Evaluation of microbial consortia for the control of *Rhizoctonia solani* kühn in serrano pepper under greenhouse conditions

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ABSTRACT

Objective: To evaluate the effectiveness of bacterial consortia and endophytic fungi for the control of *Rhizoctonia solani* in serrano pepper.

Design/methodology/approach: Two experiments were established: the first used two-month-old chili plants, and the second used one-month-old plants. The following treatments were inoculated in the root zone: T1=*Bacillus cereus* P5 + *Irpex lacteus* P7B, T2=*Paenibacillus polymyxa* BACAGA + *Bacillus glycinifermentans* CORI2 + *Bacillus subtilis* PI04 + *Xylaria feejeensis* PASI22, T3=*Bacillus* sp. SALGUA + *Trichoderma* sp. TRICOE, T4=Negative control (without microorganisms), and T5=Positive control (inoculation of *R. solani*). In the first experiment, disease severity was estimated using a diagrammatic scale. In the second, it was assessed using two methods: a diagrammatic severity scale in the first, and a millimeter ruler in the second. Using the data obtained, the area under the disease progression curve (AUCPE) was estimated for each treatment. The incidence and severity were assessed over 13 days, and the area under the disease progress curve (AUCPE) was estimated. At the end of the experiment, the fresh and dry weights of each treatment were estimated. The data were subjected to analysis of variance and means test (Tukey, $p \leq 0.05$) using SAS version 9.0. Results: The best treatment was T3, as it reduced the severity of the disease caused by *R. solani*, registering AUDPC values of 3.17 for severity in the first experiment, AUDPC of 24.90 (first method) and 10.19 (second method) in the second experiment. T5 showed the highest severity values, with an AUDPC of 11.45 in the first experiment and AUDPC of 54.70 (first method) and 23.91 (second method) in the second experiment.

Findings/conclusions: The best treatment to reduce the severity of *R. solani*-induced disease was T3 treatment.

Limitations/implications: Future research is required to evaluate the effectiveness under open-field conditions of the microbial consortia evaluated in the present study.

Keywords: *Capsicum annuum*, disease, beneficial microorganisms, severity, incidence.

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INTRODUCTION

Chili pepper (*Capsicum annuum* L.) is a crop in high global demand (Reddy *et al.*, 2016). Mexico ranks as the second-largest producer worldwide, with an annual output

of 3,681,061 metric tons (FAOSTAT, 2023). This crop is widely consumed, as its fruit is attributed with nutraceutical properties due to the presence of antioxidant compounds such as carotenoids, tocopherols, and capsaicinoids (Imran *et al.*, 2018). However, chili production is significantly impacted by the fungus *Rhizoctonia solani*, which causes root rot and wilting, affecting both seedling emergence and plant development, and can reach an incidence of up to 40% under field conditions (Varma *et al.*, 2020; Tuncer & Eken, 2023). This pathogen is particularly difficult to control due to its ability to persist in the soil for extended periods and its broad host range (Abbas *et al.*, 2022; Sam-On *et al.*, 2024). Moreover, *R. solani* exhibits high virulence and genetic diversity, complicating the development of effective management strategies (Tuncer & Eken, 2023). Traditionally, the control of *R. solani* relies on the application of synthetic fungicides. However, these have raised environmental and human health concerns (Ayaz *et al.*, 2023; Wu *et al.*, 2019). In response to this issue, there has been growing interest in evaluating more sustainable control methods that do not compromise environmental integrity. Within this context, biological control emerges as a promising alternative for disease management (Pawaskar & Kerkar, 2021). Globally, various management strategies have been assessed, including the use of biocontrol agents such as *Bacillus* spp. and *Trichoderma* spp., which have demonstrated effectiveness in reducing disease severity and promoting plant growth (Wu *et al.*, 2019; Abhinav *et al.*, 2023; Huang *et al.*, 2017). In Mexico, *R. solani* is widely distributed across all chili-producing regions, with reported incidence rates exceeding 30% (Montero-Tavera *et al.*, 2013; Vásquez-López *et al.*, 2009). Nevertheless, studies on biological control of *R. solani* under *in vivo* conditions in this crop remain scarce. In light of the above, the aim of this study was to evaluate the effectiveness of microbial consortia composed of bacteria and endophytic fungi in the control of *R. solani* in serrano pepper plants.

