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Efficacy of Chemical and Biological Nematicides against Root Knot and Sting Nematodes in Watermelon and Tomato

Presented by

Marián Nohemí Méndez Sosa

Advisors

Carolina Avellaneda, Ph. D.

Johan Desaeger, Ph. D.

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Authorities

SERGIO ANDRÉS RODRÍGUEZ ROYO

President

ANA M. MAIER ACOSTA

Vice President and Academic Dean

CELIA O. TREJO RAMOS

Director of Agricultural Science and Production Department

ANA M. MAIER ACOSTA

Secretary General a.i.

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Abstract

In agricultural ecosystems, plant-parasitic nematodes are significant pests that affect crop health and productivity. This study evaluated the efficacy of various nematicides, both biological and chemical, against root-knot nematodes (*Meloidogyne* spp.) (RKN) and sting nematodes (*Belonolaimus longicaudatus*) (SN) in Honduras and the State of Florida in the United States. Root-knot nematode experiments were conducted on watermelon (*Citrullus lanatus*) and tomato (*Solanum lycopersicum*), while the SN experiment was conducted on tomato. In Honduras, treatments included biological nematicides (i.e., *Paecilomyces lilacinus*, *Trichoderma harzianum*) and chemical nematicides (i.e., fluensulfone, oxamyl, fluopyram), and in Florida, biological nematicides (i.e., *Purpureocillium lilacinum strain PL11, Bacillus amyloliquefaciens strain D747, vegetable extracts (cinnamon, clove and thyme oil)*) and chemical nematicides (i.e., fluopyram). The variables measured were fresh and dry shoot weight, height, root gall ratings, number of eggs in roots, and second-stage juveniles in soil for RKN, and number of nematodes in soil and rhizosphere for SN. Results from the Honduras trial showed that fluensulfone and oxamyl were the most effective treatments ($P < 0.05$), significantly reducing the RKN population. In the Florida trial, fluopyram showed the highest efficacy ($P < 0.05$) in reducing RKN. No significant reduction in SN was noted for any of the treatments, but the experiment confirmed that tomato is a good host for SN.

Keywords: Biological control, chemical control, Fluensulfone, Fluopyram, greenhouse, Oxamyl, root-knot nematodes, sting nematodes.

Resumen

En los ecosistemas agrícolas, los nematodos fitoparásitos son plagas significativas que afectan la salud y la productividad de los cultivos. Este estudio evaluó la eficacia de varios nematicidas, tanto biológicos como químicos, contra los nematodos del nudo de la raíz (*Meloidogyne* spp.) (RKN) y los nematodos picadores (*Belonolaimus longicaudatus*) (SN) en Honduras y el estado de Florida en los Estados Unidos. Los experimentos con RKN se realizaron en sandía (*Citrullus lanatus*) y tomate (*Solanum lycopersicum*), mientras que el experimento con SN se realizó en tomate. En Honduras, los tratamientos incluyeron nematicidas biológicos (es decir, *Paecilomyces lilacinus*, *Trichoderma harzianum*) y nematicidas químicos (es decir, fluensulfona, oxamilo, fluopiram), y en Florida, nematicidas biológicos (es decir, *Purpureocillium lilacinum cepa PL11*, *Bacillus amyloliquefaciens cepa D747*, extractos vegetales (aceite de canela, clavo y tomillo)) y nematicidas químicos (es decir, fluopiram). Las variables medidas fueron peso fresco y seco del tallo, altura, escala de agallas en las raíces, número de huevos en las raíces y juveniles de segundo estadio en el suelo para RKN, y número de nematodos en el suelo y la rizosfera para SN. Los resultados del ensayo en Honduras mostraron que fluensulfona y oxamilo fueron los tratamientos más efectivos ($P < 0.05$), reduciendo significativamente la población de RKN. En el ensayo de Florida, fluopiram mostró la mayor eficacia ($P < 0.05$) en la reducción de RKN. No se observó una reducción significativa en SN para ninguno de los tratamientos, pero el experimento confirmó que el tomate es un buen hospedador para SN.

Palabras clave: Control biológico, control químico, Fluensulfona, Fluopiram, invernadero, Oxamilo, nematodos del nudo de la raíz, nematodos picadores.

Introduction

In agricultural ecosystems, plant parasitic nematodes lurk beneath the surface, potentially having a negative influence over crop health and productivity. As silent adversaries, they pose a persistent challenge to farmers worldwide, demanding innovative strategies for management and control (Afzal & Mukhtar, 2024).

Farmers usually identify insects, pests, and other constraints as production problems but often overlook plant parasitic nematodes. Due to their hidden nature, nematode pests are difficult to control and often disregarded (Noling, 2024).

Nematodes are classified based on their feeding habits: bacterivores, fungivores, omnivores, predators, and plant parasitic. The first four are classified as free-living nematodes (Aguilar Arévalo, 2020; Weigel, 2019). Plant parasitic nematodes cause damage to individual plants, creating disease complexes with other microorganisms that may result in loss for many important agricultural crops such as vegetables, fruits, and ornamental plants (Singh et al., 2015). More than 4,100 species of plant parasitic nematodes are responsible for an estimated economic loss of $125 billion annually in the agricultural sector. Nematodes are found worldwide and they have been reported to cause considerable crop loss in regions such as Honduras and the State of Florida in the United States (US) (Crow & Duncan, 2018; Luc et al., 2013; Mesa-Valle et al., 2020).

Nematode management systems help maintain nematode populations at a level in which crop damage does not represent an economic loss, given that complete eradication of nematodes is not an achievable goal (Cardona et al., 2009). Among the most devastating plant parasitic nematodes are root-knot nematodes (*Meloidogyne* spp.) (RKN) and sting nematodes (*Belonolaimus* spp.) (SN) because of the substantial losses they cause in different crops (Crow, 2022; Rusinque et al., 2023)

RKN belong to the *Meloidogyne* genus, which are a group of sedentary endoparasitic plant parasitic nematodes (Eisenback, 2021). These microscopic roundworms infect plant roots, leading to the formation of characteristic galls or "knots” Which disrupt nutrient and water uptake. RKN go through several stages, starting from eggs to juveniles and finally to adults. The second-stage juvenile is the infective stage, entering the plant roots and inducing gall formation (Jagdale et al., 2021).

RKN infest different crops like tomato, pepper, watermelon, cucumber and squash causing reduced crop yields and quality (Meadows et al., 2018). RKN are one of the major pests affecting tomato production all over the world, especially in tropical and subtropical regions (Regmi & Desaeger, 2020). Infested tomato plants produce fewer and smaller fruits, resulting in substantial economic losses for farmers (Pun et al., 2021). In watermelon (*Citrullus lanatus*), RKN also infect roots causing the formation of galls which disrupt the plant ability to absorb water and nutrients, leading to stunted growth, yellowing leaves, and wilting. (Varma et al., 2019).

SN belongs to the *Belonolaimus* genus and are a group of ectoparasitic plant parasitic nematodes that negatively impact agricultural crops. SN spend their entire life in soil, and adults can reach up to 3 mm in length which is large for nematode standards (Crow, 2015). SN have a slender, elongated body and are equipped with a long, robust stylet, which is a needle-like mouthpart used to puncture plant cells and extract nutrients (Crow & Han, 2005). SN are most prevalent in the southeastern region of the United States but can be found in other areas with similar soil conditions. They are found primarily in sandy soils (typically 90% or more sand) with little organic matter where they pose a serious threat to a variety of crops, including strawberries, many vegetables, turfgrasses, and ornamentals (Grabau, 2019). SN halt root growth and stimulate lateral roots, which are later damaged, leading to a stunted and stubby root appearance, sometimes with swollen root tips

(Grabau, 2023). The effect of this pest on tomato (*Solanum lycopersicum* L.) has not been studied much but the crop is considered a host for the nematode (Bielinski & James, 2019).

Control of plant-parasitic nematodes involves the use of fumigant or non-fumigant nematicides which can be of chemical or biological nature. Some of the commonly used synthetic options include Nimitz® (a.i. fluensulfone), Verango® and Velum® (a.i. fluopyram), and Vydate® (a.i. oxamyl). Organic alternatives include Nemakill® (cinnamon, clove and thyme oil), Pazam® (a.i. *Purpureocillium lilacinum* (syn. *Paecilomyces lilacinus*), Trichozam® (*Trichoderma harzianum*), NemaClean® (a.i. *Purpureocillium lilacinum* strain PL11), Double Nickel ® (a.i. *Bacillus amyloliquefaciens* strain D747).

In the United States, regulations are stricter when it comes to use of agrochemicals as compared to Honduras. The disparity in regulation and utilization of agrochemicals between Honduras and Florida represents a significant gap in the adoption of safe and sustainable agricultural practices. Until recently, Florida growers had few nematicide options beyond oxamyl. Now, with new registrations like fluensulfone, fluopyram, and upcoming fluazaindolizine, non-fumigant alternatives are available for nematode management (Regmi & Desaeger, 2020). Updating regulations for the use of agrochemicals not only ensures the health of the environment and of employees working in the fields but also promotes a more sustainable agriculture aligned with global demands for food security and ecosystem preservation.

