Adv. Hort. Sci., 2026 40(1): 61-70

DOI: 10.36253/ahsc-18118
https://oaj.fupress.net/index.php/ahs

AHS
Advances in Horticultural Science

OPEN ACCESS

The combined inoculation of *Trichoderma harzianum* and *Pseudomonas putida* as a microbial consortium enhances the growth and yield of pepper (*Capsicum annuum* L.)

L. Cejas [1], L. Guiñazú [1, 2], M. Rovera [1], J. Andrés [1, 2], A. Torres [1], N. Pastor [1 (*)]

[1] *Institute of Mycology and Mycotoxicology Research (IMICO), National Scientific and Technical Research Council (CONICET), Department of Microbiology and Immunology, Faculty of Exact, Physical, Chemical and Natural Sciences, National University of Río Cuarto (UNRC), Route 36, km 601 (X5804BYA), Río Cuarto, Córdoba, Argentina.*

[2] *Department of Agricultural Biology, Faculty of Agronomy and Veterinary Medicine, National University of Río Cuarto (UNRC), Route 36, km 601 (X5804BYA), Río Cuarto, Córdoba, Argentina.*

(*) **Corresponding author:**
npastor@exa.unrc.edu.ar

Citation:
CEJAS L., GUIÑAZÚ L., ROVERA M., ANDRÉS J., TORRES A., PASTOR N., 2026 - *The combined inoculation of Trichoderma harzianum and Pseudomonas putida as a microbial consortium enhances the growth and yield of pepper (Capsicum annuum L.).* - Adv. Hort. Sci., 40(1): 61-70.

ORCID:
TA: 0000-0002-5452-004X
PN: 0000-0002-2455-2315

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no conflict of interests.

Received for publication 27 June 2025
Accepted for publication 29 December 2025

Key words: Field assays, microbial consortium, pepper, *Pseudomonas putida, Trichoderma harzianum,* yield.

Abstract: A sustainable agricultural method to increase crop yield and reduce dependence on chemical inputs is the use of beneficial microbial species as bioinoculants, especially *Trichoderma* spp., *Pseudomonas* spp. and microbial consortia. This study evaluated the impact of single and combined inoculations of *Trichoderma harzianum* ITEM 3636 and *Pseudomonas putida* PCI2 on pepper plants in field assays. Fruits from consortium-inoculated plants were larger than those from single-strain treatments or uninoculated controls, showing pronounced increases in fruit width and fruit length. Additionally, yield gains were highest under consortium inoculation, whereas individual strain inoculations resulted in intermediate responses. These results suggest the potential use of the consortium as an effective bioinoculant for increasing pepper yield. An initial qualitative assessment of soil microbial taxonomic profiles was carried out. Results showed that root-associated soil from individual inoculations showed higher abundances of one dominant fungal taxon compared to the remaining taxa, in contrast to dual inoculation that displayed a more balanced fungal distribution. Also, bacterial taxa were more balanced in inoculated soils compared to the control. Overall, our work reports on a useful microbiological tool to improve sustainability in horticultural systems.

1. Introduction

The greatest economic importance of pepper (*Capsicum annuum* L.)

lies in the commercialization of its fruits, which are rich in provitamin A, vitamin B, vitamin C, and minerals such as calcium, phosphorus, potassium, and iron (Maboko *et al.,* 2012). This crop is among the seven most important vegetables in the world, with an estimated annual production of more than 30 million tons. It is consumed fresh, cooked, or as a condiment or «spice» in typical foods of various countries, and also in a range of industrial products (Barik *et al.*, 2022).

In order to meet the increased demand for food caused by the growing world population, crop production together with agricultural sustainability must rise. Plant growth-promoting microorganisms (PGPM) are a practical technology that can increase plant production, provide disease defense and have beneficial effects on agriculture (Vishwakarma *et al.*, 2020). Protection from pathogens, reduction of drought effects and stimulation of nutrient uptake are just a few of the many benefits that PGPM can provide. Additionally, microbial management can support the development of climate change resistant systems (Aguilar-Paredes *et al.*, 2020). The urge to look for natural methods that lessen the use of chemicals in agriculture is what has sparked interest in environmentally friendly solutions in contemporary agriculture. The employment of advantageous microorganisms together in consortia is extremely promising for enhancing crop productivity and quality, representing a dependable and eco-friendly approach that may address the issues for modern agriculture (Tabacchioni *et al.*, 2021).

Trichoderma plant symbionts live in a variety of environments, such as the rhizosphere and plant tissues (as endophytes). The use of several *Trichoderma* strains as biocontrol agents against phytopathogenic bacteria is very common (Abdullah *et al.*, 2021). *Trichoderma* spp. are also widely known for being important plant growth-promoting fungi (PGPF), as they boost the growth, development and yields of a variety of crops (Stewart and Hill, 2014; Abdullah *et al.*, 2021; Tyśkiewicz *et al.*, 2022). In prior investigations, we observed that strain ITEM 3636 of *T. harzianum* protected peanut plants against smut, caused by *Thecaphora frezii*. Additionally, *T. harzianum* ITEM 3636 increased crop yield without substantially altering the structure of soil microbial communities (Ganuza *et al.*, 2018; Ganuza *et al.*, 2019).