MATERIALS AND METHODS

Geographic location of the experimental site

Two greenhouse experiments were conducted at the Faculty of Agricultural and Environmental Sciences of the Autonomous University of Guerrero (FCAA-UAGro), located in Iguala de la Independencia, Guerrero, Mexico, at geographic coordinates 18° 21' 19.70" N and 99° 32' 54.16" W, at an elevation of 750 meters above sea level. The experiment was repeated twice at different times under greenhouse conditions. Two-month-old chili plants were used in the first experiment, and one-month-old plants in the second. In both experiments, relative humidity was recorded using a Datalogger Extech RTH10® at hourly intervals. To increase relative humidity within the experimental area, transparent plastic bags measuring 90×120 cm were placed over each treatment for approximately 72 hours.

Isolation of the phytopathogenic fungus

In San Vicente Palapa, a locality in the municipality of Tepecoacuilco de Trujano, Guerrero, a sampling was conducted in a serrano pepper field where plants showing symptoms of basal rot were collected. The estimated disease incidence was 15%. The

plants exhibited wilting, root rot, basal stem rot, and overall collapse. Symptomatic plants were processed at the Laboratory of Plant Physiology and Biotechnology (FCAA-UAGro), where isolate P5SPA was obtained and identified at the cultural and morphological level as *Rhizoctonia solani*.

Obtaining beneficial bacteria and fungi

The bacterial and fungal strains used in this study were provided by the Laboratory of Plant Physiology and Biotechnology at FCAA-UAGro. The microbial strains evaluated included: *Paenibacillus polymyxa* (BACAGA), *Bacillus glycinifermentans* (CORI2), *Bacillus subtilis* (PI04), *Bacillus cereus* (P5), *Bacillus* sp. (SALGUA), *Trichoderma* sp. (TRICOE), *Irpex lacteus* (P7B), and *Xylaria feejeensis* (PASI22).

Planting of plant material

Seeds of serrano chili were used, disinfected with 5% NaOCl for three minutes, and rinsed three times to remove excess NaOCl (Calaña-Janeiro, 2019). Seeds were sown in 200-cell seedling trays filled with Peat Moss® substrate. Nine days after germination, the seedlings were transplanted into plastic pots containing Peat Moss® and placed in a greenhouse.

Experimental design

Both experiments followed a completely randomized design with five treatments and 20 replications, totaling 100 experimental units. Each experimental unit consisted of one serrano chili plant. The treatments applied were as follows:

T1 = *B. cereus* P5 + *Irpex lacteus* P7B; T2 = *P. polymyxa* BACAGA + *B. glycinifermentans* CORI2 + *B. subtilis* PI04 + *X. feejeensis* PASI22; T3 = *Bacillus* sp. SALGUA + *Trichoderma* sp. TRICOE; T4 = Negative control (no microorganisms); T5 = Positive control (*R. solani* P5SPA inoculation)

Evaluation of *R. solani* biocontrol

Serrano pepper plants were first inoculated with beneficial microorganisms according to their respective treatments. Four days later, they were inoculated with *R. solani* P5SPA, following the methodology proposed by Villajuan-Abgona (1996). The fungi *R. solani* P5SPA, *Irpex lacteus* P7B, *Xylaria feejeensis* PASI22, and *Trichoderma* sp. TRICOE were cultured on potato dextrose agar (PDA) supplemented with 1 mL/L gentamicin and incubated for 14 days under laboratory conditions. Bacterial strains (*P. polymyxa*, *B. glycinifermentans*, *B. subtilis*, *B. cereus*, and *Bacillus* sp.) were grown on PDA (without antibiotics) and incubated at 28 °C for 48 hours. From the fungal cultures, 19 mm diameter mycelial plugs were extracted using a sterile cork borer and placed at the base of each plant stem using sterile wooden sticks (autoclaved at 121 °C for 20 minutes). Bacterial colonies were suspended in sterile water and adjusted to 10^8 CFU/mL using a hemocytometer. Then, 50 mL of each suspension was applied at the base of each plant stem.

