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Effect of rhizospheric biological products on eggs and juveniles of *Meloidogyne incognita* in tomato (*Solanum lycopersicum* L.) under greenhouse conditions

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Abstract: *Meloidogyne incognita*, known as the root-knot nematode, is one of the main pests affecting tomato crops worldwide, so its effective management is crucial to minimize economic losses and ensure crop quality. The objectives of this research were to evaluate the effectiveness of commercial biological products to estimate the root extraction and control the population of *Meloidogyne* sp. in tomato (*Solanum lycopersicum* L.), variety Rio Grande under greenhouse conditions. The following biological treatments were used: Stimplex®, Serenade®, PHC Condor®, Labrador®, PHC Lilatron®, a mix of all products (full doses) and a control (water). The treatments were applied in an experimental design of blocks completely in random with five repetitions, using 70 experimental units, each with a tomato plant inoculated with 1,227 J2 juveniles within 2 days after planting (DAP) in tomato nurseries. Three applications of the treatments were carried out every 7 days, and the experiment concluded in 86 days after the transplant (DAT). The results showed that the treatments significantly reduced the population of *Meloidogyne incognita* and improved root development. The evaluated treatments significantly reduced the gall index caused by *M. incognita*. Lilatron® (T5) achieved a 73.33% reduction in Experiment 1, while Stimplex®, Serenade®, and PHC Condor® (T1–T3) reached 83.64% in Experiment 2. No galls were observed in the non-inoculated blocks, confirming the reliability of experiment.

Keywords: Effectiveness; Galls; Inoculation; Nematodes; Tomato nurseries.

Introduction

The tomato crop *Solanum lycopersicum* L. is one of the more important in Mexico and worldwide (Seymour and Rose, 2022). It is produced in more than 100 countries for fresh consumption and for various processing purposes, such as canned purees and sauces (Morales, 2022). In 2021 cycle, world tomato production was 189,133,955.04 Mt harvested from 5,167,388 ha. It should be noted that the main producing countries were: China, the United States, India, and Turkey, while Mexico ranked 10th (FAOSTAT, 2023) and in 2022 it obtained a national production of 3,461,766.43 t harvested from 49,196.04 ha, distributed across 32 producing states; among which stood out: Sinaloa, San Luis Potosí, Michoacán, and Jalisco (SIAP, 2023).
The yield and quality of tomato crops are affected by the incidence of pests, especially root-knot nematodes of genus *Meloidogyne*; which are obligate parasites that penetrate the roots, causing deformations and galls, thereby reducing the absorption of water and nutrients in the soil, and consequently the plant delays growth, suffers general chlorosis and wilting (Hussey and Janssen, 2002), which results in

