---|
| *T. harzianum v.s P. capsici* | 12.89 d | 0.89 | 4.57 ab | 1.40 | 5.09 c | 0.20 |
| *T. koningiopsis v.s P. capsici* | 18.24 b | 3.19 | 5.14 a | 1.46 | 9.52 b | 2.79 |
| *T. lentiforme v.s P. capsici* | 21.11 a | 4.30 | 5.00 a | 1.15 | 11.02 a | 1.39 |
| *T. harzianum* | 12.83 d | 1.54 | 3.43 bc | 1.81 | 4.58 c | 0.31 |
| *T. koningiopsis* | 12.83 d | 1.21 | 3.43 bc | 1.00 | 4.58 c | 0.43 |
| *T. lentiforme* | 10.71 e | 1.62 | 3.43 bc | 0.98 | 4.97 c | 0.54 |
| Positive Control (+*P.c.*) | 8.27 f | 0.85 | 2.29 c | 0.49 | 4.38 c | 0.58 |
| Negative Control (-*P.c.*) | 10.37 e | 1.92 | 4.57 ab | 1.13 | 4.90 c | 0.24 |



**Table 4.** Effect of *Trichoderma* spp on plant height (cm), number of leaves, and root length.

Our findings suggest that the cohabitation of *Trichoderma* with the plant pathogen produces a favorable effect on root growth, contrasting with the presence of only of *Thrichoderma,* the antagonist. It has been noted that the interactions between *Trichoderma* spp. and plant pathogens may exhibit variability due to components of their secretion, including volatile organic compounds, cell wall-degrading enzymes, reactive oxygen species, and antimicrobial secondary metabolites. These interactions enhance plant fitness and biotic stress resilience by eliciting plant defense mechanisms, promoting growth, or mitigating pathogen pressure (Alfiky & Weisskopf, 2021). Additionally, researchers have postulated that the impact of *Trichoderma* species on root growth and plant development may be linked both to the displacement and regulation of root microflora. Moreover, studies have suggested that *Trichoderma* has potential direct effects on plant biochemistry (Wyndham et al., 1986; Harman, 2000). Hence, it is proposed that the outcomes observed in the *Trichoderma* spp. vs *P. capsici* treatments may be related to the activation of defense mechanisms or growth-promoting factors induced by the presence of the pathogen. This phenomenon underscores the existence of intricate interactions within the soil-plant-antagonist-phytopathogen system, warranting further detailed investigation.

## Total fresh and dry weight

*T. lentiforme* and. *T. koningiopsis*, each vs. *P.capsici* presented fresh weight values of 1.40 g ± 0.46 g and 1.23 g ± 0.21 g, respectively. No statistical differences were shown between these two treatments. In contrast, *T. harzianum* vs. *P. capsici* showed a significantly lower value of 0.42 g ± 0.13 g. This same trend was observed among plants inoculated solely with *Trichoderma* spp., where the highest fresh weight was obtained with *T. lentiforme*, at 1.06g ± 0.37 g. This was followed by *T. koningiopsis* and *T.harzianum,* at 0.41 g ± 0.10 g and 0.33 g ± 0.06 g, respectively. The positive (+*P.c.*), and negative control (-*P.c.*) presented fresh weight values of 0.16 g ± 0.02 g and 0.56 g ± 0.11 g, respectively, with the former being the lowest value among the evaluated treatments (Figure 1) Uddin et al. (2018) reported the inhibitory activity of *T. harzianum* against *P. ultimum* and *P. capsici* in tomato plants. However, unlike our results, their study observed a significant increase in the fresh weight of the plants inoculated with this species. Additionally, Larios et al. (2019) reported that treatments with *Trichoderma* spp. resulted in the highest fresh and root biomass, ranging from 0.8 g ± 0.02 to 0.12 g ± 0.01 g, with the positive control (+*P.c.*) significantly lower. It is worth noting the increase in fresh biomass attributed to the activity of *T. lentiforme* (TLSAT002) compared to other treatments

