

Article

Pre-Plant Biofumigation and Integrated Post-Plant Strategies for Management of *Nacobbus aberrans* and *Meloidogyne incognita* in Greenhouse Tomato

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Abstract

Root-knot (*Meloidogyne* spp.) and false root-knot (*Nacobbus aberrans*) nematodes limit greenhouse tomato productivity. The effectiveness of integrating pre-plant biofumigation with post-plant chemical, biological, and botanical inputs was assessed under commercial conditions. A split-plot trial (2019) contrasted biofumigated and non-biofumigated whole plots (50 t ha⁻¹ of sorghum residues plus poultry and sheep manures) and 13 subplot treatments (fluopyram, *Purpureocillium lilacinum*, *Pochonia chlamydosporia*, *Trichoderma viride*, *Tagetes erecta*, and plant oil formulations). Nematodes were sampled 0, 60, and 120 days after transplanting, and the area under the nematode population curve (AUNPC), area under the root-damage curve (AURDC), and yield were analyzed. Biofumigation reduced pre-transplant *N. aberrans* populations by 86% and lowered the AUNPC by 39% relative to the non-biofumigated treatment; the whole-plot yields did not differ. *Meloidogyne incognita* remained at a very low density throughout. Among the subplot treatments, fluopyram decreased the AURDC by ≈22% and more than doubled the yield (63 vs. 26 t ha⁻¹; +142%), while the AUNPC of *N. aberrans* was unchanged. Biological and botanical packages reduced damage indices in some cases but did not increase the yield. No whole-plot × subplot interaction was detected for the yield. The results indicate that sorghum-based biofumigation, complemented by a low-risk nematicide at transplanting, can be embedded in integrated nematode-management programs for greenhouse tomato.

Keywords: fluopyram; *Purpureocillium lilacinum*; *Pochonia chlamydosporia*; *Trichoderma viride*; *Tagetes erecta*; plant oil botanical formulations; sorghum residue amendment; suppressive soils



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1. Introduction

In Mexico, tomato (*Solanum lycopersicum* L.) represents 9% of the total value of agricultural production and 31% of horticultural exports [1]. Plant-parasitic nematodes are considered responsible for ≈30% of the yield losses attributed to biotic stresses in crops worldwide [2]. Plants attacked by nematodes typically show stunted growth, reduced root development, leaf chlorosis, and reduced fruit quality and quantity [3].

The false root-knot nematode (*Nacobbus aberrans*) and root-knot nematodes (*Meloidogyne* spp.) are among the most destructive nematode pests affecting protected tomatoes in North, Central, and South America; in México, substantial losses have been reported during greenhouse production [4,5]. *Nacobbus aberrans* is one of the most agriculturally important nematodes in Mexico and the Andean region, owing to its broad host range—notably tomato, chili (*Capsicum annuum* L.), bean (*Phaseolus vulgaris* L.), and many other crops—its wide geographical distribution, and its economic impact [5,6]. The galling it induces resembles that caused by *Meloidogyne* spp. but differs in its onset and arrangement, giving rise to the common names “false root-knot” and “rosary-gall”. More than one hundred species of root-knot nematodes are recognized within *Meloidogyne*. Infestations starting at the seedling stage can reduce tomato yields by >27% [7], and reported losses in Mexico range between 7 and 70% depending on host susceptibility and the environment [6].

Because Mexican growers have access to both synthetic (e.g., non-fumigant nematicides) and natural inputs (e.g., fungal biocontrols and botanical oil blends), commercially available products and their brand-specific formulations were prioritized to reflect on-farm practices. Biofumigation—the release of biotoxic volatiles during decomposition of organic amendments—can suppress soil-borne pathogens without relying on synthetic fumigants. Volatile isothiocyanates and other plant-derived volatile organic compounds generated as residues decompose are key drivers of nematode suppression [8,9]. Biological control agents—beneficial bacteria and fungi—may regulate plant-parasitic nematode populations via parasitism and antibiosis, production of nematicidal metabolites and lytic enzymes, niche competition in the rhizosphere, and induction of plant systemic resistance [10,11]. Several species have shown efficacy against *Meloidogyne* spp., including *Purpureocillium lilacinum* [12], *Pochonia chlamydosporia* [13], *Trichoderma* spp. [14], and *Bacillus subtilis* [15]. By contrast, peer-reviewed evidence for *N. aberrans* remains limited, with few greenhouse/field trials [5,16]. In addition, non-fumigant nematicides, such as fluopyram, have gained attention as components of integrated management programs [4,17].