The present study aims to *i)* evaluate the effectiveness of nematicides (i.e., *Trichoderma harzianum*, fluensulfone, oxamyl, fluopyram, *Purpureocillium lilacinum*, *Bacillus amyloliquefaciens strain D747*, vegetable extracts (cinnamon, clove and thyme oil)) in managing RKN on watermelon in Honduras and tomato in Florida, and *ii)* to evaluate the efficacy of selected nematicides in managing SN on tomato in Florida.

Materials and Methods

Experiment 1: Evaluation of Nematicides against Root-Knot Nematode (RKN) in Honduras

Study Location and set-up

The experiment was conducted from January 22 to April 2, 2024, in a greenhouse within the Crop Research and Development Unit (UIDC) at Zamorano University, in Francisco Morazán, Honduras. Each experimental unit consisted of a single plastic pot (each with an area of 100.17 cm^2) (**Appendix A**) and filled with pasteurized soil. The substrate used consisted of 50% sand and 50% loam soil.

Nematode Inoculation

RKN eggs were extracted from infested watermelon roots (*Citrullus lanatus* L.). Extraction of eggs from infected roots is modified from a protocol developed by Hussey and Barker (1973) (Atamian et al., 2012) (***Appendix E***). With a Pipette a total of 1 mL equivalent to 10,000 RKN eggs were inoculated in each pot. Eggs were inoculated one day after transplant and one day before application of treatments.

Treatments

The trial included 7 treatments with 5 replicates per treatment, providing a total of 35 experimental units (EUs) arranged in a complete randomized design (Figure 1). **Appendix A** Treatments evaluated consisted of three biological and three chemical nematicides. Additionally, two control treatments were included (Table ***1***).

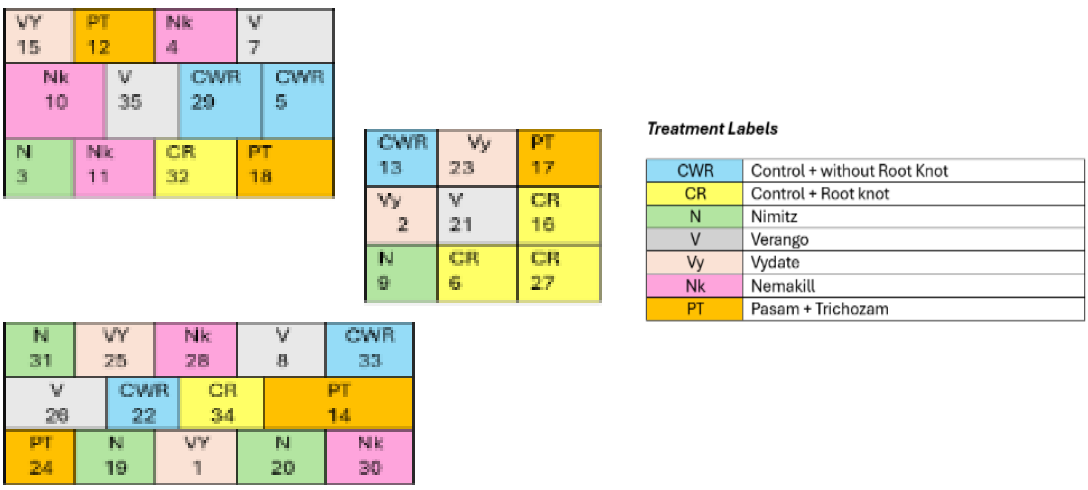
Table 1

List of treatments for the Root Knot Nematode Trial at Zamorano University, Honduras.

	Label	Treatments
Biological	PT	Pazam (a.i. *Paecilomyces lilacinus*) + Trichozam (a.i. *Trichoderma harzianum*)
	Nk	Nemakill (cinnamon, clove and thyme oil)
Chemical	N	Nimitz (a.i. Fluensulfone)
	Vy	Vydate (a.i. Oxamyl)
	V	Verango (a.i. Fluopyram)
Control	CWR	Negative Control (i.e., Control + without Root Knot)
	CR	Positive control (i.e., Control + Root Knot)

Figure 1

Location of each treatment within the greenhouse for the Root Knot Nematode Trial at Zamorano University, Honduras.



Note. Each color represents a treatment, each number represents a pot, and each rectangle represents a greenhouse bench.

Planting

Watermelon seedlings (cv. WDL3112 grafted on squash rootstock) were grown in trays for 4 weeks before transplanting. Seedlings were transplanted one day before inoculating with RKNs. Crops were kept in the greenhouse for a total of 10 weeks and transported to the laboratory for data collection. During the crop development, the plants were watered daily and fertilized weekly with 20-20-20 fertilizer.

Experimental Design and Statistical Analysis

A completely randomized design (CRD) was used with 7 treatments, each with 5 replicates, totaling 35 experimental units. Means were separated using Duncan's test, and interactions were analyzed using the analysis of variance model with the aov() function in RStudio version 2024.04.1+748, with a significance level of $P \leq 0.05$.

Experiment 2: Evaluation of Nematicides against RKN in Florida

Study Location

The experiment was conducted from March 13 to May 3, 2024, in a greenhouse within the Gulf Coast Research and Education Center, located in 14625 Co Rd 672, Wimauma, FL 33598, United States. Each experimental unit consisted of a single plastic pot (each with an area of 71.80 cm^2) (***Appendix I***) and filled with pasteurized soil. Soil type was sandy with a 1.5% of organic matter and pH of 5.1

Nematode Inoculation

RKN eggs were extracted from infested tomato roots (*Solanum lycopersicum* L.). Extraction of eggs from infected roots is modified from a protocol developed by Hussey and Barker (1973) (Atamian et al., 2012) (***Appendix E***) With a Pipette a total of 1 mL equivalent to 10,000 RKN eggs were inoculated in each pot. Eggs were inoculated one day after transplant and one day before application of treatments.

Treatments

The trial included 6 treatments with 5 replicates per treatment, providing a total of 30 experimental units (EUs) distributed in 5 blocks (Figure 2). Appendix *I* Treatments evaluated consisted of biological and chemical nematicides. Additionally, two control treatments were considered (**Table 2**).

Table 2

List of treatments for the Root Knot Nematode Trial at Gulf Coast Research and Education Center, Florida (GCREC)

Figure 2

Location of each treatment within the greenhouse for the Root Knot Nematode Trial at Gulf Coast Research and Education Center, Florida (GCREC)

BLOCK 1 (A)		BLOCK 2 (B)		BLOCK 3 (C)		BLOCK 4 (D)		BLOCK 5 (E)	
RK A4	RNC A1	RDNC B2	RC B6	RW C5	RDNC C2	RNC D1	RK D4	RC E6	RV E3
RDNC A2	RW A5	RNC B1	RV B3	RV C3	RK C4	RC D6	RDNC D2	RW E5	RNC E1
RC A6	RV A3	RW B5	RK B4	RNC C1	RC C6	RV D3	RW D5	RK E4	RDNC E2

Treatment Labels

R= Root Knot Nematode

RNC (1)	Root Knot - Nemaclean
RDNC (2)	Root Knot - Double Nickle + Nemaclean
RV (3)	Root Knot - Velum
RK (4)	Root Knot - Nemakill
RW (5)	Control Without Root Knot
RC (6)	Control - Root knot

Note. Each color and number represent a treatment, each letter represents a block, and each square represents a pot.

Planting

Tomato seedlings (cv. HM 1823) were grown in trays for 6 weeks before transplanting. Seedlings were transplanted one day before inoculating with RKNs. Crops were kept in the greenhouse for a total of 6 weeks and transported to the laboratory for data collection. During the crop development, the plants were watered daily and fertilized weekly with 20-20-20 fertilizer.

Experimental Design and Statistical Analysis

A completely randomized block design (CRBD) was used with 6 treatments, each with 5 replicates, totaling 30 experimental units. Means were separated using Duncan's test, and interactions were analyzed using the analysis of variance model with the aov() function in RStudio version 2024.04.1+748, with a significance level of $P \leq 0.05$.

Experiment 3: Evaluation of Nematicides against SN in Tomato.

Study Location

The experiment was conducted from March 13 to May 3, 2024, in a greenhouse within the Gulf Coast Research and Education Center, located in 14625 Co Rd 672, Wimauma, FL 33598, United States. Each experimental unit consisted of a single clay pot (each with an area of 112.19 cm^2) (***Appendix K***) and filled with soil from a local strawberry field that was naturally infested with sting nematodes. Soil type was sandy with a 0.55% of organic matter and pH of 5.3

Treatments

The trial included 7 treatments with 5 replicates per treatment, providing a total of 35 experimental units (EUs) distributed in 5 blocks, (Figure 3). Appendix *K* Treatments evaluated consisted of three biological nematicides and one chemical nematicide. Additionally, three control treatments were considered (Table 3).

