Interaction between arbuscular mycorrhizal fungi and rhizobacteria in chili (*Capsicum annuum* L.) plants infected with phytopathogens that cause damping off

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ABSTRACT

Objective: To determine the effect of single or combined inoculations of three rhizobacteria strains and a mycorrhizae consortium in chili seedlings infected with phytopathogens that cause damping off.

Design/methodology/approach: Guajillo chili seeds were used in this experiment. In addition, three growth-promoting rhizobacteria strains and an arbuscular mycorrhizae fungal consortium were used as beneficial microorganisms, while *Fusarium* spp. and *Rhizoctonia* spp. were used as phytopathogens. The following variables were evaluated 53 days after emergence: dry weight (mg plant^{-1}), leaf phosphorous and nitrogen content (mg plant^{-1}), and the percentage of total mycorrhizal colonization (%). Each variable was subjected to a one-way ANOVA. Afterwards, the post hoc test of the Tukey method was used to compare the results, considering that $P<0.05$ values were significant.

Results: Overall, the single or combined inoculation of the B8, B14, B23, and *Glomus* spp. (Zac-19) strains efficiently controlled damping off. These treatments recorded a higher dry matter production and nutrient content than chili plants infected with phytopathogens to which no beneficial microorganisms were applied.

Limitations on study/implications: The seedlings could have been transplanted into larger containers.

Findings/conclusions: The beneficial microorganisms had a positive effect on the growth and health of the guajillo chili seedlings. Consequently, their use is a biotechnological alternative to control damping off.

Keywords: *Capsicum annuum*, damping off, PGPR, mycorrhizae, nutrient content.

Citation: Hernández-Hernández, E. J., Hernández-Ríos, I., Peredo-Rivera, E., Soto-Estrada, A., & Torres-Aquino, M. (2024). Interaction between arbuscular mycorrhizal fungi and rhizobacteria in chili (*Capsicum annuum* L.) plants infected with phytopathogens that cause damping off. *Agro Productividad*. https://doi.org/10.32854/agrop.v17i7.2913

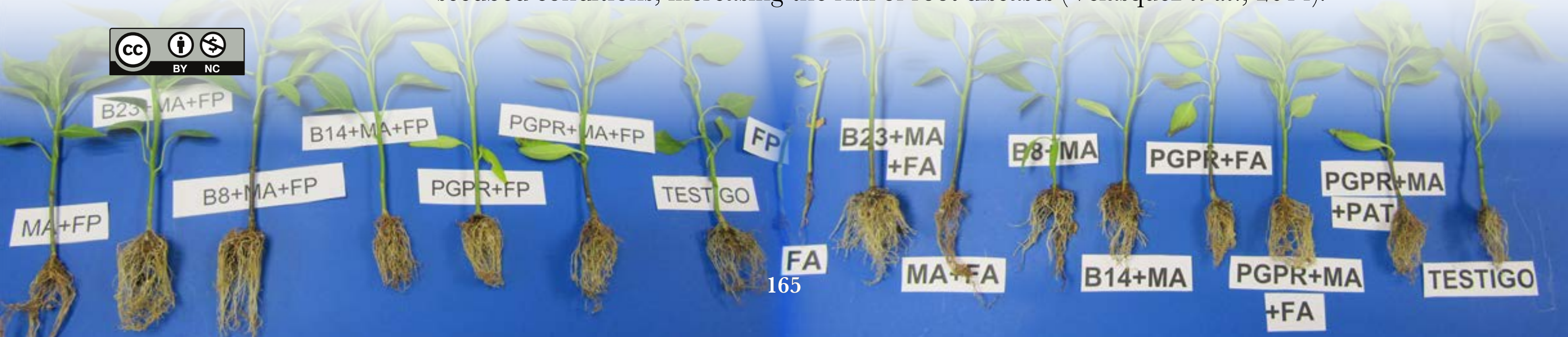
Academic Editor: Jorge Cadena Iñiguez
Guest Editor: Juan Francisco Aguirre Medina

Received: May 21, 2024.
Accepted: July 12, 2024.
Published on-line: September 18, 2024.