However, most management strategies are evaluated in isolation rather than as integrated technological packages, and few studies have tested their combined performance against mixed populations of *Nacobbus aberrans* and *Meloidogyne incognita* under commercial greenhouse conditions. As regional context, brassica-residue biofumigation has also been explored in tomato systems; these experiences lie outside the scope of the present tomato trial and are not used for inference. This study assessed the impact of combining pre-plant biofumigation with chemical, biological, and botanical strategies for management of *Nacobbus aberrans* and *Meloidogyne incognita* in greenhouse tomato.

2. Materials and Methods

2.1. Experimental Design and Treatments

This study was conducted in 2019 in a grower-managed commercial greenhouse (5000 m²) in San Felipe, Texcoco, Mexico (19°27'24" N, 98°53'50" W). Tomatoes of the cultivar “El Cid” (saladette type) were transplanted into in-ground soil beds and irrigated via a drip system. Routine crop protection followed the grower’s commercial practice but was limited to foliar disease management; no soil-applied pesticides, fumigants, or nematicides were delivered to the root zone or through the drip line during the experiment other than the designated experimental treatments.

The grower reported a recurrent history of root galling; accordingly, the trial relied on the naturally occurring populations of *N. aberrans* and *M. incognita* at this commercial greenhouse. No external inoculation was introduced. A split-plot design with four blocks (replications) was used. Whole plots (WPs) (124.8 m² each) were either biofumigated or non-biofumigated. Within each WP, 13 subplots (SPs) of 10 plants (2.4 m²) received

chemical, biological, and botanical products, applied singly or in combination as integrated packages (Table 1). Each WP and its set of SPs were replicated four times, with the SP treatments allocated randomly within each WP replicate. Treatment effects were analyzed using a split-plot ANOVA appropriate for this layout. In plain terms, this analysis tested (1) the whole-plot effect of biofumigation, (2) the subplot effects of post-plant treatments, and (3) their interactions. The model accounts for block-to-block variability and correctly recognizes that the large-scale effect of biofumigation was tested with a different level of precision than the smaller-scale subplot treatments. See Section 2.7 for the statistical model.

Table 1. Management treatments evaluated against *Nacobbus aberrans* and *Meloidogyne incognita* in greenhouse tomato.

No.	Active Ingredient/ Product Type	Trade Name	Dose (ha ^{−1})	Application Schedule
1	BA (biocontrol fungus)	BioAct®	0.8 L	BT + every 20 DAT
2	Fluopyram (nematicide)	Verango®	1 L (0.5 + 0.5)	BT (0.5 L) + 15 DAT (0.5 L)
3	<i>T. erecta</i> (botanical)	Nemacem®	3 L	BT + every 15 DAT
4	S (mixed plant oils)	Sterminar®	5 L	BT, 12 DAT, then every 30 DAT
5	<i>T. viride</i> (biocontrol fungus)	Trichomix	3 L	BT + every 20 DAT
6	LS (biocontrol fungus)	Lila-Sin®	480 g	BT + every 20 DAT
7	M (mixed plant oils)	Majesty®	12 → 2 L	BT, 7 d × 3, then 14 d (12 → 9 → 6 → 3 → 2 L)
8	K (biocontrol fungus)	Kastelo®	2 L	BT, 14 DAT × 2, then 28 DAT
9	<i>P. chlamydosporia</i> (biocontrol fungus)	Genexis PH®	250 g	BT + every 15 DAT
10	Technological package 1: M + K	—	See 7 and 8	Same schedules as 7 and 8
11	Technological package 2: BA + <i>T. erecta</i> + <i>T. viride</i> + M	—	See 1, 3, 5, and 7	BT (BA) → 7 DAT (<i>T. viride</i>) → 14 DAT (M) → 21 DAT (<i>T. erecta</i>); cycle repeats every 28 d
12	Technological package 3: <i>T. erecta</i> + S + LS + <i>P. chlamydosporia</i>	—	See 3, 4, 6, and 9	BT (S) → 7 DAT (LS) → 14 DAT (<i>P. chlamydosporia</i>) → 21 DAT (<i>T. erecta</i>); cycle repeats every 28 d
13	Untreated control	—	—	—

Abbreviations: BA (*Purpureocillium lilacinum* (BioAct®)), LS (*P. lilacinum* (Lila-Sin®)), K (*P. lilacinum* (Kastelo®)), S (botanical extracts (Sterminar®)), M (botanical extracts (Majesty®)). BT = before transplanting; DAT = days after transplanting. Manufacturers: Bayer (Mexico): BioAct®, Verango®; Plantoria (Mexico): Nemacem®, Sterminar®; Arvensis (Mexico): Kastelo®, Majesty®; Agrobionsa (Mexico): Lila-Sin®; Qualtium (Mexico): Genexis PH®; UTEFI Laboratory, Junta Local de Sanidad Vegetal del Valle del Fuerte (JLSVVF) (Mexico): Trichomix.