	Label	Treatments
Biological	SNC	NemaClean (a.i. *Purpureocillium lilacinum* strain PL11)
	SDNC	Double Nickle (a.i. *Bacillus amyloliquefaciens* strain D747) + NemaClean
	SK	Nemakill (cinnamon, clove and thyme oil)

Chemical	SV	Velum (a.i. Fluopyram)
Control	SW	Negative control (i.e., pasteurized soil)
	SC	Positive control (i.e., natural soil with sting nematodes)
	SSG	Control (i.e. natural soil with sting nematodes) + Sorghum Sudangrass†

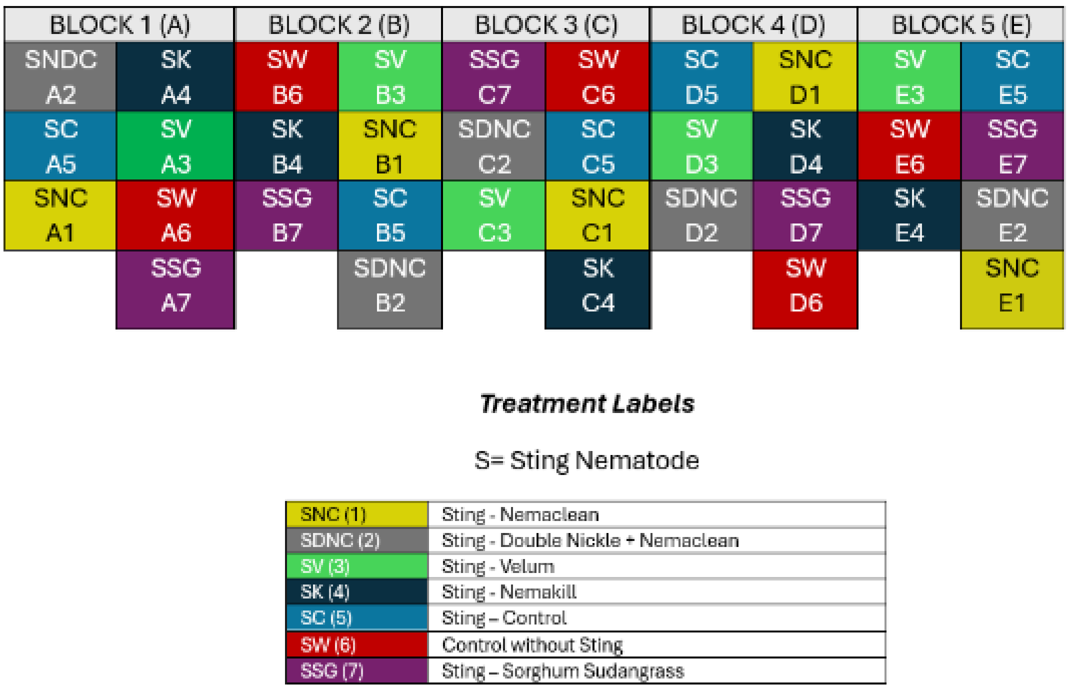
Table 3

List of treatments for the Sting Nematode Trial at Gulf Coast Research and Education Center, Florida (GCREC)

Note. † Was used as a control for nematode survival in a known host (i.e. *Sorghum × drummondii*) during the trial; therefore, data from this treatment was not considered in the analysis.

Figure 3

Location of each treatment within the greenhouse for the Sting Nematode Trial at Gulf Coast Research and Education Center, Florida (GCREC)



Note. Each color and number represent a treatment, each letter represents a block, and each square represents a pot.

Application of Treatments† Was applied during first and second week. ‡ Were applied during first, second, and fourth week.

Plant growth (Shoot, Root Weight for RKN, Root Dry Weight for SN, Height exp. 1 and

General ProceduresPlanting

Tomato seedlings (cv. HM 1823) were grown in trays for 6 weeks before transplanting. Seedlings were transplanted two days after filling pots with infested soil with SN. Crops were kept in the greenhouse for a total of 6 weeks and transported to the laboratory for data collection. During the crop development, the plants were watered daily and fertilized weekly with 20-20-20 fertilizer.

Experimental Design and Statistical Analysis

A completely randomized block design (CRBD) was used with 6 treatments, each with 5 replicates, totaling 30 experimental units. Means were separated using Duncan's test, and interactions were analyzed using the analysis of variance model with the aov() function in RStudio version 2024.04.1+748, with a significance level of $P \leq 0.05$.

General Procedures

Application of Treatments

Nematicides concentrations were prepared according to manufacturer's recommendation shown on each product label (Table 4). This recommended rate, typically provided in ounces per acre (oz/A) was converted to milliliters (mL) for volume and to square meters (m^2) for area to obtain a final rate in mL/ m^2. Finally, formula [1] was used to obtain the final volume in microliters (µL) of nematicide to be diluted in 50 mL of water and to be applied per pot, treatments were applied to the seedling.

$$\frac{Recommend\ volume\ (mL) \times Surface\ acre\ (m^2)}{Surface\ pot\ area\ (m^2)} \times 1000 = Volume\ (microliters)\ /\ 50\ mL \quad [1]$$

Table 4

Nematicides recommended rate in oz/A and rate per application per pot in µL diluted in 50 mL of water.

Nematicide	Recommended Rate (oz/A)	Rate per application per pot (µL)							
		Experiment 1†		Experiment 2‡			Experiment 3‡		
		1	2	1	2	3	1	2	3
Pazam	21	5.09	5.09	N/A	N/A	N/A	N/A	N/A	N/A
Trichozam	21	5.09	5.09	N/A	N/A	N/A	N/A	N/A	N/A
Vydate	96	11.9	5.95	N/A	N/A	N/A	N/A	N/A	N/A
Verango	6.8	1.26	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Nimitz	80	14.88	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Nemakill	48	8.93	4.46	6.4	6.4	6.4	10	10	10
NemaClean	32	N/A	N/A	4.27	4.27	4.27	6.66	6.66	6.66
Double Nickle	64	N/A	N/A	8.53	8.53	8.53	13.33	13.33	13.33
Velum	6.8	N/A	N/A	0.91	N/A	N/A	1.42	N/A	N/A

Note. † Was applied during first and second week. ‡ Were applied during first, second, and fourth week.

Root knot Nematodes (Gall Rating, Egg and J2 Counts)/ Sting Nematodes (Soil and Root Counts)

To extract SN (i.e. from soil and roots), RKN and RKN eggs (i.e. from roots) and evaluate gall rating, each pot was individually inverted into a larger container that could retain all the soil. Soil was gently shaken from the roots and collected in a plastic bag and labeled according to the corresponding treatment. Nematodes were extracted from 200 mL of soil using modified Baermann Funnel Method (Viglierchio & Schmitt, 1983) (Appendix B). Root gall rating on each root was performed using the Bridge and Page (1980) scale (Appendix C). Egg extraction was done as follows. The roots were rinsed under running water until the soil particles were washed away. The roots were cut with scissors into small pieces, and the taproot was disposed of. The chopped roots from a single plant were placed in a large plastic jar with a lid, just enough 10% bleach solution was added to cover the roots, and the lid was closed. The jar containing the roots was shaken for 2 minutes. Four prewet sieves of 500, 250, 75, and 25 µm aperture were stacked from top to bottom in the following order: 500, 250, 75, and 25 µm aperture. The eggs were collected on the 25 µm sieve at the bottom. The sieves were placed on a wire mesh supported in a sink. The jar was opened, and the roots were poured onto the top sieve and washed with a hose attached to a misting nozzle for at least 30 seconds and until all the bleach was gone. The top sieve was removed, and the debris on the second sieve was rinsed. The second sieve was removed, and the debris on the third sieve was rinsed. The third sieve was removed, and the debris on the last sieve was collected. With a gentle stream from a wash bottle, the debris was moved to one side of the sieve, and debris and eggs collected into a clean vial numbered according to each treatment and/or block. Extraction of eggs from infected roots is modified from a protocol developed by Hussey and Barker (1973) (Atamian et al., 2012) (Appendix E). For the counting of juvenile RKN nematodes, and SN counting dish was used, in which the solution previously extracted from the soil was placed. Subsequently, counting was performed under a compact inverted microscope. As for the counting of nematode eggs per gram of root, a 'blood-counting' slide that holds 1 mL of solution was used. Four squares of the plate were counted, repeating this process three times. Then, the average ($\bar{x}$) was calculated and multiplied by 6, as the nematode counting slide consists of 24 squares in total. Finally, it was multiplied by the number of mL in the container or vial and divided by the weight of the root to determine the number of eggs per gram of root.

$$\frac{(\bar{x} \times 6) \times mL\ in\ the\ vial}{Root\ weight} = eggs\ per\ gram\ of\ root \qquad [2]$$

Plant growth (Shoot, Root Weight for RKN, Root Dry Weight for SN, Height exp. 1 and 2)

Plants were cut at the base of the stem, being careful not to lose any part of the plant that might affect the weight. Immediately after cutting, each plant was weighed to measure the fresh weight of the plants. Each plant was then placed into individually numbered paper bags corresponding to each treatment, Samples were dried in a greenhouse at approximately 32 °C for 120 hours until all plant moisture dissipated. After 120 hours, samples were removed from the greenhouse and weighed again to obtain the dry weight.

