



Use of *Purpureocillium lilacinum* MTL01 and *Bacillus velezensis* 83 to reduce galling by *Meloidogyne enterolobii* and improve flowering in tomato

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ABSTRACT

Background/Objective. The aim of this study was to determine the combined effect of an environmental strain of *Purpureocillium lilacinum* (MTL01) together with *Bacillus velezensis* 83, upon infection by the root-knot nematode *Meloidogyne enterolobii*.

Experimental development. Three greenhouse experiments were conducted at different times (July–October 2022, February–June 2023, and September 2024–January 2025). In each experiment, treatments included uninoculated controls, plants inoculated only with *P. lilacinum* MTL01, with *B. velezensis* 83 only, or with both microorganisms, with and without nematode infection. Certified tomato seeds were inoculated with MTL01 spores or solution as a control and transplanted after germination. *B. velezensis* 83 was inoculated one week later, followed by two additional applications. Nematodes were introduced three weeks after transplanting, and plants were subsequently maintained in a greenhouse for an additional eight weeks. At harvest, growth parameters and infection levels were quantified (biomass, length, number of flowers, root weight, number of galls, and galling index).

Results. Data of vegetative growth varied among experiments, with no clear evidence of effects associated with microbial inoculation. However, the combination of *P. lilacinum* MTL01 and *B. velezensis* 83 showed reproducible results, reducing gall formation caused by *M. enterolobii* by more than 60% and promoting a consistent tendency toward increased flowering relative to nematode-infested control plants. These results demonstrate the potential of using combined microorganisms to improve tomato performance under greenhouse conditions.

Conclusion. Our findings highlight the importance of mitigating *M. enterolobii* infection in tomato using a combination of microorganisms, not only to control nematode populations but also to increase flower yield by up to 4.3-fold (Experiment 3) in plants without nematodes, and 1.6-fold in the presence of *M. enterolobii*. This represents a promising strategy; however, further research is needed to confirm its safety and optimize its application under real agricultural conditions.

Keywords: Fungus, Bacterium, Nematode, Biocontrol, Phenology



INTRODUCTION

Tomato (*Solanum lycopersicum*) is one of the most important crops in Mexico, and its production is affected by *Meloidogyne enterolobii*. Traditional management in Mexico relies mainly on synthetic pesticides. However, efforts to reduce their use have led to the evaluation of crop rotation and biological control strategies (Lamberti, 1997; Tapia-Vázquez *et al.*, 2022). For example, arbuscular mycorrhizal fungi such as *Acaulospora longulata* (Campos *et al.*, 2013; de Sá and Campos, 2020) and filamentous fungi such as *Pochonia chlamydosporia* (Silva *et al.*, 2017), *Paraboeremia taiwanensis* (Liang *et al.*, 2020), *Trichoderma harzianum* (Jindapunnapat *et al.*, 2013), and *Purpureocillium lilacinum* stand out for their nematicidal capacity. Likewise, bacteria from the genera *Pseudomonas*, *Paenibacillus* (Sohrabi *et al.*, 2018), and *Bacillus* spp. (Basyony and Abo-Zaid, 2018; Díaz-Manzano *et al.*, 2023; Vieira de Carvalho Júnior *et al.*, 2022) have shown potential.

In Mexico, several commercial microbial-based products exist (Tapia-Vázquez *et al.*, 2022); however, farmers show resistance to their use due to the need for frequent applications and slower action compared to synthetic nematicides. Therefore, management strategies that increase the efficacy of bioproducts are required. The use of microbial combinations may represent a valuable alternative. For instance, Nesha and Siddiqui (2017) evaluated *Paecilomyces lilacinus* and *Aspergillus niger* individually and combined in carrot (*Daucus carota*), showing significant reductions in gall formation by *M. javanica*. Additionally, *Bacillus* species are known plant growth promoters and have been reported to contribute to pest control. Suppression of *Meloidogyne* populations by *B. subtilis* has been documented in chili (*Capsicum annuum*) (Mokbel *et al.*, 2022; Soto Deza *et al.*, 2012) and tomato (Basyony and Abo-Zaid, 2018). Strains BMH e INV, related to *B. velezensis*, reduced more than 90% of galls and eggs of *M. incognita* in tomato (Cruz-Magalhães *et al.*, 2022). Likewise, *B. velezensis* Bv-25 reduced galling by 73.8% in cucumber roots (*Cucumis sativus*) through root colonization and volatile compound production (Tian *et al.*, 2022). Moreover, *B. velezensis* VB7 inhibited egg viability and caused mortality of *M. incognita* juveniles in vitro and induced innate immune responses in tomato (Kamalanathan *et al.*, 2023).

