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Research Article

Potential of Acetyl-CoA Carboxylase Inhibitors as Nematicides Against Southern Root-Knot Nematode, *Meloidogyne incognita* (Kofoid and White, 1919): *In vitro* and Semi-Field Studies

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Abstract | The management of plant-parasitic nematodes such as root-knot nematodes (*Meloidogyne* spp.) has been one of the most important research priorities over the last two decades. Chemical nematicides are the primary control method used by farmers against RKN_s. However, the available control measures are very limited. Therefore, in this study we tried to provide recent options that could be effective. Laboratory and pot trials were conducted to evaluate the effect of three spiro-tetramic acid compounds namely; spirodiclofen, spiromesifen and spirotetramat against the root-knot nematode (*Meloidogyne incognita*) on Okra plants (*Abelmoschus esculentus*) at different rates. The results of laboratory study revealed that all applied treatments showed very limited nematicidal activity on J_2 of *M. incognita* after 48 h of exposure with LC_{50} values at range of 25766.70 to 57344.90 µg ml^{-1}. Meanwhile, spirotetramat induced the highest juvenile mortality of 29.73%. On the other hand, in the pot trial, the evaluated parameters included root galls, soil population and egg masses. The general mean percentage reduction in root galls was at range from 44.21 to 56.28% and soil population at range from 46.37 to 52.68%, while egg masses at range from 50.50 to 64.09%. Meanwhile, the most of plant growth indices such as weight and length or height of shoots and roots were increased significantly.

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Introduction

Okra (*Abelmoschus esculentus* L. Moench) is a biannual crop with two growing seasons. Moreover, okra is a rich source of proteins and vitamins, A and B. as well as, contains K, Mg, Na, Zn, P, Ca and Fe (El-Khalifa *et al.*, 2021). The production of okra is still low due to attacks of several pathogens, such as fungi, viruses, bacteria, nematodes and abiotic factors. One of the most widespread pathogens in vegetables including okra is root -knot nematodes (*Meloidogyne* spp.) that cause global economic losses (Khalil *et al*., 2022; Forghani and Hajihassani, 2020). Certain practices were employed to manage root-knot nematodes (RKNs) such as organic soil amendments, plant extracts, plant resistant varieties, soil solarization and biological agents (Radwan *et al*., 2012; Renčo *et al*., 2014). Also, management practices included fumigant and non-fumigant nematicides (Abd El-Aziz and Khalil, 2020; Renčo *et al*., 2014). Using chemical nematicides (fumigant/non-fumigant) increase agricultural costs and contribute to environmental contamination (El-Marzoky *et al*., 2022). In Egypt, the availability of nematicides is very limited and costed, therefore, management of plant-parasitic nematodes is challenging and often requires complex practices (Haydock *et al*., 2013). Nevertheless, the search for new alternative nematicides is ongoing.

Spirodiclofen, spiromesifen and spirotetramat are the most recent class of acaricides/ insecticides which belong to the class of cyclic keto-enols and are used as a systemic insecticide which applied as plant foliage for crop protection. Spirotetramat (Movento®) is hydrolyzed within plants to the active enol form. This active form is able to be transported systemically in two ways through phloem and xylem (Fischer and Weib, 2008; Vermeer and Baur, 2008). In insects and mites, Spirotetramat-enol suppressed fatty acid biosynthesis by inhibiting the action of acetyl-CoA carboxylase (Brück *et al*., 2009; Nauen *et al*., 2008).

The first report on the nematicidal activity of spirotetramat was published by McKenry *et al*. (2009) who investigated the effect of spirotetramat against plant-parasitic nematode species infecting citrus, grape and walnut by foliar applications. Later, certain reports were published on various plant -parasitic nematodes namely; *Heterodera avenae*, *Pratylenchus vulnus*, *Mesocriconema xenoplax* and *Meloidogyne incognita* (Shirley *et al*., 2019; Vang *et al*., 2016; Smiley *et al*., 2011; McKenry *et al*., 2010). However, no reports have yet investigated the nematicidal impact of spirodiclofen or spiromesifen against plant-parasitic nematodes.

Materials and Methods

The tested pesticides

- Spirodiclofen (Concor® 24% SC, 3- (2, 4-dichlorophenyl) -2-oxo-1-oxaspiro [4.5] dec-3-en-4-yl 2,2-dimethylbutyrate)
- Spiromesifen (Oberon® 24% SC, 3-mesityl-2-oxo-1-oxaspiro[4.4]non-3-en-4-yl 3,3-dimethylbutyrate)
- Spirotetramat (Movento® 10% SC, Cis-4-(ethoxycarbonyloxy)-8-methoxy-3-(2,5-xylyl)-1-azaspiro [4.5] dec-3-en-2-one)
- Fosthiazate (Nemathorin® 10% G), [RS-S-sec-butyl O-ethyl 2-oxo-1, 3-thiazolidin-yl phosphonothioate; (RS)-3-[sec-butylthio(ethoxy) phosphinoyl]-1,3-thiazolidin-2-one).