Agro Productividad, *17*(8). August. 2024. pp: 165-172.

INTRODUCTION

From the cultural, agronomic, nutritional, and economic points of view, ancho, jalapeño, serrano, and mirasol (guajillo in its dry form) chilies and red pepper are the most important cultivars in Mexico since they represent 70 to 80% of the domestic production (Aguirre-Mancilla *et al.*, 2017). In the Potosino Plateau, commercial varieties of chili seedlings are produced under soil (seedbed) and greenhouse (in styrofoam trays with substrate) conditions. The disinfection of soil and seeds is not a common practice under seedbed conditions, increasing the risk of root diseases (Velásquez *et al.*, 2014).



The main root disease of seedbed chili is damping off (Ita *et al.*, 2021). This disease has been reported in several states, including San Luis Potosí (Rodríguez *et al.*, 2007; Anaya-López *et al.*, 2011; Montero-Tavera *et al.*, 2013). In Oaxaca, this disease causes significant economic losses up to 76%, particularly during the germination and seedling stages (Pérez-Acevedo *et al.*, 2017).

Damping off is caused by a complex group of pathogenic soil fungi (Reyes-Tena *et al.*, 2021). Producers use fungicides to control them; however, the excessive use of these substances impacts human health, pollutes the environment, and promotes resistance among phytopathogens (Lamichhane *et al.*, 2017).

The use of natural enemies (mainly antagonist microorganisms) stands out as an ecological and sustainable alternative for the control of phytopathogens. Duc *et al.* (2017) mentioned that the inoculation of beneficial microorganisms is an alternative to improve the resistance of the chili crops to biotic stress. For example, the inoculation of arbuscular mycorrhizae fungi and actinomycetes had synergetic effects, including growth promotion and bioprotection against chili blight (*Phytophthora capsici*) (Reyes-Tena *et al.*, 2017).

Therefore, the objective of this study was to determine the effect of single and combined inoculation of three plant growth-promoting rhizobacteria (PGPR) and an arbuscular mycorrhizae (MA) consortium on growth promotion and nutrient content of chili plants infected with pathogenic fungi that cause damping off.

MATERIALS AND METHODS

Obtaining the seedlings. Guajillo chili seeds from the previous year were provided by producers from Villa de Ramos, San Luis Potosí. The seeds were disinfected with 70% alcohol and 10% commercial bleach for 5 and 15 minutes, respectively. Subsequently, they were rinsed three times with distilled water. The seeds were sown in 200-hole Styrofoam seedling trays; each hole had 6 g of substrate (peat moss-perlite in a 1:1 ratio). Substrate was previously twice-sterilized at 121 °C for 1 h., at a 3-day interval between each other.

Microorganisms and treatments. The beneficial microorganisms consisted of three strains of *Pseudomonas chlororaphis* (B8), *Pseudomonas* sp. (B14), and *Bacillus* sp. (B23). Hernández-Hernández *et al.* (2018) reported that these strains produce indole-3-acetic acid and phosphorus solubilization. In addition, the Zac-19 mycorrhizae consortium (MA) —made up of the *Glomus claroideum* (Schenck & Sm.), *G. diaphanum* (Morton & Walker), and *G. albidum* (Walker & Rhodes) morphospecies— was used. This AM was provided by the Laboratorio de Microbiología de Suelos, Colegio de Postgraduados - Campus Montecillo. The phytopathogens used were *Fusarium* spp. and *Rhizoctonia* spp. (R) strains. They were classified according to the color produced in the culture medium: yellow phytopathogen (FA), orange phytopathogen (FN), and purple phytopathogen (FP) (Hernández-Hernández *et al.*, 2018).

Inoculation. Seven days after emergence (DAE), 1 mL culture of each bacteria strain (with 10^9 CFUs [colony forming units]) were inoculated at the base of the stems of the chili seedlings. The arbuscular mycorrhizal fungi (AMF) treatments were inoculated following the method described by Chamizo *et al.* (2009). Four days later, two 5 mm wide agar discs with mycelia from the pathogen strains were placed in the crown of each seedling. The experiment was carried out under greenhouse conditions, for 53 DAE. The seedlings were irrigated every 72 hours with 50 ml of a Long Ashton 50% phosphorus nutrient solution (Hewitt, 1966). The activation of a wet wall and the watering the soil were used to keep a <30 °C temperature inside the greenhouse.