2.2. Pre-Plant Biofumigation (Whole Plot)

Biofumigation was carried out during the hottest months for this area (May–June; average maximum ambient temperature: 26 °C). A total of 50 t ha^{−1} of organic matter—fresh chicken manure (18 t ha^{−1}), fresh sheep manure (18 t ha^{−1}), and dry sorghum (*Sorghum vulgare* L.) at flowering (14 t ha^{−1})—was incorporated to a depth of ≈30 cm, and the soil was covered for 40 d with a silver-colored polyethylene mulch (0.25 mm). The plastic was then removed, and 10 d later, four-week-old tomato seedlings were transplanted into the WPs. The non-biofumigated WP did not receive the organic amendments.

The amendment load and the 40-day tarping in late spring were selected to promote rapid decomposition and release of volatiles under warm greenhouse conditions and fell within ranges reported as effective for biofumigation/organic amendments in protected horticulture (e.g., tens of t ha^{−1} and multi-week coverage) [9,18,19].

2.3. Management Product Treatments (Subplots)

Commercial products commonly used by Mexican producers were selected. The 13 subplot treatments (SP1–SP13) are detailed in Table 1 (trade name, active ingredient, rate, timing, and application mode). For brevity, the following abbreviations are used hereafter (see Table 1 footnote): BA, LS, K (three formulations of *Purpureocillium lilacinum*), S (Sterminar®), and M (Majesty®).

According to the product datasheets, S comprises mixed plant oils (neem, “anisillo” essential oil, and cinnamon), while M is a proprietary plant-extract blend (26.46%); in both cases, the botanical species are not disclosed. The rates and timings followed the product labels. Fluopyram was applied as a split dose at transplanting and 15 DAT (1.0 L ha^{−1} total), a label-compliant schedule targeting early infection windows [17,20,21]. For the biological and botanical treatments (BA, LS, K, S, and M), when the labels provided a range, the mid-range rate was selected to avoid phytotoxicity while ensuring establishment/persistence (typical 7–28 d cycles to maintain propagule density or product presence in the rhizosphere). The treatments were applied by immersing the seedling root systems for 20 s before transplanting (BT), followed by a 50 mL drench at the stem base after transplanting according to the BT/DAT schedule in Table 1.

Three brand-specific formulations of *P. lilacinum* were included because growers in this region commonly rotate or compare these labels; benchmarking across brands was therefore of practical interest. “Technological packages” combined co-applied inputs as marketed by the respective companies, allowing evaluation of label-typical sequences rather than hypothetical mixtures.

2.4. Nematode Population Density

The population densities of *Nacobbus* sp. and *Meloidogyne* sp. were measured at four time points in the biofumigated whole plots—pre-biofumigation (baseline), post-biofumigation (−1 DAT), 60 DAT, and 120 DAT—and at three time points in the non-biofumigated treatment (−1 DAT, 60 DAT, and 120 DAT). The soil was cored to a depth of 25 cm (one core per location). Samples were collected as follows: pre-biofumigation (biofumigated WP only), 16 samples (4 per block); −1 DAT (both WPs), 32 samples (4 per block × 2 WPs); and 60 and 120 DAT (both WPs and all SPs), 104 samples per date (4 blocks × 2 WPs × 13 SPs). Nematodes were extracted from 200 g of soil per sample by the sieving–centrifugation method [22–24].

2.5. Nematode Identification, Gall Index, Root-Damage Percentage, and Tomato Yield

2.5.1. Nematode Identification and Root Assessments

Nacobbus sp. and *Meloidogyne* sp. were identified by morphological characteristics using keys produced by Manzanilla-López et al. [25] and Sasser & Carter [26], respectively. Gallings of tomato roots was rated on a 1-to-10 scale 60, 90, and 120 DAT [27]. At each assessment, one plant per subplot was completely destructively sampled and completely uprooted for root inspection (n = 104 plants per date; 13 SPs × 2 WPs × 4 blocks, totaling 312 plants across this study).

The root-damage percentage (*P*) was calculated from the gall index (GI) distribution as follows [28]:

$$P = \frac{\sum(n_i)(v_i)}{(N)(V)} \times 100$$

where *n_i* is the number of plants in the GI category (*i*), *v_i* is the category value, *N* is the total number of plants assessed, and *V* is the maximum GI value. In plain terms, *P* is the mean GI expressed as a percentage of the scale maximum.