The non-fumigant nematicide, fosthiazate used at the recommended rate of 12.5 kg/feddan. Meanwhile, spirodiclofen, spiromesifen and spirotetramat were tested at 4 L/feddan (960g a.i), 2 L/feddan (480g a.i) and 1 L/feddan (240g a.i). The evaluated pesticides were used as soil drench for one-time application.

Root-knot nematode (Meloidogyne incognita) inoculum

The inoculum of *M. incognita* was isolated from infected root of tomato plants (cv. Balady). The tomato roots were cut into small severs, then immersed in 5% sodium hypochlorite (NaOCl) and shacked for 3 min. The sieves of 200 and 400 mesh were used to separate the free eggs (Hussey and Barker, 1973). The obtained eggs were washed several times with water and counted under a stereo-microscope. The perennial patterns method of Taylor and Nelscher (1974) was used to discriminate the root-knot nematode species (*M. incognita*).

The laboratory assays

The nematicidal efficacy of namely, spirodiclofen, spiromesifen and spirotetramat was investigated against juveniles' mortality under laboratory conditions (27±2°C). Different serial concentrations from each compound were prepared and these concentrations were ranged from 200 to 3600 µg ml^{-1}. Each replicate (vial) included approximately 200 J_2s and vials included distilled water served as controls. After 48 h of exposure the dead and alive individuals of J_2 were recorded and the mortality percentages were calculated (Abbott formula, 1925). The toxicity index and relative potency were also calculated as followed:

- Toxicity index= LC_{50} of the most effective compound/ LC_{50} of the tested compound × 100. (Sun, 1950).
- Relative potency= LC_{50} of the least tested compound/LC_{50} of the most effective compound. (El-Sheikh and Aamir, 2011).

Pot trial

The performance of spirodiclofen, spiromesifen and spirotetramat was assessed against *M. incognita* on okra plants (cv. Balady). The microplots (pots) were 17.5 cm diameter filled with approximately 2 kg of autoclaved sandy soil. Each pot contained three okra seeds which were left to grow till plantlet stage, and then thinned to one plantlet. Four weeks later, the plantlets were inoculated with the isolated eggs of *M. incognita* in holes around the root system by 7000 eggs per pot. Ten treatments were implemented and two controls were used during the experiment; untreated control and untreated uninoculated control.

The pots were placed outdoors and the experimental conditions were 30 ± 2 °C, 72 ± 2 RH and photoperiod (D:L= 10:14 h). Irrigation and fertilization were done regularly. Seven weeks after inoculation, the plants were up-rooted to investigate okra growth indices such as; shoot fresh weight, root fresh weight, shoot height and root length. Moreover, the 2nd stage juveniles/250g soil, galls/ root system and egg masses/ root system were recorded. The 2nd stage juveniles were isolated with sieving and Baermann plate method according to Ayoub (1980). Meanwhile, Phloxine B stain (0.15 gl^{-1} water for approximately 15 minutes) was used on okra roots to facilitate egg masses counting according to Holbrook *et al.* (1983).

Statistical analysis and experimental design

In this study, the pot experiment was arranged in a complete randomized (CRD) design and the data were analyzed by using a computer Costat Program (2005) version 6.303. In the pot trial, each treatment was replicated five times. The analysis of variance (ANOVA) was used to compare the significant differences among means with the least significant differences (LSD) and *P*-values at 0.05 probabilities. For laboratory assay, each concentration was replicated four times and the probit analysis was used to calculate the LC_{50} of each compound according to Finney (1971).

Results

The mortality of second-stage juveniles (J_2s) of the root-knot nematode, *M. incognita* increased progressively with increasing concentrations of the tested compounds following 48 h of exposure (Figure 1). All evaluated treatments exhibited a clear rate-dependent nematicidal effect, although their efficacy varied significantly among compounds and concentrations. Among the tested compounds SPT (10% SC) consistently induced the highest juvenile mortality across all concentrations, reaching a maximum of 29.73% at 3600 µg ml^{-1}. SPD (24% SC) was ranked the second in effectiveness, achieving a maximum mortality of 26.00% at the highest concentration, whereas SPM (24% SC) exhibited comparatively lower nematicidal activity, with mortality values ranging from 2.94 to 22.22%. At lower concentrations (200 and 400 µg ml^{-1}), all compounds caused limited but statistically significant increases in juveniles' mortality. However, a pronounced increase in mortality was observed at higher concentrations (≥ 800 µg ml^{-1}), particularly SPT and SPD. The significant differences were detected among some concentrations within each compound.