The following variables were determined at 53 DAE: dry matter of the aboveground section (mg); leaf phosphorus and nitrogen (mg plant^{-1}), and the percentage of total mycorrhizal colonization (%).

Statistical analysis. The assumption of normality and homogeneity of variance were evaluated for each dependent variable, based on the Kolmogorov-Smirnov test and Bartlett's test. Therefore, the comparisons were carried out with a one-way analysis of variance (ANOVA). Afterwards, the post hoc test of the Tukey method was used to compare the results, considering as significant those values with $P<0.05$; the information was presented as error bars.

RESULTS AND DISCUSSION

Dry matter. Significant differences ($F_{34,\ 0.05}=7.84$, $p<0.01$) were detected on dry matter production among treatments. The highest values were found in the B23+MA+FA (613.33±112.89) treatment, followed by B23+MA+R (583.33±41.77) and B8+MA+FP (503.33±76.67) (Figure 1). On the other hand, the lowest dry weight was recorded with the purple *Fusarium* negative control (CONTROL-FP, Figure 1). Therefore, the PGPR+MA co-inoculation seems to provide an effective biocontrol.

On this regard, El-Feky *et al.* (2019) reported that pepper plants infected with *F. solani*, *F. oxysporum,* and *F. moniliformis* and inoculated with *P. fluorescens* (P2) and *B. subtilis* (B1) had a significantly higher dry weight than their negative control. Raio and Puopolo (2021) reported that *P. chlororaphis* is a species with a high microbial competitiveness in the soil, which can effectively control several phytopathogens. Hyder *et al.* (2021) mention that the inoculation with *Bacillus* spp. and *Pseudomonas* spp. suppresses the damping off caused by *Pythium myriotylum*, as a consequence of the promotion of defense mechanisms and the growth of chili plants. Meanwhile, Bilgili and Güldür (2018) determined that the single or combined inoculation of three different *Glomus* species significantly increased the dry weight of *Capsicum annuum* plants infected by *F. oxysporum*. For their part, Leos-Escobedo *et al.* (2019) pointed out that the HMA+PGPR (*Pseudomonas* sp. and *Bacillus* sp.) co-inoculation significantly increased dry weight and diminished the damage caused by *P. capsici* to 23 chili cultivars. In this context, Reyes-Tena *et al.* (2017) suggested that the potential synergy between beneficial microorganisms diminishes the damage caused by the *P. capsici* infection to chili plants. Consequently, biological control is an alternative measure for the management of the diseases caused by plant pathogens, given its potential biofertilizing effect which stimulates plant growth (El-Feky *et al.*, 2019).

Leaf phosphorus content. Significant differences ($F_{33,\ 0.05}=4.52$, $p<0.01$) were detected among the treatments (Figure 2). The highest leaf phosphorus (P) values were recorded by the following treatments: B14+FN (211.07±17.86), B23+MA+R (181.64±25.54), and absolute control (177.09±52.25). On the other hand, the lowest values were reported by the negative controls: FA (31.41±11.92) and FN (11.20±0.01).