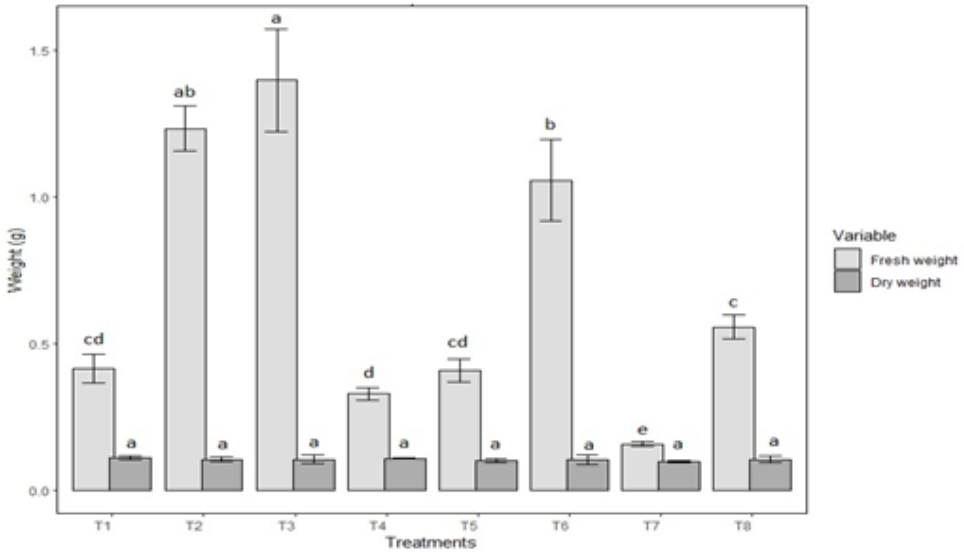


**Figure 1.** Comparison of treatments with *Trichoderma* spp., *Phytophthora capsici* on fresh and dry weight in healthy and diseased pepper plants.

Note: T1: *T. harzianum* vs *P. capsici*, T2: *T. koningiopsis* vs *P. capsici*, T3: *T. lentiforme* vs *P. capsici*, T4: *T. harzianum*, T5: *T. koningiopsis*, T6: *T. lentiforme*, T7: Positive control (+*P.c.*), T8: Negative control (-*P.c.*)

This indicates the stimulating potential that certain isolates have on plants. Viracocha & Cadena (2023), as well as Bader et al. (2020), also reported an increase in fresh weight yield in plants inoculated with *T. harzianum* compared to the positive control (+ *P.c.*), with increases of 25 % and 100 %, respectively.

Concerning dry weight, the treatments exhibited no significant differences. However, the positive control (+*P.c.*) presented a lower value, at 0.11 g ± 0.01 g. These results align with those reported by Huallanca & Cadenas (2014) and Bader et al. (2020). In these studies, the *Trichoderma* treatments showed no differences, and the positive control (+*P.c.*) value was numerically lower. In contrast, Romero et al. (2017) found that the positive control (+*P.c.*) demonstrated a significantly lower dry weight compared to the other *Trichoderma* treatments. Similarly, Sid et al. (2006) reported a 68.6 % reduction in the dry weight of inoculated plants compared to the negative control (-*P.c.*).

**Root and leaf severity**

Root severity was evaluated at the end of the trial. Treatments *T. harzianum*, *T. koningiopsis*, and *T. lentiforme*, when confronted with *P. capsici*, exhibited a root severity percentage of 27.5 % (grade 1), 33.13 % (grade 2), and 30.63 %. On the other hand, the positive control (+*P.c.*), recorded the highest values compared to the other treatments, reaching a severity percentage of 81.25 % (grade 4), showing symptoms of darkened roots, large lesions on the stem, general wilting throughout the plant, and halted growth. These results are consistent with those obtained by Romero et al. (2017), who also observed the highest severity and root incidence values in the treatment with *P. capsici* in tomato plants compared to all studied treatments. Additionally, Sid et al.(2006) mentioned that the presence of *T. harzianum* in the substrate reduced root rot caused by *P. capsici* in pepper plants. Furthermore, in a study conducted by Athafah et al. (2020), it as observed that their strain of *T. virens* managed to reduce disease severity by 64.2 % five days after inoculation, compared to the positive control (+*P.c.*), under *in vivo* conditions. Despite the infection of *P. capsici* in treatments with *Trichoderma* spp, no secondary symptoms were observed in the aerial part with 0 % foliar severity (grade 0). In contrast, the positive control (+*P.c.*), obtained a 60.63 % (grade 3), presenting leaf epinasty. The low level of foliar severity (Grade 0) in treatments with *Trichoderma* spp. could be explained by the control action exerted by the antagonist (Figure 2). According to a study conducted by Nawaz et al. (2018), the authors observed that the interaction between *Trichoderma* species and *P. capsici* strains affected the development of