To measure the root weight, the measurement was taken once at the end of the experiment before the egg's extraction, a scale in grams was used (***Appendix H***).

To measure the height, a ruler in centimeters was used; the measurement was taken once at the end of the experiment (Appendix G).

To measure the dry root weight, the roots previously used for the extraction of SN from the roots were dried for one week at room temperature, a scale in grams was used.

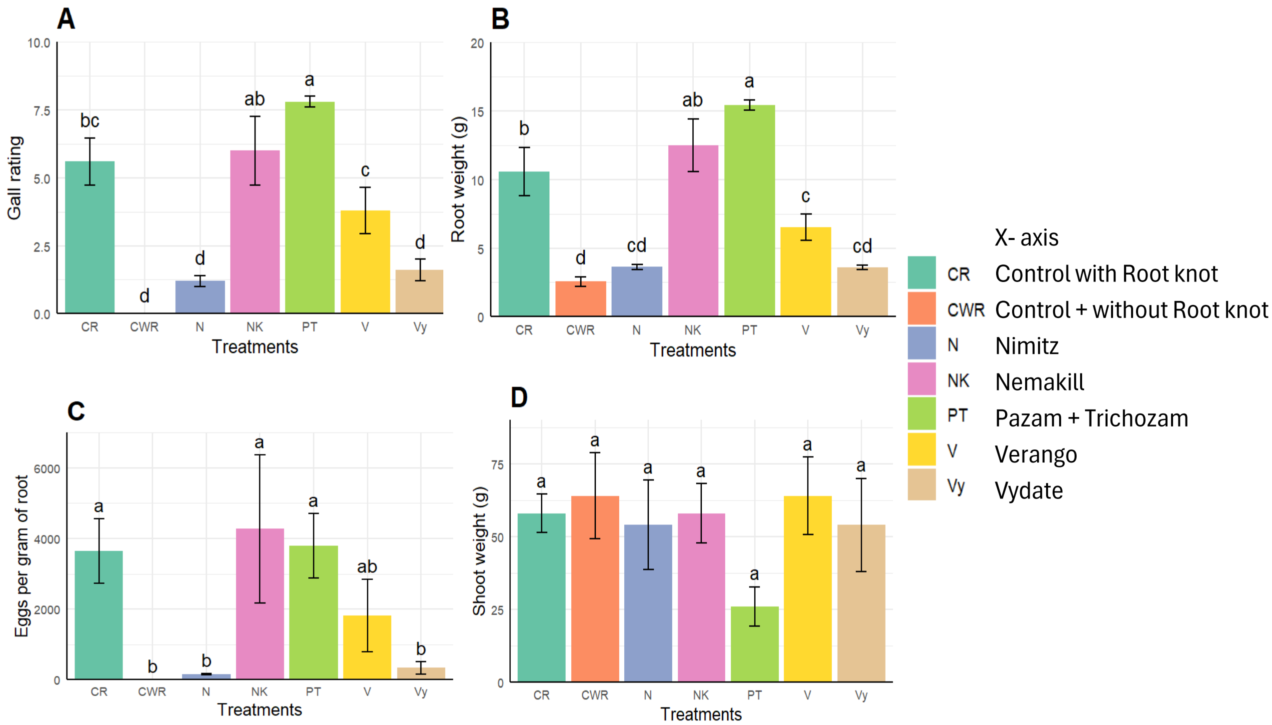
Results and Discussion

Experiment 1: Evaluation of Nematicides against RKN in Honduras

It was found that shoot weight did not show significant differences among treatments (*P* > 0.05; Figure 4D). However, gall rating, root weight, and eggs per gram of root, did show significant differences among treatments, (*P ≤ 0.05*).

Figure 4

Plots of Duncan test in gall rating, root weight, eggs per gram of the root, shoot weight in watermelon plants infested with Root knot Nematode, Escuela Agrícola Panamericana, Honduras.



Note. Means with different letter indicate statistical difference *(P ≤ 0.05)*.

Gall Formation and Root Weight

Data showed that watermelon treated with N (Fluensulfone) and Vy (Oxamyl) resulted in the lowest formation of galls (*P* <0.05) while watermelon treated with Pazam+ Trichozam increased the formation of galls as compared to the positive control (CR). Similarly, treatments N (Fluensulfone), Vy

(Oxamyl) and V (Fluopyram) resulted in the lightest root weight, while Nk (cinnamon, clove and thyme oil) and Pazam + Trichozam showed the heaviest root weight ($P < 0.05$). These results are due to Root-knot nematodes (*Meloidogyne* spp.) promoting the formation of galls in the root system, which can increase the weight of the roots. Therefore, a heavier root system may indicate a higher nematode infestation (Figure 4A and 4B).

Number of Egg formation in root system

Nematode eggs density in watermelon treated with Nemakill (cinnamon, clove and thyme oil), Pazam + Trichozam and V (Fluopyram) showed no statistical difference as compared to the positive control (CR), while N (Fluensulfone) and Vy (Oxamyl) did show a statistically lower number of eggs in the root system (Figure 4C). Results are consistent with the data from gall formation and root weight as a higher number of galls typically correspond to an increased density of nematode eggs within the root system.

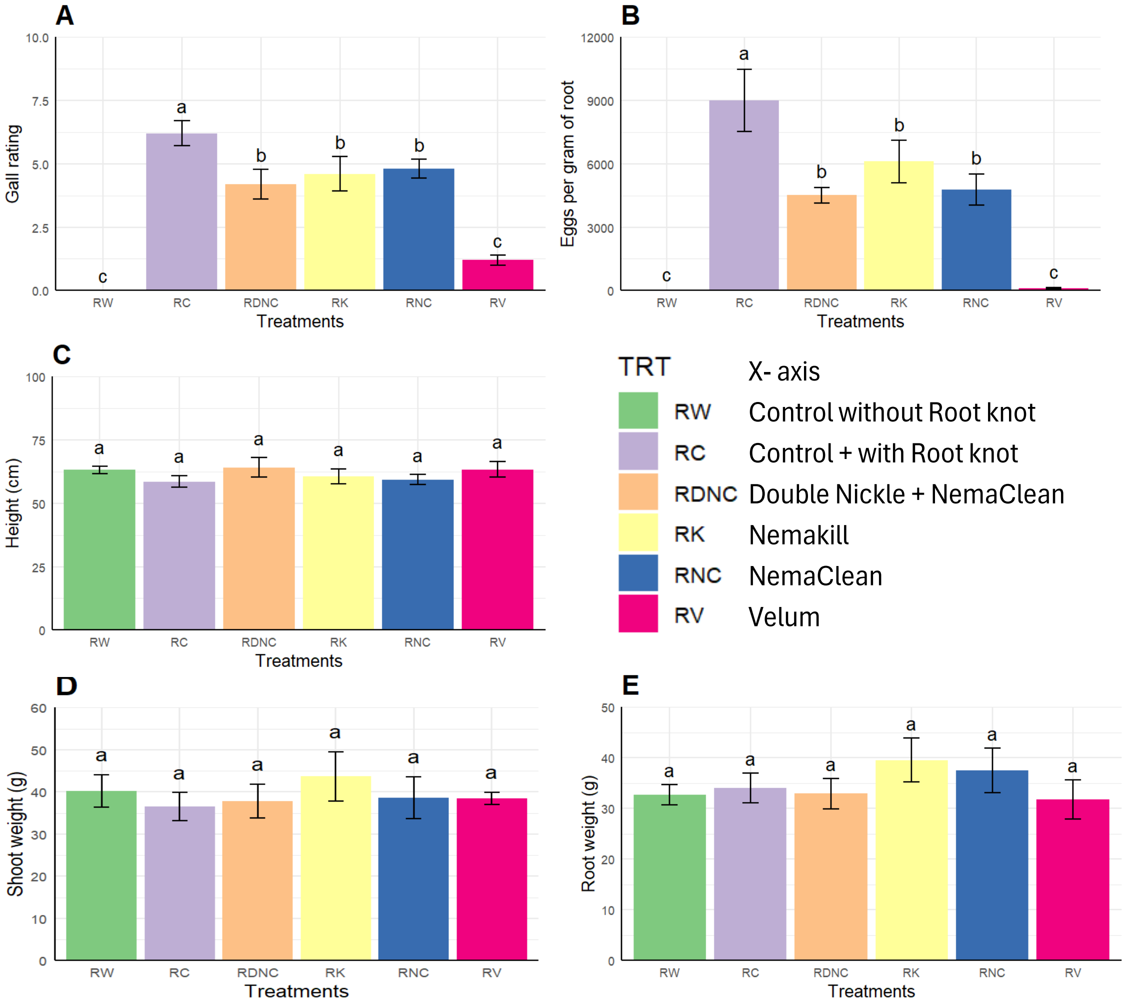
Results suggest that the most effective treatments that significantly reduced root-Knot nematode population density were N (Fluensulfone) and Vy (Oxamyl), since both showed no statistical difference as compared to the negative control treatment (Figure 4). Other studies (Aguilar Arévalo, 2020; Silva Zegarra, 2021) have evaluated the effect of Fluensulfone and Oxamyl in strawberry and pepper and found them to be effective in reducing root-Knot Nematodes populations.