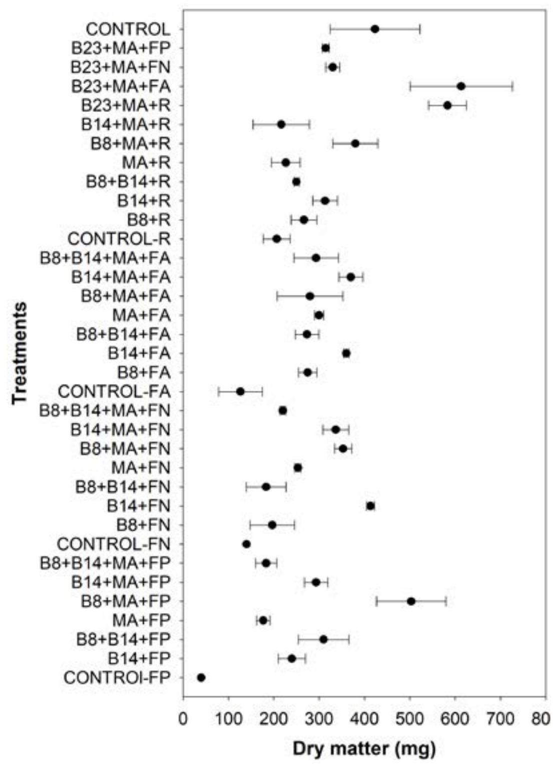


Figure 1. Comparison of the different treatments, based on the mean ± standard error (SE) of the dry weight response variable. The *post hoc* test of the Tukey method was used for contrasting purposes, considering as significant those values with $p<0.05$.

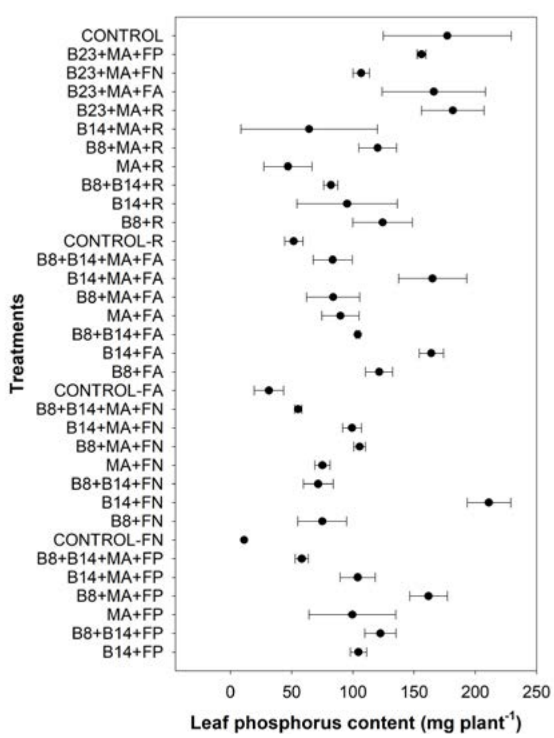


Figure 2. Effect of the individual or combined (PGPR and HMA consortium) inoculation on leaf P content in chili plants infected with the fungi that cause damping off (53 DAE).

AMF are widely known to improve the growth of plants, mainly through a greater phosphate nutrition (Thilagar *et al.*, 2015). Meanwhile, PGPRs promote plant growth, both directly —through the solubilization of phosphorus and the control of plant hormone levels— and indirectly —through the inhibition of the development of phytopathogens- (Rodríguez *et al.*, 2006; Emmanuel and Babalola, 2020). Kim *et al.* (2010) reported that the combined inoculation of *Methylobacterium oryzae* strains and AM fungi resulted in significantly higher nitrogen (N) accumulation in the roots and shoots of red pepper plants compared to uninoculated controls.

Leaf nitrogen content. Significant differences ($F_{34,\ 0.05}$=4.13, $p<0.01$) were detected between treatments (Figure 3). The highest values were obtained with the following treatments: B8+MA+FP (2632.00±624.14), B23+MA+FA (2230.33±719.86), B23+MA+R (1799.00±222.72), and B8+MA+FN (1578.83±107.72). On the other hand, the lowest values were recorded by the FN (350.00±0.01) and FP (160.00±0.01) negative controls. The combined inoculation of PGPR+AMF does not only promote the resistance to root diseases, but it also increases the nutrition content of nitrogen, phosphorus, potassium, calcium, magnesium, iron, and zinc (Weng *et al.*, 2022). Meanwhile, red peppers inoculated with rhizobacteria (*Azotobacter chroococcum*+*Azospirillum lipoferum*) absorb more N, while plants inoculated with mycorrhizal species (*Rhizophagus irregularis*+*Funneliformis mosseae*) absorb more P (Sini *et al.*, 2024).