Beneficial microorganisms were inoculated according to their respective treatment, and four days later, *R. solani* P5SPA was introduced, following the aforementioned procedure. The positive control consisted of *R. solani* P5SPA disks, while the negative control consisted of PDA disks without mycelium.

Estimation of incidence and severity

Disease incidence and severity caused by *R. solani* were evaluated every 24 hours for 13 days in both experiments. Incidence was calculated using the following formula:

$$(\textit{number of diseased plants} \mid \textit{total number of plants per treatment}) \times 100$$

In the first experiment, disease severity was assessed using the scale proposed by Horsfall and Barratt (1945). In the second experiment, two methods were used:

Method 1: Modified scale from Andrade-Luna *et al.* (2017).

where: 0 = no symptoms, 1 = initial basal stem rot, 2 = moderate basal stem rot, 3 = stem constriction, 4 = collapsed plant with turgid leaves, 5 = collapsed plant with wilted leaves, 6 = advanced stem rot and leaf drop, 7 = severe stem rot, 8 = generalized rot.

Method 2: Severity was measured using a millimeter ruler.

Severity and incidence data over time were used to calculate the Area Under the Disease Progress Curve (AUDPC) as described by Campbell and Madden (1990). Analysis of variance and mean separation (Tukey, $p \leq 0.05$) were performed using SAS software version 9.0.

Fresh and dry biomass weight

Following the final incidence and severity evaluation, both fresh and dry biomass weights were measured for each experimental unit/treatment in both experiments. For fresh weight, plants were removed from the soil, washed with running water to remove substrate, and weighed using a compact OKAYA® scale. For dry weight, plants were placed in kraft paper bags and dried in a NOVATECH® oven at 65 °C for 72 hours. The resulting data were analyzed by ANOVA and Tukey's test ($p \leq 0.05$) using SAS software version 9.0.

RESULTS AND DISCUSSION

Environmental conditions of the experiment

In the first experiment, conducted between October and November, the average temperature and relative humidity inside the greenhouse were 25 °C and 68%, respectively. In the second experiment, carried out from November to December 2024, the average temperature and relative humidity were 21 °C and 55%, respectively.

Disease incidence

Experiment one

In the first experiment, symptoms of the disease caused by *R. solani* P5SPA appeared six days after inoculation. The highest incidence was recorded in the positive control (T5) at 45%, while the lowest incidence was observed in T3 at 5%. By the end of the experiment, T5 reached 50% incidence, whereas T3 remained the lowest at 15%. The negative control remained asymptomatic, with 0% incidence (Figure 1).

Experiment two

In the second experiment, initial symptoms of the disease appeared five days after inoculation with *R. solani*. The positive control exhibited 25% incidence, while T3 showed the lowest incidence at 10%. At the end of the experiment, the positive control (T5) recorded the highest incidence at 90%, while T3 maintained the lowest at 65%. The negative control remained asymptomatic throughout the experiment (0% incidence) (Figure 2).

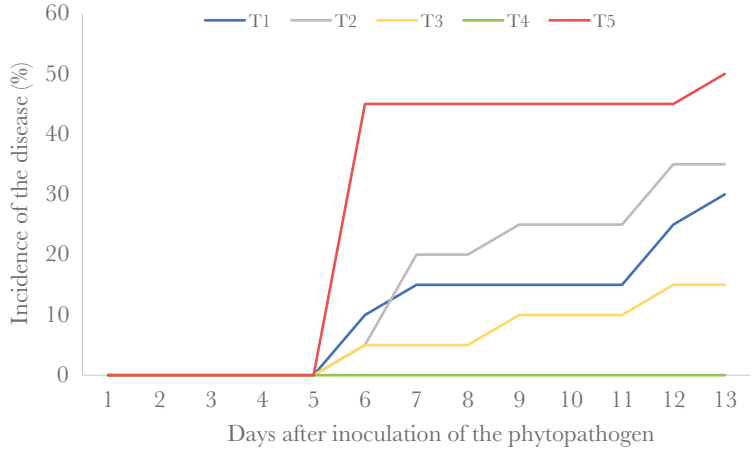

Figure 1. Disease incidence caused by *R. solani* in two-month-old serrano pepper plants (Experiment One).

