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Efficacy of bacterial strains isolated from tomato rhizosphere against Meloidogyne hapla, Chitwood, 1949.

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Article Title

Efficacy of bacterial strains isolated from tomato rhizosphere against *Meloidogyne hapla,* Chitwood, 1949.

Abstract

Background

Root-knot nematodes (Meloidogyne spp.) remain a major constraint to tomato production worldwide, causing substantial yield losses and necessitating effective control solutions. This study assessed the nematicidal activity of rhizosphere bacteria isolated from tomato plants infected with Meloidogyne spp.

Results

Four bacterial strains belonging to the genera Bacillus and Pseudomonas demonstrated high mortality of second-stage juveniles of Meloidogyne hapla Chitwood, 1949 (86.2–90.6%) under laboratory conditions. In two independent greenhouse experiments, these strains reduced gall formation by 79.3–85.4%, improved biometric parameters, increased yield by 46.3–61.4% relative to the infected control, and maintained fruit biochemical quality at levels comparable to the chemical and biological standards.

Conclusions

The findings confirm that selected indigenous bacterial strains can effectively suppress root-knot nematodes and enhance tomato growth under nematode pressure, supporting their potential as active agents for the development of effective biocontrol products.

Keywords: root-knot nematodes, tomato, biological control, bacteria, *Meloidogyne hapla*

Background

Tomato (*Solanum lycopersicum* L.) is among the world's most important vegetable crops. In 2024, the global tomato processing volume reached 45.8 million tons, up by 1.43 million tons from 2023 (World Processing Tomato Council 2024; World Processing Tomato Council 2025). In the

Russian Federation, open-field tomato output increased 1.2-fold (to 1.1 million tons) and greenhouse production 1.7-fold (to 724 thousand tons) over the last five years (Russian Agricultural News Service 2024). Intensification of cultivation and expansion of planted areas lead to increased abundance and impact of key pests. Among the key pest groups affecting tomato are root-knot nematodes (*Meloidogyne* spp.), which can reduce tomato yield by up to 68% (Padilla-Hurtado et al. 2022). In temperate regions, *Meloidogyne hapla* is particularly problematic, causing losses of up to 29% (Rawal 2020).

Timely identification of plant damage caused by root-knot nematodes is difficult due to the hidden nature of the early stages of invasion and the presence of similar symptoms caused by phytopathogens. Due to this, broad-spectrum plant protection products are often applied, but they lack specific activity against nematodes, reducing control efficacy (Nekoval et al. 2024a). For targeted control of root-knot nematodes, chemical nematicides are traditionally used, providing rapid suppression, but their application is limited by ecological and toxicological risks (Obiazikwor et al. 2025).

Antagonistic rhizobacteria suppress *M. hapla* through various molecular mechanisms that affect nematode physiology, development, and behavior. These include the production of pore-forming toxins, hydrolytic enzymes, and volatile neuroactive compounds. The key mechanisms underlying the nematicidal activity of three representative bacterial species are illustrated in Figure 1 (Fig. 1) (Bel et al. 2023; Siddiqui et al. 2005; Díaz-Manzano et al. 2023).

[figure 1 near here]

In global practice, biological nematicides based on bacterial strains such as *Bacillus firmus*, *Burkholderia rinojensis*, *Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Burkholderia cepacia* are widely used (Ayaz et al. 2024; Subedi et al. 2020). In Russia, the only registered biological nematicide is «Nematophagin-Micopro» based on *Arthrobotrys oligospora*, approved for potato, and no products are available for tomato (State Catalog of Pesticides and Agrochemicals. Nematophagin-Micopro, P 2025 accessed 2025-06-15).

Due to this, there remains a need in Russia to identify bacterial strains with high nematicidal activity. Such strains can be used as active agent in the development of new nematicides capable of effectively controlling populations of *Meloidogyne* spp., including *M. hapla*. Considering the mechanisms by which bacteria act against root-knot nematodes, the aim of this study was to identify rhizosphere bacteria with high nematicidal activity against *M. hapla* and to evaluate their effects on the biometric and biochemical parameters of tomato plants.

Methods

Location of the research

The study was conducted at the laboratory of bio-rational means and technologies of plant protection for ecologically friendly, resource-saving and organic agriculture, Federal Research Center of Biological Plant Protection (FSBSI FRCBPP), Krasnodar (45.035656° N, 38.975106° E; WGS84), Russia.

Sampling and microbiological analysis of soil samples

Soil and tomato plant samples infected with root-knot nematodes were collected in farms of Krasnodar Krai (45.206196° N, 37.286695° E; WGS84), southern Russia. Tomato plants with rhizosphere soil were placed in polyethylene bags and transported to the laboratory at 4 °C. Rhizosphere soil was separated from roots, passed through a sterile 2 mm sieve (Lychagina et al., 2018), and used for microbiological analysis. Ten grams of soil were suspended in 90 mL of distilled water (1:10) and shaken on an orbital shaker for 5 min. A 0.1 mL aliquot of the suspension was plated on fish meal hydrolysate agar (FMH/GRM-agar; State Research Center for Applied Microbiology and Biotechnology, Obolensk, Russia; TU 9398-021-78095326-2006) and incubated inverted at 25 °C for 72 h. medium composition per litre ($g \cdot L^{-1}$): pancreatically digested fish-meal hydrolysate 8.0, fermentative peptone 8.0, NaCl 4.0; pH 7.0-7.4. Agar plates were prepared by adding agar to 10 $g \cdot L^{-1}$ to the same base and sterilizing by autoclaving (121 °C, 15 min). Morphologically distinct colonies were re-streaked to pure culture using the depletion-streak method, transferred to slanted FMH agar, and stored at 5-8 °C (Pokhilenko et al., 2009).

Species identification of bacterial isolates

To determine the species affiliation of the studied microorganisms, the 16S rRNA gene was analyzed. Identification was conducted using the Sanger sequencing method with an ABI 3500xl analyzer and the BrilliantDye kit from Nimagen (Janda and Abbott, 2007). The following bacterial species were identified and used in this study: three strains of *Pseudomonas silesiensis*, as well as single strains of *Bacillus thuringiensis*, *Bacillus mycoides*, *Metabacillus idriensis*, *Bacillus cereus*, *Lysinibacillus fusiformis*, and *Brucella intermedia*.

Obtaining a suspension of the northern root-knot nematode (*M. hapla*)

A laboratory population of *M. hapla* maintained on the susceptible tomato hybrid Prima Dona since 2022 was used for experiments (Nekoval et al. 2025). Egg sacs were separated from tomato roots under a stereomicroscope with a dissecting needle (Konrat et al. 2021), transferred to Petri dishes with water, and incubated at 25±3 °C. After 3 days, hatched second-stage juveniles (J2) larvae were collected with a pipette and used in subsequent assays.

Effect of the bacterial strains on *M. hapla* under laboratory conditions.

The laboratory experiment was performed in 35 mm Petri dishes containing 50 J2 of *Meloidogyne hapla* in 1 mL suspension, with five replicates per treatment (Migunova et al., 2015). Prior to assays, bacterial suspensions were grown in FMH/GRM broth (same manufacturer; TU 9398-021-78095326-2006) at 25 °C, 180 rpm for 72 h and then standardized. Broth composition per litre ($g \cdot L^{-1}$): fish-meal hydrolysate 8.0, fermentative peptone 8.0, NaCl 4.0; pH 7.0–7.4. Inocula were standardized by plate counts/OD_{600} calibration to 1.0×10^8 $CFU \cdot mL^{-1}$ and applied at 1.0 mL per dish. Reference nematicides were Nematophagin-Micopro (*Arthrobotrys oligospora* F-1303 ≥ 3×10^6 spores/g) and Verango (fluopyram 400 g L^{-1}), prepared according to manufacturer instructions (State Catalog of Pesticides and Agrochemicals. Nematophagin-Micopro, P 2025, accessed 2025-06-15; State Catalog of Pesticides and Agrochemicals. Verango, KC, 2025, accessed 2025-06-15). Control dishes received 1.0 mL of sterile water. J2 mortality was assessed at 24, 48, and 72 h after the start of exposure at 25 °C. At 72 h, nematodes were separated

from the bacterial suspension using a Baermann-type funnel, allowed to migrate for 24 h (96 h), and then counted under a microscope; immobilized, straightened juveniles with visible damage were recorded as dead (Konrat et al., 2021). Nematicidal efficacy (%) was calculated according to the Schneider–Orelli formula (Talavera-Rubia et al., 2020).

$$M_{corr} = \frac{M_t - M_c}{100 - M_c} \times 100 \quad (1)$$

where,

M_{corr} – corrected mortality (%);

M_t – mortality in the treatment (%);

M_c – mortality in the control (%).

Effect of the bacterial strains on *M. hapla* under the greenhouse conditions.