Experiment 2: Evaluation of Nematicides against RKN in Florida

Height, shoot and root weight did not show significant differences among treatments ($P > 0.05$; Figure 5C, 5D and 5E). However, gall rating, and eggs per gram of root, did show significant differences among treatments, ($P \leq 0.05$).

Figure 5

Plots of Duncan test in gall rating, eggs per gram root, height, shoot weight, root weight in tomato plants infested with Root Knot Nematode, Gulf Coast Research and Education Center, Florida.



Note. Means with different letter indicate statistical difference *(P ≤ 0.05)*.

Gall Formation

Data indicated that all treatments showed reduction in formation of galls (Figure 5A). However, RV (Fluopyram) resulted in the lowest by showing no statistical difference as compared to the negative control (RW) (P <0.05).

Number of Egg formation in root system

Results indicated that all treatments reduced RKN eggs density (Figure 5B). However, RV (Fluopyram) resulted in the lowest by showing no statistical difference as compared to the negative control (RW) ($P < 0.05$). Results are consistent with the data from gall formation as a higher number of galls typically correspond to an increased density of nematode eggs within the root system. **Figure *5***

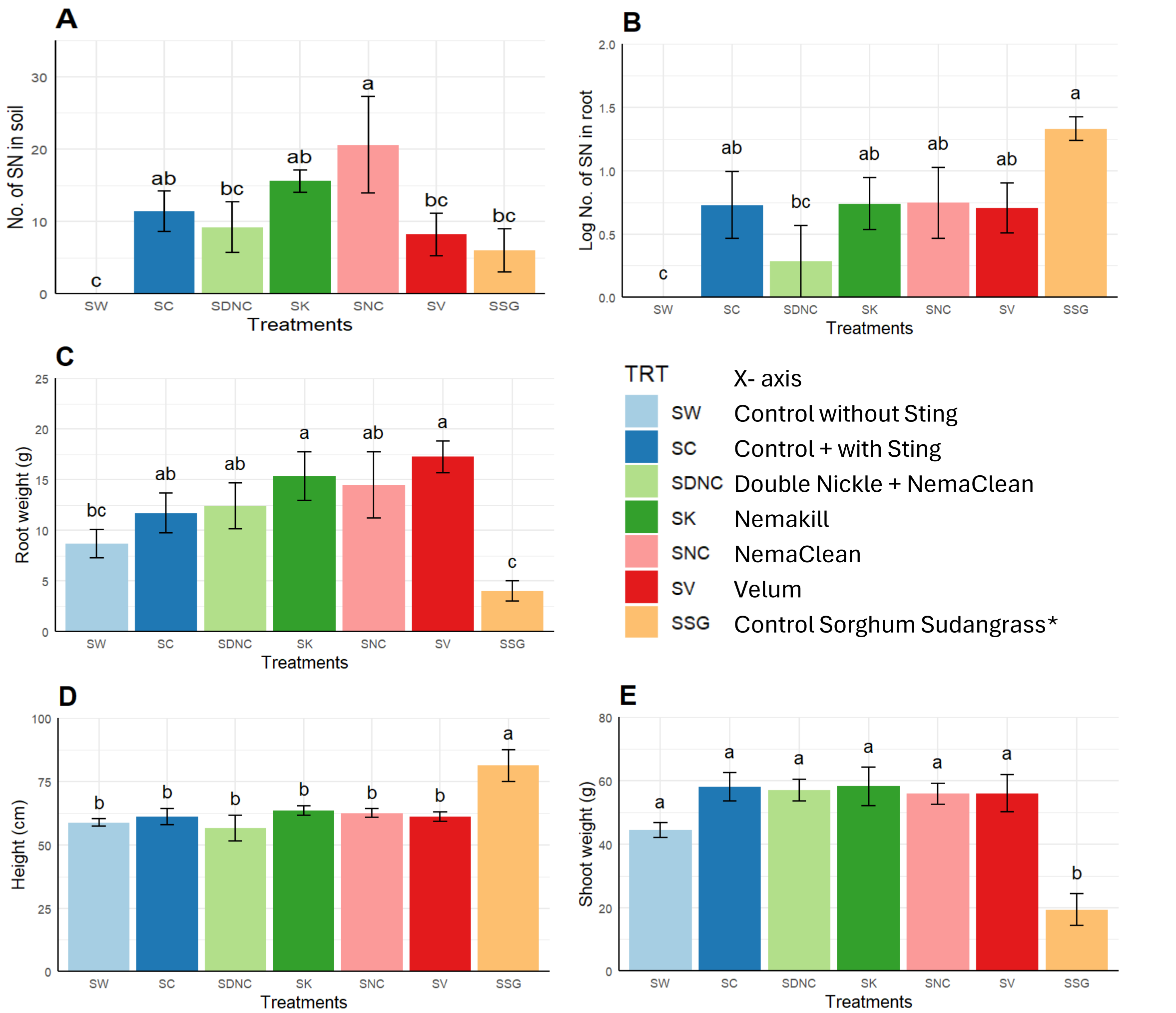
Experiment 3: Evaluation of Nematicides against SN in Tomato.

Height and Shoot Weight did not show significant differences among treatments ($P > 0.05$; Figure 6D and 6E). However, the number of SN in soil, number of SN in root, and root weight, did show significant differences among treatments, ($P \leq 0.05$).

Figure 6

Plots of Duncan test in Number of Sting Nematodes in Soil, Number of Sting Nematodes in the Roots, Root Weight and Height in tomato plants, Gulf Coast Research and Education Center, Florida.

Note. *Was used as a control for nematode survival in a known host (i.e. *Sorghum × drummondii*) during the trial; therefore, data from this



treatment was not considered in the analysis. Means with different letter indicate statistical difference *($P \leq 0.05$)*.

Results from the number of SN in the soil and roots of tomato plants confirm that tomatoes are a host for sting nematodes. Figure 6A shows the number of SN in soil of all treatments, indicating no statistical difference ($P < 0.05$). Whereas Figure 6B shows the number of SN in the roots of all treatments. Treatments showed no statistical difference as compared to the positive control and they ranged from 1 to 49 No. of SN in root. Treatments SK (cinnamon, clove and thyme oil), SV (Fluopyram) and SNC (NemaClean®) were statistically different when compared to the negative control ($P < 0.05$) but not compared to the positive control ($P>0.05$). Treatment SDNC (Double Nickle® + NemaClean®) showed no statistical significance as compared to either control ($P >0.05$).

Impact of SN on Root Weight

Tomatoes treated with SV (Fluopyram) showed a higher root weight as compared to the negative control ($P < 0.05$), but not as compared to the positive control ($P > 0.05$). All other treatments showed no statistical significance ($P > 0.05$; Figure 6A and 6B). SN cause significant root damage that leads to a reduced root mass in infested plants; thus, a lower overall root weight can be related to a higher infestation of SN (Crow, 2015). Nonetheless, the positive and negative controls showed no statistical difference (P > 0.05; Figure 6C).Conclusions

Fluensulfone and Oxamyl were the most effective nematicides against root-knot nematodes in watermelon in Honduras, while *Paecilomyces lilacinus* + *Trichoderma harzianum* and Vegetables Extracts (cinnamon, clove and thyme oil) showed the lowest effectiveness.

All nematicides evaluated in tomatoes in Florida were effective in reducing root-knot populations with Fluopyram showing the greatest reduction. No significant nematicide effect was noted on sting nematodes in tomato in Florida, but the crop showed to be a good host for sting nematode.

Recommendations

More research is needed to further evaluate these products in field trials in Honduras and Florida and for other important crops. Increase duration of trials to see effectiveness of nematicides in mature stages of the crops.