lower crop yields (Lizardo et al., 2022; Díaz-Manzano et al., 2016). It has been estimated that they cause losses of $80 to $157 billion dollars per year (Nicol et al., 2011; Elling, 2013; Mokrini et al., 2018).
The control of *Meloidogyne* spp. is difficult due to the wide range of hosts. They are hidden in the soil, there is lack of resistant varieties, the prohibition of many chemical nematicides due to their high toxicity, environmental contamination and damage to non-target organisms (Xiang et al., 2018; Subedi et al., 2020;). Therefore, biological control and botanical pesticides are increasingly being used as sustainable alternatives (Díaz-Manzano et al., 2016; Subedi et al., 2020; Riyaz et al., 2022).
In biological control, rhizobacteria such as *Bacillus* and *Pseudomonas*, nematophagous fungi such as *Purpuriocillium*, *Mirothecium* and *Trichoderma*, and marine algae: *Ascophyllum nodosum* (Lahlali et al., 2022), are used separately or in consortia to combat root-knot nematodes in different crops of global economic importance (Tapia-Vázquez *et* al., 2022). Rhizobacteria of the genus *Bacillus* have a direct effect against nematodes through the production of enzymes (Lian et al., 2007; Corrales-Ramírez et al., 2017; Mazzuchelli et al., 2020), antibiotics (Nadeem et al., 2021), volatile organic compounds and diffusible secondary metabolites in the rhizosphere (Oliveira et al., 2014). They also form a biofilm that covers the roots and physically protects them from attack by bacteria, fungi and phytopathogenic nematodes (Ansari and Ahmad, 2019; Mazzuchelli et al., 2020). They have the ability to suppress nematode populations in soil (Engelbrecht et al., 2018; Topalović et al., 2020).
On the other hand, *Purpureocillium lilacinum* (=*Paecilomyces lilacinus*) is a cosmopolitan fungus with potential applications in agriculture as a biocontrol agent and biofertilizer (Ahmad et al., 2018; Baron et al., 2020; Barbosa et al., 2022). It is the most effective parasite of *Meloidogyne* spp. eggs. The genus *Trichoderma* is one of the most widely used fungi in the biological control of phytopathogens and to stimulate plant growth (Vinale et al., 2008; Ferreiría et al., 2021). *Trichoderma*, like *Purpureocillium*, traps and kills root-knot nematodes in soil and root system; which attacks eggs, juveniles, and adults (Yao et al., 2015; Schouten, 2016).
In addition to the use of rhizospheric bacteria and fungi, the effectiveness of seaweed extract derivatives such as *Ascophyllum nodosum* is recently being investigated to reduce damage caused by *Meloidogyne* spp. and promote crop nutrition and growth (Williams et al., 2021). These algae contain high levels of bioactive organic and inorganic compounds, including mannitol, laminarin, alginic acid, poly and oligosaccharides, vitamins, antioxidants, phytohormones (auxins, cytokinins, gibberellins and betaine) and low concentrations of minerals (potassium, phosphorus, calcium, boron, magnesium, zinc and other trace elements) (Klarzynski et al., 2000). Therefore, the objectives of this research were to evaluate the effectiveness of commercial biological products to estimate the root extraction and control the population of *Meloidogyne* sp. in tomato (*Solanum lycopersicum* L.), variety Rio Grande under greenhouse conditions.

Results

Fresh root weight
Treatments did not cause any significant effect on this variable in Experiment 1 (p= 0.2714^{NS}, Table 2), but did in Experiment 2 (p=<.0001**, Table 2).

Experiment 1. In the witness treatment with *M. incognita* (T7), an average of 18.14 g was recorded, which is 110.44% higher than 8.62 g of the witness without *M. incognita* (T14). Likewise, in the group of treatments inoculated with *M. incognita* (T1 to T7), treatment T6 (Consortium: T1+T2+T3+T4+T5), tended to develop greater weight of the fresh root, with an average of 98.98 g, which implied an increase of 445.64%, compared to the control with *M. incognita* (T7). However, in treatments without *M. incognita*, the plants treated with Labrador® (T11) were the heaviest, with 20.17 g, which is 133.99% greater than 8.62 g of the control without *M. incognita* (T14) (Table 2).

Experiment 2. In the block with *M. incognita*, the T7 witness showed an average of 31.36 g, which is 297.97% higher than 7.88 g of T14 witness without the nematode. Furthermore, in the block with J2 juveniles of the nematode, with treatments T1 to T7, it was observed that the highest weight of fresh root was obtained in treatment T4 (Labrador®), with 56.01 g, that is, 78.60% higher than 31.36 g of the control (T7). On the other hand, plants without *M. incognita*, inoculated with Lilatron® (T12) obtained the highest average with 20.77 g. In other words, it increased 163.58%, with respect to the control without *M. incognita* (T14) with 7.88 g (Table 2).

Dry root weight
The treatments did not have any significant effect on this variable in experiment 1 (p = 0.1112^{NS}; Table 2). However, in experiment 2 highly significant differences were observed (p = 0.0002**) (Table 2).

Experiment 1. Treatment T7, inoculated with *M. incognita*, had an average fresh root weight of 3.61 g, which represents an increase of 56.28% compared to the control T14 (without nematode), whose weight was 2.31 g. Within the group of treatments inoculated with *M. incognita* (T1 to T7), treatment T5 (Lilatron®) showed the highest root weight, with an average of 8.33 g, which is equivalent to an increase of 130.75% compared to 3.61 g in control T7. In contrast, in the block without *M. incognita*, the treatment with the consortium: T1+T2+T3+T4+T5 (T13) presented the highest root weight, with an average of 4.71 g, which represents an increase of 103.90% compared to the control T14 (2.31 g) (Table 2).