Two independent greenhouse experiments ("first" and "repeat") were conducted using the susceptible tomato hybrid 'Prima Dona' (State Register of Breeding Achievements Approved for Use in the Russian Federation 2025, accessed 2025-06-15), grown in 5-L pots with five replicates, each consisting of five plants (Nekoval et al., 2023). Tomato seedlings were transplanted on day 0 and treated with bacterial suspensions on day 0 (root drench). Liquid bacterial cultures were prepared as for laboratory assays in FMH/GRM broth (25 °C, 180 rpm, 72 h; composition per litre: fish-meal hydrolysate 8.0, fermentative peptone 8.0, NaCl 4.0; pH 7.0–7.4) and then standardized to the working density. Inocula were applied as a root drench at transplanting at 1.0×10^8 CFU·mL^{-1}; each plant received 20 mL of suspension (2.0×10^9 CFU per plant). Control plants received an equal volume of sterile water. Reference nematicides were Nematophagin-Micopro (*Arthrobotrys oligospora* F-1303 ≥ 3×10^6 spores/g) and Verango (fluopyram 400 g L^{-1}), prepared and applied according to manufacturer instructions (State Catalog of Pesticides and Agrochemicals. Nematophagin-Micopro, P 2025, accessed 2025-06-15; State Catalog of Pesticides and Agrochemicals. Verango, KC 2025, accessed 2025-06-15). Nematode inoculation (500 J2 per plant) was performed on day 7 after treatment. Galling was evaluated 60 days after

transplanting under greenhouse conditions (Udalova et al., 2023; Nekoval, 2024a). The repeat experiment reproduced the first with identical treatments, doses, layout and timing; datasets from the two experiments were analyzed separately and are presented side-by-side in the Results.

Effect of bacterial strains on biometric and biochemical parameters of tomato plants

Biometric parameters were recorded at the fruiting stage. Plant height (cm) was measured from the soil surface to the shoot apex; the number of fruits per plant was counted manually. Average fruit weight (g) was calculated from ten randomly selected ripe fruits per plant. Total fruit mass per plant (g) was obtained by summing the weight of all harvested fruits, and yield (kg m^{-2}) was calculated relative to the cultivation area (FSBSI Rosinformagrotech 2018).

For biochemical analysis, ripe tomato fruits were evaluated for soluble sugars (%) using an Atago PAL-1 refractometer (GOST 8756.13-87), and ascorbic acid (vitamin C, mg/100 g) by high-performance liquid chromatography (HPLC) on an Ultimate 3000 SD system after extraction with metaphosphoric acid (GOST 24556-89). Titratable acidity (%) was determined by titration of aqueous fruit extracts with NaOH using phenolphthalein as indicator (GOST ISO 750-2013).

Statistical analysis

All data were expressed as means of five independent replicates ± SE. Model assumptions (normality of residuals and homogeneity of variances) were verified prior to inference. Percentage variables (J2 mortality, biological efficacy/galling reduction) were analyzed after arcsine$\sqrt{(x/100)}$ transformation; count data (number of galls per plant) were $\log_e(x+1)$ – transformed before analysis. For greenhouse trials, analyses were conducted separately for each of the two independent experiments. Treatment effects were tested by one-way ANOVA within each experiment. For J2 mortality dynamics, two-way ANOVA (factors: Treatment and Time) was additionally applied for 24-72 h, and a separate two-way ANOVA was run for 72-96 h to evaluate post-filtration persistence. When overall effects were significant ($\alpha = 0.05$), Duncan's multiple range test was used for post-hoc pairwise comparisons. For clarity, tables and figures present

155 untransformed means ± SE, while statistical inferences were based on the transformed data. Each
156 replicate represented an independent experimental unit (Petri dish or individual plant).

157 # Results

158 **Selection and analysis of bacteria isolated from the tomato rhizosphere**

159 From rhizosphere samples, we initially obtained 37 colony-forming isolates. After dereplication
160 based on colony morphology and growth traits, 9 non-redundant isolates were retained. These nine
161 isolates were purified and designated IB16-IB25. The partial 16S rRNA gene of each isolate was
162 amplified and sequenced, and species identity was determined using BLAST searches of the
163 GenBank database (NCBI). The following species were identified: IB16 – *Pseudomonas*
164 *silesiensis*, IB17 – *Bacillus thuringiensis*, IB18 – *Pseudomonas silesiensis*, IB19 – *Bacillus*
165 *mycoides*, IB20 – *Metabacillus idriensis*, IB21 – *Bacillus cereus*, IB22 – *Lysinibacillus fusiformis*,
166 IB24 – *Pseudomonas silesiensis*, and IB25 – *Brucella intermedia*. Nucleotide sequences of all
167 isolates were deposited in GenBank under accession numbers OR758847.1-OR758856.1 (NCBI
168 Nucleotide 2025a-2025i).

169 According to published reports, *M. idriensis*, *B. cereus*, *L. fusiformis*, and *B. intermedia* are
170 potentially pathogenic to humans and may cause allergic or toxic effects (Ko et al. 2006; Bottone
171 2010; Wu et al. 2021; Bartlett et al. 2022; Morioka et al. 2022). Therefore, isolates IB20, IB21,
172 IB22, and IB25 were excluded from further experiments for biosafety reasons.

173 **Screening of the bacterial strains to determine nematicidal activity**

174 Five strains *P. silesiensis* IB16, *B. thuringiensis* IB17 *P. silesiensis* IB18, *B. mycoides* IB19, *P.*
175 *silesiensis* IB24 and two standards Verango, Nematophagin-Mycopro were tested in laboratory
176 assays (Fig. 2, 4).

177 Mortality data (%) were subjected to arcsine square-root transformation to stabilize variances.
178 For each time point (24, 48, 72, and 96 h), a one-way analysis of variance (ANOVA) with the
179 factor “Treatment” was performed, followed by Duncan’s post hoc test ($\alpha = 0.05$). To assess the
180 overall dynamics (24-72 h), a two-way ANOVA (factors «Time» and «Treatment») was applied,

along with the Kruskal-Wallis test as a non-parametric verification. For the interval 72-96 h, the persistence of the effect was additionally evaluated using two-way ANOVA and pairwise comparisons (t-test, Wilcoxon test), which allowed classification of the treatments as nematicidal or nematostatic. Visualization was carried out in R (ggplot2) using mean ± SE plots and boxplots. [figure 2 near here].

Already after 24 h of treatment, most preparations provided a significant increase in nematode mortality compared with the control. The strongest early effect was observed for Nematophagin-Micopro and strain IB18 (81.6; 81.5 %), whereas Verango showed maximum activity at 72 h, reaching nearly complete lethality (98.4 %). At this time point, strains IB24 and IB19 also maintained high efficacy (95.4; 95.8 %). Strain IB16 demonstrated the lowest mortality level (42.6-62.7 %), being statistically different from the others. The control remained consistently low (5.9-14.8 %) across all time points and formed a separate significance group.

[figure 3 near here]

A comparison of 72 h and 96 h showed that, for most treatments, mortality levels remained high and did not change significantly, indicating a stable nematicidal effect (Fig. 4). For Nematophagin-Micopro and IB24, the reduction in mortality did not exceed 5 percentage points and was not statistically significant. At the same time, IB18 showed a significant decline of more than 7 percentage points, indicating a partial nematostatic effect. For Verango, IB17, and IB19, a moderate decrease was observed, which was statistically ambiguous and requires additional replications.

[figure 4 near here]

Strain IB16 showed the lowest efficacy – 57.8 % mortality after filtration (i.e., at 96 h), was therefore not included in subsequent greenhouse experiments.

Effect of the bacterial strains on gall formation in the tomato plants

205 The analysis included Control (infected plants, water), the standards (Verango, Nematophagin-
206 Mycopro), and four strains (IB17, IB18, IB19, IB24), Control (uninfected plants, water) served as
207 a clean control and was not entered into pairwise tests (Fig. 5, 7).

208 For each greenhouse experiment separately, a one-way analysis of variance (ANOVA; factor
209 Treatment) was performed on raw data. Assumptions of residual normality and homogeneity of
210 variances were checked using standard procedures. When significant effects were detected,
211 Duncan's multiple range test ($\alpha = 0.05$) was applied. The treatment effect was highly significant
212 in both the first experiment ($F(6.28) = 136.8$; $p<2\times10^{-16}$) and the repeat experiment ($F(6.28) =$
213 104.7; $p<2\times10^{-16}$). Untransformed means ± SE are reported; letter codes are interpreted within
214 each experiment. Results were consistent with a nonparametric Kruskal-Wallis check (first
215 experiment: $x^2(6) = 17.74$; $p = 0.0069$; repeat experiment: $x^2(6) = 17.03$; $p = 0.0092$).