References

Afzal, A., & Mukhtar, T. (2024). Revolutionizing nematode management to achieve global food security goals - An overview. *Heliyon*, *10*(3), e25325. https://doi.org/10.1016/j.heliyon.2024.e25325

Aguilar Arévalo, I. M. (2020). *Evaluación de nematicidas no-fumigantes para el control del nematodo Belonolaimus longicaudatus en el cultivo de fresa Fragaria × ananassa var. Sequoia en invernadero* [Proyecto Especial de Graduación, Escuela Agrícola Panamericana Zamorano]. bdigital.zamorano.edu. https://bdigital.zamorano.edu/server/api/core/bitstreams/8f8aeabd-eb35-4213-b59f-d2f3bfeaead8/content

Atamian, H. S., Roberts, P. A., & Kaloshian, I. (2012). High and low throughput screens with root-knot nematodes Meloidogyne spp. *Journal of Visualized Experiments : JoVE.* Advance online publication. https://doi.org/10.3791/3629

Bielinski, S., & James, G. (2019). Tomato Nutrient Absorption and Nutsedge and Sting Nematode Management with Propylene Oxide. https://www.cabidigitallibrary.org/doi/pdf/10.5555/20193084298

Cardona, C., Hincapié, J., & Loaiza, M. (2009). Evaluación biológica del manejo de picudos y nematodos fitopatógenos en plátano (Musa AAB) Biological evaluation of the management of borers and phytopathogenic nematodes of plantain (Musa AAB). *Acta Agronomica (Palmira)*, *58*(4), 260–269. http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S0120-28122009000400005

Crow, W. T. (2015). *sting nematode - Belonolaimus longicaudatus.* UFL. https://entnemdept.ufl.edu/creatures/nematode/sting_nematode.htm

Crow, W. T. (2022). *EENY618/IN395: Belonolaimus longicaudatus Rau (Nematoda: Secernentea: Tylenchida: Tylenchina: Belonolaimidae: Belonolaiminae).* Entomology and Nematology. https://edis.ifas.ufl.edu/publication/IN395

Crow, W. T., & Duncan, L. (2018). Management of Plant Parasitic Nematode Pests in Florida. In S. A. Subbotin & J. J. Chitambar (Eds.), *Sustainability in Plant and Crop Protection. Plant parasitic nematodes in sustainable agriculture of North America* (pp. 209–246). Springer. https://doi.org/10.1007/978-3-319-99588-5_9

Crow, W. T., & Han, H. (2005). Sting Nematode. *The Plant Health Instructor.* Advance online publication. https://doi.org/10.1094/PHI-I-2005-1208-01

Eisenback, J. D. (2021). *Meloidogyne incognita (root-knot nematode)*. CABI International. https://doi.org/10.1079/ISC.33245.20210200734

Grabau, Z. (2019). *Recognizing and Managing Sting Nematode Problems in Peanuts* [Nematodes, Peanut, Pest Management]. https://nwdistrict.ifas.ufl.edu/phag/2019/04/26/recognizing-and-managing-sting-nematode-problems-in-peanuts/

Grabau, Z. (2023). *Is That Bad Patch in My Field Caused by Sting Nematodes?* Universsity of Florida: IFAS Extenion. https://nwdistrict.ifas.ufl.edu/phag/2023/08/25/is-that-bad-patch-in-my-field-caused-by-sting-nematodes/

Jagdale, S., Rao, U., & Giri, A. P. (2021). Effectors of Root-Knot Nematodes: An Arsenal for Successful Parasitism. *Frontiers in Plant Science*, *12*, 800030. https://doi.org/10.3389/fpls.2021.800030

Luc, V., Jean-Louis, S., Hernández, A., Bertrand, B., Anthony, F., Lashermes, P., Pierre, C., Anzueto, F., Dechechi, R. M., & Gomes, C. (2013). Research/Investigación Diversity of Root-Knot Nematodes Parasitizing Coffee in Central America. https://journals.flvc.org/nematropica/article/view/82708/79652

Meadows, I., Averre, C., Duncan, H., & Baker, K. (2018). Control of root knot nematodes in the home vegetable garden. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Control+of+Root-Knot+Nematodes+in+the+Home+Vegetable+Garden&btnG=

Mesa-Valle, C. M., Garrido-Cardenas, J. A., Cebrian-Carmona, J., Talavera, M., & Manzano-Agugliaro, F. (2020). Global Research on Plant Nematodes. *Agronomy*, *10*(8), 1148. https://doi.org/10.3390/agronomy10081148

Noling, J. W. (2024, June 6). *ENY-032/NG032: Nematode Management in Tomatoes, Peppers, and Eggplant.* Entomology and Nematology. https://edis.ifas.ufl.edu/publication/NG032

Pun, T. B., Neupane, A., & Koech, R. (2021). Quantification of Root-Knot Nematode Infestation in Tomato Using Digital Image Analysis. *Agronomy*, *11*(12), 2372. https://doi.org/10.3390/agronomy11122372

Regmi, H., & Desaeger, J. (2020). Integrated management of root-knot nematode (Meloidogyne spp.) in Florida tomatoes combining host resistance and nematicides. *Crop Protection*, *134*, 105170. https://doi.org/10.1016/j.cropro.2020.105170

Rusinque, L., Camacho, M. J., Serra, C., Nóbrega, F., & Inácio, M. L. (2023). Root-knot nematode assessment: Species identification, distribution, and new host records in Portugal. *Frontiers in Plant Science*, *14*, 1230968. https://doi.org/10.3389/fpls.2023.1230968

Silva Zegarra, J. A. (2021). *Eficiencia de cinco especies de hongos nematófagos sobre el control de Meloidogyne spp. en pimiento del piquillo (Capsicum annuum L.) bajo condiciones de casa malla* [Tesis de Pregrado, Universidad Privada Antenor Orrego; PE, Perú]. repositorio.upao.edu.pe. https://repositorio.upao.edu.pe/handle/20.500.12759/8354

Singh, S., Singh, B., & Singh, A. P. (2015). Nematodes: A Threat to Sustainability of Agriculture. *Procedia Environmental Sciences*, *29*, 215–216. https://doi.org/10.1016/j.proenv.2015.07.270

Varma, A., Tripathi, S., & Prasad, R. (Eds.). (2019). *Springer eBook Collection*. *Grafting for Management of Southern Root-Knot Nematode, Meloidogyne incognita, in Watermelon: State of the Art* (1st ed. 2019). Springer. https://doi.org/10.1007/978-3-030-26657-8

Viglierchio, D. R., & Schmitt, R. V. (1983). On the methodology of nematode extraction from field samples: Baermann funnel modifications. *Journal of Nematology*, *15*(3), 438–444.

Weigel, K. (2019). *Soil-inhabiting nematodes - Phylum Nematoda*. https://entnemdept.ufl.edu/creatures/nematode/soil_nematode.htm

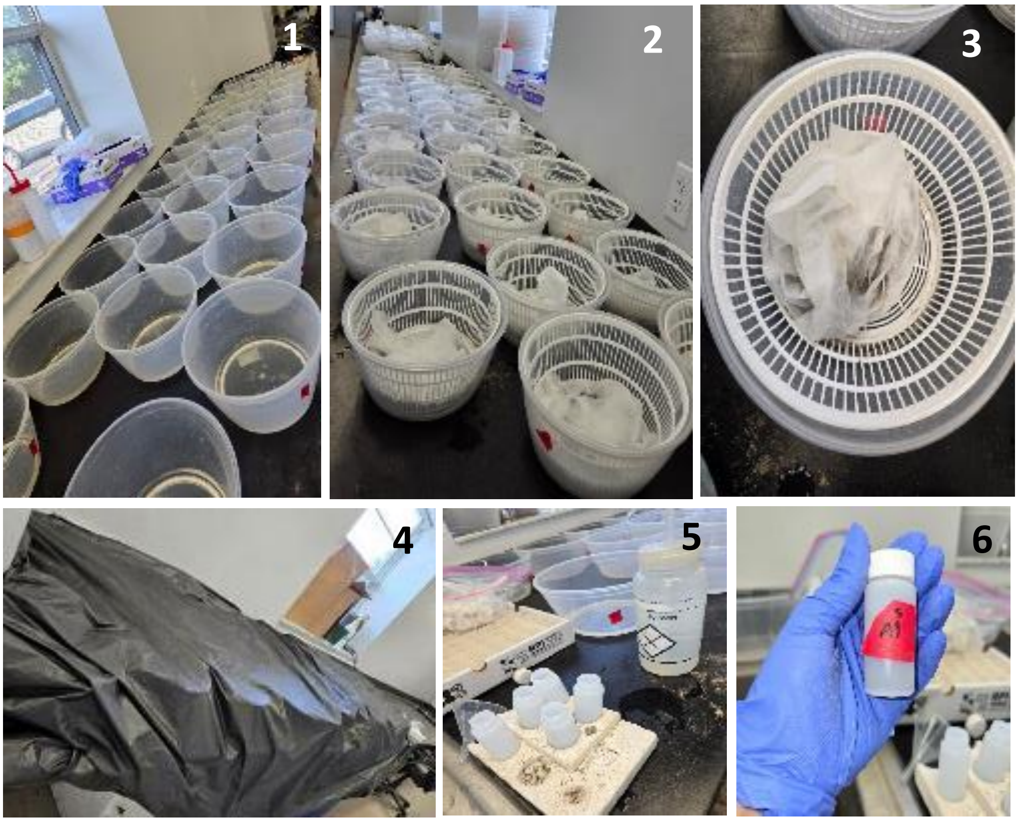
Appendices

Appendix A

Location of each pot within the greenhouse for the Root Knot Nematode Trial at Zamorano University Honduras.

