

Biological control of vascular wilt by *Trichoderma* spp. for peanut crops

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Abstract

In Mexico, peanut crops are a productive activity in rural areas; nevertheless, they are threatened by fungal diseases, such as vascular wilt, caused by *Fusarium incarnatum*, recently reported in the country. The study assessed the antagonistic capacity of five *Trichoderma* species against the 'MA-PET-03' strain of *F. incarnatum* in peanut crops in Buenavista de Benito Juárez, Chietla, Puebla. It was highlighted that *T. koningiopsis* showed the highest growth rate and the highest percentage of radial growth inhibition (PRGI) of *F. incarnatum* in *in vitro* tests. Under field conditions, treatments with *Trichoderma* spp. had more peanut pods per plant and lower incidence of the disease, improving crop yield. These results confirm the effectiveness of *Trichoderma* spp. for managing vascular wilt in the region.

Keywords:

Trichoderma spp., antifungal activity, basal necrosis.



Introduction

Peanut (*Arachis hypogaea* L.) crops are of great importance worldwide due to their economic and nutritional benefits (Akram *et al.*, 2018). In addition, they are a vital source of protein, healthy fats, and essential vitamins and minerals, such as iron, calcium, phosphorus, magnesium, selenium, and zinc, as well as vitamin E, B6, riboflavin, thiamine and niacin (Montero-Torres, 2020). Beyond their nutritional value, peanuts play a crucial role in the food security and economy of many regions, providing sources of employment and direct income to farmers in local communities (Desmae *et al.*, 2018).

The Food and Agriculture Organization of the United Nations (FAO) mentions that the main producers of peanuts are China, India, Nigeria, the United States, Argentina, and South Africa (FAO, 2023). In Mexico, the largest peanut-producing states are Chihuahua, Sinaloa, Chiapas, Puebla, and Oaxaca, representing 77.2% of the total cultivated area for the 2022-2023 agricultural cycle, where it reached 47 532 ha, with a production of 81 413 t.

The state of Puebla ranked third in national production with 9.31 t (SIAP, 2024). Despite the success of the crop, peanuts are vulnerable to various diseases caused by fungi, bacteria, and viruses that negatively impact yield, with fungal diseases being of particular concern due to significant economic losses (Thirumalaisamy *et al.*, 2019).

Fungal diseases affecting peanut crops include wilt caused by *Fusarium oxysporum* Schlecht, pod rot caused by *Fusarium equiseti* (Corda) and *Fusarium solani* (Mart.), and vascular wilt caused by *F. incarnatum*, recently reported in Mexico (Romero-Arenas *et al.*, 2024). *F. incarnatum* is classified within the *F. incarnatum-equiseti* species complex (FIESC), which comprises 33 phylogenetic species in a wide range of habitats and hosts worldwide (Wang *et al.*, 2019).

It is characterized by an initially white aerial mycelium that with age produces orange sporodochia. As a phytopathogen, it can infect peanut plants, causing symptoms of chlorosis, leaf wilt, and stem and root rot, leading to premature death of the host plant (Romero-Arenas *et al.*, 2024). The control of phytopathogenic fungi is mainly based on the use of fungicides.

Nonetheless, the application of chemical compounds is not recommended for diseases caused by soil-dwelling fungi due to their high costs, low efficiency, and possible toxicity to humans and the environment, which could lead to the development of fungicide-tolerant strains in short periods of time (Andrade-Hoyos *et al.*, 2019). In this context, *Trichoderma* species have emerged as potential agents that can play a fundamental role in integrated disease management in agriculture (Bokade *et al.*, 2021).

The *Trichoderma* genus is recognized for its antagonistic ability against various pathogens. It can interact synergistically with fungicides, improve plant resistance to diseases, and minimize the risk of developing resistance to different fungicides (Asad *et al.*, 2022). Therefore, the objective was to evaluate the antagonistic capacity of five species of *Trichoderma* against the 'MA-PET-03' strain of *F. incarnatum*, as well as the reduction of the incidence of the disease and the effect on the productivity of peanut crops, in the rural community of Buenavista de Benito Juárez, located in the municipality of Chietla, Puebla, Mexico.