216 [figure 5 near here]

217 In both experiments, the infected control showed the highest number of galls, whereas the
218 reference treatments produced the lowest values.

219 First experiment. The lowest means were observed for Verango (5.58 ± 2.23; c) and
220 Nematophagin-Mycopro (7.18 ± 1.73; bc). The indigenous strains formed an intermediate band
221 with overlapping groups: IB19 (8.14 ± 0.72 bc), IB18 (9.18 ± 0.86 bc), IB24 (10.48 ± 0.89 bc),
222 while IB17 was at the upper end among strains (11.28 ± 1.91; b). The infected control had the
223 highest mean (56.60 ± 1.83; a).

224 Repeat experiment. The ranking was reproduced: Verango (5.26 ± 2.41; c) formed the group
225 with the lowest values. In this experiment, Nematophagin-Mycopro (8.10 ± 1.76; bc) did not differ
226 statistically from several bacterial strains, namely IB19 (9.10 ± 0.89; bc), IB18 (9.68 ± 2.50; bc),
227 and IB17 (10.64 ± 2.62; bc). IB24 (12.20 ± 0.92; b) occupied a higher level among the bacterial
228 variants. The infected control had the maximum value (58.90 ± 0.56; a).

229 In all cases, every treatment significantly outperformed the infected control in reducing gall
230 formation; Duncan letter codes indicating significant differences are shown above the bars in

panels first experiment and repeat experiment of Figure 5. These trends are also evident in the root system images (Fig. 6).

[figure 6 near here]

Biological efficacy (BE, %) was calculated relative to the infected control. For each experiment separately, a one-way ANOVA (factor Treatment) was performed on arcsine square-root–transformed values (arcsine$\sqrt{(x/100)}$); untransformed means ± SE are reported in the text and figures. When significant effects were detected, Duncan's multiple range test ($\alpha = 0.05$) was applied. The treatment effect was highly significant in both the first experiment ($F(6,28) = 148.0$; $p < 2\times10^{-16}$) and the repeat experiment ($F(6,28) = 82.88$; $p < 2\times10^{-16}$). These results were consistent with a nonparametric check (first experiment: Kruskal–Wallis $x^2(6) = 18.20$; $p = 0.0057$; repeat experiment: $x^2(6) = 17.23$; $p = 0.0085$).

[figure 7 near here]

In both experiments, the reference treatments provided the highest biological efficacy (BE), whereas the bacterial strains showed high but somewhat lower efficacy. All treatments significantly outperformed the infected control.

First experiment. The highest BE was observed for Verango 90.48 ± 3.49 (a). Nematophagin-Mycopro 87.28 ± 3.14 (ab) and IB19 85.46 ± 1.56 (ab) formed an upper tier of efficacy comparable to each other. Values for IB18 83.70 ± 1.68 (b), IB24 81.30 ± 1.91 (b), and IB17 80.18 ± 3.17 (b) were statistically lower, yet remained high in absolute terms.

Repeat experiment. The ranking of treatments was reproduced: Verango 91.08 ± 4.14 (a) maintained the highest BE; Nematophagin-Mycopro 86.30 ± 2.94 (ab), IB19 84.60 ± 1.53 (ab), IB18 83.60 ± 4.19 (ab), and IB17 81.76 ± 4.53 (ab) formed an intermediate tier; comparatively lower efficacy was recorded for IB24 79.30 ± 1.56 (b). For some treatments (notably IB17 and IB18), SE values were somewhat larger (up to 4.53), indicating higher biological variability without altering the overall group order.

Taken together, the data confirm the reproducible superiority of the reference treatments in BE and the consistently high efficacy of all bacterial strains relative to the infected control.

Effect of bacterial strains on biometric and yield parameters of tomato plants

Biometric and yield parameters (plant height, number of fruits per plant, single fruit weight, total fruit mass and yield) were evaluated in two independent greenhouse experiments («first» and «repeat»). Each experiment was analyzed separately using one-way ANOVA with "Treatment" as the factor. In both experiments, ANOVA revealed significant treatment effects ($p < 0.05$). For multiple comparisons, Duncan's post hoc test ($\alpha = 0.05$) was applied within each experiment; different letters indicate significant differences among treatments within the same column and experiment. Data are presented as mean ± SE. The overall ranking of treatments observed in the first experiment was reproduced in the repeat experiment (Table 1).

[Table 1 near here].

Plant height. In the first experiment, uninfected plants reached 88.22 ± 3.54 cm (group a). All treatments, including Verango, Nematophagin-Mycopro, IB17, IB18, IB19 and IB24, did not differ from the healthy control and belonged to group a. Infected plants were shorter at 71.12 ± 2.45 cm (group b). In the repeat experiment, the pattern was reproduced. Uninfected plants had the tallest canopy at 90.08 ± 2.20 cm (group a). Verango, Nematophagin-Mycopro, IB17 and IB19 formed group ab and did not differ from the healthy control within this experiment. IB18 and IB24 formed group b and remained significantly taller than the infected control, which showed the minimum height at 68.40 ± 2.78 cm (group c).

Number of fruits per plant. In the first experiment, uninfected plants produced 23.00 ± 0.32 fruits per plant (group a). All treatments including Verango, Nematophagin-Mycopro, IB17, IB18, IB19 and IB24 did not differ from the healthy control and belonged to group a. The infected control showed the minimum value at 15.80 ± 0.20 fruits per plant (group b).

In the repeat experiment, the patterns were reproduced. Uninfected plants had the maximum number of fruits at 23.80 ± 0.20 per plant (group a). The chemical reference Verango did not differ

from the healthy control at 23.20 ± 0.37 per plant (group a). The biological reference Nematophagin-Mycopro formed a lower level at 21.40 ± 0.40 per plant (group b). Indigenous strains IB19 20.20 ± 0.80, IB18 19.60 ± 0.81, IB24 19.40 ± 0.68 and IB17 19.20 ± 0.58 also belonged to group b and exceeded the infected control at 14.00 ± 0.32 per plant (group c).

Average fruit weight. In the first experiment, uninfected plants showed the highest average fruit weight at 196.38 ± 4.70 g (group a). All treatments – Verango, Nematophagin-Mycopro, IB17, IB18, IB19, and IB24 – did not differ from the healthy control and belonged to group a. The infected control had a lower weight of 171.62 ± 3.89 g (group b).

In the repeat experiment, the patterns were reproduced. Uninfected plants again exhibited the maximum value at 197.60 ± 2.60 g (group a). Verango, Nematophagin-Mycopro, IB17, IB18, and IB19 formed group ab and did not differ from the healthy control within this experiment. IB24 belonged to group b and provided an intermediate level. The lowest average fruit weight was recorded in the infected control at 168.40 ± 4.16 g (group c).

Fruit weight per plant. In the first experiment, uninfected plants showed the maximum total fruit mass at 4512.06 ± 71.24 g (group a). The reference treatments Verango and Nematophagin-Mycopro did not differ from the healthy control at 4267.70 ± 53.42 g and 4379.28 ± 149.06 g, respectively (group a). Indigenous strains provided an intermediate level: IB17 3964.08 ± 151.57 g and IB18 4023.56 ± 79.54 g (group b); IB19 and IB24 likewise formed an intermediate group and exceeded the infected control at 2711.36 ± 68.79 g (group c).

In the repeat experiment, the patterns were reproduced. Uninfected plants again had the maximum fruit mass at 4701.40 ± 44.02 g (group a). Nematophagin-Mycopro did not differ from the healthy control at 4483.98 ± 174.48 g (group a), whereas Verango yielded a somewhat lower value of 4086.94 ± 111.89 g (group b). Strains IB17 3573.98 ± 144.01 g and IB18 3671.66 ± 184.39 g belonged to group b; IB19 and IB24 occupied a comparable intermediate level and were significantly higher than the infected control at 2360.00 ± 97.10 g (group c).

Yield. In the first experiment, uninfected plants showed the highest yield at 18.05 ± 0.29 kg·m^{-2} (group a). The reference treatments Verango (17.07 ± 0.21 kg·m^{-2}) and Nematophagin-Mycopro (17.52 ± 0.60 kg·m^{-2}) did not differ from the healthy control and belonged to group a. The strain B. mycoides IB19 also fell into group a at 16.71 ± 0.38 kg·m^{-2}. The indigenous strains IB17 (15.86 ± 0.60 kg·m^{-2}), IB18 (16.09 ± 0.32 kg·m^{-2}) and IB24 (16.29 ± 0.30 kg·m^{-2}) formed an intermediate level (group b) and exceeded the infected control at 10.84 ± 0.27 kg·m^{-2} (group c).