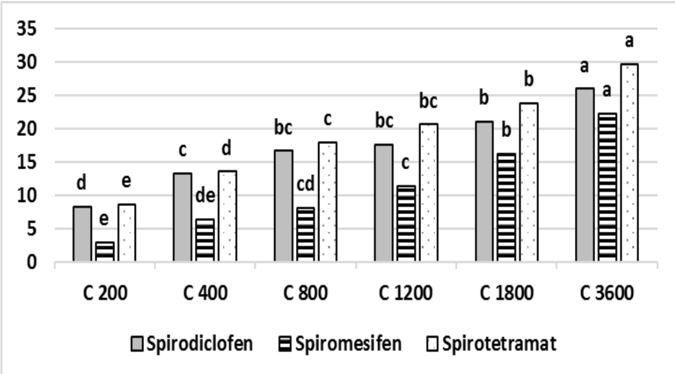

Figure 1: *Mortality (%) of 2nd stage juveniles (J_2s) of the root-knot nematode, Meloidogyne incognita after 48 h of exposure to different concentrations (C 200 to C 3600 µg ml^{-1}) of Spirodiclofen, Spiromesifen and Spirotetramate under laboratory conditions.*

The exposure (48 h) of *M. incognita* juveniles to spirodiclofen (SPD) and spiromesifen (SPM), in addition to spirotetramat (SPT) under laboratory conditions exhibited fair nematicidal activity (Table 1). According to the LC_{50} values, SPT and SPM exhibited the highest rates of J_2s mortality with LC_{50} values of 25766.70 and 26380.02 µg ml^{-1} after 48h of exposure. Meanwhile, the least toxicity was recorded with SPD by LC_{50} value of 57344.90 µg ml^{-1}. Based on the LC_{50} values the nematicidal toxicity in descending order was SPT > SPM > SPD.

In respect to toxicity index, SPT came in the 1st rank with 100% followed by SPM and SPD which came in 2nd and 3rd rank with values of 97.68 and 44.93%, respectively. Furthermore, the relative potency showed that the highest activities of SPT and SPM were almost the same with values of 1.00 and 1.02, while SPD was less effective with recorded value of 2.22.

The present study revealed that all tested compounds significantly suppressed root galls formation, soil populations and egg masses of root-knot nematode, *M. incognita* on okra plants under pot experimental conditions, compared with the untreated control. However, their nematicidal performance varied according to the active ingredient and application rate (Table 2).

All applied treatments caused statistically significant reductions in root galls, soil populations and egg masses showing a clear rate-response relationship, where higher application rate resulted in greater nematode suppression. Fosthiazate significantly reduced the root galls (90.14%), soil population (95.25 %) and egg masses (89.16%). The application of SPD, SPT and SPM suppressed okra root galls with general mean reductions of 56.28, 53.44 and 44.21%, respectively. In soil, the population of *M. incognita*, was minimized by 52.68, 47.96 and 46.37% with SPM, SPD and SPT, respectively. Meanwhile, the formed egg masses were reduced by 64.09, 59.08 and 50.50% with SPT, SPD and SPM, respectively. Generally, it was obvious that the high rate (4L/ feddan) recorded the highest reductions in root galls, soil population and egg masses, vice versa with the low rate (1L/feddan). No significant differences were noticed between the rates of 4L and 2L in galls and J_2s with SPD and SPM. Also, the high rate (4 L/ feddan) of SPT recorded the highest reductions in all recorded characterizations of root-knot nematode among estimated tetramic compounds.

The effects of SPD, SPM and SPT, in comparison with fosthiazate, on key growth parameters of okra plants namely: shoot fresh weight (SFW), root fresh weight (RFW), shoot height (SH) and root length (RL) of okra plants were assessed at the termination of the experiment (Table 3). The untreated uninoculated control exhibited substantial increases in all measured parameters, with SFW, RFW, SH and RL, rising by 271.15, 240.00, 58.14 and 132.16%, respectively.

Table 1: *Nematicidal activity of Spirodiclofen, Spiromesifen and Spirotetramate against J_2s of root-knot nematode (Meloidogyne incognita) after 48 h of exposure under laboratory conditions.*

Treatments	LC_{50} (µg ml^{-1})	Fiducial limits (Lower-Upper)	Slope ± SE	Toxicity index	Relative potency
Spiromesifen	26380.02	10255.42 – 237550	0.88 ± 0.18	97.68	1.02
Spirodiclofen	57344.90	12896.41 – 9254700	0.54 ± 0.15	44.93	2.22
Spirotetramat	25766.70	8654.30- 490720	0.62 ± 0.15	100.00	1.00

Table 2: *The nematicidal effect of Spirodiclofen, Spiromesifen and Spirotetramate on root galls, soil population (J_2s) and egg masses of root-knot nematode (Meloidogyne incognita) under pot trial on okra plants.*