A group of bacteria known as plant growth promoting rhizobacteria (PGPR) interacts closely with the roots of plants to affect plant health and soil fertility. They provide a fantastic blend of qualities that are advantageous for promoting plant growth and controlling diseases. With their rapid growth, straightforward nutritional needs, capacity to utilize a variety of organic substrates, and mobility, fluorescent pseudomonads have emerged as the most prevalent and possibly the most promising group of PGPR. Numerous characteristics of *Pseudomonas* species make them suitable for use as PGPR. They are also a common type of bacteria in agricultural soils (Dorjey *et al.*, 2017). We isolated *Pseudomonas putida* PCI2 from the rhizosphere of a healthy tomato plant. *P. putida* PCI2 proved to be positive for phosphatase activity, solubilizes a high amount of P in Sperber broth medium and promotes tomato growth (Pastor *et al.*, 2014).

Microorganisms associated with plants have a significant positive impact on agricultural productivity. Several experiments have been conducted on inoculating seeds or plants with microorganisms, either individually or in consortia, to aid in the growth and development of plants. Even though multiple studies have shown that a single microorganism can benefit plants, it is becoming abundantly clear that when two or more microorganisms are participating in a microbial consortium, additive or synergistic effects can be anticipated (Vishwakarma *et al.*, 2020; Tabacchioni *et al.*, 2021). Agriculture can benefit from microbial consortia, which include bacteria and fungi combinations, in several ways. They can reduce the need for chemical pesticides by successfully controlling plant diseases and pathogens. Microbial consortia components can work together in a synergistic way to boost crop productivity and prevent disease in a more thorough and effective manner. By utilizing microbial consortia, farmers might support sustainable agricultural practices and reduce their reliance on chemical fertilizers. Since microbial consortia can affect the nutrient cycle and soil health, their effects on soil microbial populations should also be taken into account (Pastor *et al.*, 2023). Diverse microbial consortia (with different bacterial or fungal compositions) are regularly being released on the market as beneficial products. Thus, the impact of such formulations on agronomic and qualitative performance requires evaluation in open field experiments (Fusco *et al.*, 2023). In a previous study, we tested *T. harzianum* ITEM 3636 and *P.*

putida PCI2, alone and combined, for their effects on growth and yield of tomato under field conditions and observed that inoculation with the microbial consortium resulted in increases in yield, compared to single inoculation and the control treatment (Pastor *et al.*, 2024).

The effects of inoculating pepper plants during transplantation to the field with a *Trichoderma* strain and a *Pseudomonas* strain, alone or combined, have not been extensively studied. Duc *et al.* (2017), for instance, explored the impact of arbuscular mycorrhizal fungi alone or combined with *Trichoderma* and plant growth-promoting bacteria on defense enzymes and yield in the field. Additionally, Chemeltorit *et al.* (2017) tested a *Trichoderma* strain combined with a *Pseudomonas aeruginosa* strain in peppers, but with biocontrol purposes against *Phytophthora capsici*. The goal of this research was to assess the effectiveness of *T. harzianum* ITEM 3636 and *P. putida* PCI2, alone and combined, to promote pepper growth and fruit yield in a field setting.

2. Materials and Methods

Plant cultivar

Pepper seeds from the Fyuco INTA variety were used. The seeds were superficially disinfected using a 70% ethanol solution for 1 minute, followed by a 2% NaOCl solution for another minute. After disinfection, the seeds were washed with sterile distilled water (SDW) 8 times. The disinfected seeds were placed one per cell in trays containing a sterile soil:perlite mixture (2:1). To guarantee proper germination, trays were covered with a transparent nylon bag. Germination trays were placed in a growth chamber under controlled cycles of 14 h of light at 25°C and 10 h of dark at 20°C. The nylon bags were taken off once the seedlings had germinated, and they were maintained in the same environment until field tests.

Microorganisms

T. harzianum ITEM 3636, originally isolated from soil cropped with peanuts by researchers from the Plant Pathology Department, at UNRC, was deposited at the Institute of Toxins and Mycotoxins from Plant Parasites (Rojo *et al.*, 2007). The fungus was maintained in 15% glycerol at -80°C. To create the *T. harzianum* ITEM 3636 inocula, 7-day-old cultures on Petri plates with malt extract agar (MEA), at 28°C, were used. Each of the 10 plates was covered with 10 mL of SDW before the conidia suspensions were collected by scraping the surfaces of the cultures with a sterile glass spatula. The filtrates were obtained by filtering the suspensions through four layers of sterile gauze and the inoculum density was adjusted to 1 x 10^5 conidia mL^{-1} by adding SDW and counting with a hemocytometer. *P. putida* PCI2 was regularly cultivated on King's B medium at 28°C (King *et al.*, 1954) or Tryptic Soy Broth (TSB) and preserved at -20°C in TSB amended with 20% (v v^{-1}) glycerol. Bacterial cells were centrifuged after being incubated in liquid medium and then resuspended in sterile 0.9% saline solution to the adequate concentration (1 x 10^6 CFU mL^{-1}).

Field assays. Location of the assays

The field assays were conducted at the experimental field belonging to the National University of Río Cuarto, Córdoba, Argentina (33°06'26.0'' S, 64°17'52.5'' W) during the summer seasons of 2021-2022 and 2023-2024. Two days prior to transplanting, plots were abundantly watered to minimize tress and ensure good hydration of plants. The soils where seedlings were transplanted into during the experiments had, on average, a pH of 6.3, and 2.6% organic matter, 0.026 $mg \cdot g^{-1}$ of extractable phosphorus, 0.072 $mg \cdot g^{-1}$ of nitrates, 0.014 $mg \cdot g^{-1}$ of sulphates, 0.15% of total nitrogen, 7 meq 100 g^{-1} soil of Ca^{++}, 3 meq 100 g^{-1} soil of Mg^{++}, 0.12 meq 100 g^{-1} soil of Na^+ and 1.8 meq 100 g^{-1} soil of K^+.