In the repeat experiment, the patterns were reproduced. Uninfected plants again had the maximum yield at 18.81 ± 0.18 kg·m^{-2} (group a). The biological reference Nematophagin-Mycopro did not differ from the healthy control at 17.94 ± 0.70 kg·m^{-2} (group a). The chemical reference Verango provided a somewhat lower yield at 16.35 ± 0.45 kg·m^{-2} (group b). Strains IB19 (15.24 ± 0.59 kg·m^{-2}), IB18 (14.69 ± 0.74 kg·m^{-2}), IB17 (14.29 ± 0.57 kg·m^{-2}) and IB24 (14.24 ± 0.50 kg·m^{-2}) belonged to group b and were significantly higher than the infected control at 9.44 ± 0.39 kg·m^{-2} (group c).

Across both experiments, nematode infection substantially reduced the number of fruits, fruit mass per plant, and overall yield, indicating a marked loss of productivity. All bacterial treatments consistently increased these metrics relative to the infected control and shifted them toward levels comparable to the chemical and biological standards (Verango and Nematophagin-Mycopro) and to the uninfected control. Among the bacterial strains, IB19 most frequently delivered the highest values, whereas IB17, IB18, and IB24 produced intermediate yet statistically significant improvements. The ranking of strains and the magnitude of improvements were reproduced in the repeat experiment, indicating stable, experiment-independent effects of the bacterial treatments.

Effect of bacterial strains on biochemical parameters of tomato plants

In addition to biometric and yield traits, the biochemical composition of tomato fruits was assessed. The parameters analyzed included titratable acidity, soluble sugar content, ascorbic acid (vitamin C) concentration (Fig. 8, 9, 10), which are key indicators of fruit quality and nutritional value.

The proportion of acidity (%) was calculated separately for each experiment. Prior to analysis, data were transformed using arcsine$\sqrt{(x/100)}$; untransformed means ± SE are reported in the text and figures. One-way ANOVA (factor Treatment) revealed significant effects in both experiments: first experiment – $F(7.32) = 9.26$; $p = 3.2\times10^{-6}$ (Shapiro-Wilk $p = 0.045$; Levene $p = 0.723$); repeat experiment – $F(7.32) = 30.74$; $p = 1.79\times10^{-12}$ (Shapiro-Wilk $p = 0.605$; Levene $p = 0.515$). Multiple comparisons were performed using Duncan's test ($\alpha = 0.05$). The conclusions were consistent with a nonparametric check (first experiment: Kruskal-Wallis $x^2 = 22.31$; $p = 0.0023$; repeat experiment: $x^2 = 33.36$; $p = 2.27\times10^{-5}$).

[figure 8 near here]

First experiment. The highest acidity was recorded in infected plants (0.328 ± 0.008; a). Among the reference treatments, Verango provided the lowest value (0.290 ± 0.0105; c), whereas Nematophagin-Mycopro showed an intermediate level (0.300 ± 0.0055; bc) comparable to IB19 0.300 ± 0.0045 (bc). The bacterial strains overall formed an intermediate range: IB18 0.310 ± 0.0063 (abc) and IB24 0.310 ± 0.0063 (abc) occupied mid-positions, while IB17 0.318 ± 0.0049 (ab) had the highest value among the strains. The lowest acidity was observed in uninfected plants (0.260 ± 0.0071; d).

Repeat experiment. Infected plants again showed the maximum acidity (0.360 ± 0.0099; a), whereas uninfected plants had the minimum (0.240 ± 0.0059; e). Among the reference treatments, Verango (0.270 ± 0.0089; d) had lower values than Nematophagin-Mycopro (0.290 ± 0.0040; c). The bacterial strains formed a homogeneous group b: IB19 0.310 ± 0.0059, IB18 0.320 ± 0.0049, IB17 0.320 ± 0.0049, and IB24 0.330 ± 0.0081, with IB24 showing the highest value among the strains.

Overall, nematode infection was associated with increased fruit acidity, whereas all treatments reduced this parameter; the reference treatments produced the lowest values, and the bacterial strains consistently occupied intermediate positions.

Sugar percentage was calculated separately for each experiment. Prior to analysis, data were transformed using arcsine$\sqrt{(x/100)}$; untransformed means ± SE are reported in the text and figures. One-way ANOVA (factor Treatment) detected significant effects in both series: first experiment – $F(7.32) = 93.22$; $p < 2\times10^{-16}$ (Shapiro-Wilk $p = 0.540$; Levene $p = 0.967$); repeat experiment – $F(7.32) = 73.52$; $p < 2\times10^{-16}$ (Shapiro-Wilk $p = 0.322$; Levene $p = 0.981$). The nonparametric analysis confirmed the presence of between-treatment differences (first experiment: Kruskal-Wallis $x^2 = 32.65$; $p = 3.08\times10^{-5}$; repeat experiment: $x^2 = 31.58$; $p = 4.86\times10^{-5}$).

[figure 9 near here]

First experiment. Values ranged from 6.10 ± 0.10 (f) in uninfected plants to 10.50 ± 0.13 (a) in infected plants, i.e., infection increased sugar content by about 4.4 percentage points (pp). Among the treatments, values were within 6.80–7.72%. The highest value occurred in IB18 (7.72 ± 0.08; b), followed by IB17 and IB24 (7.58 ± 0.16 and 7.40 ± 0.12; bc). IB19 occupied an intermediate position (7.20 ± 0.16; cd). The reference treatments formed the lower bound among treated variants: Nematophagin-Mycopro 7.00 ± 0.10 (de) and Verango 6.80 ± 0.14 (e).

Repeat experiment. The range extended from 6.04 ± 0.12 (e) in uninfected plants to 10.46 ± 0.16 (a) in infected plants, replicating the first series. Among treatments, values fell within 6.60–7.80%. The maximum was observed for IB24 (7.80 ± 0.15; b); strains IB17, IB18, and IB19 formed a homogeneous level (7.60 ± 0.18, 7.50 ± 0.10, 7.40 ± 0.14; bc). Among the references, Nematophagin-Mycopro reached 7.20 ± 0.16 (c), whereas Verango showed 6.60 ± 0.16 (d).

Vitamin C was analyzed without transformation. For each experiment, a one-way ANOVA (factor Treatment) was conducted; model assumptions were met (first experiment: Shapiro-Wilk $p = 0.239$, Levene $p = 0.302$; repeat experiment: Shapiro-Wilk $p = 0.568$, Levene $p = 0.778$). The treatment effect was highly significant in both series (first: $F(7.32) = 40.93$; $p = 3.36\times10^{-14}$; repeat: $F(7.32) = 56.88$; $p = 2.93\times10^{-16}$). Duncan's test ($\alpha=0.05$) was used for multiple comparisons. A nonparametric analysis confirmed between-treatment differences (first experiment: Kruskal-Wallis $x^2 = 32.995$; $p = 2.65\times10^{-5}$; repeat experiment: $x^2 = 34.286$; $p = 1.523\times10^{-5}$).

[figure 10 near here]

Across both series, nematode inoculation was associated with an increase in vitamin C, whereas treated variants shifted the values to intermediate levels bounded by the infected and uninfected controls.

First experiment. Infected plants showed the maximum vitamin C 14.30 ± 0.321 (a); uninfected plants had the minimum 10.40 ± 0.152 (f). Among treatments, IB17 displayed the highest value 12.20 ± 0.105 (b); IB18 12.00 ± 0.161 (bc) and IB24 11.88 ± 0.080 (bc) were slightly lower, and IB19 11.60 ± 0.045 (cd) occupied an intermediate position. For the references, Nematophagin-Mycopro 11.28 ± 0.208 (de) exceeded Verango 10.92 ± 0.224 (ef).

Repeat experiment. Infected plants again had the highest value 14.50 ± 0.212 (a), whereas uninfected plants had the lowest 10.10 ± 0.122 (e). Among treatments, IB24 12.50 ± 0.141 (b) ranked highest; IB17 12.30 ± 0.170 (bc) and IB18 12.20 ± 0.152 (bc) were close, and IB19 11.90 ± 0.130 (c) was lower. Among the references, Nematophagin-Mycopro 11.10 ± 0.240 (d) exceeded Verango 10.60 ± 0.230 (de).

Across both experiments, nematode infection altered titratable acidity, soluble sugars, and vitamin C in tomato fruit. For all three metrics, the bacterial strains produced values comparable to the chemical and biological standards and to the uninfected control, while remaining significantly different from the infected control. The treatment ranking and effect sizes were reproduced in the repeat experiment, confirming the robustness of these biochemical responses.

Discussion

The nematicidal activity of five bacterial strains (*P. silesiensis* IB16, *B. thuringiensis* IB17, *P. silesiensis* IB18, *B. mycoides* IB19, *P. silesiensis* IB24) against *M. hapla* was assessed in vitro; subsequently, four strains (IB17, IB18, IB19, IB24) were evaluated for effects on tomato biometric and biochemical parameters in two independent greenhouse experiments.