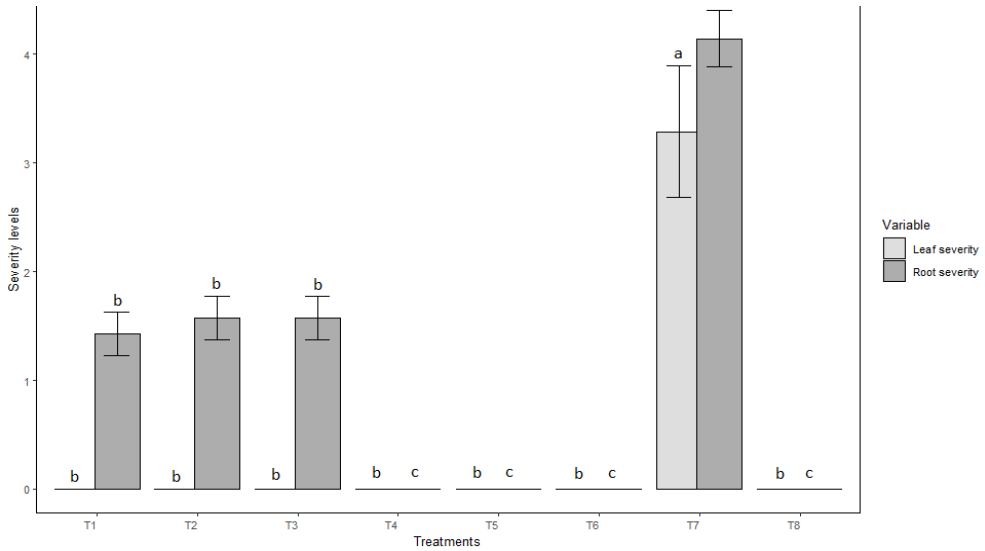


**Figure 2.** Comparison of the severity in the leaf area and roots in diseased cans of pepper between treatments.

Note: T1: *T. harzianum* vs *P. capsici*, T2: *T. koningiopsis* vs *P. capsici*, T3: *T. lentiforme* vs *P. capsici*, T4: *T. harzianum*, T5: *T. koningiopsis*, T6: *T. lentiforme*, T7: Positive control (+*P.c.*), T8: Negative control (-*P.c.*)

zoospore content and hyphal lysis. Additionally, this reduction can be attributed to competition for nutrients and space, negatively impacting the pathogen's development. It has been reported that species like *T. harzianum* exhibit high cellulase activity, with this enzyme being important for the degradation of the cell walls of phytopathogenic fungi during their infection process (Cherkupally et al., 2017; Kamala & Indira, 2014).

Finally, the results obtained, along with other studies, support the potential of *Trichoderma* spp. in the control of plant pathogens. Furthermore, they underscore the need to investigate the mechanisms of action of this genus for its eventual application in an integrated crop management approach.

## Conclusion

At the *in vitro* level, the isolates of *T. lentiforme* and *T. koningiopsis* obtained a PRGI of 51.47 % and 51.60 %, respectively, showing significant differences compared to *T. harzianum*, which had a value of 50.02 %. under greenhouse conditions, treatment with *T. lentiforme* against *P. capsici*, as well as treatment with *T. lentiforme* alone, showed superior results compared to both positive (+ *P.c.*), and a negative control (- *P.c.*) in various variables, including plant height, root length, and total fresh and dry weight. These findings highlight the efficacy of *Trichoderma lentiforme* against *P. capsici* and its effect on the growth of pepper plants. Based on these results, the use of native *Trichoderma* spp. strains isolates from Peruvian rainforest areas demonstrate potential in biocontrol and growth promotion in pepper cultivation, both *in vitro* and greenhouse conditions.

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## Conflict of Interest

The authors declare no conflict of interest regarding the publication of this manuscript.

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## Authors contribution

All authors contributed to all stages of manuscript preparation.

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