Efficacy of Plant Extracts, Biological Control Agents, and Nematicide on the Management of Plant-Parasitic Nematodes in Beefsteak Tomato (*Solanum lycopersicum*) under Greenhouse Conditions

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Abstract

Plant-parasitic nematodes are among the major constraints affecting the production of beefsteak tomato (*Solanum lycopersicum* L.). Traditionally, their management has depended on chemical nematicides: however, their effectiveness has progressively declined due to the development of resistance and increasing environmental concerns. This has driven the search for sustainable alternatives. The objective of this study was to evaluate the efficacy of plant extracts and biological agents for the management of nematodes in beefsteak tomato under greenhouse conditions. The experiment was confirmed using a randomized complete block design (RCBD) with six treatments three plant extracts: garlic {*Allium sativum*}, marigold {*Tagetes* spp.}, and neem {*Azadirachta indica*}; two biological agents {*Trichoderma* spp. and *Bacillus* spp.}; and one commercial chemical nematicide {Solvigo®}. Each treatment was used replicated four times. Parameters assessed included incidence, severity, nematode population in roots, root biomass, and treatment efficacy were evaluated. Ninety days after application, the *A. sativum* extract significantly reduced incidence (26.50%), severity (30%), and nematode population (159 individuals/10 g root), achieving an efficacy of 68.57%, which surpassed that of the chemical nematicide (65.28%). Furthermore, it promoted greater root development (24.45 g). In contrast, *Tagetes* spp. and neem extracts, as well as *Trichoderma* spp. and *Bacillus* spp. treatments, exhibited low to moderate efficacy. These results highlight *A. sativum* extract as a promising and eco-friendly alternative for sustainable nematode management in protected tomato production systems.

Keywords: Biological control, efficacy, *Solanum lycopersicum*, Plant-parasitic nematodes.

Introduction

Beefsteak tomato (*Solanum lycopersicum* L.) is one of the most widely cultivated and consumed vegetables worldwide, produced intensively year-round, mainly under greenhouse conditions **(López, 2017)**. In Ecuador, tomato cultivation has expanded significantly **(Castro, 2022)**, particularly in the provinces of Tungurahua, Imbabura, Pichincha, Azuay, and Cotopaxi, with Cotopaxi being the leading production area. Currently, 1,399 ha are cultivated, with an estimated yield of 40,003 t **(INEC, 2023)**.

Plant-parasitic nematodes are among the most important global threats to agriculture, causing substantial damage to various crops **(Jones et al., 2013)**. These microscopic organisms infect plant roots, impairing water and nutrient uptake, which results in reduced growth and yield **(Rodiuc et al., 2014**). Foliar symptoms include wilting, chlorosis, flower abortion, and reduction in fruit size and number **(Youssef Banora, 2024)**. In severe cases, infections can lead to plant death **(Gusqui Vilema et al., 2014)**. Moreover, nematode infestations increase plant susceptibility to both biotic and abiotic stress factors, such as drought, nutrient deficiencies, and secondary diseases, thereby exacerbating economic losses (Jones et al., 2013). Globally, yield losses may reach up to 85% (Surendar et al., 2020), while in Ecuador reductions of up to 47% have been reported by **Vivanco (2014)**.

In response to this problem, producers have mainly relied on chemical nematicides **(Bakr et al., 2015; Mavuze et al., 2021**), many of which belong to toxicological categories I and II (e.g., methyl bromide, carbofuran, 1,3-dichloropropene). However, increasing environmental regulations, residual toxicity, and the emergence of resistant nematode populations have restricted their use **(Moens et al., 2009; Mavuze et al., 2021**). Consequently, alternative strategies have been proposed, including plant extracts, essential oils, and antagonistic microorganisms **(Kouamé, 2021)**, which not only provide nematicidal effects but also contribute to soil fertility conservation (Suárez Díaz, 2015). Although preliminary studies have shown promising results, rigorous evaluations are needed to determine their efficacy, technical feasibility, and economic viability before being adopted in commercial production systems **(Gusqui Vilema et al., 2014)**.

Therefore, the aim of this study was to evaluate the effect of different biological control agents, plant extracts, and nematicides on the management of plant-parasitic nematodes in beefsteak tomato under greenhouse conditions, the study further sought to identify effective and sustainable alternatives that reduce dependency on synthetic nematicides and promote environmentally responsible agricultural practices. Based on this objective, it is hypothesized that plant extracts and biological control agents exhibit differential efficacy in nematode suppression.