Materials and methods

Biological material

The strains used for this study were: *Trichoderma harzianum* (T-H4), *Trichoderma koningiopsis* (T-K11), *Trichoderma asperellum* (T-AS1), and *Trichoderma hamatum* (T-A12), isolated from the rhizosphere of avocado (*Persea americana*). The sequences of these strains are available in the National Center for Biotechnology Information (NCBI) database with accession numbers: MK779064, MK791648, MK778890 and MK791650, respectively.

Additionally, a strain of *T. harzianum* (T-Ah) MW227646 native to the study region, isolated from the rhizosphere of the peanut crop (*Arachis hypogaea* L.), was included. The pathogen used was the MA-PET-03 strain of *Fusarium incarnatum*, recently reported in Mexico (Romero-Arenas *et al.*, 2024), associated with peanut vascular wilt, with accession numbers: OQ679820, OQ679821. All the strains are safeguarded in the Phytopathology Laboratory 204 of the Center for Agroecology, Institute of Sciences-Meritorious Autonomous University of Puebla (BUAP, for its acronym in Spanish).

***In vitro* evaluation of the antagonistic capacity of *Trichoderma* spp.**

To assess mycelial growth, 5 mm diameter fragments of 10-day-old *Trichoderma* and *F. incarnatum* strains were inoculated in Petri dishes with potato dextrose agar medium (PDA: Bioxon, Becton Dickinson, Mexico City, Mexico) and incubated in the dark at 28 °C for 10 days. Mycelial diameter was measured every 12 hours with a digital Vernier (CD-6 Mitutoyo, Naucalpan de Juárez, Mexico). The growth rate (cm day^{-1}) was calculated using the growth equation $y = mx + b$. Where: y = distance; m = slope; x = time; b = constant.

The experiment was conducted in duplicate in a completely randomized experimental design, with three replications per treatment. To determine the percentage of radial growth inhibition (PRGI), the dual culture technique was used according to Andrade-Hoyos *et al.* (2019). Where: $\text{PRGI}(\%) = (R1 - R2) / R1 \times 100$. Where: PRGI= percentage of radial growth inhibition, $R1$ = radial growth (mm) of *F. incarnatum* without *Trichoderma* spp. and $R2$ = radial growth (mm) of *F. incarnatum* with *Trichoderma* spp. Five-millimeter diameter fragments of 10-day-old *Trichoderma* and *F. incarnatum* strains were inoculated at opposite ends of Petri dishes containing PDA, with a separation of 7.5 cm between them (antagonist-phytopathogen) and incubated at 28 °C for 10 days.

The radial growth of the fungal colony was assessed every 12 h until the first contact between the mycelia of the antagonist and *F. incarnatum*, using the scale proposed by Bell *et al.* (1982), where: I) the growth of *Trichoderma* spp. covered the entire surface of the medium and reduced the colony of *Fusarium* sp.; II) the growth of *Trichoderma* spp. covered at least $\frac{2}{3}$ of the medium; III) *Trichoderma* spp. and *F. incarnatum* grew $\frac{1}{2}$ and $\frac{1}{2}$ of the surface of the medium; IV) *F. incarnatum* grew at least $\frac{2}{3}$ of the medium and resisted invasion of *Trichoderma* spp. and V) growth of *F. incarnatum* covered the entire surface of the medium.

Evaluation of disease incidence and effect on crop productivity

An *in situ* test was conducted under open field conditions in the community of Buenavista de Benito Juárez (18° 27' 39" north latitude; 98° 37' 11" west longitude), located in the municipality of Chietla, state of Puebla, Mexico. 1 500 peanut seeds of the 'Virginia Champs' variety, provided by community producers, were used and disinfected with 0.3% (v/v) sodium hypochlorite for 10 min, then rinsed three times with sterile water and dried with sterile paper (Illa *et al.*, 2019).

Seeds were sown using a standardized mechanical procedure in germination trays with Peatmoss and Agrolite (1:1 v/v), previously sterilized at 121 °C and 15 psi pressure (Mannai *et al.*, 2018). One seed per cavity was placed at a depth of 1 cm. Mancozeb 80[®] was used as a chemical control agent, following the manufacturer's recommendations for seedling production (1 kg of seed per 5 g of product). Finally, the trays were covered with black plastic for 10 days and kept at a temperature of 27 °C to facilitate germination.