Treatments and plant handling

Raised ridges were prepared and then, 4-week-old plants were transplanted into double raised ridges (rows) with a spacing of 20-30 cm between plants and 70-100 cm between rows. Each treatment's surface area on the test plots was 8-10 m^2. The following treatments were tested: (1) immersion of roots in SDW (Control); (2) immersion of roots in a suspension of ITEM 3636 conidia; (3) immersion of roots in a suspension of PCI2 cells (4) immersion of roots in a mixed suspension containing ITEM 3636 conidia + PCI2 cells (Consortium). At the time of transplant, seedlings were inoculated by immersion of roots for 1 min in the corresponding single suspension or mixture. The concentration used for *Trichoderma* was 10^5 conidia mL^{-1} and that for *Pseudomonas* was 10^6 cfu mL^{-1}. SDW was applied to the roots of control seedlings. To supplement the water acquired by precipitation and meet the summertime water needs of plants, a furrow irrigation system was implemented. In order to keep the plants adequately turgid and hydrated during the whole growth cycle, they were watered at regular intervals. After ransplanting, weeds were removed manually. No chemical herbicides were applied. Additionally, neither chemical fungicides nor insecticides were used. Plants from all treatments received a unique dose of 150 g (per furrow between rows) of a commercial fertilizer (TRIPLE 15), applied 1 month after transplanting. The fertilizer was incorporated through successive irrigations and with the water from precipitations. The composition of the applied fertilizer was as follows: nitrogen (15%), phosphorus (15%), Sulfur (3%), Calcium (6%), and potassium (15%). In experiment 1, fruits were collected when mature and plants (15 per treatment) were harvested at 120 days post transplanting (DPT) to the field. Assessments included: length and width of peppers, yield, dry weights of shoots, dry weights of roots and lengths of roots. In experiment 2, fruits were collected when mature and plants (20 per treatment) were harvested at 140 DPT. The parameters evaluated were: size of fruits, fruit yield, shoot area (using *ImageJ*), shoot dry weight and root dry weight.

Qualitative analysis of soil microbial profiles

Five plants were randomly selected and safely removed from their respective growing rows during harvest. Soil samples, including pits and soil detached from roots, were then collected from those zones. These samples were combined into 500 g samples using polyethylene bags, and promptly stored at 4 °C. For the following procedures, one sample per treatment was used. Following the guidelines and recommendations given, the commercial Highway® DNA PuriPrep-SOIL kit (K1210-50) was used to extract DNA from 250 mg of soil for each sample. After lyophilization, soil DNA samples were shipped to CD Genomics (Shirley, NY) for sequencing, PCR amplification, purification, and DNA library creation. The ITS1-1F-F and ITS1-1F-R primers were used to examine the spectrum of fungi, while a primer set that amplifies the hypervariable regions V3 and V4 of the 16S rRNA gene was used to investigate bacterial profiles. The Illumina Mi-Seq platform (Illumina Inc., San Diego, CA, USA) was used to sequence the amplicons. For the following bioinformatic analysis, the Quantitative Insights Into Microbial Ecology 2 (QIIME2) software tool was utilized (Bolyen *et al.*, 2019; Estaki *et al.*, 2020).

Statistical analyses

All statistical analyses were conducted using R, *version 4.1.2*. Analyses of data from the field assays were performed using ANOVA, and differences were calculated using the Fisher's least significant difference (LSD) test ($P \leq 0.05$). The Kruskal-Wallis test was used when necessary.

3. Results

Field assays. Experiment 1

Plants were kept in the field for 120 DPT. At harvest, plants from all the treatments were collected, removing them individually being careful so as not to damage their root systems. Growth parameters were measured. There were statistically significant differences in shoot dry weight between treatments. The control presented the lowest average value, while inoculation with the consortium showed the highest. However, there was no statistically significant difference in root weight between the treatments. Plants inoculated with ITEM 3636 alone showed the highest values, generating an average value of 11.4 g. The rest of the treatments presented very similar values, slightly lower than those from ITEM 3636. Lastly, we found that the consortium inoculation resulted in the highest average root length value (19.3 cm). The control and PCI2 inoculation showed the lowest root length values, which did not differ significantly (Table 1).

Statistically significant differences were observed between treatments for average length of peppers. Peppers from plants treated with PCI2 showed the

Table 1 - Growth parameters of inoculated pepper plants from field assay 1

Treatment	Shoot dry weight (g)	Root dry weight (g)	Root length (cm)
Control	28.1 b	10.3 a	13.6 c
ITEM 3636	37.2 ab	11.4 a	17.3 ab
PCI2	40.7 a	10.7 a	14.5 bc
Consortium	44.5 a	10.4 a	19.3 a

The LSD test indicates that the mean values from each column with a distinct letter are significantly different (P≤0.05). Control= non-inoculated plants; PCI2= inoculated with *P. putida* PCI2 alone; ITEM 3636= inoculated with *T. harzianum* ITEM 3636 alone.

highest mean value (133.1 mm). The other treatments had values ranging between 115 and 121 mm and did not differ significantly. For fruit width, the treatment with the highest mean value was inoculation with the consortium. The other treatments did not present significant statistical differences between them. We also observed statistically significant differences between conditions regarding yield. Inoculation with the consortium generated the highest average value. The control and inoculation with ITEM 3636 alone presented very similar values, lower than inoculation with PCI2 alone and with the consortium, although without significantly differing from the first (Table 2).