The strain *B. velezensis* 83 is commercialized in Mexico as a foliar biofungicide (Fungifree AB®), used for biological control of phytopathogenic fungi in various crops (Galindo *et al.*, 2013). Additionally, Balderas-Ruiz *et al.* (2021) demonstrated its capacity to promote tomato growth and control *Botrytis cinerea*, improving yield and quality.

This study aimed to evaluate individually and in combination *B. velezensis* 83 and the environmental strain *P. lilacinum* MTL01 against *M. enterolobii* infection in tomato, as well as their efficiency in increasing yield.

EXPERIMENTAL DEVELOPMENT

Nematode isolation. A *Meloidogyne enterolobii* population was established in the laboratory for biological control studies. *M. enterolobii* nematodes (GenBank OM721664) were obtained from infected tomato roots intended for nematode propagation, originating from a chard crop (*Beta vulgaris* var. *cicla*) and collections from Veracruz (Salinas-Castro *et al.*, 2022). Egg masses were extracted from infected roots, and second-stage juveniles (J2) were recovered by a modification of the Baermann funnel method (Tintori *et al.*, 2022).

These juveniles were used to establish laboratory populations on tomato cv. Calafia F1 (Harris Moran), due to its susceptibility to this nematode.

Fungal isolation and identification. During independent propagation experiments of *Meloidogyne paranaensis* in a tomato microculture system, the survival of one of five seedlings inoculated with egg masses was observed. In the surviving plant, J2s failed to hatch because of a fungal infection. An axenic fungal culture was obtained on potato dextrose agar (PDA) supplemented with ampicillin ($100 \mu\text{g mL}^{-1}$) and kanamycin ($50 \mu\text{g mL}^{-1}$). The isolate was characterized morphologically and identified molecularly by amplification and sequencing of intergenic ITS region of ribosomal DNA gene with the primers ITS1 (5'-TCCGTAGGTGAACCTGCG-3') and ITS4 (5'-TCCTCCGCTTATTGATATG-3') (White *et al.*, 1990), followed by phylogenetic comparison with related strains of the same genus and species. Phylogenetic reconstruction was performed using the TN93 model (Tamura and Nei, 1993), with branch support values calculated using the aLRT (SH-like) algorithm and visualized with the SeaView software.

Interaction between *M. enterolobii* and *P. lilacinum* MTL01. To evaluate the interaction and possible parasitism of *P. lilacinum* MTL01 on *M. enterolobii*, *in vitro* assays and observations of organisms recovered from infected tomato plants were carried out. First, a confrontation assay was performed on PDA medium supplemented with kanamycin ($50 \mu\text{g mL}^{-1}$) and ampicillin ($100 \mu\text{g mL}^{-1}$), in which *M. enterolobii* egg masses were placed alongside a *P. lilacinum* colony, approximately 5 cm apart. After 5 days, egg parasitism was observed. In addition, at the end of the greenhouse experiments, females from galls collected from tomato roots treated with *P. lilacinum* were recovered in water, and fungal mycelium emerged after 48 h. Egg masses obtained from the same treatment were disinfected with 3% NaOCl and washed four times with sterile water. The recovered eggs were incubated on antibiotic-supplemented PDA to evaluate fungal colonization.