Preparation of the land and planting

The peanut producers prepared the land three months before starting the experiment, ploughing the soil to a depth of 50 cm to reduce compaction and level the soil, then ploughing it two more times at a depth of 30 cm to improve aeration and ensure a uniform texture.

The transplant was carried out on July 10, 2022, using 30-day-old peanut seedlings, planted at a depth of 8 cm. It was fertilized with 40 kg ha⁻¹ of phosphorus (P) and 60 kg ha⁻¹ of potassium (K) 15

days after transplant (DAT). The planting density was 4 plants m^{-2} , 35 cm apart, distributed in 26 rows 60 cm apart, forming experimental blocks of two rows each in a straight line.

The experimental design was done in completely randomized blocks with eight treatments, using 100 plants for each with eight replications, including the control group. Four plants were left on the edges, which were not considered, specifying 800 seedlings established for the present study.

Inoculation of *F. incarnatum* was performed 15 DAT at the base of each peanut plant (100 seedlings per treatment) with 2 ml of sterile saline solution at a concentration of 1×10^8 conidia ml^{-1} , obtained using a Neubauer chamber (Paul Marienfeld). After 36 h, the plants were inoculated with a solution of spores of the strains *T. harzianum* (T-H3), *T. asperellum* (T-AS1), *T. hamatum* (T-A12), *T. koningiopsis* (T-K11) and the native strain (T-Ah) at the same concentration as the pathogen (1×10^8 conidia ml^{-1}) for each treatment. Cercobin[®] (thiophanate-methyl) was applied as a chemical control group, following the manufacturer's recommendations (500 g in 400 L of water ha^{-1}). Finally, for the control group, only 2 ml of sterile water was applied.

The expected incidence of the disease was measured in relation to the total length of the root and was classified according to the scale proposed by Bokhari and Perveen (2012), where: 0 \leq 25% severity, 1= 26 to 50% severity, 2= 51 to 75% severity and 3 \geq 76% severity. In addition, complementary variables were recorded, such as the total fresh weight of each plant, the dry weight of the pods per plant, the number of pods per plant, and the weight of 100 peanut grains per treatment. Likewise, the yield was calculated according to Zamurrad *et al.* (2013) at the end of the production cycle.

Statistical analysis

Radial growth inhibition (PRGI) data were expressed in percentages and transformed using the angular expression $\sqrt{x+1}$. A Bartlett homogeneity test was performed, followed by a Tukey-Kramer comparison of means test, with a significance level of $p \leq 0.05$, using the SPSS Statistics statistical package, version 17 for Windows (Stehlik-Barry and Babinec, 2017). In the field stage, the variables were analyzed using a two-way analysis of variance (Anova) followed by a Tukey-Kramer comparison of means test, using the same statistical package.

Results

The results of the study on the rate of development and growth rate showed statistically significant differences ($p = 0.0021$). *T. koningiopsis* (T-K11) reached the highest values, with 2.2 ± 0.15 mm h and 2.33 ± 0.01 cm day, respectively. In addition, it was classified as class I on Bell's antagonism scale. In addition, there were areas of interaction between *T. koningiopsis* (T-K11), *T. harzianum* (T-H3), *T. hamatum* (T-A12), *T. asperellum* (T-AS1) and the native strain (T-Ah) against *F. incarnatum*, where a parasitism of more than 80% was achieved at 240 h.

The highest percentage of radial growth inhibition (PRGI) obtained for the phytopathogenic fungus MA-PET-03 was 92.34% when faced with *T. koningiopsis* during the 10 days of evaluation (Table 1), followed by *T. harzianum* (84.44%), showing statistically significant differences ($p < 0.05$). The native strain 'Th-Ah' of *T. harzianum* presented the lowest PRGI, with 74.11% for this study (Figure 1).