Table 2 - Effect of *T. harzianum* ITEM 3636, P. putida PCI2 and their combination on pepper fruits and yield in field assay 1

Treatment	Length of peppers (mm)	Width of peppers (mm)	Yield (g/plant)
Control	120.6 b	77.2 b	352.3 b
ITEM 3636	115.3 b	77.8 b	299.7 b
PCI2	133.1 a	73.6 b	565.1 ab
Consortium	120.9 b	85.2 a	631.2 a

Significance in the differences between groups was assessed using the Fisher's LSD test. A different letter within each column indicates a significant difference between treatments at the P≤0.05 level.

Field assays. Experiment 2

We measured the shoot area at 60 DPT. It was observed that all variants of inoculation resulted in higher values compared with the control treatment. Additionally, the values obtained from plants inoculated with single microorganisms were higher at the time of measurement (Fig. 1A). Figure 1A also shows the average number of fruits per plant at that time. As shown, the highest average value was obtained from the inoculation with PCI2 treatment while the lowest was from the control. The other treatments showed intermediate average values. For shoot dry weight, no statistically significant differences were recorded between treatments at 140 DPT, the time of measurement after harvest (Fig. 1B). On the other hand, statistically significant differences were observed between treatments for average root dry weight. Inoculation with ITEM 3636 caused the highest value and the control the lowest (Fig. 1B). For the length of fruits, it was observed that peppers from plants inoculated with the consortium showed the highest mean value (114 mm). The rest of the treatments presented values that oscillated around 100 mm and did not differ significantly. For fruit width, the treatments did not present significant statistical differences between them. Additionally, we observed that there were statistically significant differences between treatments regarding average yield. Specifically, the highest yield was caused by plants inoculated with the microbial consortium, compared to the control, while the treatments of inoculation with the

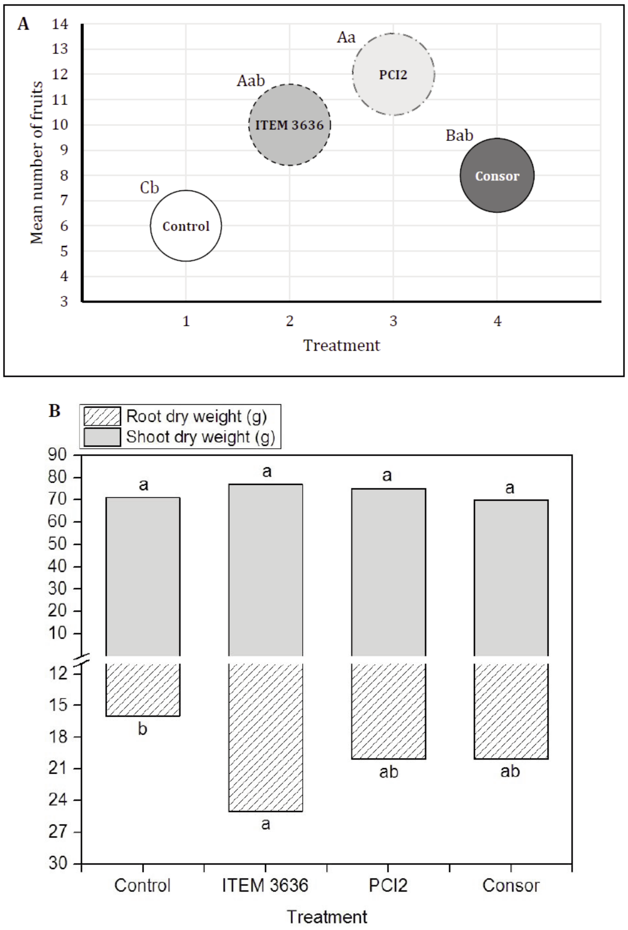

Fig. 1 - Growth parameters of inoculated pepper plants during experiment 2. (A) Bubbles with a different capital letter and size have a significantly different shoot area according to the LSD test (P≤0.05); Bubbles with a different lower case letter evidence a significantly different number of fruits per plant according to the LSD test (P≤0.05); (B) Mean values from each column with a different letter are significantly different according to the LSD test (P≤0.05). Control= non-inoculated plants; PCI2= inoculated with *P. putida* PCI2 alone; ITEM 3636= inoculated with *T. harzianum* ITEM 3636 alone. Shoot area and number of fruits values were assessed at 60 days. Dry weights were registered after harvest, at 140 days.

individual microbial strains caused intermediate values (Table 3).

Table 3 - Effect of *T. harzianum* ITEM 3636, *P. putida* PCI2 and their combination on pepper fruits and yield during experiment 2

Treatment	Length of peppers (mm)	Width of peppers (mm)	Yield (g/plant)
Control	103.32 b	76.16 a	480.31 b
ITEM 3636	103.84 b	76.28 a	639.68 ab
PCI2	99.54 b	78.04 a	594.36 ab
Consortium	114.47 a	79.85 a	703.42 a

The Kruskal-Wallis test was used to determine whether the differences between the groups were significant. A different letter within each column indicates a significant difference between treatments.

Global effects of treatments

After normalizing the values, the analysis of the overall impact of treatments on the various assessed parameters suggests that the development of the root system significantly improved in response to inoculation with strain ITEM 3636, both alone and in combination with PCI2 as a microbial consortium. The root development values were lowest in the control. On the other hand, inoculation in all its forms was successful in stimulating the aerial growth of pepper plants, especially in the case of strain PCI2. The consortium was superior in terms of enlarging peppers. When compared to fruits from plants treated with single strains or uninoculated controls, these fruits showed increased width and length, particularly width. Furthermore, yield measurements showed the highest gains under consortium inoculation, followed by individual strain inoculation (Fig. 2).