Evaluation of the combination of *P. lilacinum* MTL01 and *B. velezensis* 83 as control agents against *M. enterolobii* in tomato plants. We aimed to determine whether there was a synergistic effect on *M. enterolobii* control when *P. lilacinum* MTL01 and *B. velezensis* 83 (commercial product Fungifree AB®, LMG S-30921) are inoculated together in tomato plants, compared with the effects of each microorganism applied separately. The experimental design was intended to favor early colonization of the MTL01 fungus in tomato seedlings during germination, and to introduce *B. velezensis* 83 treatments two weeks after germination, followed by two additional applications every 20 days. For the biocontrol assays, tomato seeds were surface-disinfected with 10% NaOCl and 70% ethanol, followed by washes with sterile distilled water. Then seeds were incubated for 24 h in a suspension of 1×10^6 spores mL^{-1} of MTL01 in 10% sterile glycerol before sowing. After germination and transplanting, *B. velezensis* 83 was applied to the substrate as a suspension containing 1×10^8 CFU per pot, with two additional applications every 20 days. Three weeks after transplanting, plants were inoculated with a combination of J2s and eggs of *M. enterolobii* to apply between 1,200 and 3,400 nematodes per plant, depending on each experiment.

Experiments were repeated three times over time in a greenhouse under a completely randomized design, at different seasons of the year: Experiment 1, July to October 2022; Experiment 2, February to June 2023; and Experiment 3, September 2024 to January 2025. In each experiment, four treatments were evaluated (with and without *M. enterolobii*): 1) no microorganisms; 2) *P. lilacinum* MTL01 only; 3) *B. velezensis* 83 only; and 4) the

combination of *P. lilacinum* MTL01 + *B. velezensis* 83. Six plants were included per treatment. Plants were monitored for eight weeks after nematode inoculation, and the following variables were quantified: total length, aboveground biomass (fresh and dry weight), number of flowers, root fresh weight, number of galls, and galling index (Taylor and Sasser, 1978). Data were analysed by one-way ANOVA, and means were compared using Tukey's test ($p \leq 0.05$) in GraphPad Prism.

Characterization of *P. lilacinum* MTL01 and interaction with *M. enterolobii*. Based on morphological and molecular characteristics of the fungal isolate obtained from *M. paranaensis* eggs (Figure 1A), *Purpureocillium lilacinum* identification was confirmed, and the isolate was designated as strain MTL01, with 100% identity and 100% coverage for the ITS region (GenBank OR470716). On PDA medium, the colony was initially white-beige, with a spongy-cottony texture, and gradually became lilac to violet as sporulation progressed (Figure 1C and 1D). Microscopic observation showed branched conidiophores, elongated phialides, and oval conidia (Figure 1B) (Nguyen Thi H *et al.*, 2025).