Total mycorrhizal colonization. Differences between treatments ($F_{18,\ 0.05}$=2.70, $p<0.01$; Table 1) were determined based on the comparison of their total mycorrhizal colonization (MC) percentage. The highest values were obtained with the following

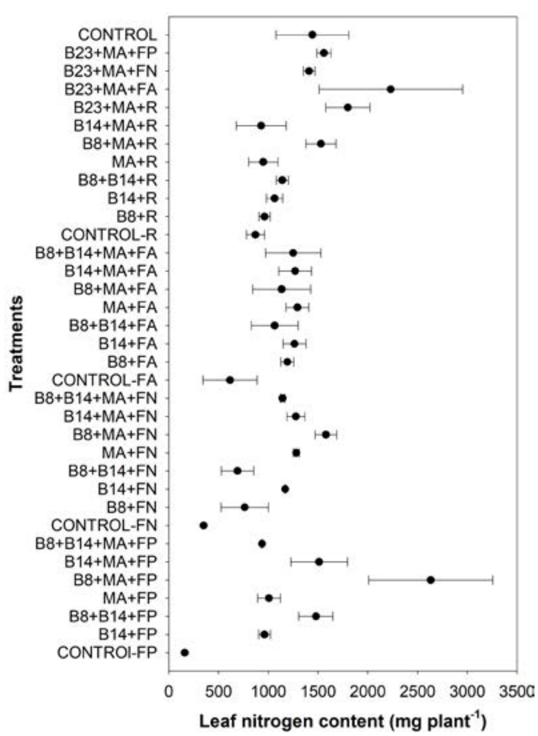


Figure 3. Effect of the individual or combined (rhizobacteria and mycorrhizal consortium) inoculation on N content of chili seedlings infected with fungi species that cause damping off (53 DAE).

Table 1. Mycorrhizal colonization (MC) in chili seedlings infected with root phytopathogens and inoculated with three strains of rhizobacteria (B8, B14 and B23) and the mycorrhizal consortium Zac-19 (MA), the average and its respective standard error ($\bar{x}$ ±S.E.). Multiple comparisons were carried out using the post hoc test of the Tukey, considering that $P<0.05$ values were significant.

Treatments	$\bar{x}$ (%)	± S.E.	Post hoc Tukey
MA+FP	79.07	2.98	abc
B8+MA+FP	60	14.72	abc
B14+MA+FP	74.26	7.58	abc
B8+B14+MA+FP	52.32	1.76	bc
MA+FN	75.55	3.06	abc
B8+MA+FN	67.5	11.08	abc
B14+MA+FN	70.09	16.72	abc
B8+B14+MA+FN	75.19	1.52	abc
MA+FA	89.44	6.24	ab
B8+MA+FA	63.44	16.73	abc
B14+MA+FA	59.58	12.27	abc
B8+B14+MA+FA	75.56	5.34	abc
MA+R	97.96	0.98	a
B8+MA+R	73.33	3.06	abc
B14+MA+R	67.04	2.13	abc
B23+MA+R	39.63	2.25	c
B23+MA+FA	51.3	7.49	bc
B23+MA+FN	57.41	3.96	abc
B23+MA+FP	83.69	9.82	abc

treatments: MA+R (97.96±0.98), MA+FA (89.44±6.24), and B23+MA+FP (83.69±9.82).

The MC percentage of the AMF-PGPR inoculation surpassed the AMF treatment in guajillo chili plants infected by *P. capsici* (Leos-Escobedo *et al.*, 2019). Under natural conditions, AMF can be found in the same ecological niche and colonization site as soilborne patogens.

CONCLUSIONS

The beneficial microorganisms used in this study had a positive effect on the growth and health of guajillo chili plants. Seedlings coinoculated with *Pseudomonas chlororaphis* (B8) and *Bacillus* sp. (B23) combined with the Zac-19 mycorrhizae consortium accumulated a higher amount of dry biomass than seedlings that were only infected by *Fusarium* spp. isolates. The same trend was observed regarding the content of P and N on leaves.

ACKNOWLEDGMENTS

The authors are grateful to the Consejo Nacional de Ciencia, Humanidades y Tecnología (CONAHCyT), for the MSc scholarship awarded to the first author.

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