2.5.2. Yield Assessment

Six harvests of ripe fruit were collected from each SP at 7-day intervals once maturity began. Each SP initially contained 10 plants (1040 plants in total across the trial). Because one plant per SP was destructively sampled to determine the GI 60, 90, and 120 DAT, the marketable yield was recorded based on the remaining plants within each SP. The yield was expressed as t ha^{-1} by scaling the sum of the marketable fruit weight to the effectively harvested SP area on each date; culls were excluded according to commercial standards.

2.6. Complementary Greenhouse Bioassay (Context Only)

In addition to the split-plot trial reported here, an independent greenhouse bioassay was conducted on tomato in 2018 at a different commercial site in the same region. Pre-plant biofumigation was implemented by incorporating broccoli residues and sheep manure and covering the soil with plastic mulch, followed by biological inputs with and without fluopyram. The resulting data are not statistically combinable with those from the present trial; this note is provided strictly as regional context and is not used for inference. Qualitative consistency is addressed in Section 4.

2.7. Statistical Analysis and Cumulative Indices

Data were analyzed with a split-plot ANOVA, with biofumigation (whole plot, A) and the post-plant treatments (subplots, B) as fixed effects and block as a random effect; the $A \times B$ interaction was tested. The linear model was

$$y_{ijk} = \mu + \beta_k + A_i + (A \times \beta)_{ik} + B_j + (A \times B)_{ij} + \epsilon_{ijk};$$

where μ is the overall mean; β_k is the random block effect ($k = 1, \dots, 4$); A_i is the whole-plot factor (two levels; $i = 1, 2$); B_j is the subplot factor (13 levels; $j = 1, \dots, 13$); $(A \times \beta)_{ik}$ is the whole-plot error term; and ϵ_{ijk} is the subplot residual. The whole-plot effect (A) was tested against $(A \times \beta)_{ik}$; B and $A \times B$ were tested against ϵ_{ijk} . Assumptions (normality and homoscedasticity) were verified using residuals. All tests were two-sided with $\alpha = 0.05$. When the factor levels were compared, the mean separations used Tukey's HSD. The results are reported as means $\pm$ SEs on the original scale. Analyses were performed in R v4.0.5 (macOS).

The area under the nematode population curve (AUNPC) and the area under the root-damage curve (AURDC) were determined for each SP by the trapezoidal method from 0 to 120 DAT [29], providing integrated measures of nematode dynamics and cumulative damage over time.

3. Results

3.1. Identification of *Nacobbus* and *Meloidogyne*

Adults and immature females were dissected from galls for identification. The tomato roots showed the "rosary" galling typical of *Nacobbus* (Figure 1A). The adult females had a spindle- to sac-shaped body with a short neck (Figure 1C), and the immature females presented a subterminal vulva ($\approx 95\%$); the males bore spicules and a peloderan bursa—characteristics consistent with *N. aberrans*. A smaller fraction of the galls exhibited root-knot symptoms (Figure 1B). The females from these galls were globose with an $\approx 15 \mu\text{m}$ stylet (Figure 1D) and a perineal pattern of smooth striae with a high, quadrate dorsal arch (Figure 1E); the males showed a large round labial disc and a smooth to weakly annulated cephalic region. The male stylet averaged $23 \mu\text{m}$. Spicules were present; bursae were absent. These observations are compatible with *M. incognita*.