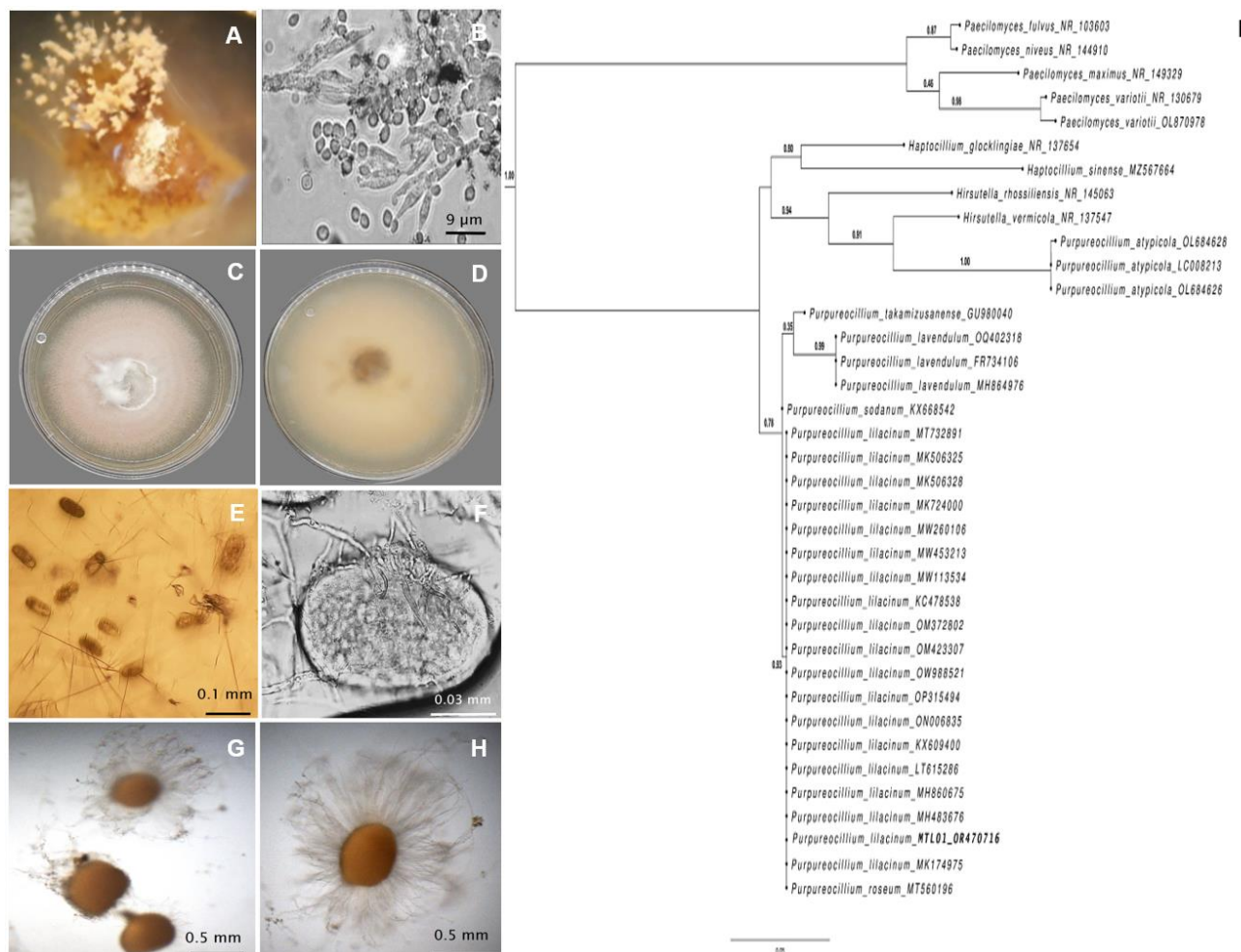


Figure 1. Isolation and characterization of *P. lilacinum* MTL01. **A)** MTL01 mycelium growing on an egg mass of *M. paranaensis*. **B)** Reproductive structures of MTL01: conidiophores, phialides, and conidia. **C and D).** Appearance of MTL01 on PDA medium: upper and lower views. **E)** *M. enterolobii* eggs parasitized by MTL01 on PDA medium. **F)** Parasitized egg from roots in Experiment 1. **G and H)** Parasitized adult females from roots in Experiment 1. **I)** Phylogeny of MTL01 compared with other strains and related species.

Meloidogyne enterolobii successfully established and completed its life cycle in the roots of Calafia F1 tomato, causing the formation of characteristic galls associated with root-knot nematode infection (Figure 2A), which led to overall plant decline. These structures contained adult females and egg masses associated with the nematode reproductive cycle (Tapia-Vázquez *et al.*, 2022). A close-up of a root knot showed an egg mass protruding from the tissue (Figure 2B), while observations under a stereomicroscope revealed adult females with their characteristic pear-shaped morphology (Tapia-Vázquez *et al.*, 2022), accompanied by egg masses (Figure 2C). Recently hatched J2s were motile and survived in water for up to two weeks (Figure 2D).