At 96 h after filtration, laboratory assays showed that *P. silesiensis* IB16 had low nematicidal activity (57.8%) and was therefore not progressed to greenhouse trials. By contrast, the remaining

strains (IB17, IB18, IB19, IB24) achieved 86.2-90.6% in vitro mortality of *M. hapla* J2. In the greenhouse, gall reduction was reproduced in two independent experiments, ranging 79.3-85.5% for the indigenous strains, 86.3-87.3% for the biological standard Nematophagin-Mycopro, and 90.5-91.1% for the chemical standard Verango. These results indicate that the selected indigenous strains approached the efficacy of the standards, with treatment ranking and effect sizes remaining stable between experiments.

The strain *Bacillus thuringiensis* IB17 exhibited high nematicidal activity against *Meloidogyne hapla*. Under laboratory conditions, J2 mortality was 86.2% at 96 h (post-filtration). In greenhouse trials, IB17 substantially reduced gall formation – 80.2% in the first experiment and 81.76% in the repeat experiment – confirming reproducibility. Treatment with this strain improved plant biometric traits and increased yield by 46.3% in the first experiment and by 51.4% in the repeat experiment relative to the infected control, which is attributed both to the reduction of nematode pressure and to the growth-promoting properties of *Bacillus* spp., including the synthesis of indole-3-acetic acid and gibberellins (Sorokan et al. 2023; Banik and Gilson 2024). The biochemical parameters of tomato fruits in treated plants were lower than in the infected control, which can be explained by reduced stress load and restoration of metabolic homeostasis in plants (Ma et al. 2019). The antagonistic activity of the strain is associated with the production of δ-endotoxins (Cry proteins). After activation in the nematode intestine, they bind to enterocyte receptors, induce pore formation, and cause parasite death (Bel et al. 2023).

The effectiveness of *B. thuringiensis* against root-knot nematodes is further supported by Wang et al. (2024), who reported that strains of this species caused 100% mortality of *M. incognita* J2 under laboratory conditions and reduced the number of egg masses by 79.4% in tomato greenhouse trials.

The strain *B. mycoides* IB19 demonstrated high nematicidal activity against *M. hapla*. Under laboratory conditions, J2 mortality was 90.6% at 96 h (post-filtration). In greenhouse experiments, IB19 reduced root galling by 85.46% in the first experiment and 84.60% in the repeat experiment,

confirming reproducibility. Application of IB19 improved plant biometric traits and increased yield by 54.2% in the first experiment and 61.4% in the repeat experiment relative to the infected control. For fruit biochemical parameters, values in the IB19 treatment were comparable to those of the uninfected control and the reference standards, while remaining significantly different from the infected control, which may be explained by the reduction of metabolic stress caused by nematode infection and the normalization of physiological processes.

The antagonistic effect of the strain is associated with the production of volatile organic compounds, primarily styrene, which exhibits repellent and neurotoxic activity. Styrene has been shown to act on nematode sensory neurons by activating G-protein–related signaling pathways, leading to loss of mobility and juvenile death (Koga et al. 2002; Luo et al. 2018). Due to its high volatility, styrene functions as a biofumigant in soil, ensuring effectiveness even in the absence of direct contact with the phytoparasite. In addition, *B. mycoides* can synthesize acetoin and other VOCs that modulate plant signaling pathways through effects on hormonal status and reactive oxygen species, thereby enhancing growth-promoting activity (Bakker et al. 2007; Yahyaoui et al. 2024), as well as induce systemic resistance (ISR) in plants against phytoparasites (Hofmann et al. 2010). Another important factor of sustainability is the ability of the strain to stably colonize the rhizosphere and form biofilms, which provide long-term protection of the root system (Ryu et al. 2003).

These findings are consistent with Luo et al. (2018), who demonstrated that a styrene-producing *B. mycoides* strain caused 100 % mortality of *M. incognita* J2 under laboratory conditions and reduced gall formation by 90.9 % when plants were grown in pots.

The *P. silesiensis* IB18 and *P. silesiensis* IB24 strains demonstrated pronounced nematicidal activity against *M. hapla*. Under laboratory conditions, J2 mortality was 86.3% for IB18 and 90.5% for IB24 at 96 h (post-filtration). In greenhouse experiments, these strains reduced root gall formation by 83.70% (first experiment) and 83.57% (repeat) for IB18, and by 81.30% (first experiment) and 79.29% (repeat) for IB24, confirming reproducibility. Application of the isolates

improved plant biometric traits and increased yield by 48.43% (first) and 55.61% (repeat) for IB18, and by 50.28% (first) and 50.85% (repeat) for IB24 relative to the infected control. The biochemical parameters of fruits were lower than in the infected control, which may be explained by a reduction of metabolic stress and restoration of the antioxidant balance under the influence of rhizobacterial signaling molecules (Yahyaoui et al. 2024).

The antagonistic effect of the strains is associated with the production of extracellular enzymes, such as alkaline protease AprA and chitinases that degrade the nematode cuticle and eggshells, thereby preventing juvenile emergence and reducing parasite viability (Siddiqui et al. 2005). In addition to direct antagonism, *Pseudomonas* spp. are capable of inducing systemic resistance (ISR) in plants, which enhances defense responses and reduces tomato damage caused by root-knot nematodes (Bakker et al. 2007).

The role of AprA protease in the nematicidal activity of *Pseudomonas* spp. was previously confirmed in *P. fluorescens* CHA0, where this enzyme was identified as a key factor in the biological control of *M. incognita*. Under laboratory conditions, culture filtrates inhibited egg hatching by up to 80 % and reduced juvenile viability by up to 75 % (Siddiqui et al. 2005).

In tomato plants infected with *M. hapla*, an increase in the content of sugars, vitamin C, and acids was recorded in the infected control. Such changes are considered a manifestation of the plant's metabolic restructuring in response to biotic stress. According to Expósito et al. (2020), nematode invasion is accompanied by the activation of genes regulating carbohydrate metabolism and the biosynthesis of ascorbic acid, which contributes to the mobilization of internal resources and the protection of cellular structures from oxidative damage. The increase in biochemical indicators in the infected control, on the one hand, reflects the activation of defense mechanisms, but on the other hand, indicates a high level of stress that can negatively affect yield (Hofmann et al. 2010; Nekoval et al. 2024b). The application of the studied bacterial strains, as well as reference preparations, reduced these indicators compared with the infected control, which can be explained by a decrease in stress load and normalization of metabolism.

Conclusion

Four indigenous bacterial strains, *B. thuringiensis* IB17, *P. silesiensis* IB18, *B. mycoides* IB19, and *P. silesiensis* IB24, demonstrated high activity against *M. hapla* under laboratory and greenhouse conditions. In two independent greenhouse experiments, these strains consistently reduced gall formation, improved plant biometric traits, increased yield relative to the infected control, and maintained fruit biochemical quality at levels comparable to the reference treatments. Among the bacterial isolates, *B. mycoides* IB19 showed the most stable and pronounced positive effect. The obtained results indicate that the selected rhizosphere bacteria are promising candidates for the development of biological products for the control of root-knot nematodes in tomato.

List of Abbreviations

BE – Biological efficacy

CFU – Colony-forming units

HPLC – High-performance liquid chromatography

J2 – Second-stage juveniles

spp – Species

ANOVA - Analysis of variance

FMH/GRM – Fish meal hydrolysate/glucose-peptone medium

WGS84 – World Geodetic System 1984

FSBSI FRCBPP - Federal State Budgetary Scientific Institution Federal Research Center of Biological Plant Protection

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data supporting the findings of this study are included in the article and its supplementary materials. The 16S rRNA gene sequences of the bacterial isolates have been deposited in GenBank under accession numbers OR758847.1-OR758856.1. Additional datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author Contributions

S.N. conceived the study. M.C., O.M. conducted experiments. M.C., A.Z. and Z.T. wrote, reviewed and edited the manuscript. K.K. and V.I. analyzed the data. All the authors have read and approved the manuscript.

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713 Yahyaoui H, El Allaoui N, Aziz A, Hafidi M, Habbadi K (2024) Minimizing the adverse
714 impacts of soil salinity on maize and tomato growth and productivity through the application of
715 plant growth-promoting rhizobacteria. Crops 4:463–479. https://doi.org/10.3390/crops4040033

716 **Table 1.** The influence of the studied bacterial strains on the growth and development of the Prima Dona tomato hybrid.