Experiment 2. Within the block with *M. incognita*, witness T7 recorded an average fresh root weight of 4.46 g, which represents an increase of 38.08% compared to witness T14 without nematode (3.23 g). In this same block, treatment T5 (Lilatron®) reached the highest root weight with an average of 8.76 g, which represents an increase of 96.41% compared to the control T7 (4.46 g). Likewise, in the block without *M. incognita*, treatment T12 (Lilatron®) had the highest average weight, with 7.88 g, exceeding the control T14 (3.23 g) by 143.96% (Table 2).

Galling index
According to the data from the analysis of variance, in both experiments there were significant differences due to the effect of the microorganisms evaluated as treatments (p=<0.0001**) (Table 3). The control treatment with *M. incognita* (T7) of experiment 1 recorded an average of 83.33% of galls, compared to the control with *M. incognita* of experiment 2 with an average of 91.66%, which is an increase of 8.33% galls.

Experiment 1. In the treatment group with *M. incognita*, it was observed that the gall index of T5 (Lilatron®) achieved a gall percentage of 10%, highlighting a 73.33% decrease compared to the control, which reached 83.33%.

Experiment 2. In the treatments with *M. incognita*, it was analyzed that the gall index of T1 (Stimplex®), T2 (Serenade®) and T3 (PHC Condor®) managed to obtain 15% gall while the control generated 91.66%. Finally, in the blocks without *M. incognita* of both experiments (T8 to T14), the percentage of galls was 0%, since the plants were not inoculated with *M. incognita* (Table 3).

Number of eggs on the root

According to the data from the analysis of variance, only in experiment 1, there were no significant differences ($p= 0.22^{NS}$) due to the effect of the microorganisms evaluated as treatments. However, in experiment 2 significant differences were observed ($p=0.016^{**}$) (Figure 1). The control treatment with *M. incognita* from experiment 1 (T7) recorded an average of 36.09 eggs g^{-1} of fresh root, compared to the control with *M. incognita* from experiment 2 with an average of 27.29 eggs g^{-1} of fresh root, meaning a decrease of 24.38% (Figure 1).

Experiment 1. The control treatment (T7) with *M. incognita* recorded an average of 36.09 eggs g^{-1} of fresh root. However, it was observed that the number of eggs in the T3 treatment (PHC Condor®) managed to obtain an average of 0 eggs g^{-1} of fresh root, influencing a 100% decrease compared to the control (Figure 1).

Experiment 2. It was determined that the number of eggs of T3 (PCH Condor®) managed to obtain an average of 2.58 eggs g^{-1} of fresh root, which reflected a decrease of 90.55% compared to the control that obtained an average of 27.29 eggs g^{-1} of fresh root (Figure 1).

Number of J2 juveniles in the substrate

According to the analysis of variance, only in experiment 1, there were significant differences between the treatments with microorganisms evaluated ($p = 0.0035^{**}$). In contrast, in experiment 2 no significant differences were observed ($p = 0.3441^{NS}$) (Figure 2). The control treatment with *M. incognita* in experiment 1 presented an average of 63.90 juveniles per 50 cm^3 of substrate, while in experiment 2 the control recorded 31.70 juveniles in the same amount of substrate, which represented a reduction of 49.60% (Figure 2).

Experiment 1. The control treatment with *M. incognita* (T7) recorded an average of 63.90 juveniles in 50 cm^3 of substrate. However, in the T4 treatment (Labrador®) it was observed that the number of juveniles was 5.09 juveniles in 50 cm^3 of substrate (Figure 2).

Experiment 2. It was found that the number of T3 larvae (PCH Condor®) managed to obtain an average of 4.62 juveniles in 50 cm^3 of substrate, while the control treatment generated an average of 31.70 juveniles in 50 cm^3 of substrate (Figure 2).

Discussion

Fresh root weight

Hernández-Ochandía et al. (2015) reported that in the treatment with *Trichoderma* sp., an average of 7.27 g of fresh root was obtained, compared to 5.76 g of tomato plants without inoculation of *Meloidogyne* sp. On the other hand, Krif et al. (2022) validated the effect of the consortium of microorganisms *(A. nodosum*, *B. amyloliquefaciens* and *T. harzianum*), in tomato plants and determined that this treatment significantly improved the fresh root weight by more than 58.13%. Furthermore, Khan (2021) described that the association of different microorganisms (from the genera *Bacillus*, *Trichoderma* and *Paecilomyces*) have an important role as promoters of growth and increase in root and leaf volume in different horticultural plants, since rhizogenic bacteria and fungi produce metabolites such as hydrogen cyanide, 2,4-diacetylphloroglucinol, antibiotics and volatile compounds that benefit plant growth; likewise, they release phytohormones that influence the physiological processes of plants.