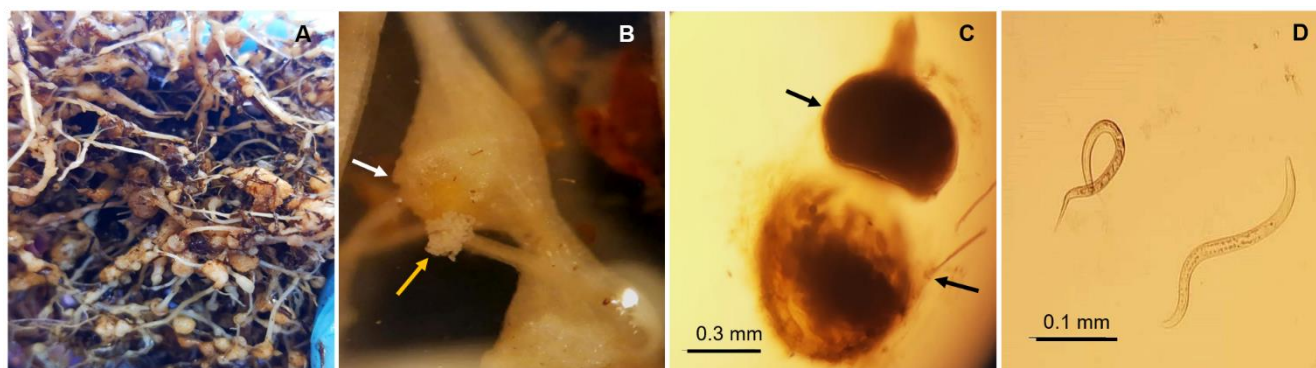


Figure 2. Laboratory population of *M. enterolobii*. **A)** Gallling roots after *M. enterolobii* propagation 90 days after J2 inoculation in a 4-month-old tomato plant. **B)** Gall with an exposed egg mass (white arrow) and eggs (yellow arrow). **C)** Pear-shaped female body (upper arrow) and egg mass (lower arrow). **D)** J2-stage nematodes hatched in water.

In the *in vitro* confrontation assays, *M. enterolobii* eggs placed in the presence of *P. lilacinum* MTL01 were progressively colonized by the fungus, preventing juvenile hatching (Figures 1E and 1F). Meanwhile, females recovered from galls from roots treated with MTL01 showed visible mycelial growth after incubation in sterile distilled water (Figures 1G and 1H). Likewise, eggs obtained from those galls produced *P. lilacinum* colonies when incubated on PDA supplemented with kanamycin and ampicillin, confirming the nematophagous potential of this strain.

In the three greenhouse experiments, variable responses were observed in vegetative growth parameters (Tables 1, 2, and 3), possibly due to the weather conditions of the season of the year. In Experiment 1, the combined treatment of *P. lilacinum* MTL01 and *B. velezensis* 83 increased shoot length in the absence of nematodes; however, this effect was not observed in plants inoculated with *M. enterolobii*. Differences in root weight were not significant; nevertheless, nematode-infected roots were heavier, possibly due to gall formation (Table 1). In Experiment 2, no consistent effects on plant growth associated with any treatment were detected (Table 2). In Experiment 3, individual inoculation with *P. lilacinum* MTL01 reduced root fresh weight (27 g) in nematode-infested plants, while the other parameters remained comparable among treatments (Table 3). This suggests that the growth-promoting responses induced by *Purpureocillium lilacinum* MTL01 and *Bacillus velezensis* 83 were influenced by the specific greenhouse conditions, as has been reported for tomato plants (Jeong *et al.*, 2026).

Table 1. Phenological response variables in the presence and absence of *M. enterolobii* under different treatments, Experiment 1 (July to October 2022).

Treatment	Height (cm)		Shoot fresh weight (g)		Shoot dry weight (g)		Root fresh weight (g)	
	Control	+ <i>M. enterolobii</i>	Control	+ <i>M. enterolobii</i>	Control	+ <i>M. enterolobii</i>	Control	+ <i>M. enterolobii</i>
Control	122.3±12.2 ^a	118.3±12.7 ^a	38.7±5.0 ^a	27.9±7.8 ^a	5.0±0.6 ^{ab}	3.7±1.0 ^b	17.6±5.4 ^{abc}	22.8±8.4 ^{ab}
MTL01	116.3±9.9 ^a	104.8±8.5 ^a	32.3±6.1 ^a	30.6±7.8 ^a	4.34±0.7 ^{ab}	4.0±0.9 ^b	12.5±2.2 ^c	24.5±6.8 ^a
<i>B.v.</i> 83	109.8±8.4 ^a	116.8±8.0 ^a	27.4±8.1 ^a	36.3±5.8 ^a	4.0±1.2 ^b	5.3±0.8 ^{ab}	15.6±4.7 ^{abc}	19.7±3.7 ^{abc}
MTL01 + <i>B.v.</i> 83	121.7±12.0 ^a	108.5±3.9 ^a	39.1±12.3 ^a	39.0±5.8 ^a	6.1±1.6 ^a	5.4±0.8 ^{ab}	14.2±2.2 ^{bc}	23.0±3.6 ^{ab}

*Different letters indicate statistically significant differences (one-way ANOVA, Tukey's test; $p < 0.05$).