Treatment	Plant height, cm		Number of fruits per plant, pcs.		Fruit weight,g		Fruit weight per plant, g		Yield, kg m^{-2}	
	first experiment	repeat experiment	first experiment	repeat experiment	first experiment	repeat experiment	first experiment	repeat experiment	first experiment	repeat experiment
Control (uninfected plants)	88.22 ± 3.54 a	90.08 ± 2.20 a	23.00 ± 0.32 a	23.80 ± 0.20 a	196.38 ± 4.7 a	197.6 ± 2.60 a	4512.06 ±71.24 a	4701.40 ± 44.02 a	18.05 ± 0.29 a	18.81 ± 0.18 a
Control (infected plants)	71.12 ± 2.45 b	68.4 ± 2.78 c	15.80 ± 0.20 b	14.00 ± 0.32 c	171.62 ± 3.89 b	168.4 ± 4.16 c	2711.36 ± 68.79 c	2360.00 ± 97.10 c	10.84 ± 0.27 c	9.44 ± 0.39 c
Chemical standard Verango	85.78 ± 1.39 a	84.1 ± 3.67 ab	22.20 ± 0.49 a	21.40 ± 0.40 b	192.5 ± 3.53 a	191.1 ± 5.11 ab	4267.70 ± 53.42 a	4086.94 ± 111.89 b	17.07 ± 0.21 a	16.35 ± 0.45 b
Biological standard	85.3 ± 3.31 a	87.6 ± 3.90 ab	22.80 ± 0.58 a	23.20 ± 0.30 a	191.98 ± 2.97 a	193.04 ± 4.87 ab	4379.28 ± 149.06 a	4483.98 ± 174.48 a	17.52 ± 0.60 a	17.94 ± 0.70 a

Nematophagin-Mycopro										
B. thuringiensis IB17	83.0 ± 2.07 a	82.7 ± 2.75 ab	21.20 ± 0.58 a	19.20 ± 0.59 b	187.1 ± 5.93 a	185.9 ± 2.79 ab	3964.08 ± 151.57 b	3573.98 ± 144.01 b	15.86 ± 0.60 b	14.29 ± 0.57 b
P. silesiensis IB18	83.9 ± 1.12 a	81.40 ± 1.27 b	21.40 ± 0.25 a	19.60 ± 0.81 b	187.98 ± 2.37 a	187.18 ± 3.58 ab	4023.56 ± 79.54 b	3671.66 ± 184.39 b	16.09 ± 0.32 b	14.69 ± 0.74 b
B. mycoides IB19	84.38 ± 1.11 a	84.50 ± 1.69 ab	22.00 ± 0.55 a	20.20 ± 0.80 b	190.0 ± 3.41 a	188.70 ± 3.03 ab	4176.32 ± 96.05 a	3809.46 ± 146.80 b	16.71 ± 0.38 a	15.24 ± 0.59 b
P. silesiensis IB24	83.4 ± 1.85 a	80.1 ± 1.48 b	21.6 ± 0.25 a	19.4 ± 0.68 b	188.6 ± 4.04 a	183.5 ± 2.29 b	4071.72 ± 76.07 b	3559.08 ± 124.90 b	16.29 ± 0.30 b	14.24 ± 0.50 b

Biometric and yield traits of «Prima Dona» tomato in two independent greenhouse experiments (first and repeat). Values are mean ± SE (n = 5 replicates; 5 plants per replicate). Different letters within a column denote significant differences among treatments according to Duncan's test (α = 0.05); post-hoc tests were run separately for each experiment. Uninfected plants served as the healthy control; infected plants (water) as the nematode control. The treatment ranking observed in the first experiment was reproduced in the repeat experiment.

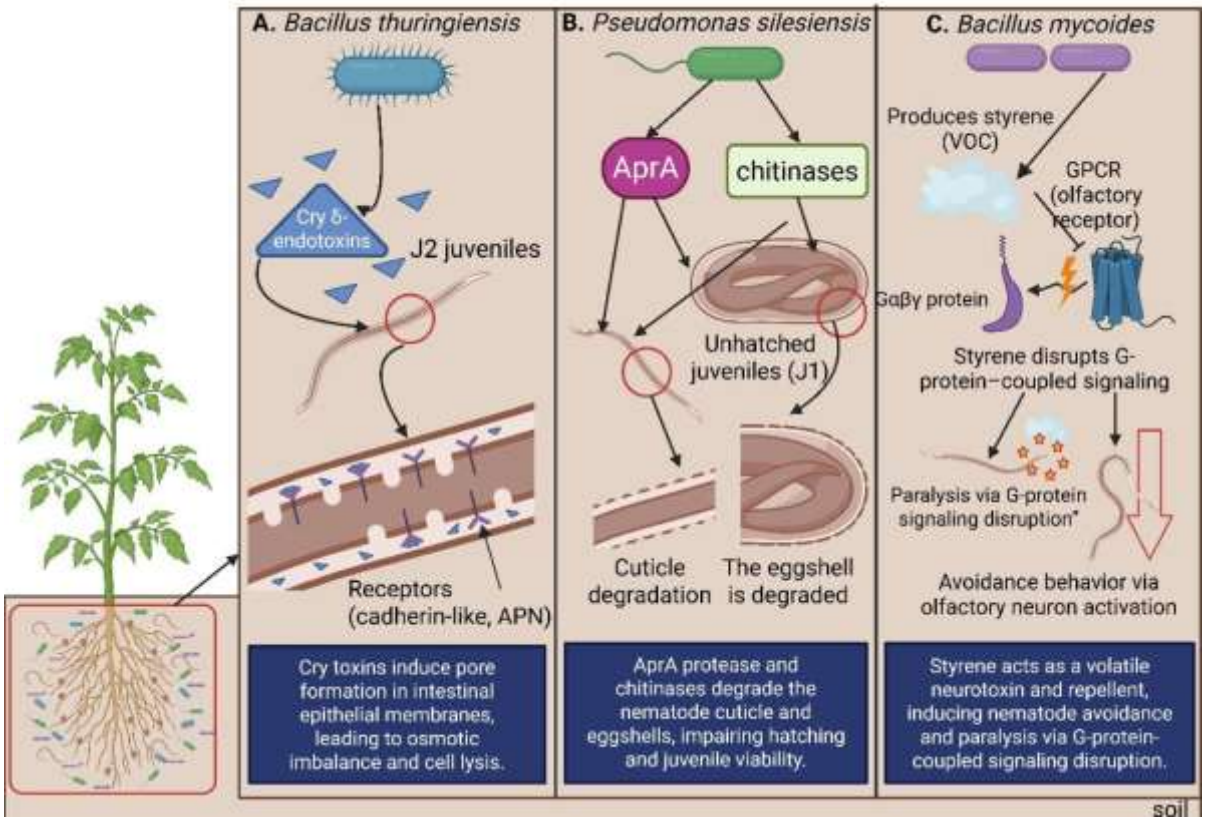


Fig. 1 Antagonistic mechanisms of rhizobacteria against *M. hapla* in the tomato rhizosphere. (a) *B. thuringiensis* produces δ-endotoxins (Cry proteins) that, upon ingestion by second-stage juveniles (J2), are activated by nematode intestinal proteases. The activated toxins bind to cadherin-like receptors and APN in the epithelial membrane, causing pore formation, osmotic imbalance, and cell lysis. (b) *P. silesiensis* secretes extracellular enzymes including alkaline protease AprA and chitinases, which degrade the nematode eggshell and cuticle. This action impairs egg hatching and juvenile viability. (c) *B. mycoides* emits styrene, a volatile aromatic hydrocarbon that acts as both a neurotoxic compound and repellent. Styrene disrupts G-protein-coupled signaling via GPCR–Gβγ binding, resulting in nematode avoidance and paralysis https://BioRender.com/t6vch8m

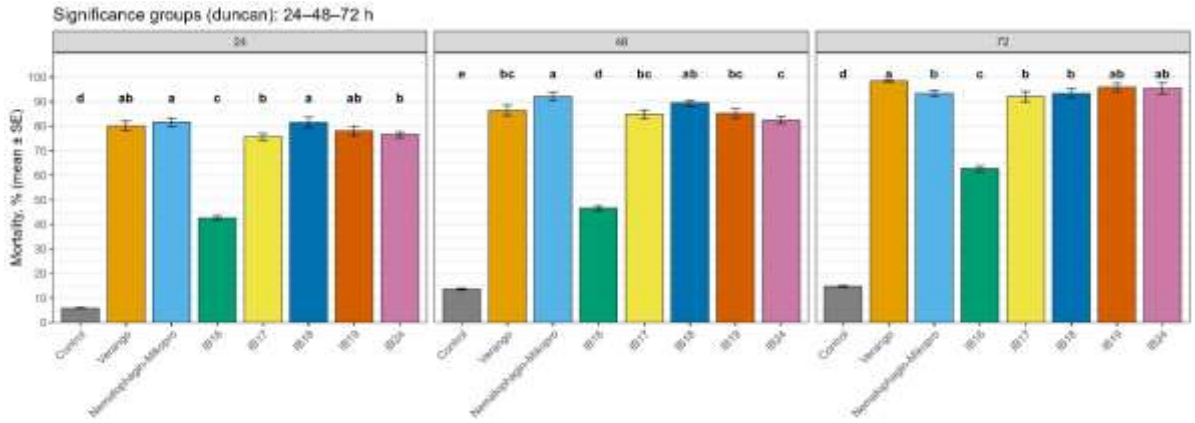


Fig. 2 Nematode mortality (%) under the effect of the tested treatments after 24, 48, and 72 h in vitro (mean ± SE). Significance groups are indicated above the bars according to Duncan's post-hoc test (different letters denote significant differences, α = 0.05).