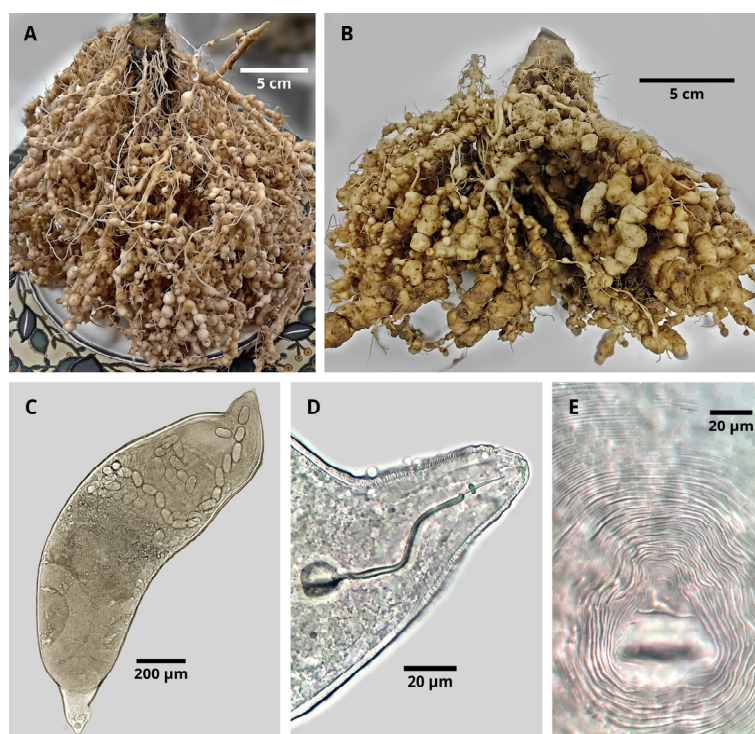


Figure 1. Symptoms and key characters of *Nacobbus aberrans* and *Meloidogyne incognita* in tomato. Rosary-type galling (*N. aberrans*) (A); root-knot galling (*M. incognita*) (B); adult female of *N. aberrans* (C); anterior region of *M. incognita* female with stylet (D); perineal pattern of *M. incognita* (E). Photo credits: (A) I. Cid del Prado-Vera; (B–E) authors.

3.2. Interaction Between Biofumigation and Management Product Treatments

For the area under the *N. aberrans* population curve (AUNPC), the combination of control + non-biofumigated had the highest average value, whereas *Tagetes erecta* + biofumigation had the lowest; however, the ANOVA did not detect differences among the WP × SP combinations. For the area under the root-damage curve (AURDC), the interaction was significant ($p = 0.000389$): under non-biofumigated conditions, the most damage occurred in the control, BA, S, and *T. erecta* treatments; under biofumigation, fluopyram, M, and *T. erecta* had the least damage. The fluopyram + biofumigation combination showed an ~28% lower AURDC than control + non-biofumigated (454 vs. 630 units; Table 2). For the marketable yield, no differences were detected among the WP × SP combinations, although fluopyram (with or without biofumigation) had the highest mean values (Table 2).

Table 2. Split-plot interaction (WP × SP): the effects of the biofumigation × post-plant management treatments on the area under the *Nacobbus aberrans* population curve (AUNPC), the area under the root-damage curve (AURDC), and the marketable tomato yield.

No.	Management Treatments	AUNPC (Units)		AURDC (Units)		Tomato Yield (t ha ^{−1})	
		B	NB	B	NB	B	NB
1	<i>P. lilacinum</i> (BA)	13,830 ^a	16,072 ^a	551 ± 26 ^{abc}	615 ± 6 ^a	32 ^a	30 ^a
2	Fluopyram	6375 ^a	27,690 ^a	454 ± 17 ^c	491 ± 25 ^{bc}	48 ^a	78 ^a
3	<i>T. erecta</i>	2325 ^a	12,015 ^a	536 ± 25 ^{abc}	607 ± 10 ^a	32 ^a	37 ^a
4	S	15,638 ^a	10,027 ^a	570 ± 6 ^{ab}	611 ± 17 ^a	34 ^a	37 ^a

Table 2. Cont.

No.	Management Treatments	AUNPC (Units)		AURDC (Units)		Tomato Yield (t ha ^{−1})	
		B	NB	B	NB	B	NB
5	<i>T. viride</i>	7725 ^a	13,192 ^a	559 ± 15 ^{abc}	607 ± 13 ^a	31 ^a	38 ^a
6	<i>LS</i>	25,342 ^a	37,942 ^a	566 ± 9 ^{ab}	600 ± 20 ^a	32 ^a	39 ^a
7	<i>M</i>	20,715 ^a	21,007 ^a	532 ± 16 ^{abc}	589 ± 25 ^{ab}	37 ^a	22 ^a
8	<i>K</i>	12,600 ^a	20,032 ^a	566 ± 17 ^{ab}	596 ± 9 ^a	38 ^a	35 ^a
9	<i>P. chlamydosporia</i>	9150 ^a	10,155 ^a	562 ± 23 ^{abc}	592 ± 52 ^{ab}	45 ^a	24 ^a
10	Technological package 1	4957 ^a	15,135 ^a	562 ± 13 ^{ab}	577 ± 14 ^{ab}	33 ^a	27 ^a
11	Technological package 2	20,280 ^a	4867 ^a	540 ± 29 ^{abc}	592 ± 10 ^a	36 ^a	31 ^a
12	Technological package 3	8107 ^a	23,055 ^a	574 ± 15 ^{ab}	577 ± 29 ^{ab}	35 ^a	46 ^a
13	Control	14,400 ^a	77,430 ^a	585 ± 30 ^{ab}	630 ± 0 ^a	19 ^a	32 ^a

Product abbreviations as in Table 1. B = biofumigated; NB = non-biofumigated. Means (original scale). Different lowercase letters within a column indicate Tukey's HSD groupings at $\alpha = 0.05$ (tested within B and within NB separately); values sharing a letter do not differ. Interaction (biofumigation × post-plant treatment, WP × SP) *p* values: AURDC, 0.000389; AUNPC, n.s.; yield, n.s.