Materials and Methods

Experimental location

The study was conducted in a greenhouse located in the Quilajalo community, Salcedo canton, Cotopaxi province, Ecuador (01°01′32″ S, 78°36′59″ W; 2638 m.a.s.l.). The area has a mean annual temperature of 14 °C, an average relative humidity of 84%, and an annual precipitation of 187 mm **(PDOT Cantón Salcedo, 2023)**.

Plant material and experimental design

Beefsteak tomato (*Solanum lycopersicum* L.), hybrid Pietro grafted onto the Enpower rootstock, approximately 30 days old at the start of the trial and showing visible symptoms of nematode infestation, was used. The experiment was established on 24 experimental beds of 17.5 m² each (35 m × 0.5 m), each experimental bed had 70 beefsteak tomato plants. A randomized complete block design (RCBD) was employed with six treatments and four replications per treatment.

Treatments

The evaluated treatments included three plant extracts, two biological agents and one commercial chemical nematicide. Applications were performed every 30 days during the experimental period (Table 1).

The treatments whit plant extracts corresponded to the commercial formulation Vigga® (*Allium sativum*, 80.0% p/v), Microcat GOLD® (*Tagetes* spp., 80.0 p/p) and Neem-X® (*Azadirachta indica*, 4 g/L); each product contained standardized concentration as indicated by the manufacturer, and the recommended doses were adjusted to the experimental design. Likewise, the biological control agents consisted of commercial formulations Tricho Vit® (*Trichoderma* spp., 7.0 x 10^8 CFU g^{-1}) and BrugNem® (*Bacillus* spp., 7.0 x 10^8 CFU mL^{-1}), whose microbial identity and concentration were guaranteed by the manufacturer. The chemical control corresponded to Solvigo® (abamectin 36 g L^{-1} and thiamethoxam 72 g L^{-1}), also applied according to the manufacturer's doses.

Table 1. Treatments evaluated in the greenhouse nematode management trial.

Treatment	Description	Dose (per liter)	Concentration
T1	*Allium sativum* extract	0.5 mL L^{-1}	80.0 p/v
T2	*Tagetes* spp. extract	2 mL L^{-1}	80.0 p/p
T3	*Azadirachta indica*	0.63 mL L^{-1}	4 g L^{-1}
T4	*Trichoderma spp.*	0.7 g L^{-1}	7.0 x 10^8 CFU g^{-1}
T5	*Bacillus* spp.	1.25 mL L^{-1}	7.0 x 10^8 CFU mL^{-1}
T6	Commercial chemical control (abamectin + thiamethoxam)	0.45 mL L^{-1}	Abamectina = 36 g L^{-1} Thiamethoxan = 72 g L^{-1}

Evaluated variables

Identification and quantification of nematode genera

Root samples were collected prior to the first application (day 0) and 90 days after application (90 DAA). Nematode extraction was performed in the laboratory using the Baermann funnel technique combined with centrifugal flotation. Extracted nematodes were identified to genus level using optical microscopy and standard taxonomic keys **(Taipe Lema, 2018)**.

Incidence of nematode infection

Incidence was determined as the percentage of plants with visible infection symptoms (galls, root swellings) relative to the total number of plants evaluated per experimental unit, both at the initial sampling and at 90 DAA **(Chiliquinga, 2015)**. Incidence was calculated as:

$$\text{Incidence (\%)} = \frac{n}{N} \times 100$$

where n is the number of infected plants and N is the total number of plants evaluated (Llerena & Llerena, 2010).

Nematode population in roots

Ten grams of fresh roots were collected per experimental unit at each sampling date. Samples were processed in the laboratory and nematodes were quantified and expressed as individuals per 10 g of root **(Gusqui Vilema et al., 2014)**.

Severity of root damage

Severity was assessed according to Miller's scale as cited by **Llerena & Llerena** (2010) shown in Table (2), which is based on nematode density per 10 g of root. The severity index was calculated following **Taylor & Sasser (1978)**:

$$\text{Severity (\%)} = \frac{\sum(n_i \times g_i)}{N \times G_{\max}} \times 100$$

where n_i is the number of plants in grade i, g_i is the grade value, N is the total number of plants, and $G_{\max}$ is the maximum grade value (4).

Table 2. Miller severity scale for nematode damage as cited by Llerena & Llerena, (2010).

Grade	Nematodes / 10 g root	Severity index	Rating
0	0	0%	Free
1	1 - 150	1 - 25%	Low
2	151 - 300	26 - 50%	Moderate
3	300 - 600	51 - 75%	High
4	> 600	76 - 100%	Very high

Root fresh weight

Root fresh weight (g) of three plant per experimental unit was recorded at day 0 and at 90 DAA using a precision balance (±0.01 g).