Table 2. Phenological response variables in the presence and absence of *M. enterolobii* under different treatments, Experiment 2 (February to June 2023).

Treatment	Height (cm)		Shoot fresh weight (g)		Shoot dry weight (g)		Root fresh weight (g)	
	Control	+ <i>M. enterolobii</i>	Control	+ <i>M. enterolobii</i>	Control	+ <i>M. enterolobii</i>	Control	+ <i>M. enterolobii</i>
Control	104.9±7.5 ^{ab}	106.7±4.4 ^{ab}	36.6±2.4 ^a	36.6±2.4 ^a	7.2±1.0 ^a	5.9±1.3 ^{abc}	18.5±1.1 ^{ab}	18.6±4.6 ^{ab}
MTL01	108.8±4.4 ^a	107.0±8.0 ^a	31.1±3.0 ^{ab}	31.1±3.0 ^{ab}	5.0±0.9 ^{bc}	5.4±0.4 ^{abc}	16.2±4.2 ^{bc}	13.6±1.2 ^{bc}
<i>B.v.</i> 83	108.4±3.8 ^a	95.2±6.2 ^b	32.9±4.0 ^{ab}	32.9±4.0 ^{ab}	6.0±1.5 ^{ab}	5.3±0.4 ^{bc}	22.8±4.6 ^a	13.6±1.5 ^{bc}
MTL01 + <i>B.v.</i> 83	104.5±7.9 ^{ab}	104.1±6.0 ^{ab}	27.4±3.9 ^b	27.4±3.9 ^b	4.3±0.9 ^{bc}	4.2±0.7 ^c	16.2±2.3 ^{bc}	11.6±2.1 ^c

* Different letters indicate statistically significant differences (one-way ANOVA, Tukey's test; $p < 0.05$).

Table 3. Phenological response variables in the presence and absence of *M. enterolobii* under different treatments, Experiment 3 (September 2024 – January 2025).

Treatment	Height (cm)		Shoot fresh weight (g)		Shoot dry weight (g)		Root fresh weight (g)	
	Control	+ <i>M. enterolobii</i>	Control	+ <i>M. enterolobii</i>	Control	+ <i>M. enterolobii</i>	Control	+ <i>M. enterolobii</i>
Control	120.4±18.9 ^{ab}	121.3±10.1 ^{ab}	45.5±11.1 ^a	48.1±3.8 ^a	8.2 ± 2.3 ^a	8.4±0.8 ^a	31.6±17.3 ^{ab}	35.8±3.3 ^a
MTL01	126.7±6.8 ^a	94.7±14.4 ^b	39.0±4.9 ^{ab}	29.8±7.7 ^b	6.9±0.9 ^a	5.5±1.9 ^a	11.9±0.5 ^d	27.0±3.9 ^{abc}
<i>B.v.</i> 83	107.8±13.9 ^{ab}	104.1±12.7 ^{ab}	40.9±7.8 ^{ab}	41.6±4.0 ^{ab}	8.2±1.8 ^a	7.9±0.9 ^a	20.4±7.4 ^{bcd}	32.43±4.1 ^{ab}
MTL01 + <i>B.v.</i> 83	115.5±18.9 ^{ab}	106.3±21.9 ^{ab}	40.3±10.1 ^{ab}	37.0±9.7 ^{ab}	7.6±2.0 ^a	6.4±1.9 ^a	16.4±2.8 ^{cd}	30.1±7.2 ^{ab}

*Different letters indicate statistically significant differences (one-way ANOVA, Tukey's test; $p < 0.05$).