Dry root weight

In the experiment carried out by Guiri et al. (2022), it was proven that treatment with *Paecilomyces variotti* in chili plants (*Capsicum annuum* L.) with and without *M. incognita* inoculation significantly increased the weight of the dry root by 66.87% in plants with the nematode. Furthermore, Moreno-Gavíra et al. (2020) reported that root weight increased by 18.2–6.7% in pepper plants treated with *P. variotti* and inoculated with J2 juveniles of *M. incognita.*

Galling index

Oclarit and Cumagun (2009) evaluated the gall index in tomato plants infested with *Meloidogyne* spp., using *P. lilacinus* as treatment. Their results were similar to the present investigation, having significantly higher data in tomato plants not treated with *P. lilacinus*, obtaining a gall reduction percentage of 98%. Similarly, Niño-Arteaga et al. (2023) evaluated the behavior of *Paecilomyces* with the gall index in tomato plants infected with *M. incognita*. Their results demonstrated that these fungi significantly reduced the presence of galls in the roots of the plants by 80%. Similarly, Moreno-Gavíra et al. (2020), working with the *P. variotti* strain on chili plants inoculated with J2 larvae of *Meloidogyne* spp., explained that after the recognition and pathogen-antagonist interaction, this fungi undergoes (mechanical) penetration and/or secretion of enzyme complexes, through the release of cellulase, glucanase, laccase, leucinoxin, lipase, pectinase, protease, chitinase or xylanase, which are involved in the infection process, leading to the growth of the fungi at the expense of its host.

Number of eggs on the root

The results reported by Chahal and Chahal (2020) on *B. thuringiensis* on pea roots (*Pisum sativum* L.) infected by *Meloidogyne* spp., significantly inhibited the nematode eggs by more than 89.5 and 100%. Similar data were found in the work of Leong et al. (2021), when working with *B. thuringiensis*, demonstrating that these beneficial bacteria exhibited a 78.8% reduction in eggs in black pepper roots. This is because *Bacillus* species generate nematophagous crystalline proteins (delta endotoxins, or Cry proteins) which, when in contact with this pathogen, cause the eggs to disintegrate (Ramalakshmi et al., 2020). Similarly, Yolanda et al. (2022) determined the activity of seven classes of *B. turingensis* Cry toxins (Cry5, Cry6, Cry12, Cry13, Cry14, Cry21 and Cry55) in

Table 1. Study treatments with and without inoculation of J2 juveniles of *M. incognita* and with application of beneficial microorganisms with nematicidal action.

No. Treat.	*M. incognita*	Commercial product	Active ingredient	mL or g per pot
T1	With 1,227 J2 juveniles	Stimplex®	*Ascophyllum nodosum* (99.588%)	7.5
T2		Serenade®	*Bacillus subtilis* (1.34%)	3
T3		PHC Condor®	*Bacillus thuringiensis* (15%)	3
T4		Labrador®	*Trichoderma harzianum* (14%)	2.25
T5		Lilatron®	*Paecilomyces lilacinus* (3%)	2.25
T6		Consortium: T1+T2+T3+T4+T5	*A. nodosum* + *B. subtilis* + *B. turingensis* + *T. harzianum* + *P. lilacinus*	7.5+3+3+2.25+2.25
T7		Control (water)	-	10
T8	Without J2 juveniles	Stimplex®	*Ascophyllum nodosum* (99.588%)	7.5
T9		Serenade®	*Bacillus subtilis* (1.34%)	3
T10		PHC Condor®	*Bacillus thuringiensis* (15%)	3
T11		Labrador®	*Trichoderma harzianum* (14%)	2.25
T12		Lilatron®	*Paecilomyces lilacinus* (3%)	2.25
T13		Consortium: T1+T2+T3+T4+T5	*A. nodosum* + *B. subtilis* + *B. turingensis* + *T. harzianum* + *P. lilacinus*	7.5+3+3+2.25+2.25
T14		Control (water)	-	10

Nº Treat.= Treatment number.