3.3. Effects of Biofumigation on Nematode Population, Cumulative Root Damage, and Yield

Nematode populations were sampled on four dates, a pre-biofumigation baseline and −1, 60, and 120 DAT, yielding a total of 256 observations. Time series were summarized as the AUNPC for *N. aberrans*, and the cumulative root damage was integrated as the AURDC. Analyses followed the split-plot ANOVA described in Section 2.7.

Across the dates, *M. incognita* remained at a very low density (J2 only when detected), whereas the *N. aberrans* populations were dominated by J2. By 120 DAT, the *N. aberrans* populations were ~9.8% lower in the biofumigated plots than in the non-biofumigated plots, consistent with the overall trend (Table 3).

Table 3. The population densities of *Nacobbus aberrans* and *Meloidogyne incognita* at the pre-biofumigation baseline and −1, 60, and 120 days after transplanting in the biofumigated and non-biofumigated plots.

Nematode	Pre-Biofumigation (B Only)	−1 DAT		60 DAT		120 DAT	
		B	NB	B	NB	B	NB
<i>N. aberrans</i>	3166	435	3627	1869	7330	16,218	17,984
<i>M. incognita</i>	0	0	0	35	0	105	135

B = biofumigated; NB = non-biofumigated; DAT = days after transplanting. Values are counts per 200 g of soil.

Biofumigation significantly reduced the AUNPC for *N. aberrans* (12,472 vs. 20,402 units; −39%; $p = 0.00315$) and the AURDC (548 vs. 590 units; $p = 0.0228$) relative to the non-biofumigated whole plots; the marketable yields did not differ (35 vs. 37 t ha^{−1}) (Table 4).

Table 4. Main effects of pre-plant biofumigation (whole-plot factor) on AUNPC (*Nacobbus aberrans*), AURDC, and marketable tomato yield.

Whole-Plot Treatments	AUNPC (Units)	AURDC (Units)	Tomato Yield (t ha ^{−1})
Biofumigation	12,472 ± 1881 ^b	548 ± 6.39 ^b	35 ^a
Non-biofumigated	20,402 ± 2256 ^a	590 ± 9.11 ^a	37 ^a

AUNPC: the area under the *N. aberrans* population curve; AURDC: the area under the root-damage curve. Means ± SEs (original scale). Different lowercase letters within a column indicate Tukey's HSD groupings at $\alpha = 0.05$ for the main effect of biofumigation; values sharing a letter do not differ. Model *p* values for the main effect of biofumigation: AUNPC, 0.00315; AURDC, 0.0228; Yield, n.s. Note: For the AURDC, the whole-plot × subplot interaction was significant in the full model (see Table 2); the values here are whole-plot means averaged across the subplot levels.

3.4. Effects of Management Product Treatments on *Nacobbus aberrans* Population, Cumulative Root Damage, and Yield

Across the subplot treatments (means across the whole-plot levels), the AUNPC did not differ among the programs ($B, p > 0.05$), although the lowest means occurred with *T. erecta* and *P. chlamydosporia* and the highest occurred in the untreated control (Table 5). For the AURDC, the subplot factor was significant ($B, p < 0.001$): fluopyram showed the least cumulative damage (472 units), $\approx 22\%$ below the control (607 units; Tukey's HSD, $p = 4.06 \times 10^{-7}$), while the remaining treatments grouped with the control (Table 5). The yields also differed among the programs: fluopyram achieved the highest marketable yield (63 t ha^{-1}), exceeding the control (26 t ha^{-1} ; $+37 \text{ t ha}^{-1}$; $+142\%$) ($p = 0.000341$), whereas no other treatment increased the yield relative to the control (Table 5). These patterns are consistent with the $WP \times SP$ interaction analysis: only the AURDC exhibited a significant interaction, whereas the AUNPC and yield did not (Table 2).

Table 5. Main effects of post-plant management treatments (subplot factor) on AUNPC (*N. aberrans*), AURDC, and marketable tomato yield.