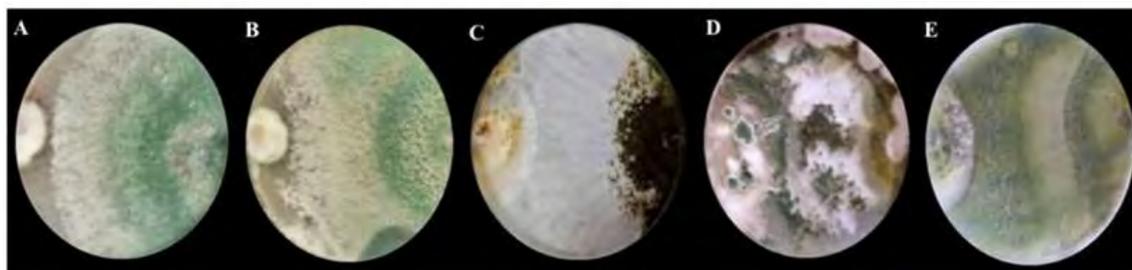
Table 1. Development rate, growth rate, and PRGI.

Strains	Development rate (mm h ⁻¹) [*]	Growth rate (cm d ⁻¹) [*]	PRGI (%) [*]
<i>T. harzianum</i> (T-H3)	1.8 ±0.02 ^c	2.4 ±0.01 ^a	84.44 ^b
<i>T. asperellum</i> (T-AS1)	1.82 ±0.01 ^c	2.09 ±0.03 ^d	82.78 ^c
<i>T. hamatum</i> (T-A12)	1.95 ±0.04 ^b	1.3 ±0.01 ^e	79.52 ^d
<i>T. koningiopsis</i> (T-K11)	2.2 ±0.15 ^a	2.33 ±0.01 ^b	92.34 ^a
<i>T. harzianum</i> (Th-Ah) 'Native'	1.79 ±0.03 ^c	2.16 ±0.2 ^c	74.11 ^e
<i>F. incarnatum</i> (MA-PET-03)	0.56 ±0.02 ^d	1.08 ±0.03 ^f	-

^{*} = different letters indicate a significant difference between treatments ($p < 0.05$).

Figure 1

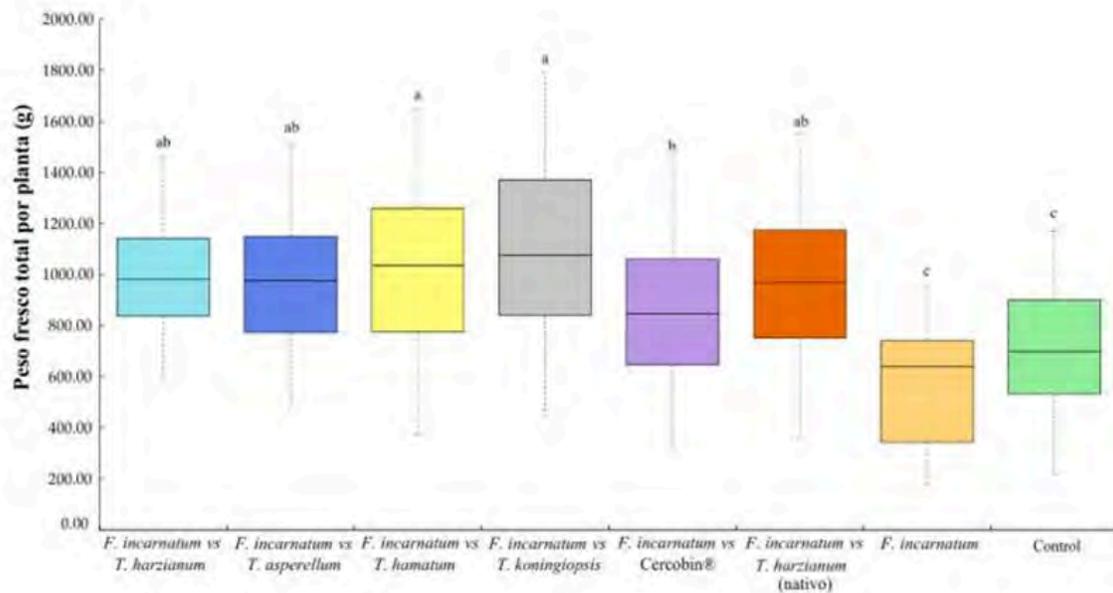
Antagonism of *T. harzianum* (A); *T. asperellum* (B); *T. hamatum* (C); *T. koningiopsis* (D) and native *T. harzianum* (E) with *F. incarnatum* on Bell *et al.* (1982) scale after 240 h in dishes with PDA medium, incubated at 28 °C. Class I antagonism (D) and class II antagonism (A, B, C and E).