In contrast to the variability observed in vegetative growth parameters, some effects showed more reproducible trends across experiments. In particular, two key parameters stand out: a) number of flowers and b) number of galls. Although the flowering response was also variable among experiments, combined inoculation with *P. lilacinum* MTL01 and *B. velezensis* 83 showed a consistent tendency toward higher flowering levels in all cases (Figures 3A, 3C, and 3E). In Experiment 1, the combination of both microorganisms

resulted in an increase of 1.3-fold more flowers than the uninoculated control plants without nematodes, and 2.3-fold more flowers in plants infected with *M. enterolobii* (Figure 3A).

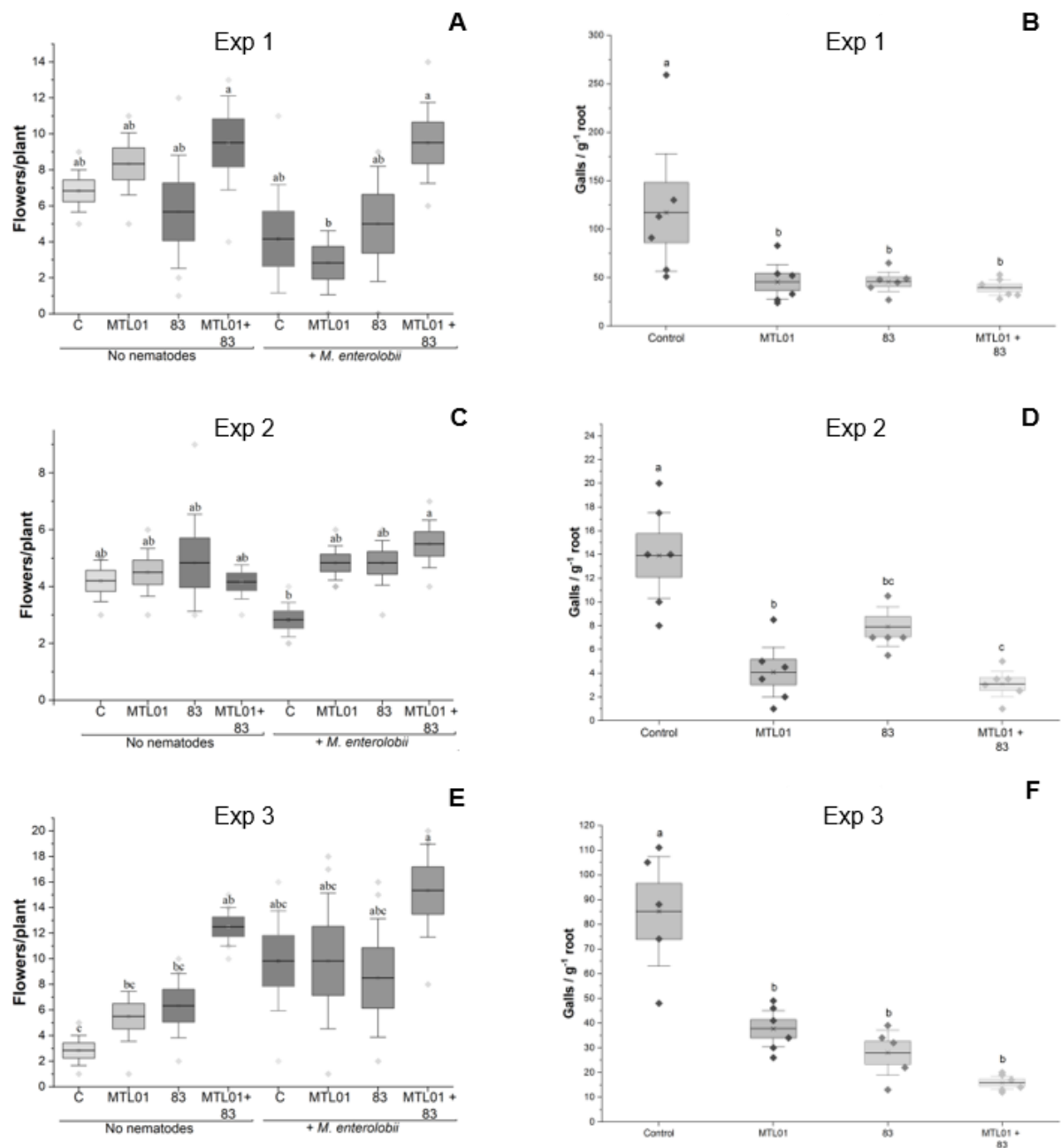


Figure 3. The combination of *P. lilacinum* MTL01 and *B. velezensis* 83 promotes flowering and reduces galling in tomato plants infected with *M. enterolobii*. **A), C), and E):** Flower counts throughout the life cycle before the end of each experiment. **B), D), and F):** Root galling by experiment. Different letters indicate statistically significant differences (ANOVA, Tukey's test; $p < 0.05$).

In Experiment 2, differences among treatments were less pronounced in the absence of nematodes (1.3-fold increase); however, the combination of both microorganisms produced almost twice as many flowers (1.94-fold increase) as the control in the presence of *M. enterolobii* (Figure 3C). Experiment 3 showed the largest differences under the

combined microbial treatment, with a 4.3-fold increase in flower number in the absence of nematodes and a 1.6-fold increase in plants with nematodes (Figure 3E).

Galling index decreased significantly in each experiment. In Experiment 1, *P. lilacinum* MTL01 and *B. velezensis* 83, individually, reduced gall formation by approximately 60%, while the combined treatment reduced it by 66%, based on one gram of peripheral roots (Figure 3B). In Experiment 2, reductions were approximately 71% (MTL01), 43% (*B. velezensis* 83), and 78% for the combination (Figure 3D). Finally, in Experiment 3, galling was reduced by approximately 56% (MTL01), 67% (*B. velezensis* 83), and 81% (combination), confirming the reproducibility of the effect across assays (Figure 3F and Table 4). These results emphasize that both *P. lilacinum* MTL01 and *B. velezensis* 83 contribute to improved biocontrol of *M. enterolobii*, with their combined application being the most effective strategy, in agreement with other reports (Díaz-Manzano *et al.*, 2023; de Paula *et al.*, 2024; Huang *et al.*, 2016).