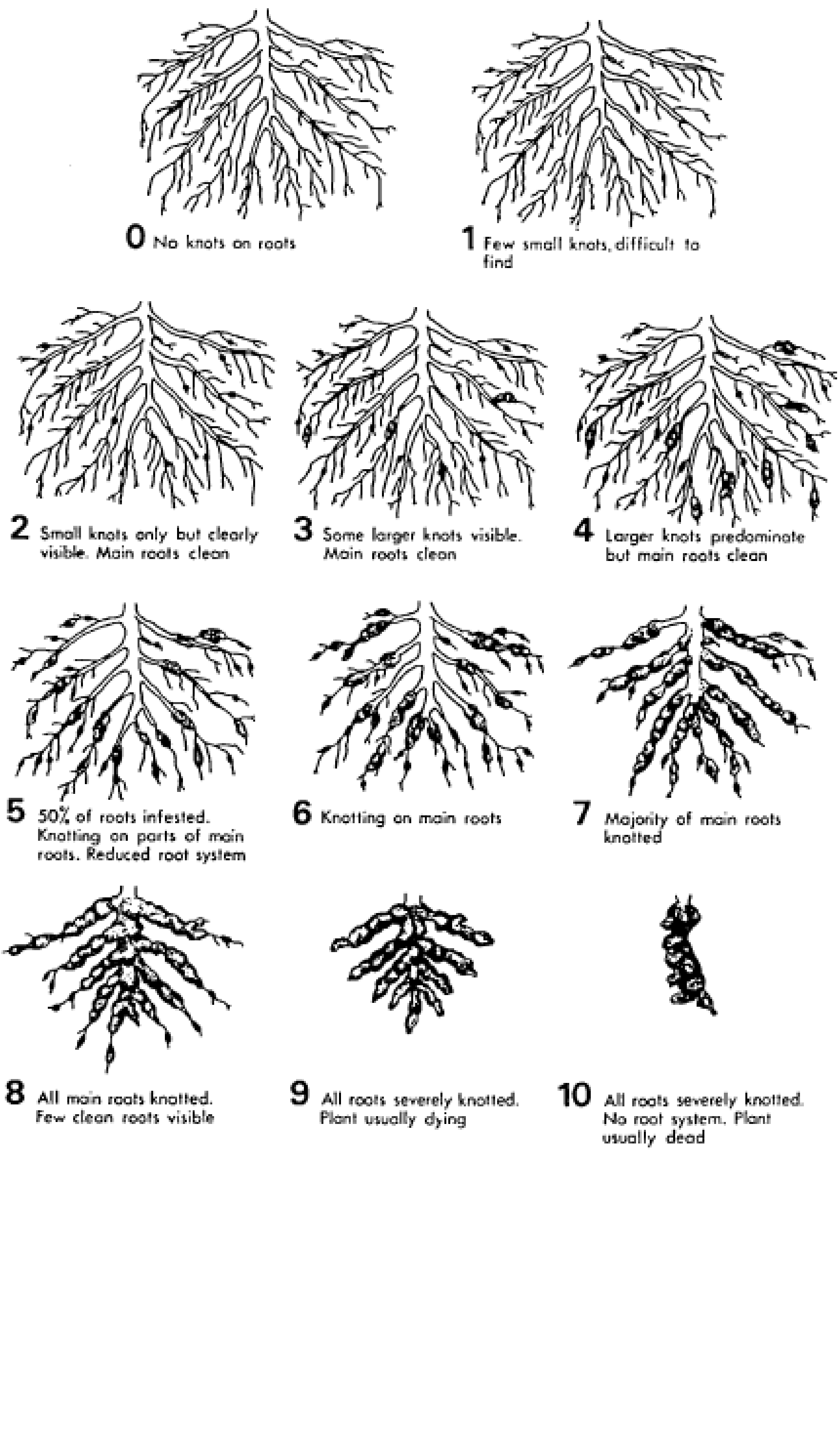
Appendix B

Modified Baermann Funnel Method for nematode soil extraction.



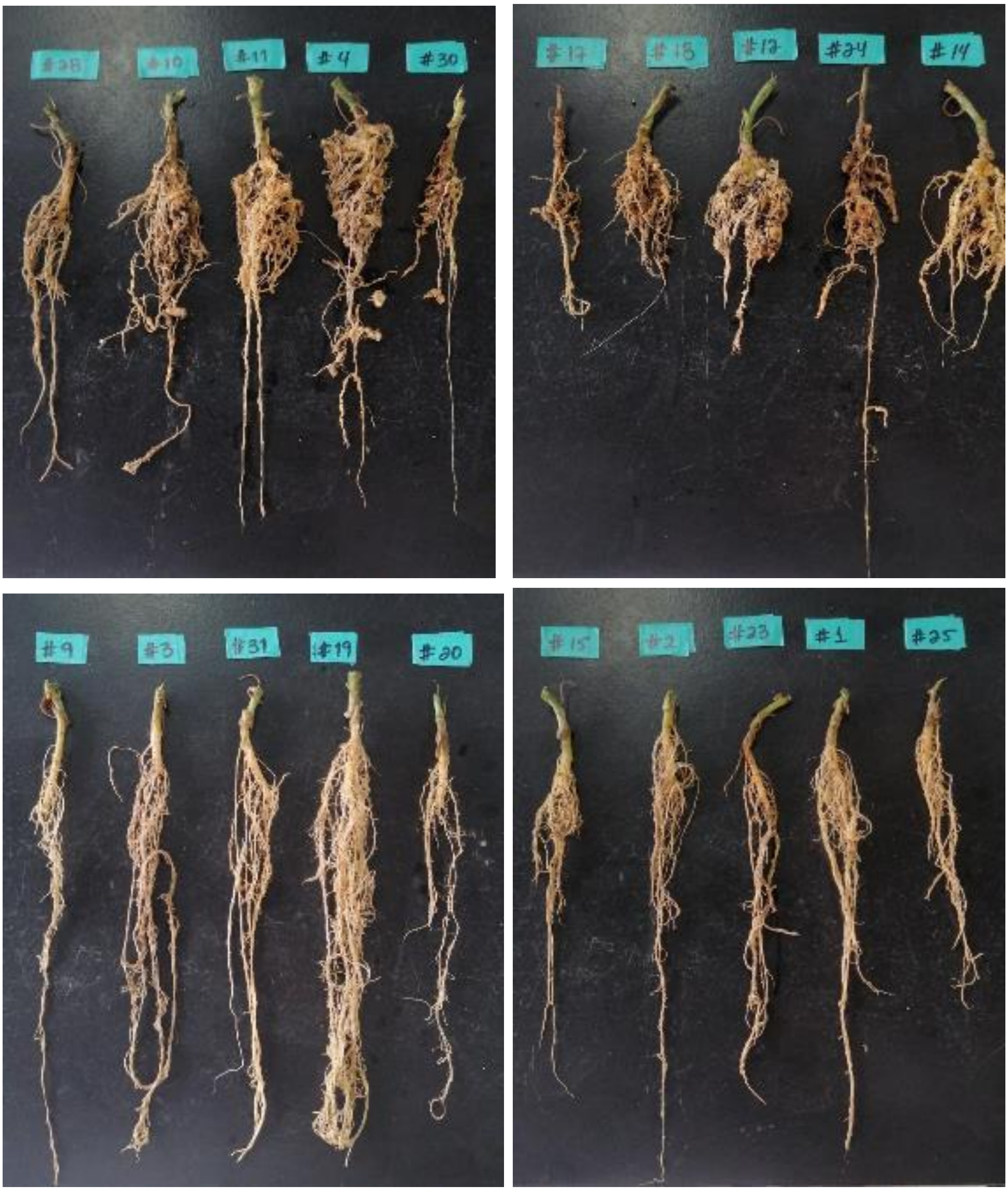
Appendix C

Gall rating scale (Bridge y Page 1980)



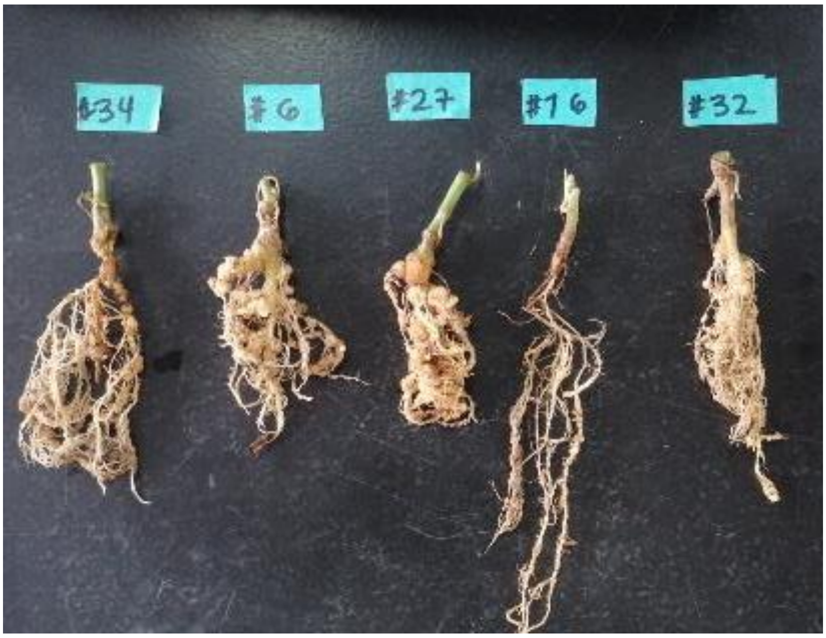
Appendix D

Experiment 1 watermelon roots at the end showing galls on the roots.