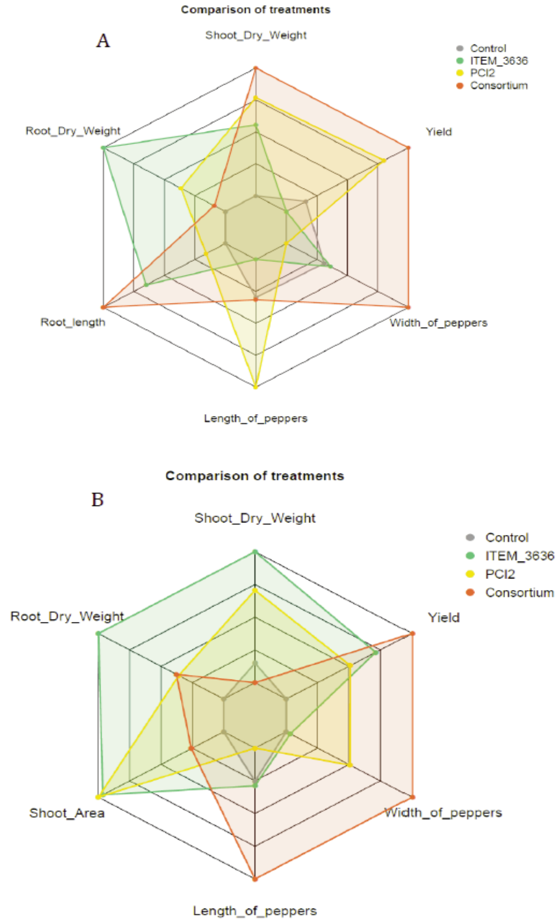

Fig. 2 - Global inoculation effects on pepper growth and yield in (A) Field assay 1 and (B) Field assay 2. Consortium = PCI2 + ITEM 3636; Control = non-inoculated plants; ITEM 3636 = inoculated with *T. harzianum* ITEM 3636 alone; PCI2 = inoculated with *P. putida* PCI2 alone.

Qualitative analysis of pepper soil microbial profiles

For this preliminary qualitative analysis, we used a single sample per treatment to obtain fungal and bacterial taxonomic profiles. In terms of fungal profiles, we focused on the 10 classified genera with the highest relative frequencies. Results are presented in figure 3A. Despite all profiles being composed of the same fungal genera, results suggest some differences, such as higher relative frequencies of one taxon in single inoculation treatments and a more balanced composition in the case of co-inoculation. The treatment of inoculation with ITEM 3636 showed the highest relative frequency for *Cladorrhinum* (49%), whereas the treatment of inoculation with PCI2 presented the highest for *Mortierella* (42.7%). Inoculation with ITEM 3636 also showed the lowest relative frequency for *Fusarium* (13.6%). The control profile suggests a dominant role of *Mortierella* (27.7%), *Fusarium* (23.4%) and *Thelonectria* (19.9%). Besides *Mortierella* (26.5%), the fungal profile for the treatment of inoculation with the consortium was also dominated by *Dichotomopilus* (20%) and *Trichoderma* (19.4%). For bacterial Class, it can be observed in figure 3B. that the detected taxa would exhibit a homogenous distribution in all of the inoculation treatments. Also, the control treatment appears to be most influenced by Actinobacteria (relative frequency of 36%). The taxonomic profiles of soil communities linked to

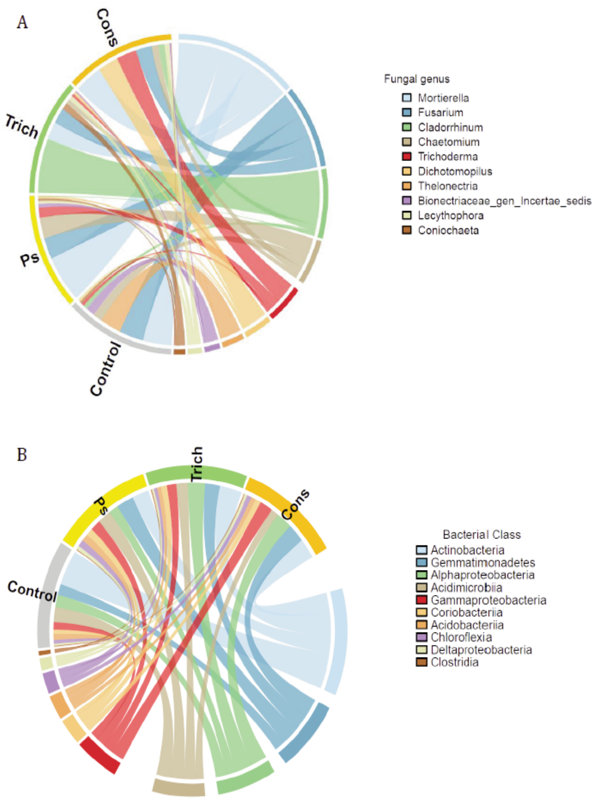

Fig. 3 - Circos/String plot of soil (A) fungal and (B) bacterial communities in the different treatments. Cons= consortium (PCI2 + ITEM 3636); Control= non-inoculated plants; Ps= inoculated with *P. putida* PCI2 alone; Trich= inoculated with *T. harzianum* ITEM 3636 alone.

inoculated pepper, as well as tomato, are currently the subject of more thorough and in-depth work based on these first explorations.