Table 4. Number and percentage of gall reduction by treatment in three independent experiments.

Treatment	Experiment 1		Experiment 2		Experiment 3	
	No. of galls	Reduction (%)	No. of galls	Reduction (%)	No. of galls	Reduction (%)
Control	117.0±76.0 ^a	-	13.9±4.5 ^a	-	85.2±25.4 ^a	-
<i>P. lilacinum</i> MTL01	45.5±22.3 ^b	61 %	4.1±2.6 ^b	71 %	37.7±9.1 ^b	56 %
<i>B. velezensis</i> 83	45.7±12.4 ^b	61 %	7.9±2.1 ^{bc}	43 %	28.0±10.4 ^b	67 %
MTL01+ 83	39.5±10.0 ^b	66 %	3.1±1.3 ^c	78 %	15.8±3.3 ^b	81%

* Different letters indicate statistically significant differences (one-way ANOVA, Tukey's test; $p < 0.05$).

The positive impact of the combined treatment on flowering and gall reduction across the experiments demonstrated a more stable and reproducible biological response. These results indicate that although growth promotion may depend on environmental conditions, the protective and reproductive effects conferred by the microbial combination are more consistent.

CONCLUSION

The combination of *P. lilacinum* MTL01 and *B. velezensis* 83 proved to be an effective strategy for the biocontrol of *Meloidogyne enterolobii* in tomato plants under greenhouse conditions. Combined inoculation of both microorganisms reduced galling by more than 60% compared with plants infected with the nematode and consistently promoted an increase in flowering ranging from 1.3- to 4.3-fold relative to the control, both in the presence and absence of the parasite. These results provide a foundation for further studies to evaluate their effectiveness under field conditions and to optimize the formulation and dosage of this treatment for sustainable agricultural applications.

Limitations. The main limitation of this study was the small sample size used in the experiments. Nevertheless, the use of certified seeds helped maintain low variability among the technical replicates of each experiment, making it possible to perform reliable statistical comparisons of the results obtained.

Conflict of interest. The authors declare that they have no conflicts of interest regarding the research, authorship, and/or publication of this note.

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