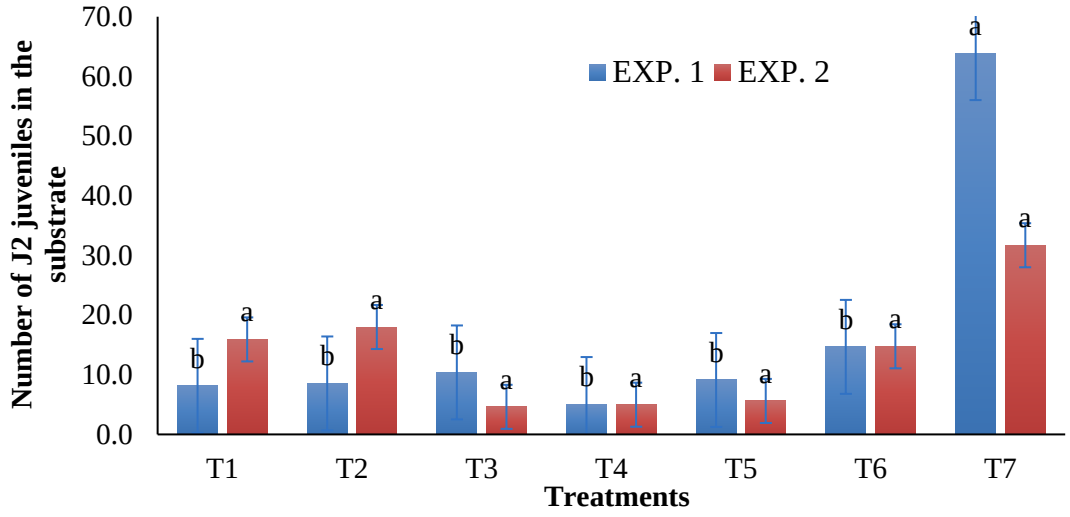

Fig. 1. Effect of inoculated microorganisms on root egg count. T1= Stimplex®, T2= Serenade®, T3= PHC Condor®, T4= Labrador®, T5= PHC Lilatron®, T6= Consortium: T1+T2+T3+T4+T5, and T7 = control. EXP. 1= Experiment 1, EXP. 2= Experiment 2. DMS = least significant difference. Values with the same letters above the columns are not statistically different (Tukey, $\alpha \leq 0.05$).

Table 2. Effect of treatments with and without *M. incognita*, and inoculation of rhizogenic microorganisms, on the root weight (g) of tomato plants in experiments 1 and 2.

Nº Treat.	Study factor		FRW		DRW	
	M. incognita	Commercial product	Exp. 1	Exp. 2	Exp. 1	Exp. 2
T1	With 1,227 J2 juveniles	Stimplex®	53.19 a	27.47 abcd	5.80 a	4.87 abc
T2		Serenade®	40.14 a	30.38 abcd	6.66 a	5.25 abc
T3		PHC Condor®	30.26 a	43.99 abc	5.42 a	7.08 abc
T4		Labrador®	38.97 a	56.01 a	5.87 a	6.99 abc
T5		Lilatron®	42.17 a	46.71 ab	8.33 a	8.76 a
T6		Consortium: T1+T2+T3+T4+T5	98.98 a	34.80 abcd	7.24 a	7.20 ab
T7		Control	18.14 a	31.36 abcd	3.61 a	4.46 abc
T8	Without J2 juveniles	Stimplex®	11.83 a	11.48 cd	3.03 a	2.93 bc
T9		Serenade®	8.80 a	6.04 d	2.52 a	2.08 bc
T10		PHC Condor®	11.60 a	7.88 d	2.89 a	2.02 bc
T11		Labrador®	16.43 a	12.26 cd	3.49 a	2.53 bc

T12	Lilatron®	20.17 a	20.77 cd	4.01 a	7.88 d
T13	Consortium: T1+T2+T3+T4+T5	17.78 a	12.61 cd	4.71 a	3.82 abc
T14	Control	8.62 a	7.88 d	2.31 a	3.23 bc
Prob F		0.2714^{NS}	<.0001**	0.1112^{NS}	0.0002**
DMS		111.53	33.735	7.4471	5.228

Nº Treat.= Treatment number. Exp. 1= experiment 1, Exp. 2= experiment 2, FRW= fresh root weight, DRW= dry root weight. DMS= Least Significant Difference. Values with the same letters within columns are not statistically different (Tukey, α ≤ 0.05).