Field results showed that treatments with antifungal activity were effective in reducing vascular wilt in the root, stem, and fruit of Virginia Champs peanut plants. It was confirmed that *T. koningiopsis* (T-K11) was the most effective treatment in promoting total plant growth, reaching a weight of 1 080.08 ±25.69 g (Figure 2), reducing the incidence of vascular wilt by 90%, similar to *T. hamatum* (T-A12). Nevertheless, the *T. harzianum* (T-H3) and *T. asperellum* (T-AS1) showed a 50% reduction in disease.

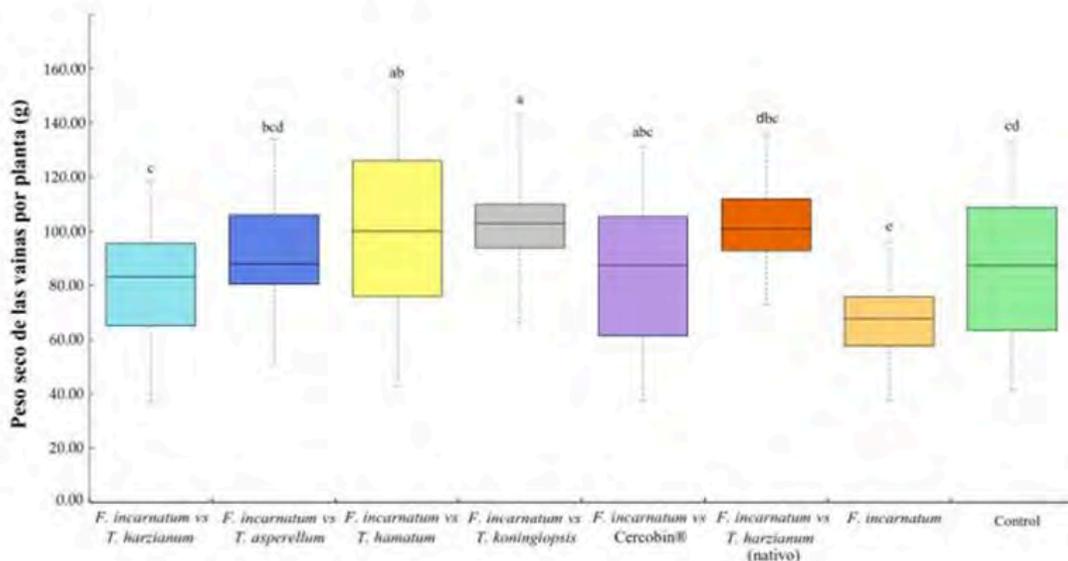


Figure 2. Fresh weight per peanut plant inoculated with different treatments. ^{abc}= different letters indicate a significant difference between treatments ($p < 0.05$).