No.	Management Treatments	AUNPC (Units)	AURDC (Units)	Tomato Yield (t ha^{-1})
1	BA	14,951 ^a	583 ± 17.3^a	31 ± 4.8^b
2	Fluopyram	17,032 ^a	472 ± 15.5^b	63 ± 7.0^a
3	<i>T. erecta</i>	7170 ^a	572 ± 18.2^a	34 ± 7.7^b
4	S	12,832 ^a	591 ± 11.3^a	36 ± 6.4^b
5	<i>T. viride</i>	10,459 ^a	583 ± 13.1^a	34 ± 6.7^b
6	LS	31,642 ^a	583 ± 12.1^a	36 ± 5.5^b
7	M	20,861 ^a	560 ± 17.2^a	30 ± 7.0^b
8	K	16,316 ^a	581 ± 10.5^a	37 ± 5.3^b
9	<i>P. chlamydosporia</i>	9652 ^a	577 ± 24.8^a	34 ± 6.6^b
10	Technological package 1	10,046 ^a	570 ± 9.4^a	30 ± 5.6^b
11	Technological package 2	12,574 ^a	566 ± 17.4^a	33 ± 5.3^b
12	Technological package 3	15,581 ^a	575 ± 15.2^a	40 ± 11.4^b
13	Control	45,915 ^a	607 ± 17.8^a	26 ± 5.9^b

Abbreviations as in Table 1. Means $\pm$ SEs (original scale); values are averaged across whole-plot levels. Different lowercase letters within a column indicate Tukey's HSD groupings at $\alpha = 0.05$ for the subplot factor (post-plant treatment); values sharing a letter do not differ. Model p values: AUNPC = n.s.; AURDC < 0.001 ; yield = 0.000341.

4. Discussion

The morphological diagnosis confirmed *Nacobbus aberrans* as the dominant species, with *Meloidogyne incognita* at a very low density; this baseline frames the interpretation of the biofumigation and subplot responses presented below.

4.1. Effect of Pre-Plant Biofumigation on Nematode Population, Root Damage, and Yield

Pre-plant biofumigation with sorghum residues plus fresh chicken and sheep manure reduced *N. aberrans* populations by 86.3% before transplanting. By 120 DAT, the populations in the biofumigated plots were 9.8% lower than in the non-biofumigated plots, and the time-integrated population index (the AUNPC) was 39% lower. Galling and cumulative root damage (the AURDC) were consistently lower under biofumigation. Sorghum residues can release hydrogen cyanide via hydrolysis of dhurrin, a cyanogenic glycoside, which contributes to suppression of soil-borne pests [30]. Prior work shows that biofumigation with crucifer or sorghum residues, often supplemented with organic amendments, reduces *Meloidogyne* populations and galling in solanaceous crops; when integrated with *Pochonia chlamydosporia*, additional suppression has been reported in greenhouse tomato, even during mixed *N. aberrans*–*M. incognita* infestations [6,18,19,30,31].

Despite lower populations and damage, the marketable yields did not differ between the whole-plot levels. Biofumigation performance is known to depend on soil properties, residue chemistry and dose, and deployment intensity [19]. Studies recommending higher loads and/or repeated cycles—e.g., 80 t ha^{−1} of sorghum (*S. bicolor*) + 40 t ha^{−1} of chicken manure—have achieved stronger suppression of *Meloidogyne* spp. [6]. Here, a single cycle totaling 50 t ha^{−1} (14 t of sorghum; 18 t of chicken manure; and 18 t of sheep manure incorporated to 30 cm) was used, which may explain the decoupling between the reduced damage and the unchanged yield in this setting.

4.2. Post-Plant Management: Main and Interaction Effects

Among the subplot programs, fluopyram produced the smallest AURDC and increased the marketable yield by 142% relative to the untreated control (63 vs. 26 t ha^{−1}), whereas the AUNPC of *N. aberrans* was not reduced. This absence of an AUNPC effect for *N. aberrans* contrasts with reports of large decreases in *M. incognita* populations and galling under fluopyram [17,20]. Given the naturally mixed infestation dominated by *N. aberrans*, the yield gains under fluopyram likely reflect protection of early infection sites and/or activity against low-density *M. incognita* rather than measurable changes in *N. aberrans* population dynamics. Consistent with recent field studies in processing/greenhouse tomato, fluorinated nematicides (including fluopyram) can reduce galling and improve yields, even when population indices decline modestly or remain stable, likely by protecting fine roots and early infection sites [4,21].

The split-plot analysis indicated a significant interaction for the AURDC but not for the AUNPC or the yield. Thus, the cumulative damage responses varied with biofumigation status, while the population data over time and yields were largely additive across factors.

The biological and botanical inputs, applied alone or bundled as integrated technological packages, showed non-significant tendencies in some comparisons but did not increase the yield under these soil and pressure conditions. This outcome accords with the context-dependence documented for bio-disinfestation and biocontrol, which are driven by soil properties, amendment regimes, and microbiome interactions [19,32]. These observations are consistent with prior work showing that organic amendments and biofumigation/biosolarization, as well as antagonistic microbes (bacteria and fungi), can reduce *Meloidogyne* damage but yield responses are variable and strongly site-dependent [6,31,33,34].