4. Discussion and Conclusions

In this paper, we propose that a consortium consisting of *T. harzianum* ITEM 3636 and *P. putida* PCI2 as a biostimulant could boost pepper yield. In a previous work, we observed that the strains under study are compatible and, in field experiments, contributed to increase tomato yield (Pastor *et al.*, 2024). When used in the field, potential biofertilizers that commonly perform well in the lab and greenhouse may not have the expected effects on plant development. Effective plant growth promotion under field settings cannot be ensured by merely screening axenic culture isolates for features that promote plant growth (Basu *et al.*, 2021). Bader *et al.* (2020) reported on *Trichoderma* strains that promoted the growth of tomato plants and hypothesized that this ability might be related to their capacity to produce phytohormones and to increase accessible phosphorus. Unpublished evidence suggests that *T. harzianum* ITEM 3636 solubilizes phosphates and raises the amount of phosphorus in the shoots of inoculated peanut plants. Conversely, we documented *P. putida* PCI2's capacity to produce indole-3-acetic acid (IAA) and to solubilize different sources of phosphate (Pastor *et al.*, 2012; Pastor *et al.*, 2014).

Inoculation with *P. putida* PCI2 alone during the first year of experimentation caused significant increases of 10% and 40% in the length of peppers and shoot dry weight, respectively, compared with control plants. Additionally, inoculation with *T. harzianum* ITEM 3636 alone resulted in a significant increase of 27% in root length when compared with the control during the first assay under field conditions. During experiment 2, we observed significant increases in shoot area values from single inoculation treatments, compared to the control. Results from this assay also demonstrated that inoculation with the consortium had a positive effect on fruit length, producing the largest fruits among all treatments. The dominance of inoculation with the consortium in this parameter underscores its potential as a promising treatment for enhancing fruit size and, hence, yield. Several studies have reported positive relationships between fruit size attributes and overall yield, suggesting that improvements in these parameters may translate into enhanced productive performance (Usman *et al.*, 2017; Sharma *et al.*, 2019; Deresa *et al.*, 2023).

Combinations of several *Trichoderma* species and bacteria have been shown to be more effective at promoting plant growth than single microorganisms (Pastor *et al.*, 2023). Our results showed that pepper yield increased significantly after inoculation under field conditions, particularly after inoculation with the microbial consortium. Results from this study showed that co-inoculation of pepper roots during transplant to the field produced significant increases in parameters such as root length and fruit size, compared with control plants, during the different experiments under field conditions. These findings concur with earlier studies on the advantages of inoculating horticultural crops with a beneficial microbial consortium. A seed-coating formulation was created by Kumar *et al.* (2015) based on a microbial consortium that included *T. harzianum* OTPB3 and *B. subtilis* OTPB1 and exhibited significant improvements in growth parameters of many horticultural crops in greenhouse and field conditions. He *et al.* (2019) carried out a greenhouse study on tomato to compare the effects of co-inoculation with combinations of *B. pumilus*, *B. amyloliquefaciens*, *B. mojavensis* and *P. putida* on plant development and yield to single microbial treatments. According to the authors, the co-inoculation of beneficial microorganisms, at particular stages of plant development, improved plant performance more quickly than any other treatment. To promote tomato growth under drought stress, Krishna *et al.* (2022) created what they called a Hexa-PGPM consortium based on *B. megaterium* BHUPSB14, *P. fluorescens* BHUPSB06, *P. aeruginosa* BHUPSB01, *P. putida* BHUPSB04, *P. polymyxa* BHUPSB17 and a *T. harzianum* strain. This consortium was proven to enhance several growth and yield indices. The effectiveness of a consortium based on *B. pumilus* YSPMK11 and *B. subtilis* MK5 for promoting growth in bell pepper plants as well as for preventing the diseases damping off and anthracnose was assessed by Kaushal *et al.* (2019) in field conditions.

The consortium significantly improved growth, reduced the incidence of damping off and anthracnose disease, and increased fruit yield. Singh *et al.* (2019) also assessed *T. harzianum* and PGPRs as a microbial consortium for promoting mint growth and came to the result that using these microbes combined was more effective than using them individually for the improvement of plant growth. The yield measures from this study showed that consortium inoculation produced the greatest gains. According to these results, favorable benefits may be amplified by synergistic interactions among the microorganisms in the consortium, which could improve fruit set and overall productivity. More specifically, the improved performance observed under consortium inoculation may be associated with complementary effects of the microbial partners. In this sense, both *T. harzianum* ITEM 3636 and *P. putida* PCI2 have been reported to have phosphorus-solubilizing activity, and *P. putida* PCI2 has been demonstrated to produce IAA. Through improved plant-microbe interactions, these combined characteristics may affect fruit development and plant growth, potentially contributing to the observed increases in fruit size and yield-related traits under field conditions.

On the whole, the microbial consortium treatment displayed more root length. Increased root development enables plants to absorb more water and nutrients by growing their roots deeper. More nutrient and water absorption allows plants to develop biomass more rapidly and count with a fitter supply of assimilates for fruit production. Although further work is needed, our results suggest that pepper root-associated soil from the co-inoculation treatment contained higher abundances of fungal genera known to include beneficial members that lead to healthier plants and more resilient ecosystems, namely *Mortierella*, *Trichoderma* and *Dichotomopilus*. Worldwide research findings highlight the value of rhizospheric plant growth-promoting fungi by demonstrating their environmentally beneficial characteristics and potential for enhancing germination, the development of plant roots and shoots and crop yields (Adedayo and Babalola, 2023).

This study provides new information on the inoculation and co-inoculation of pepper roots with *T. harzianum* and *P. putida* strains during transplanting. Our results show that the strains enhance physiological processes that boost pepper yield, acting as effective biostimulants, especially when combined. This strategy reduces reliance on chemicals by providing an effective alternative to synthetic inputs. Future studies will examine how these studied microorganisms affect soil microbial populations and plant metabolic profiles in an effort to clarify the relationships and processes underlying enhanced pepper growth. Our goal is to further the creation of agricultural practices based on microorganisms that balance environmental care with productivity.