Fig. 3 Infected second-stage juveniles (J2) of *M. hapla* after 24 h exposure to culture filtrates: (a) *P. silesiensis* IB18; (b) *P. silesiensis* IB24; (c) *B. mycoides* IB19. Scale bars = 100 µm (a-c).

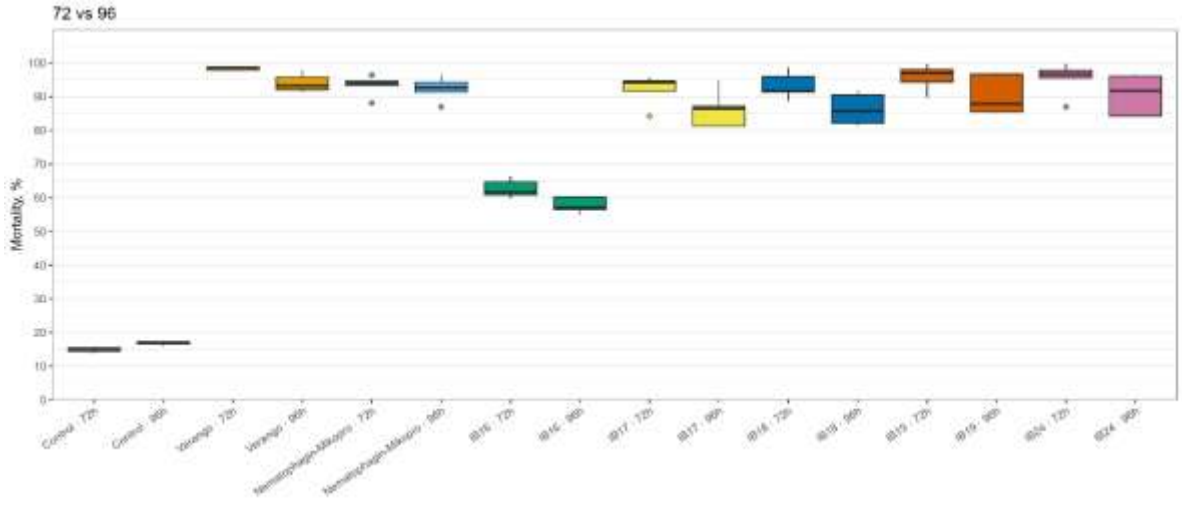
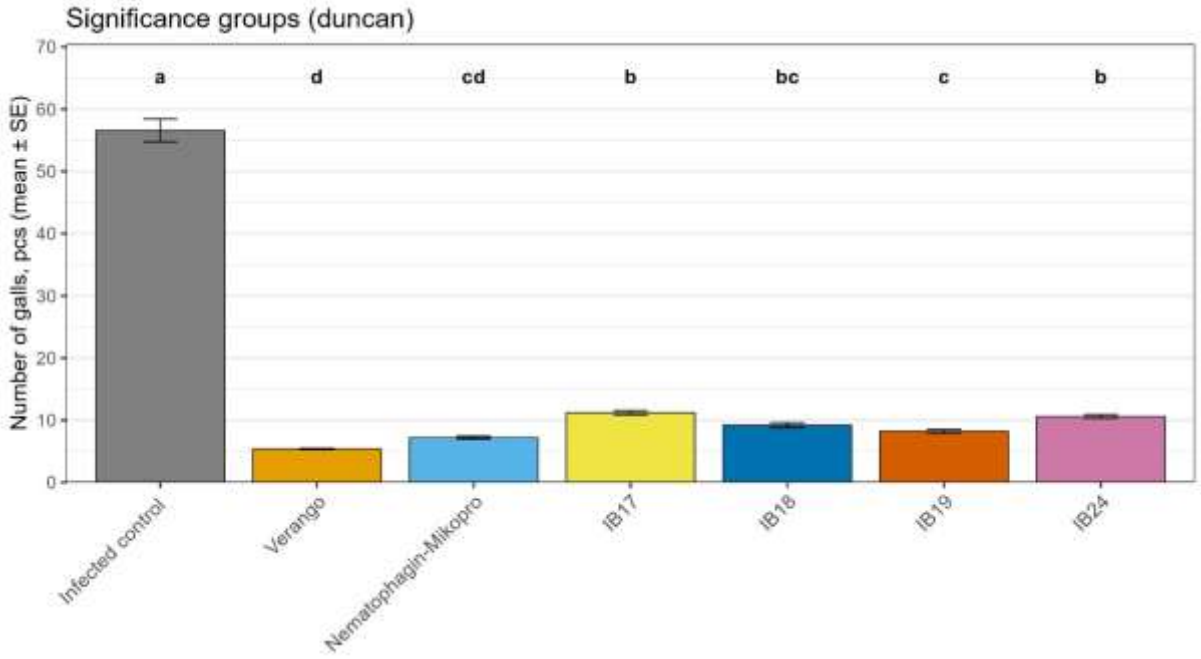


Fig. 4 Comparison of nematode mortality (%) at 72 and 96 h. Values are presented as boxplots for each treatment; sustained high levels indicate a nematicidal effect, whereas a decline after filtration suggests a possible nematostatic effect.



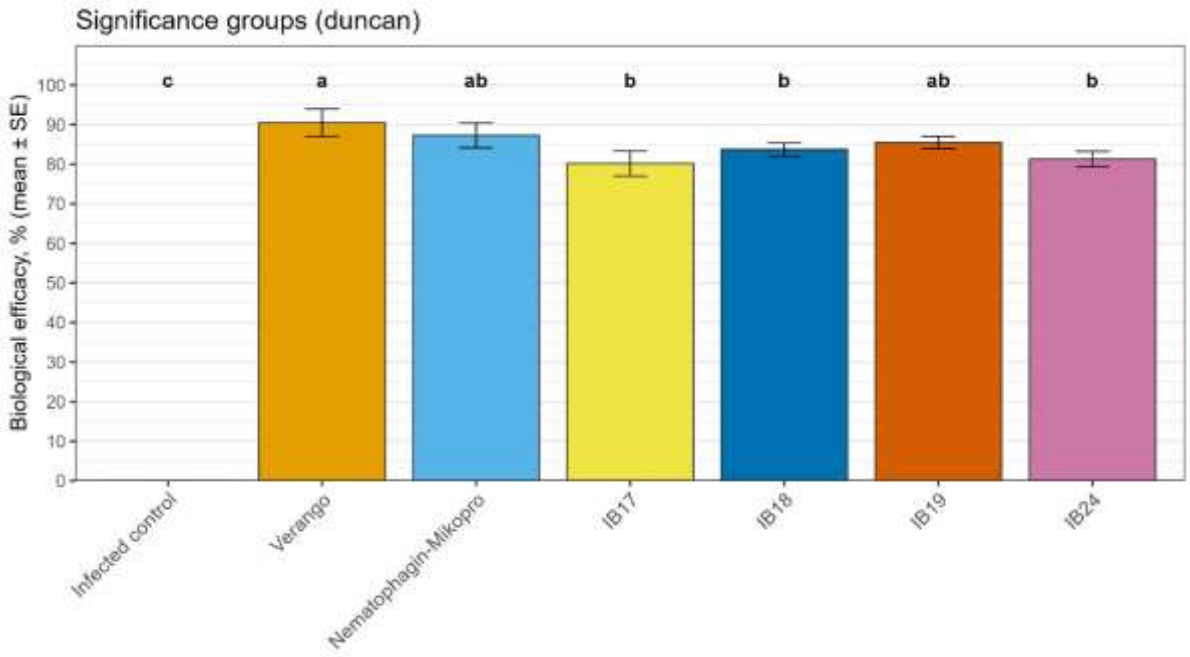
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744 Fig. 5. Number of root galls per plant in two independent greenhouse experiments: first
745 experiment; repeat experiment. Bars show mean ± SE (n = 5). Different letters above bars indicate
746 significant differences among treatments according to Duncan's test (α = 0.05), performed within
747 each experiment.