4.3. Species Composition, Anhydrobiosis, and Putative Soil Suppressiveness

Throughout this study, *M. incognita* remained at a very low density, while *N. aberrans* dominated symptom expression (prevalent “rosary” galls). Two non-exclusive mechanisms may explain this pattern. First, life-history traits of *N. aberrans* (including survival of late juveniles/adults under desiccation via anhydrobiosis) can favor early infection after rewetting, enabling rapid establishment ahead of *M. incognita* [35,36]. Second, this site may exhibit partial soil suppression of *M. incognita* mediated by the resident microbiome. Disease-suppressive soils are well documented in root-knot systems [37], and rhizosphere microbiota can cooperatively suppress plant-parasitic nematodes [32]. Classical antagonists, such as *Pasteuria* spp., can also reduce *Meloidogyne* performance under specific conditions [38]. In this investigation, biological inputs (*Pochonia*, *Purpureocillium*, and *Trichoderma*) reduced damage indices in some combinations but did not translate into yield gains under the observed pressure and schedule.

4.4. Practical Implications, Limitations, and Qualitative Consistency Across Trials

In this study, inferences are limited to one production cycle at a single commercial site; multi-season, multi-site validation is recommended. A single pre-plant biofumigation cycle

lowered the baseline *N. aberrans* pressure, and a split fluopyram program (at transplanting and 15 DAT) minimized cumulative root damage and improved the marketable yield. In contrast, multi-input biological/botanical packages reduced damage indices in some cases but did not translate into yield gains under the observed pressure and application schedule.

Although the marketable yields did not differ between the biofumigated and non-biofumigated whole plots, biofumigation reduced the baseline *N. aberrans* population and lowered the cumulative damage (the AURDC), indicating a suppressive effect on epidemic build-up. In high-value greenhouse crops, such pre-plant suppression can stabilize risk and enhance the effects of low-risk nematicides within integrated programs, even when single-season yield responses are neutral.

Two caveats warrant caution when generalizing these findings: first, the evaluation was conducted in a single season at one commercial site; second, the total amendment load (50 t ha⁻¹) may be below the thresholds reported elsewhere for stronger, yield-detectable suppression. Additionally, destructive GI subsampling reduced the plant number by up to three plants per subplot by 120 DAT; because the removals were uniform across the treatments and dates, and the yields were standardized to the effective harvested area, bias was minimized in the treatment comparisons. Notwithstanding these constraints, qualitative consistency was observed in an independent 2018 greenhouse bioassay conducted in the same region, which likewise showed biofumigation-driven reductions in *N. aberrans* and fluopyram-linked decreases in cumulative root damage, with very low *M. incognita* populations. Although the datasets are not statistically combinable, the direction of the effects aligns across the trials.

5. Conclusions

Under naturally infested commercial greenhouse conditions, sorghum-based pre-plant biofumigation suppressed the baseline *Nacobbus aberrans* pressure and reduced cumulative root damage (area under the root-damage curve, AURDC) but did not change the overall yield. Among the post-plant options, a label-compliant split fluopyram program carried out at transplanting consistently translated damage mitigation into a higher marketable yield, whereas the biological/botanical inputs—alone or bundled—sometimes lowered damage indices but did not increase the yield. Taken together, the evidence supports embedding pre-plant biofumigation within integrated nematode-management programs. When warranted early in the season, a targeted, low-risk nematicide can be included at transplanting. Further work should validate these findings across seasons and sites and directly quantify root colonization by *Purpureocillium lilacinum*, *Pochonia chlamydosporia*, and *Trichoderma* spp., together with the egg parasitism rates of *N. aberrans* and *Meloidogyne incognita*, linking these mechanistic endpoints to the AURDC and yield under commercial fertigation.

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Abbreviations

The following abbreviations are used in this manuscript:

AUNPC	Area under the nematode population curve
AURDC	Area under the root-damage curve
DAT	Days after transplanting
BT	Before transplanting
GI	Gall index
WP	Whole plot
SP	Subplot
J2	Second-stage juvenile
B	Biofumigated (whole plots)
NB	Non-biofumigated (whole plots)
BA	BioAct [®] (<i>P. lilacinum</i> , proprietary strain)
LS	Lila-Sin [®] (<i>P. lilacinum</i> , proprietary strain)
K	Kastelo [®] (<i>P. lilacinum</i> , proprietary strain)
S	Sterminar [®] (plant oil blend)
M	Majesty [®] (plant oil blend)

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