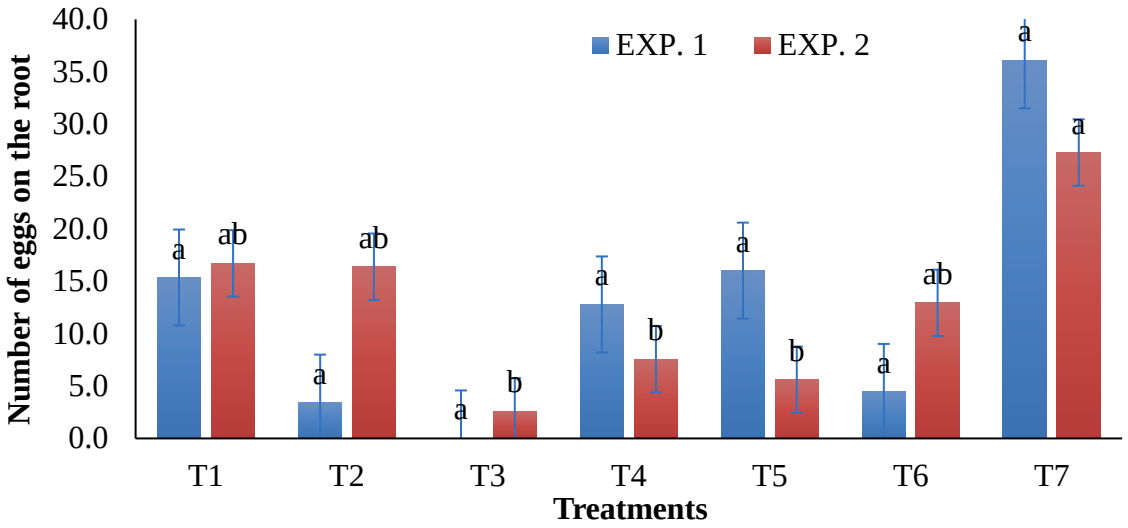

Fig. 2. Effect of inoculated microorganisms on the number of J2 juveniles in the root. T1= Stimplex®, T2= Serenade®, T3= PHC Condor®, T4= Labrador®, T5= PHC Lilatron®, T6= a mixture of all treatments (T1–T5), and T7 = control. EXP. 1= Experiment 1, EXP. 2= Experiment 2. DMS= least significant difference. Values with the same letters above the columns are not statistically different (Tukey, α ≤ 0.05).

Table 3. Effect of treatments with and without *M. incognita* and inoculation of rhizogenic microorganisms on the Galling Index in tomato plants in experiments 1 and 2.

Nº Treat.	Study factor		Galling index	
	M. incognita	Commercial product	Exp. 1	Exp. 2
T1	With 1,227 J2 juveniles	Stimplex®	28.67 b	15.0 bc
T2		Serenade®	15.0 b	15.0 bc
T3		PHC Condor®	15.0 b	15.0 bc
T4		Labrador®	28.33 b	45.33 b
T5		Lilatron®	10.0 b	29.0 bc
T6		Consortium: T1+T2+T3+T4+T5	15.67 b	20.66 bc
T7		Control (water)	83.33 a	91.66 a
T8	Without J2 juveniles	Stimplex®	0.0 b	0.0 c
TT9		Serenade®	0.0 b	0.0 c
T10		PHC Condor®	0.0 b	0.0 c
T11		Labrador®	0.0 b	0.0 c
T12		Lilatron®	0.0 b	0.0 c
T13		Consortium: T1+T2+T3+T4+T5	0.0 b	0.0 c
T14		Control (water)	0.0 b	0.0 c
Prob F			<.0001**	<.0001**
DMS			39.969	36.656

Nº Treat.= Treatment number. Exp. 1= experiment 1, Exp. 2= experiment 2. DMS= Least Significant Difference. Values with the same letters within columns are not statistically different (Tukey, α ≤ 0.05).

nematodes, concluding that they have nematicidal activity, and although the mode of action of these toxins is not well established, while carbohydrates are known to be essential for toxicity.