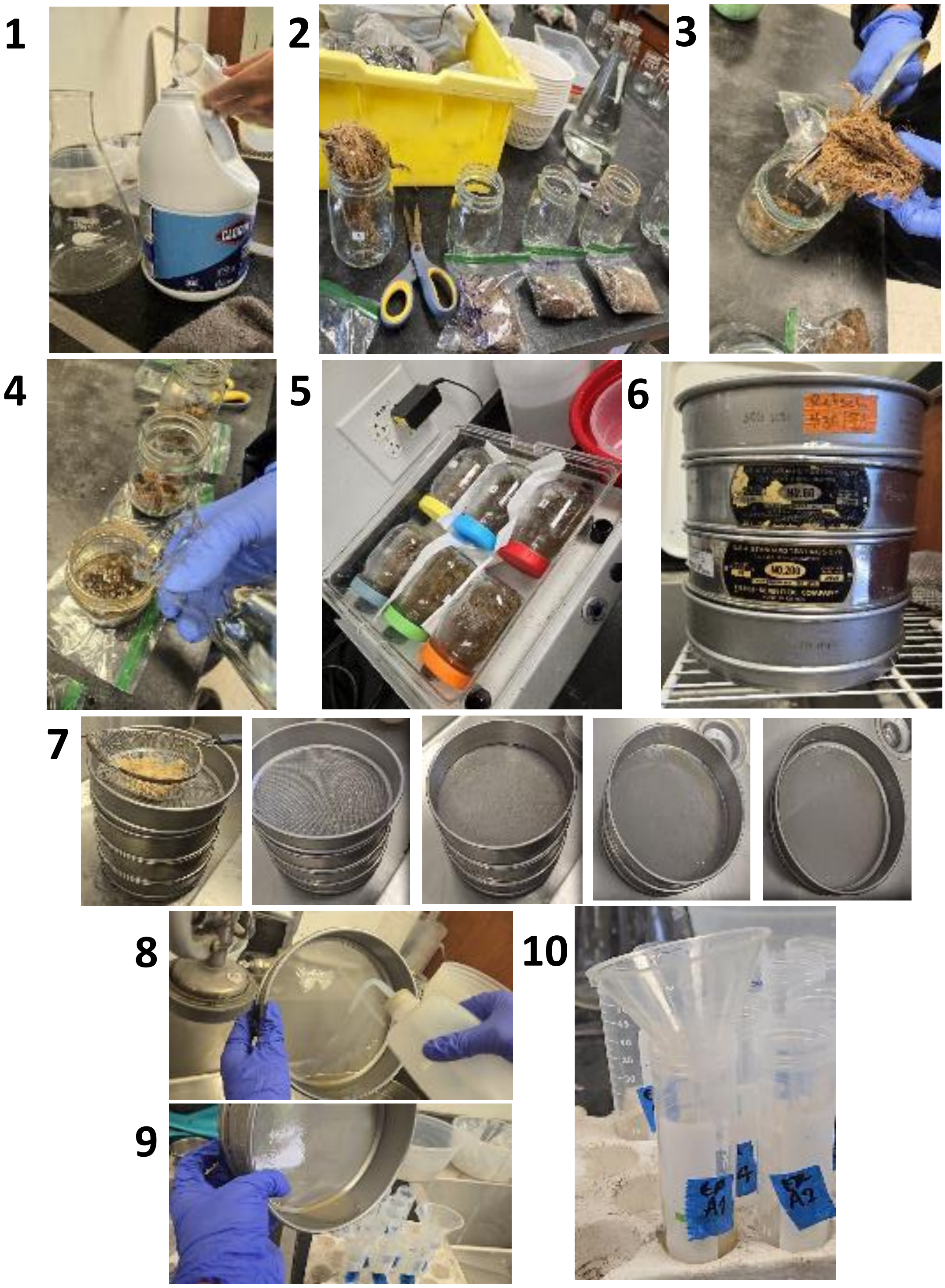

#5
#29
#13
#33
#22


#7
#35


#34
#6
#27
#16
#32

Appendix E

Root knot nematode eggs extraction procedure.



Appendix F

Measuring tomato shoot weight.

Appendix G

Measuring tomato shoot height.

Appendix H

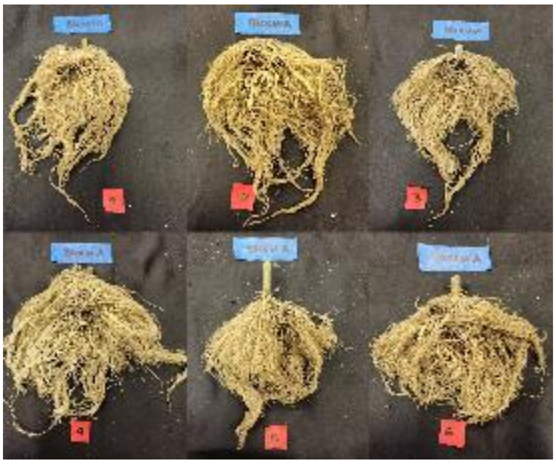
Root weight collection.

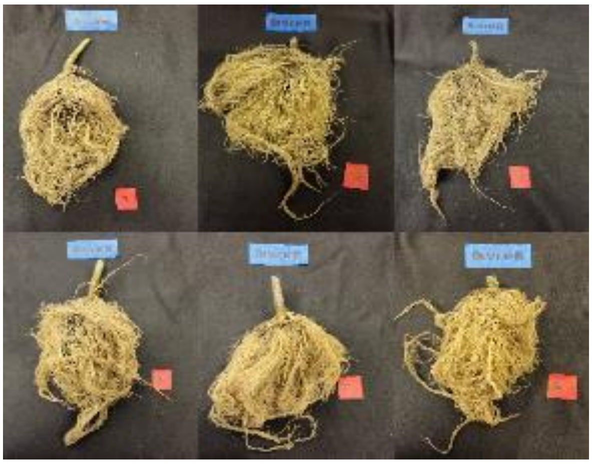
Appendix I

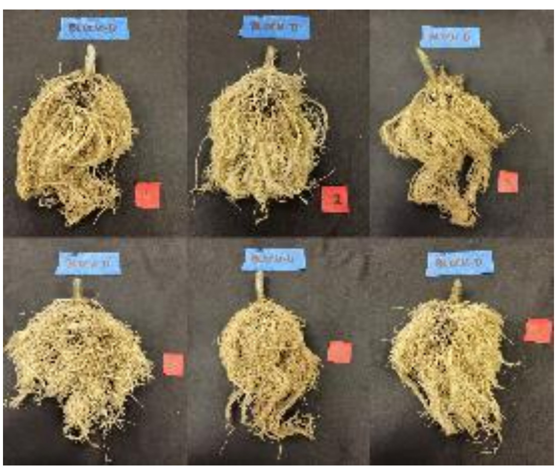
Location of each pot within the greenhouse for the Root Knot Nematode Trial at University of Florida, Florida (GCREC)

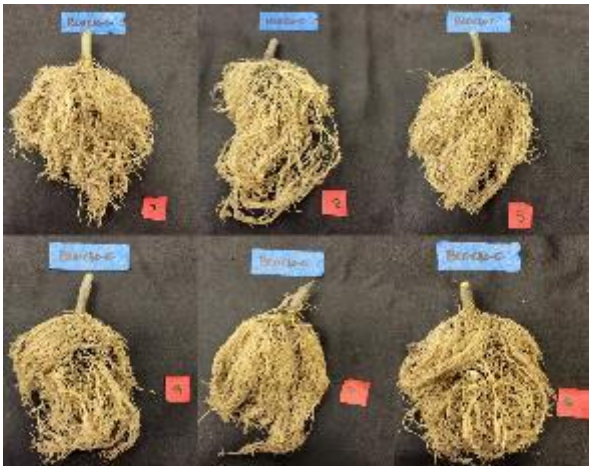
Appendix J

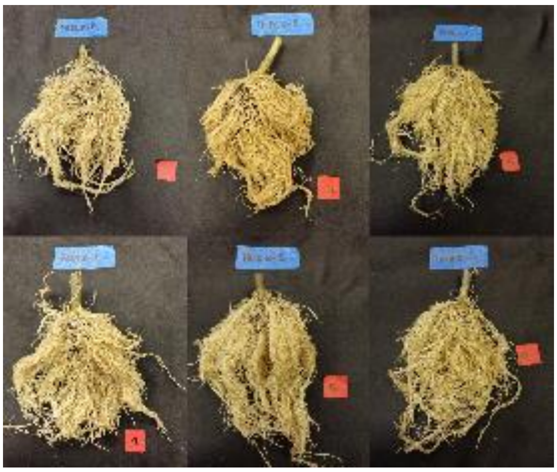
Experiment 2 tomatoes roots at the end showing galls on the roots.











Appendix K

Location of each treatment within the greenhouse for the Sting Nematode Trial at University of Florida, Florida (GCREC)