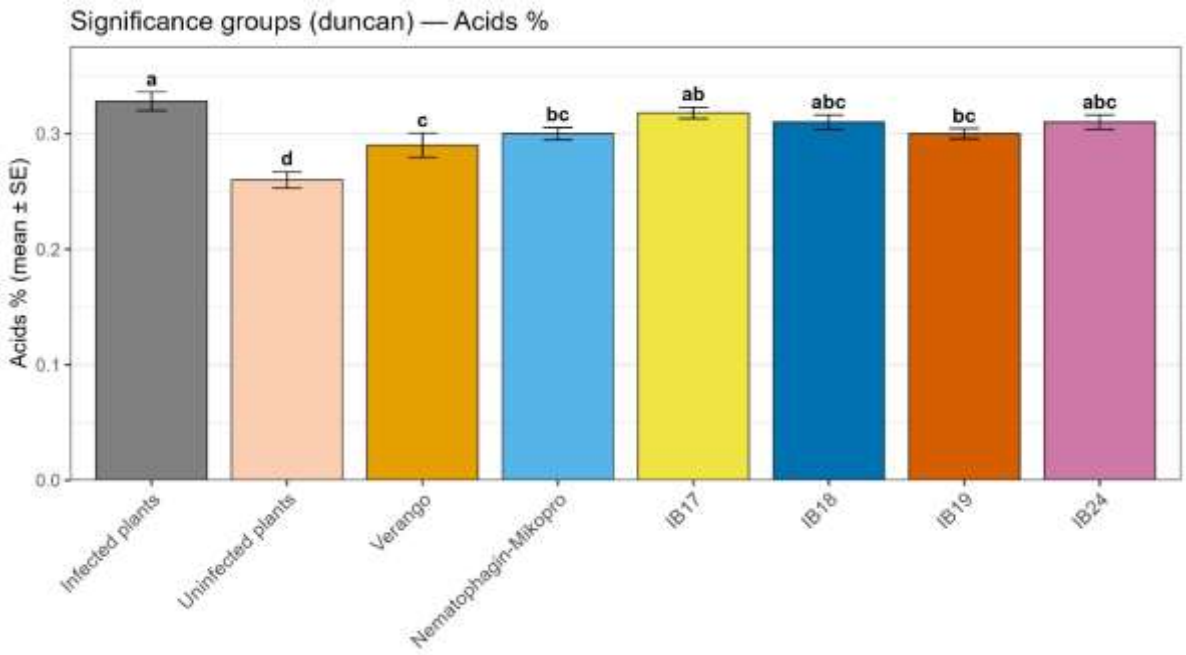
748

749 Fig. 6 Root system of the tomato hybrid Prima Dona plants infected with *M. hapla* from the
750 first experiment: (a) control (infected plants, water); (b) treatment with the *P. silesiensis* IB24; (c)
751 treatment with the *B. mycoides* IB19.



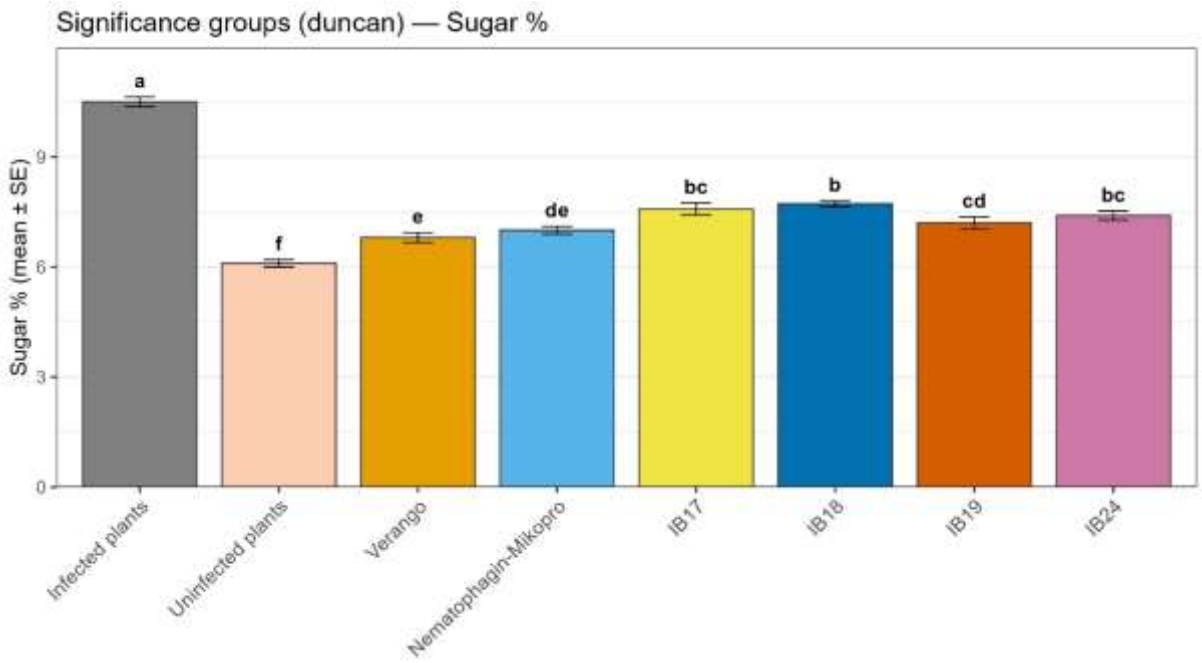
752

753 Fig. 7 Biological efficacy (%) of treatments against *M. hapla* in two independent greenhouse
754 experiments: first experiment; repeat experiment. Bars show mean ± SE (n = 5). Different letters
755 above bars indicate significant differences among treatments according to Duncan's test (α = 0.05),
756 performed within each experiment.



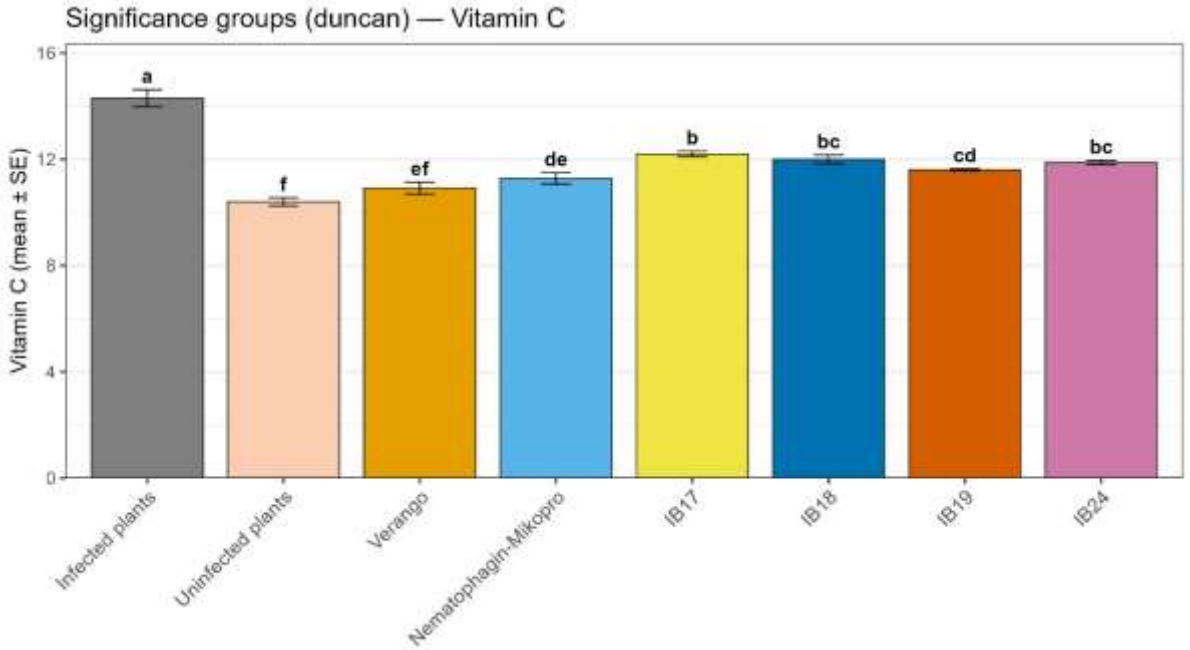
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758 Fig. 8 Acid content (%) in tomato fruit in two independent greenhouse experiments: first
759 experiment; repeat experiment. Mean ± SE (n = 5). Different letters above bars denote Duncan
760 groupings (α = 0.05) within each experiment.



761

762 Fig. 9 Sugar content (%) in tomato fruit in two independent greenhouse experiments: first
763 experiment; repeat experiment. Mean ± SE (n = 5). Different letters above bars denote Duncan
764 groupings (α = 0.05) within each experiment.



765

766 Fig. 10 Vitamin C (mg·100 g^{-1}) in tomato fruit in two independent greenhouse experiments:
767 first experiment; repeat experiment. Mean ± SE (n = 5). Different letters above bars denote Duncan
768 groupings (α = 0.05) within each experiment.