Treatment efficacy

Efficacy was calculated according to **Abbott (1925):**

$$\text{Efficacy (\%)} = \frac{(C - T)}{C} \times 100$$

where C is the initial nematode population (control or initial average) and T is the final population in the treatment. Because an absolute untreated control was not included, the initial mean population was used as reference, following adapted methodologies from similar studies **(Gusqui Vilema et al., 2014)**.

Sampling procedure and nematological analysis

Observations and sampling were conducted at day 0 and 90 DAA (after three applications). A systematic sampling approach was used: one plant per experimental unit was sampled at each date. Roots were placed in labeled polyethylene bags (25.4 × 40.6 cm) and transported to the laboratory under refrigerated conditions (4 °C) for immediate processing. Nematodes were extracted using the Baermann funnel method and centrifugal flotation.

Statistical analysis

Residual normality was assessed with the Shapiro–Wilk test and homogeneity of variances with Levene's test. Subsequently, analysis of variance (ANOVA) was performed for each variable. Means were compared using Tukey's HSD test ($\alpha = 0.05$). All analyses and graphics were produced in R statistical software (version 4.5.1; R Core Team, 2025).

Results and Discussion

Nematode genera identified

Nematological analysis identified multiple genera of plant-parasitic nematodes in tomato roots, including *Criconemella* (12.50%), *Helicotylenchus* (91.67%), *Criconemoides* (8.33%), *Meloidogyne* (100%) and *Pratylenchus* (91.67%), as well as saprophytic nematodes (12.50%) *Meloidogyne* was the predominant genus, indicating that root-knot nematode were the main contributors to the observed root damage in greenhouse-grown beefsteak tomato.

These findings were consistent with previous regional reports. **Taipe Lema (2018)** identified eight genera associated with beefsteak tomato in Cotopaxi, with *Meloidogyne*, *Criconemoides* and *Telotylenchus* among the most frequent. **Curay et al. (2021)** also reported *Meloidogyne*, *Criconemoides*, *Xiphinema*, *Tylenchorhynchus* and *Trichodorus* in tomato production systems, noting a population dynamic dependent on crop phenology (early dominance of *Xiphinema* and *Meloidogyne*, and increased *Criconemoides*, *Tylenchorhynchus* and *Trichodorus* at later stages).

Incidence of nematode infection

At the start of the trial (day 0), incidence was 100% across all treatments, with no significant differences ($p > 0.05$), confirming uniform initial infestation throughout the experimental area. At 90 DAA, significant differences among treatments were observed ($p < 0.05$). The *Allium sativum* extract (T1) reduced incidence to 26.50%, followed by the chemical nematicide (T6) at 30.75%. Conversely, the *Trichoderma* spp. treatment (T4) exhibited the highest incidence (49.50%) (Figure 1A). These results indicate that both garlic extract and the chemical product exerted a significant suppressive effect on visible nematode infection.

These observations partially contrasted with **Kouamé (2021)**, who reported 50% of plants exhibiting severe symptoms, a pattern attributed to cultivar susceptibility. In the present study a grafted hybrid purportedly resistant to nematodes was used, suggesting that inoculum pressure was high enough to partially overcome rootstock resistance. The preference of *Meloidogyne* spp. for solanaceous hosts **(Mokrini et al., 2016**) likely contributed to the high incidence observed in the study area.

Nematode population in roots

Initial root nematode populations ranged from 507 to 558 individuals per 10 g of root, with no significant differences between treatments ($p > 0.05$). At 90 DAA, highly significant differences were detected ($p < 0.05$). The *A. sativum* extract reduced population to 159 individuals/10 g root, followed by the chemical control with 176 individuals/10 g root. In contrast, *Tagetes* spp. (T2) and *Trichoderma* spp. (T4) treatments presented the highest counts (283 and 272 individuals/10 g root, respectively) (Figure 1B). These data confirmed that garlic extract had nematicidal activity comparable to, and in this case superior to, the evaluated chemical control.

These results supported the hypothesis that appropriately timed application of plant extracts could maintain nematode populations below economic thresholds **(Bárcenas-Huazano, 2025**). However, efficacy may have been modulated by factors such as crop age, since nematode density typically increased as phenology advanced **(Inés Vásquez, 2016)**.

Severity of nematode damage

At the initial evaluation, all treatments registered a mean severity of 60% (rating “high”), with no significant differences ($p > 0.05$). At 90 DAA, *A. sativum* extract reduced severity to 30% (rated “moderate”), followed by the chemical nematicide at 35%. Other treatments remained around 40% severity (Figure 1C), indicating limited capacity to mitigate root damage. These findings agreed with **Llerena & Llerena (2010)**, who reported that effective treatments reduced severity and preserved root architecture.