Number of J2 juveniles in the substrate

Mena et al. (2020) in their research working with banana plants treated with *B. subtilis* showed an 89.3% reduction in *Meloidogyne* larvae. Similarly, Choi et al. (2020) mentioned that by using *B. thuringiensis* in tomato plants infected by *Meloidogyne* spp., this bacterium reduced almost 100% of J2 larvae mortality. On the other hand, Cetz et al. (2017) reported that *Trichoderma* in tomato plants inoculated with *Meloidogyne* reduced larval formation from 23.44 to 31.21%, respectively. Bhat et al. (2023) tested *A. nodosum* on horticultural crops such as tomato and cucumber (*Cucumis sativus* L.) infested with *Meloidogyne*, arguing that they can control these pathogens through

various mechanisms, such as competition (intraspecific and interspecific), mainly for space, nutrients, water, etc., reducing the activity and reproduction of these organisms and affecting the fitness of the nematodes.

Materials and Methods

Study area

This study was conducted at Colegio Superior Agropecuario del Estado de Guerrero (CSAEGRO), located at km 14.5 of the Iguala-Cocula Highway, between coordinates 18° 14' 20" north latitude and 99° 39' 39" west longitude, at an altitude of 640 meters above sea level. The climate here is warm, subhumid, with temperatures ranging from 13 to 37 °C and an average of 28 °C.

Germination and transplanting of tomato seedlings

Clay loam soil was used as a substrate, obtained from the town of San Vicente Palapa, municipality of Tepecoacuilco de Trujano (longitude -99.411389 and latitude 18.324167) in Guerrero. First, the soil was sieved to remove stones and debris, and homogenized before filling the pots. The substrate was left to rest for two weeks to lose moisture. It was then disinfected with a solution composed of benomyl (50%) + carbendazim (50%) + manzate (80%) at a dose of 8 g L^{-1}, which was applied using a manual backpack sprayer (20 L) to moisten the substrate until it reached its saturation point.

Fifty-cavity polyethylene trays were filled with substrate, then one seed was placed per cavity and subsequently, uniform irrigation was applied. Transplanting was done 17 DAP, placing a plant in the center of the pot. After transplanting, irrigation was provided every two days for two weeks, with an expenditure of 500 mL of water per pot. Irrigation was then adjusted to every four days for the remainder of the experiment, with an expenditure of 1 L of water per pot at each irrigation.

Collection and application of J2 juveniles of M. incognita

Collection was carried out in the greenhouse of the Plant Pathology area of CSAEGRO, from a breeding of *M. incognita* established in tomato variety Rio Grande. Root samples collected in the greenhouse were washed with water to remove all soil residues and cut into pieces of ≈ 2.0 cm. Subsequently, they were disinfected by immersion for 30 min in a solution of mancozeb (80%) and copper oxychloride (85%), in doses of 8 g L^{-1} of water each and rinsed in distilled water. For the extraction of J2 juveniles of *M. incognita*, the extraction tray technique described by Coyne et al. (2007) was used, so the pieces of gall root were placed semi-submerged on a mosquito net in a tub with 3 L of distilled water, where the end of a hose connected to a small air pump (HydroPlus) was placed with the capacity to oxygenate 19 L of water, with a power of 120 volts, 1.5 watts and 60 Hertz for permanent oxygenation.

Kept was carried out at 28 ºC and photoperiod of 12 h natural light. The water with the nematode juveniles in the extraction tray was passed through a series of sieves No. 100, 200 and 400 (sizes of 150, 75 and 38 microns opening). The material retained on the last sieve was collected and made up to 200 mL in distilled water in a beaker. From the suspension obtained, 1 mL was taken, placed well distributed in a rectangular box (7.5 × 5 × 0.2 cm), with a grid bottom. The count was carried out in a stereoscopic microscope and the number of juveniles per mL^{-1} was determined. Three replicates were performed, and an average of 214 juveniles mL^{-1} was determined. A total of 1,227 juveniles were inoculated with two DAT distributed in four 4-cm-deep holes made around the center of the pot. The holes were covered and lightly watered.