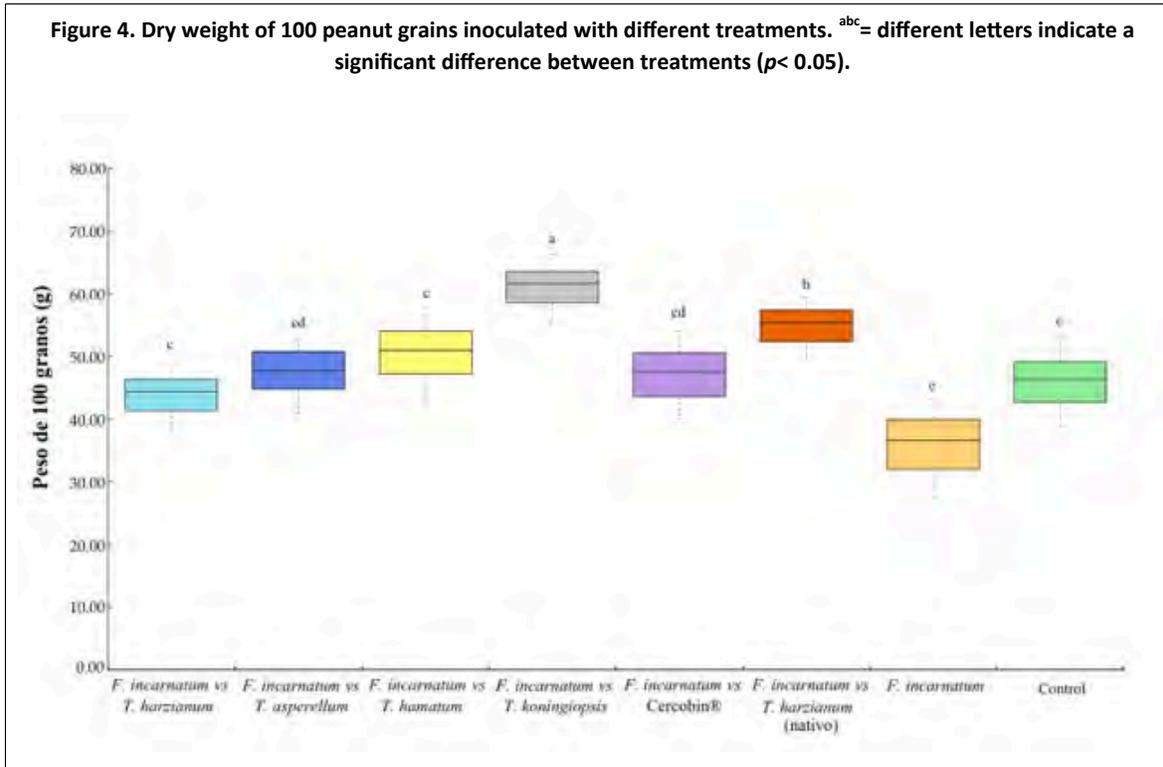


The chemical treatment (Cercobin®) reduced the impact of *F. incarnatum* by 20%, with a total weight of 91.06 ± 32.18 g. However, *T. koningiopsis* (T-K11) was the most effective treatment, achieving a dry weight of pods of 103.82 ± 4.11 g, compared to the lowest weight (Figure 3) in treatment with *F. incarnatum* (69.82 ± 3.28 g).

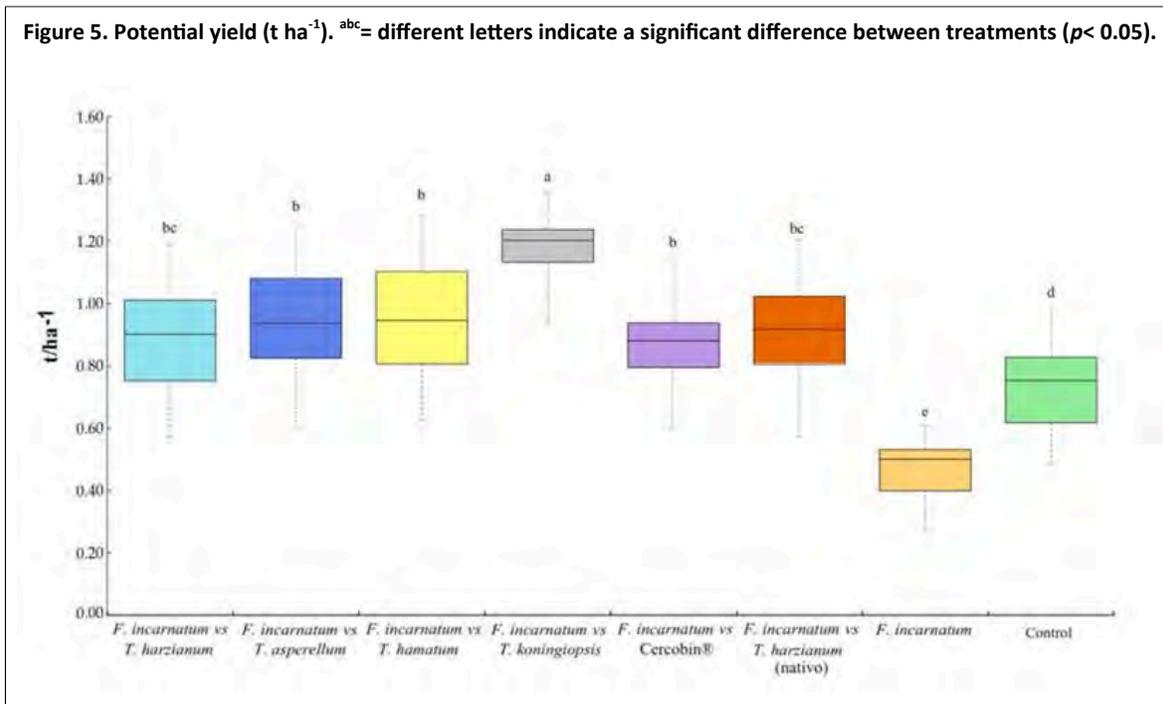
Figure 3. Dry weight of pods per peanut inoculated with different treatments. ^{abc}= different letters indicate a significant difference between treatments ($p < 0.05$).