Root fresh weight

No significant differences in root weight were detected at the start of the trial (means ranged from 11.43 to 11.58 g; $p > 0.05$), confirming uniformity of the plant material. At 90 DAA, the *A. sativum* extract induced the greatest root development (24.45 g), followed by *Trichoderma* spp. (23.20 g), while the chemical control recorded the lowest root weight (19.50 g) (Figure 1D). This suggests that, in addition to nematicidal effects, garlic extract may have exerted a bio-stimulant effect that favors root regeneration and proliferation.

A dual effect (nematicidal + biostimulant) had been documented previously: **Abo-Elyousr (2010)** reported that garlic extract not only reduced *Meloidogyne incognita* populations but also stimulated root growth, a response attributed to allicin, an organosulfur compound with antimicrobial and phytostimulatory properties. Similarly, **Hernández-Ochandía et al. (2024**) observed that interaction between *Trichoderma asperellum* and nematodes increased root weight (6.17–15.30 g), corroborating the potential of certain microorganisms as root development promoters.

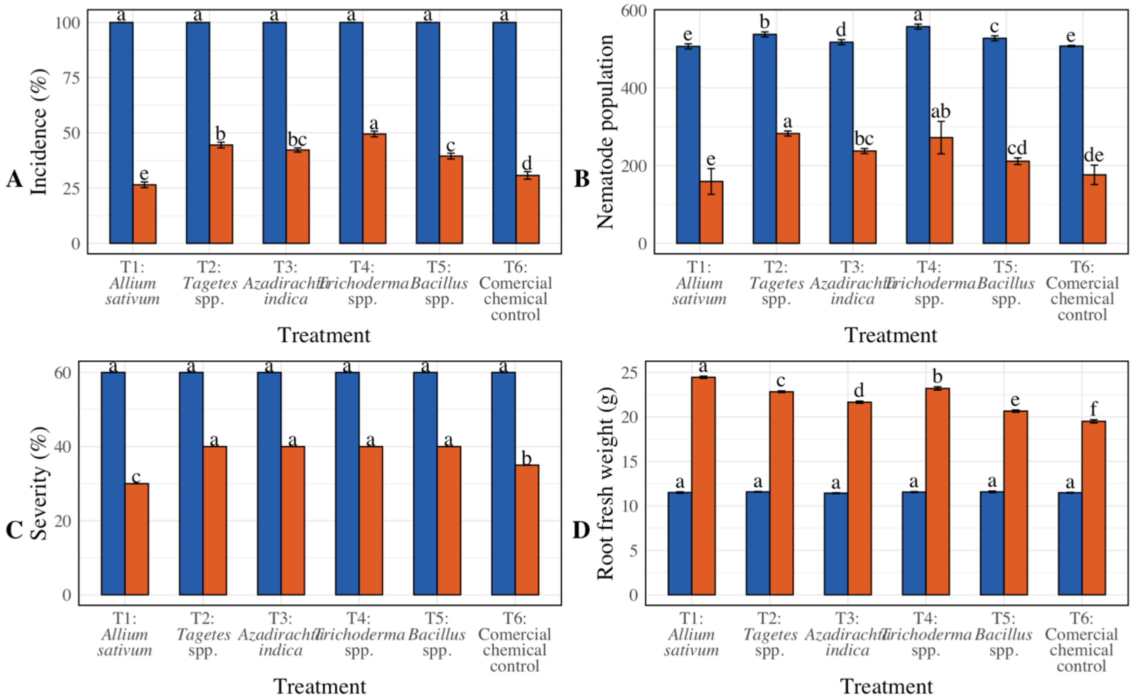

Figure 1. Effect of treatments on evaluated variables: (A) Incidence of nematode infection (%), (B) Nematode population in roots (nematodes/10g of root), (C) Severity of nematode attack (%), and (D) Root fresh weight (g). Different letters above bars indicate statistically significant differences among treatments (Tukey's HSD, $p < 0.05$). Error bars represent the standard deviation (SD). Blue bars correspond to samples taken before treatment application and orange bars correspond to samples taken 90 days after application.