Acknowledgements

This work was supported by grants from Agencia Nacional de Promoción Científica y Tecnológica (MINCyT; PICT 2017-4418-Préstamo BID; PICT 00963/2021-Préstamo BID), and Secretaría de Ciencia y Técnica de la Universidad Nacional de Río Cuarto (SECyT-UNRC).

References

ABDULLAH N.S., DONI F., MISPAN M.S., SAIMAN M.Z., YUSUF Y.M., OKE M.A., SUHAIMI N.S.M., 2021 - *Harnessing* Trichoderma *in agriculture for productivity and sustainability*. - Agronomy, 11: 2559.

ADEDAYO A.A., BABALOLA O.O., 2023 - *Fungi that promote plant growth in the rhizosphere boost crop growth*. - J. Fungi, 9: 239.

AGUILAR-PAREDES A., VALDÉS G., NUTI M., 2020 - *Ecosystem functions of microbial consortia in sustainable agriculture*. - Agronomy, 10: 1902.

BADER A.N., SALERNO G.L., COVACEVICH F., CONSOLO V.F., 2020 - *Native* Trichoderma harzianum *strains from Argentina produce indole-3 acetic acid and phosphorus solubilization, promote growth and control wilt disease on tomato (*Solanum lycopersicum *L.)*. - J. King Saud Univ. - Sci., 32: 867-873.

BARIK S., PONNAM N., REDDY A.C., REDDY DC L., SAHA K., ACHARYA G., REDDY M., 2022 - *Breeding peppers for industrial uses: Progress and prospects.* - Ind. Crop. Prod*.,* 178: 114626.

BASU A., PRASAD P., DAS S.N., KALAM S., SAYYED R.Z., REDDY M.S., EL ENSHASY H., 2021 - *Plant growth promoting rhizobacteria (PGPR) as green bioinoculants: Recent developments, constraints, and prospects*. - Sustainability, 13: 1140.

BOLYEN E., RIDEOUT J.R., DILLON M.R., BOKULICH N.A., ABNET C.C., AL-GHALITH G.A., ALEXANDER H., ALM E.J., ARUMUGAM M., ASNICAR F., BAI Y., BISANZ J.E., BITTINGER K., BREJNROD A., BRISLAWN C.J., BROWN C.T., CALLAHAN B.J., CARABALLO-RODRÍGUEZ A.M., CHASE J., CAPORASO J.G., 2019 - *Reproducible, interactive, scalable and extensible microbiome data science using QIIME2*. - Nat. Biotechnol., 37: 852-857.

CHEMELTORIT P.P., MUTAQIN K.H., WIDODO W., 2017 - *Combining* Trichoderma hamatum *THSW13 and* Pseudomonas aeruginosa *BJ10-86: a synergistic chili pepper seed treatment for* Phytophthora capsici *infested soil.* - Eur. J. Plant Pathol., 147: 157-166.

DERESA D., GIRMA S., ASSEFA G., 2023 - *Genetic variability, heritability, genetic advance, and association of characters in small pod hot pepper (*Capsicum annuum *L.) landraces in West Hararghe, Eastern Ethiopia*. - Int. J. Bot. Hor. Res., 1: 31-37.

DORJEY S., DOLKAR D., SHARMA R., 2017 - *Plant growth promoting rhizobacteria Pseudomonas: A Review.* - Int. J. Curr. Microbiol. Appl. Sci., 6: 1335-1344.

DUC N., MAYER Z., PÉK Z., HELYES L., POSTA K., 2017 - *Combined inoculation of arbuscular mycorrhizal fungi,* Pseudomonas fluorescens *and* Trichoderma *spp. for enhancing defense enzymes and yield of three pepper cultivars.* - Appl. Ecol. Environ. Res., 15: 1815-1829.

ESTAKI M., JIANG L., BOKULICH N.A., McDONALD D., GONZÁLEZ A., KOSCIOLEK T., MARTINO C., ZHU Q., BIRMINGHAM A., VÁZQUEZ-BAEZA Y., DILLON M.R., BOLYEN E., CAPORASO J.G., KNIGHT R., 2020 - *QIIME 2 enables comprehensive end-to-end analysis of diverse microbiome data and comparative studies with publicly available data.* - Curr. Protoc. Bioinform., 70: e100.

FUSCO G.M., BURATO A., PENTANGELO A., CARDARELLI M., NICASTRO R., CARILLO P., PARISI M., 2023 - *Can microbial consortium applications affect yield and quality of conventionally managed processing tomato?* - Plants, 12: 14.

GANUZA M., PASTOR N., BOCCOLINI M., ERAZO J., PALACIOS S., ODDINO C., REYNOSO M.M., ROVERA M., TORRES A.M., 2019 - *Evaluating the impact of the biocontrol agent* Trichoderma harzianum *ITEM 3636 on indigenous microbial communities from field soils.* - J. Appl. Microbiol., 126(2): 608-623.

GANUZA M., PASTOR N., ERAZO J., ANDRÉS J., REYNOSO M.M., ROVERA M., TORRE A.M., 2018 - *Efficacy of the biocontrol agent* Trichoderma harzianum *ITEM 3636 against peanut smut, an emergent disease caused by* Thecaphora frezii. - Eur. J. Plant Pathol., 151: 257-262.

HE Y., PANTIGOSO H.A., WU Z., VIVANCO J.M., 2019 - *Co-inoculation of* Bacillus *sp. and* Pseudomonas putida *at different development stages acts as a biostimulant to promote growth, yield and nutrient uptake of tomato.* - J. Appl. Microbiol., 127: 196-207.