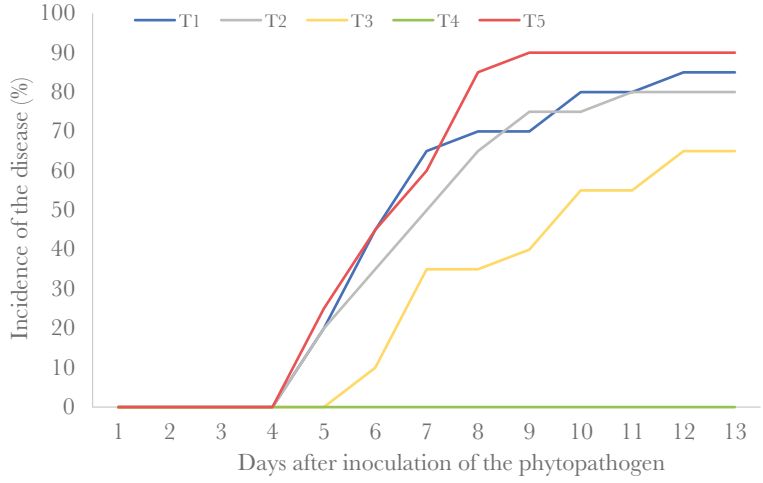

Figure 2. Disease incidence caused by *R. solani* in one-month-old serrano pepper plants (Experiment Two).

Analysis of AUDPC and biomass

Experiment one

The analysis of the Area Under the Disease Progress Curve (AUDPC) for disease incidence revealed that treatment T3 resulted in the lowest disease incidence, with an AUDPC of 3.37. In contrast, the positive control inoculated with *R. solani* P5SPA exhibited an AUDPC of 17.00 (Table 1). Regarding disease severity, treatments T1, T2, and T3 showed no significant differences, with AUDPC values of 3.12, 5.02, and 3.17, respectively. The positive control recorded a severity value of 11.45, while the negative control remained asymptomatic (Table 1). Significant differences were observed in plant biomass. The negative control produced the highest fresh and dry weights, with 5.90 g and 0.62 g, respectively. In contrast, the positive control yielded the lowest biomass, with 2.57 g (fresh weight) and 0.46 g (dry weight). Treatments T1, T2, and T3 produced intermediate values (Table 1).

Experiment Two

In the second experiment, significant differences were also observed in AUDPC values for disease incidence. T3 recorded the lowest incidence with 34.12 AUDPC, while the positive control exhibited the highest with 63.25 AUDPC (Table 1). Regarding severity estimated by Method 1, significant differences were found among treatments. T3 and T2 recorded values of 24.90 and 40.62 AUDPC, respectively, while the positive control reached 54.70 AUDPC (Table 1). Using Method 2, T3 showed the lowest disease severity with 0.19 AUDPC, whereas the positive control had the highest with 23.9 AUDPC. The negative control remained asymptomatic throughout the experiment (Table 1). Biomass data in the second experiment also showed statistical differences. The negative control produced the highest biomass, with 0.66 g (fresh) and 0.10 g (dry). The positive control recorded the lowest values, with 0.02 g and 0.01 g of fresh and dry weight, respectively (Table 1).

In the present study, the most effective treatment in reducing the incidence and severity of *R. solani*-induced disease in chili was T3 (*Bacillus* sp. SALGUA + *Trichoderma* sp. TRICOE). In contrast, the positive control exhibited the highest values for both variables (Table 1). Similarly, Abeysinghe (2009) reported that the application of *Bacillus*

Table 1. Mean comparison of AUDPC for disease incidence and severity, and plant biomass.