Efficacy of the nematicides

Efficacy differed significantly among treatments ($p < 0.05$). *A. sativum* extract achieved the highest efficacy (68.57%), slightly surpassing the chemical control (65.28%). Treatments with *Bacillus* spp. (T5), neem (T3) and *Trichoderma* spp. (T4) showed intermediate efficacies, while *Tagetes* spp. extract (T2) exhibited the lowest efficacy (Figure 2). These results positioned garlic extract as a viable and potentially superior alternative to conventional synthetic nematicides under the conditions evaluated. The efficacy demonstrated by *A. sativum* shows us that a more effective reduction in nematode pressure limits damage to the roots, allowing the plant to recover and allocate resources to the formation of new tissues. Therefore, the increase in root weight is consistent with the action of the nematicide.

Comparable studies supported these findings: **Martinotti et al. (2013)** reported up to 94.70% control of nematodes with garlic extract in vitro; **Jaramillo (2019)** observed mortalities above 77% with essential oils; **Abuslin & Vaca (2017)** reported 84–100% reductions using *Tagetes patula* and entomopathogenic fungi. Variability in efficacy may have been due to differences in formulation, concentration, application method, or edaphoclimatic conditions. Nevertheless, plant extracts provided additional advantages, such as biodegradability, low toxicity and reduced risk of resistance development **(Avila, 2022)**, making them strategic options for sustainable cropping systems.

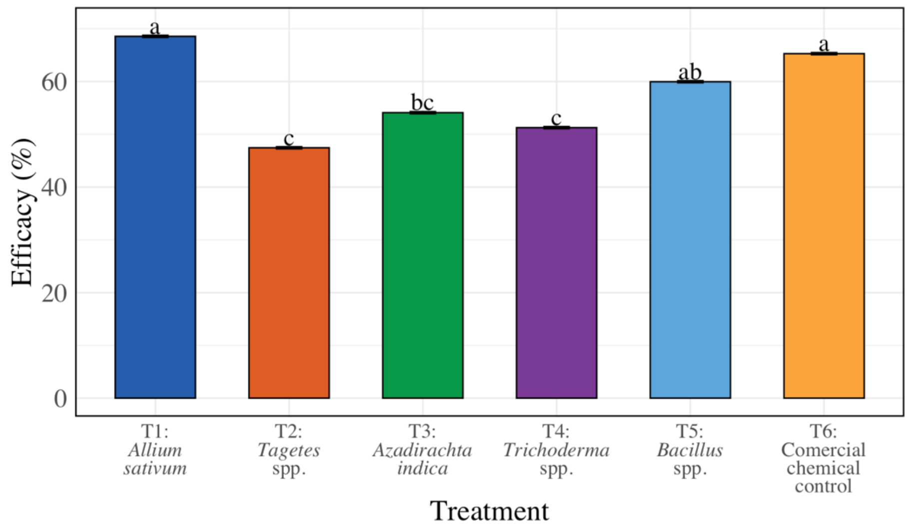

Figure 2. Efficacy of the tested nematicides. Different letters denote statistically significant differences (Tukey's HSD, $p < 0.05$).

Conclusion

Nematological analysis confirmed the presence of multiple plant-parasitic genera in greenhouse-grown beefsteak tomato in Cotopaxi province, with *Meloidogyne* as the predominant genus. The high incidence observed is associated with gall formation, which compromises vegetative development and physiological efficiency of the plants.

The *Allium sativum* extract was the most effective management strategy, producing a significant reduction in incidence (26.50%), severity (30%) and root nematode population (159 individuals/10 g root), with an efficacy of 68.57%, outperforming the commercial chemical nematicide evaluated. Additionally, it induced a significant biostimulant effect, increasing root fresh weight (24.45 g), which suggests a dual benefit: phytosanitary control and improved vegetative growth. This effect is likely attributable to bioactive compounds such as allicin, known for nematicidal and phytostimulatory properties.

In contrast, *Tagetes* spp. and neem extracts, as well as biological agents *Trichoderma* spp. and *Bacillus* spp., exhibited low to intermediate efficacy, indicating limited capacity to suppress nematode populations under high inoculum pressure. These findings underscore the importance of selecting alternatives based on demonstrated field efficacy rather than origin alone.

Overall, *Allium sativum* extract emerges as a viable, effective and environmentally sustainable alternative for integrated management of plant-parasitic nematodes in protected tomato production systems. Its implementation could contribute to reduced reliance on synthetic nematicides, promote more responsible agricultural practices and enhance crop resilience to soil-borne pests.

Although the results under greenhouse conditions are promising, one limitation of the study is the direct extrapolation of these effects to open field conditions, where environmental factors such as temperature, soil moisture, and microbiological composition are more variable. Therefore, it is strongly recommended that future research focus on validating the efficacy of Allium sativum extract under large-scale production scenarios and in different soil types.

Validation in various cultivars and regions is recommended to confirm the generalization of these findings.

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