KAUSHAL M., MANDYAL P., KAUSHAL R., 2019 - *Field based assessment of* Capsicum annuum *performance with inoculation of rhizobacterial consortia.* - Microorganisms, 7: 89.

KING E.O., WARD M.K., RANEY D.E., 1954 - *Two simple media for the demonstration of pyocyanin and fluorescin.* - J. Lab. Clin. Med., 44: 301-307.

KRISHNA R., JAISWAL D.K., ANSARI W.A., SINGH S., SOUMIA P.S., SINGH A.K.,·KUMARI B., SINGH M., VERMA J.P., 2022 - *Potential microbial consortium mitigates drought stress in tomato (*Solanum lycopersicum *L.) plant by up-regulating stress-responsive genes and improving fruit yield and soil properties.* - J. Soil Sci. Plant Nutr., 22: 4598-4615.

KUMAR S.M., CHOWDAPPA P., KRISHNA V., 2015 - *Development of seed coating formulation using consortium of* Bacillus subtilis *OTPB1 and* Trichoderma harzianum *OTPB3 for plant growth promotion and induction of systemic resistance in field and horticultural crops.* - Indian Phytopathol., 68: 25-31.

MABOKO M., DU PLOOY C., CHILOANE S., 2012 - *Effect of plant population, stem and flower pruning on hydroponically grown sweet pepper in a shadenet structure.* - Afr. J. Agric. Res., 7: 1742-1748.

PASTOR N., CARLIER E., ANDRÉS J., ROSAS S.B., ROVERA M., 2012 - *Characterization of rhizosphere bacteria for control of phytopathogenic fungi of tomato.* - J. Environ. Manage., 95: S332-S337.

PASTOR N., PALACIOS S., TORRES A.M., 2023 - *Microbial consortia containing fungal biocontrol agents, with* *emphasis on* Trichoderma *spp.: Current applications for plant protection and effects on soil microbial communities.* - Eur. J. Plant Pathol., 167: 593-620.

PASTOR N., ROSAS S., LUNA V., ROVERA M., 2014 - *Inoculation with* Pseudomonas putida *PCI2, a phosphate solubilizing rhizobacterium, stimulates the growth of tomato plants.* - Symbiosis, 62: 157-167.

PASTOR N.A., CEJAS L.G., GUIÑAZÚ L.B., ROVERA M., TORRES A.M., 2024 - *Inoculation of tomato roots with single and mixed suspensions of* Trichoderma harzianum *ITEM 3636 conidia and* Pseudomonas putida *PCI2 cells.* - Int. J. Veg. Sci., 30: 56-73.

ROJO F.G., REYNOSO M.M., FEREZ M., CHULZE S.N., TORRES A.M., 2007 - *Biological control by* Trichoderma *species of* Fusarium solani *causing peanut brown root rot under field conditions.* - Crop Prot., 26: 549-555.

SHARMA A., KUMAR M., KUMAR N., DOGRA R., KUMARI S., 2019 - *Studies on interrelationships among yield and yield contributing traits in bell pepper (*Capsicum annuum *L. var. grossum)*. - J. Pharmacogn. Phytochem., 8: 646-649.

SINGH S., TRIPATHI A., MAJI D., AWASTHIA A., VAJPAYEEB P., KALRAA A., 2019 - *Evaluating the potential of combined inoculation of* Trichoderma harzianum *and* Brevibacterium halotolerans *for increased growth and oil yield in* Mentha arvensis *under greenhouse and field conditions.* - Ind. Crops Prod., 131: 173-181.

STEWART A., HILL R., 2014 - *Applications of* Trichoderma *in plant growth promotion*, pp. 415-428. - In: GUPTA V.K., M. SCHMOLL, A. HERRERA-ESTRELLA, R.S. UPADHYAY, I. DRUZHININA, and M.G. TUOHY (EDS.) *Biotechnology and biology of* Trichoderma. Elsevier, Amsterdam, The Netherlands, pp. 549.

TABACCHIONI S., PASSATO S., AMBROSINO P., HUANG L., CALDARA M., CANTALE C., HETT J., DEL FIORE A., FIORE A., SCHLÜTER A., SCZYRBA A., MAESTRI E., MARMIROLI N., NEUHOFF D., NESME J., SØRENSEN S.J., APREA G., NOBILI C., PRESENTI O., GIOVANNETTI G., GIOVANNETTI C., PIHLANTO A., BRUNORI A., BEVIVINO A., 2021 - *Identification of beneficial microbial consortia and bioactive compounds with potential as plant biostimulants for sustainable agriculture*. - Microorganisms, 9: 426.

TYŚKIEWICZ R., NOWAK A., OZIMEK E., JAROSZUK-ŚCISEŁ J., 2022 - Trichoderma: *The current status of its application in agriculture for the biocontrol of fungal phytopathogens and stimulation of plant growth*. - Int. J. Mol. Sci., 23: 2329.

USMAN M.G., RAFII M.Y., MARTINI M.Y., OLADOSU Y., KASHIANI P., 2017 - *Genotypic character relationship and phenotypic path coefficient analysis in chili pepper genotypes grown under tropical condition*. - J. Sci. Food Agric., 97: 1164-1171.

VISHWAKARMA K., KUMAR N., SHANDILYA C., MOHAPATRA S., BHAYANA S., VARMA A., 2020 - *Revisiting plant-microbe interactions and microbial consortia application for enhancing sustainable agriculture: A review*. - Front. Microbiol., 11: 560406.