Treat[†]	Experiment 1				Experiment 2				
	Inc	Sev	FBW	DBW	Inc	SevM1	SevM2	FBW	DBW
T1	6.25 ab[¶]	3.12 ab	3.52 b	0.62 ab	57.37 ab	46.70 ab	18.77 ab	0.06 b	0.02 bc
T2	8.62 ab	5.02 ab	3.92 ab	0.66 ab	53.50 ab	40.62 b	15.11 ab	0.10 b	0.03 bc
T3	3.37 b	3.17 ab	4.30 ab	0.75 ab	34.12 b	24.90 b	10.19 bc	0.13 b	0.05 b
T4	0.00 b	0.00 b	5.90 a	0.96 a	0.00 c	0.00 c	0.00 c	0.66 a	0.10 a
T5	17.00 a	11.45 a	2.57 b	0.45 b	63.25 a	54.70 a	23.91 a	0.02 b	0.01 c

[†]Treat=Treatment; Inc=Incidence; Sev=Severity; FBW=Fresh biomass weight; DBW=Dry biomass weight; SevM1=Severity of method one; SevM2=Severity of method two.
[¶]Different literals in the same column indicate statistical differences ($p \leq 0.05$).

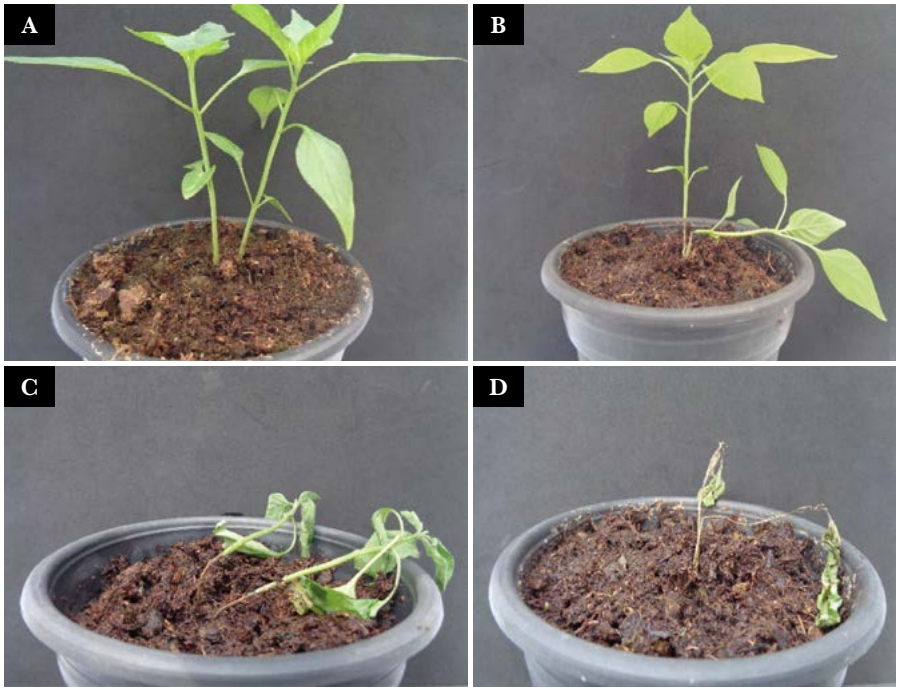

Figure 3. Representation of selected severity levels from the scale used in Experiment 2, Method 1: A) 0=no symptoms, B) 3=stem constriction, C) 5=collapsed plant with wilted leaves, D) 8=generalized rot.

subtilis CA32 (seed-inoculated) and *Trichoderma harzianum* (soil-inoculated) significantly reduced disease severity in chili, recording a severity value of 1.55 compared to 4.75 in the untreated control. Likewise, Espinoza-Ahumada *et al.* (2019) demonstrated that a combination of *Trichoderma* spp. and *Bacillus* spp. reduced disease severity in chili plants to 8.33%, compared to 19.45% in the positive control.