In addition, the dry weight of 100 peanut grains (Figure 4) in treatment with *T. koningiopsis* showed highly significant differences ($p \leq 0.05$).

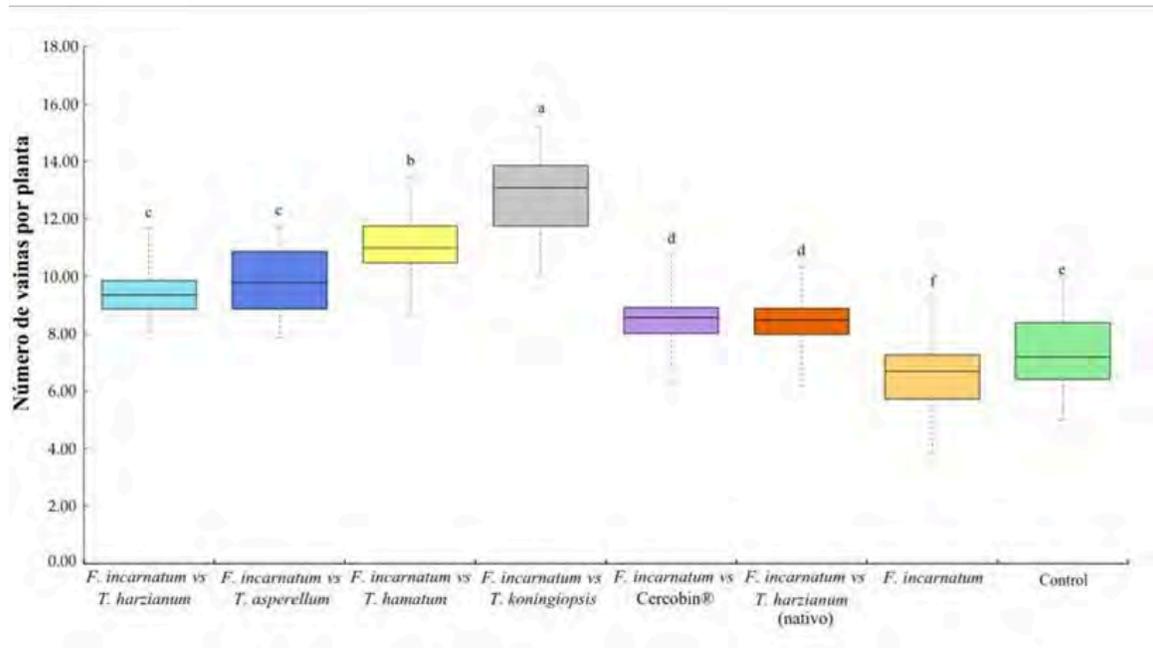


The potential yield per hectare showed highly significant differences between treatments ($p = 0.00012$), with *T. koningiopsis* (T-K11) presenting the highest production (1.2 t ha^{-1}), with 13.1 pods per plant (Figure 5). This indicates that *T. koningiopsis* holds promise to improve peanut productivity.