Treatments under study

Fourteen treatments were evaluated, with and without inoculation of J2 juveniles, and were distributed under a completely randomized block design with five replicates (Table 1). Each experimental unit consisted of one pot, and the experiment was conducted in duplicate to ensure repeatable results.

Treatments were applied three times, with a 7-day interval between each application, both with and without inoculation of J2 juveniles. The doses of the products were dissolved in 1.5 L of water. Of this solution, 300 mL were applied per pot, distributed in four holes made around the plant, oriented toward the cardinal points, located 5 cm from the neck of the plant, and 75 mL of the solution was placed in each hole.

Study variables

The experiments ended at 86 DAT and the following variables were measured:

Weight of fresh and dry root. The entire root was removed from the potting medium, washed with water, and allowed to drain. It was then weighed on a digital granataria scale and placed in paper bags. The roots were left to dry at room temperature in the greenhouse for two weeks to obtain the dry root weight.

Galling index. The galling index was determined using the 0 to 6 grade scale proposed by Taylor and Sasser (1978), where: 0= 0 galls, 1= 1-2 galls, 2= 3-10 galls, 3= 11-30 galls, 4= 31-100 galls and 5= more than 100 galls. In addition, the final population of eggs and second-stage J2 juveniles in the root system was quantified to calculate the reproduction factor.

Number of eggs on the root. This practice was carried out using the Hussey and Baker technique (1973), so the fresh root was cut into small segments (≈ 1-2 cm), which were placed in the glass of an electric mixer, 10 mL of 6% sodium hypochlorite and 190 mL of distilled water were added. It was beaten for 1 sec and passed through the series of sieves No. 100, 200 and 400. The material retained on the last sieve was collected in a beaker and made up to 200 mL with distilled water. Egg counts were performed using the procedure described for the J2 juvenile's count.

Number of J2 juveniles in the substrate. For this activity, the sieving technique described by Coyne et al. (2007) was carried out. To begin, 100 g of substrate from each inoculated experimental unit was weighed, dissolved in 1 L of water, the mixture was beaten to homogenize it and then, it was left to stand for 1 min, and finally it was passed through serial sieves, No. 100, 200 and 400. The filtered water was re-screened three times through the final sieve. The collected material was transferred to a beaker and made up to 200 mL with distilled water. J2 juveniles counting was performed using the procedure described in the *M. incognita* inoculum preparation section.

Statistical analysis
With the data obtained, an ANOVA was performed per variable and a multiple comparison of means was made with Tukey ($\alpha \leq 0.05$). Likewise, a correlation analysis was performed between the variables evaluated using SAS 9.4 SAS software (Institute, 2016).

Conclusions

M. incognita infection decreased the fresh weight of the root by (445.64 and 113.99%) and (78.60 and 163.58%) in experiments 1 and 2. The application of treatments T5 (Lilatron®) in Experiment 1 and T1 (Stimplex®), T2 (Serenade®) and T3 (PHC Condor®) in Experiment 2, reduced the gall index in fresh roots by 88 and 83.64%, respectively, in experiments 1 and 2.
Treatment T3 (PHC Condor®) was effective in reducing the number of eggs per gram of fresh root, influencing (100 and 90.55%) in experiments 1 and 2.
Treatments T4 (Labrador®) and T3 (PCH Condor®) were effective in decreasing the number of juveniles in 50 cm^3 of substrate, influencing (92.03%) and (85.42)%, in experiments 1 and 2.

Author Contributions
SAS, JLAV, MVH, JFDN and MATR Investigation; SAS, GSP, JLAV, JFDN, MVH, JTS and MAB Methodology; JLAV, MVH, OJP, JTS, MAB, JFDA and DACZ Data curation; JTS, MAB, MATR, JFDN, MFV and DACZ Visualization; SAS, JLAV, LCMA, MAB, MATR, MFV and DACZ Writing - original draft; SAS, JLAV, JFDN, JTS, MAB, LCMA, OJP and DACZ Writing - review and editing. All authors read and approved the final manuscript.

Conflict of Interest
Authors declare no conflict of interest.

Data Availability
All datasets presented in this study will be available on a fair request to the corresponding authors.

Ethics Approval
This research does not involve ethical approval.

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