These findings are consistent with the results of the present study and may be attributed to the biocontrol capabilities of *Trichoderma* spp. This fungus employs multiple mechanisms, including mycoparasitism (Wang *et al.*, 2024), which involves direct contact and penetration of *R. solani* hyphae, as well as competition for nutrients and space, and antibiosis through the secretion of antifungal metabolites and enzymes such as chitinases, glucanases, and proteases (Abbas *et al.*, 2022; Kaur *et al.*, 2021; Halifu *et al.*, 2020). Additionally, there is evidence that *Bacillus* spp. can suppress *R. solani* by producing broad-spectrum antimicrobial compounds, including volatile organic compounds (VOCs) that disrupt the pathogen's cellular structure and induce oxidative stress (Ali *et al.*, 2023; Abbas *et al.*, 2019). The combined application of *Bacillus* and *Trichoderma* may also result in synergistic effects against *R. solani*, depending on environmental conditions and the specific strains used (Zhou *et al.*, 2021; Poveda & Eugui, 2022). Treatment T1 (*B. cereus* P5 + *I. lacteus* P7B) showed a moderate reduction in disease severity caused by *R. solani* (Table 1). Notably, *I. lacteus* P7B was recently documented as a mycoparasite capable of antagonizing 100% of 22 phytopathogenic fungi tested (Palemón-Alberto *et al.*, 2024). Therefore, its combination with *B. cereus* may have contributed to the disease suppression observed in this treatment. In Mexico, Pineda-Suazo *et al.* (2021) demonstrated the *in vitro* antagonistic activity of

I. lacteus against *Fusarium* spp., *Colletotrichum* spp., and *Phytophthora* spp., with growth inhibition percentages ranging from 16.7% to 46.3%. Moreover, White and Traquair (2006) reported that *I. lacteus* reduced the germination of *Botrytis cinerea* sclerotia by 100%, while in untreated controls, all sclerotia germinated. Regarding treatment T2 (*P. polymyxa* BACAGA + *B. glycinifermentans* CORI2 + *B. subtilis* PI04 + *X. feejeensis* PASI22), it also showed a moderate reduction in disease severity (Table 1). Chávez-Ramírez *et al.* (2020) reported that *P. polymyxa* exhibited *in vitro* antagonistic activity, inhibiting *R. solani* and *Pythium ultimum* by 70-80%. *B. glycinifermentans* has also been identified as an antagonist of pathogens such as *Fusarium* spp., *F. graminearum*, *Alternaria alternata*, and *F. oxysporum* f. sp. *radicis-lycopersici* (Afordoanyi *et al.*, 2023). As for *X. feejeensis*, there are no prior reports of its antagonistic effect against *R. solani*. This study presents the first evidence of its use in combination with other microbes (*P. polymyxa*, *B. glycinifermentans*, and *B. subtilis*), which resulted in a moderate reduction of disease severity (Table 1). Brooks *et al.* (2022) did report *X. feejeensis* as a potent antagonist under *in vitro* and *in vivo* conditions against *F. oxysporum* and *A. alternata*. The highest biomass was recorded in the negative control (T4), while the lowest was observed in the positive control (T5). The remaining treatments showed intermediate biomass values (Table 1). These results clearly indicate the extensive damage caused to the overall plant system by the pathogen, a phenomenon well documented in the literature (Sharma *et al.*, 2017; Huang *et al.*, 2017).

CONCLUSIONS

The most effective treatment for controlling *R. solani* in serrano pepper was T3 (*Bacillus* sp. SALGUA + *Trichoderma* sp. TRICOE). Treatments T1 (*B. cereus* P5 + *Irpex lacteus* P7B) and T2 (*P. polymyxa* BACAGA + *B. glycinifermentans* CORI2 + *B. subtilis* PI04 + *X. feejeensis* PASI22) exhibited moderate effects in disease control. Further studies under open-field conditions are required to evaluate the biocontrol efficacy of these microbial consortia, particularly that of T3, which proved to be the most effective combination for reducing both the incidence and severity of *R. solani*.

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