The chemical treatment (Cercobin®) was characterized by no statistically significant differences with *T. harzianum*, *T. asperellum*, *T. hamatum*, and native *T. harzianum*, obtaining a yield between 0.88 and 0.93 t ha⁻¹ for this research (Figure 6). The lowest production was observed in plants inoculated only with *F. incarnatum* (0.51 t ha⁻¹), with 6.67 pods plant⁻¹ (25.48 pods m⁻²).

Figure 6. Number of pods per peanut plant inoculated with different treatments. ^{abc}= different letters indicate a significant difference between treatments ($p < 0.05$).



Discussion

The genus *Fusarium* is known to include filamentous fungi comprising numerous phytopathogenic species that cause significant losses in agricultural production (Diabankana *et al.*, 2024). Among these pathogenic species, *F. incarnatum* stands out for its ability to produce mycotoxins, such as fumonisins (Wonglom *et al.*, 2020) and is also a significant pathogen in several crops, including rice (Tralamazza *et al.*, 2021), pineapple (Blanco *et al.*, 2022) and more recently, peanuts (Thirumalaisamy *et al.*, 2019; Romero-Arenas *et al.*, 2024).

Numerous studies have provided strong evidence indicating that *Trichoderma* species possess high potential in the management of several plant pathogens in commercially important crops (Abdullah *et al.*, 2021). Likewise, Andrade-Hoyos *et al.* (2023) have discussed the mechanisms underlying the antagonistic activity of *Trichoderma*, such as mycoparasitism and induction of plant resistance.

In addition, *Trichoderma* can penetrate the root system harmlessly and improve the plant's defense capabilities by increasing the enzymatic activity of peroxidase and chitinase. This improves the overall health of the plant and increases growth and yield (Nawrocka *et al.*, 2019). It was observed that most of the strains presented parasitism rates above 80% at 240 h, with the *T. koningiopsis* strain (T-K11) standing out with 92%.

This parasitism can be attributed to competition for space and nutrients, as evidenced by studies by Wonglom *et al.* (2020); Andrade-Hoyos *et al.* (2023). In addition, Husseina *et al.* (2022) reported that *T. harzianum* had an inhibition rate of 85% against *F. incarnatum* as in the present study, where the 'T-H3' strain of *T. harzianum* showed a comparable effect (84.4%). In this sense, Intana *et al.* (2021) identified that volatile organic compounds (VOCs) emitted by *T. asperellum* (T76-14) inhibited 81% of *F. incarnatum* growth.

Several studies have attributed the antagonistic properties of *Trichoderma* spp. to the production of secondary metabolites and volatile substances, including 6-nonyl alcohol, harzianolide, palmitic acid, terpenes, and acetaldehyde (Dubey *et al.*, 2011). These compounds play a crucial role in the degradation of the fungal cell wall. Specifically, Kong *et al.* (2022) noted that *T. koningiopsis* (PSU3-2) produces 3-methyl-1-butanol, 3-octanone and butanoic acid ethyl ester, compounds associated with antibiosis and inhibition of mycelial growth of *F. incarnatum*.

The strains of *T. koningiopsis* (T-K11) and *T. hamatum* (T-A12) stood out by significantly reducing the incidence of vascular wilt and lesions in peanut roots and pods under field conditions. Similar results were described by Intana *et al.* (2021), who observed antifungal activity of *T. asperellum* against *F. incarnatum* in melon.

In contrast, plants inoculated only with the 'MA-PET-03' strain showed characteristic symptoms of *F. incarnatum*, as reported by Thirumalaisamy *et al.* (2019); Romero *et al.* (2014). Therefore, plants treated with *Trichoderma* species are more resistant to *F. incarnatum* attacks, as demonstrated in the results of this research.

Conclusions

The *T. koningiopsis* strain (T-K11) presented the highest class I antagonism on Bell's scale. In addition, it demonstrated a higher development rate, growth rate, and percentage of radial growth inhibition (PRGI) than the 'MA-PET-03' strain of *F. incarnatum* under *in vitro* conditions. Under field conditions, *T. koningiopsis* (T-K11) and *T. hamatum* strains proved to be the most effective in promoting overall plant growth, reducing the incidence of vascular wilt caused by *F. incarnatum* and improving plant health and productivity in peanut crops.

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