Treatments	Doses per feddan ($4200 M^2$)	Galls / root system			J_2s/ 250g soil			Egg masses / root system		
		Mean	R %	GMR%	Mean	R %	GMR%	Mean	R %	GMR%
Spirodiclofen 24% SC	4 L (960g a.i/F)	99.33f	68.73	56.28	6440.00de	52.94	47.96	73.67ef	70.05	59.08
	2 L (480g a.i/F)	123.33ef	61.18		7220.00d	47.24		92.00d	62.60	
	1 L (240g a.i/F)	194.00bc	38.93		7703.33cd	43.70		136.33b	44.58	
Spiromesifen 24% SC	4 L (960g a.i/F)	146.00de	54.04	44.21	4406.50ef	67.80	52.68	100.50cd	59.08	50.50
	2 L (480g a.i/F)	172.00cd	45.86		5175.67def	62.24		110.50c	55.01	
	1 L (240g a.i/F)	213.67b	32.74		9850.00bc	28.01		154.00b	37.40	
Spirotetramat 10% SC	4 L (960g a.i/F)	95.67f	69.88	53.44	3823.33f	72.06	46.37	60.33f	75.47	64.09
	2 L (480g a.i/F)	151.33de	52.36		7526.67cd	44.99		87.67de	64.36	
	1 L (240g a.i/F)	196.67bc	38.09		10666.67b	22.05		117.00c	52.44	
Fosthiazate	12.5 k (1250g a.i/F)	31.33g	90.14	---	650.00g	95.25	---	26.67g	89.16	---
Untreated control	---	317.67a	0.00	---	13683.33a	0.00	---	246.00a	0.00	---

Within a column, numbers followed by different letter(s) are significantly different using LSD at p = 0.05, Means are the average of five replicates. R%= reduction percent, GMR%= general mean reduction percent.

Table 3: *The influence of Spirodiclofen, Spiromesifen and Spirotetramate on okra roots and shoot growth characteristics infected with root-knot nematode (Meloidogyne incognita) under pot trial.*

Treatments	Doses per feddan (4200 M²)	SFW			RFW			SH			RL		
		Mean	I%	GMI %	Mean	I%	GMI %	Mean	I%	GMI %	Mean	I%	GMI %
Spirodiclofen 24% SC	4 L (960g a.i/F)	48.33b	178.85	155.77	4.00cd	20.00	16.67	82.00abc	43.02	32.17	37.33bc	89.83	66.10
	2 L (480g a.i/F)	46.00bc	165.38		4.33cd	30.00		74.00cde	29.07		33.67cd	71.19	
	1 L (240g a.i/F)	38.67cd	123.08		3.33de	0.00		71.33def	24.42		27.00ef	37.29	
Spiromesifen 24% SC	4 L (960g a.i/F)	57.67a	232.69	162.18	7.33b	120.00	56.67	84.00ab	46.51	28.88	41.00ab	108.47	80.79
	2 L (480g a.i/F)	41.67bcd	140.38		5.00c	50.00		73.33cdef	27.91		33.33cd	69.49	
	1 L (240g a.i/F)	37.00d	113.46		3.33de	0.00		64.33fg	12.21		32.33cd	64.41	
Spirotetramat 10% SC	4 L (960g a.i/F)	42.00bcd	142.31	111.54	4.00cd	20.00	-6.67	77.00bcd	34.30	27.71	29.67de	50.85	33.90
	2 L (480g a.i/F)	34.33d	98.08		2.33e	-30.00		75.33bcde	31.40		25.00ef	27.12	
	1 L (240g a.i/F)	33.67d	94.23		3.00de	-10.00		67.33ef	17.44		24.33fg	23.73	
Fosthiazate	12.5 k (1050g a.i/F)	59.00a	240.38	---	7.67b	130.00	---	88.33a	54.07	---	41.67ab	111.86	---
Untreated control	-----	17.33e	0.00	---	3.33de	0.00	---	57.33g	0.00	---	19.67g	0.00	---
Untreated uninoculated control	-----	64.33a	271.15	---	11.33a	240.00	---	90.67a	58.14	---	45.67a	132.16	---

Within a column, numbers followed by different letter(s) are significantly different using LSD at p = 0.05, Means are the average of five replicates. SFW= shoot fresh weigh; RFW= root fresh weight; SH= shoot height and RL= root length. I%= increase percent, GMI%= general mean increase percent.

Fosthiazate treatment significantly enhanced SFW (240.38%), RFW (130.00%), SH (54.07%) and RL (111.86%) compared with the untreated control. Similarly, treatments with SPM, SPD and SPT led to pronounced improvements in SFW with GMI of 162.18, 155.77 and 111.54%, respectively. Regarding RFW, significant relative GMI values of 56.67 and 16.67% were recorded following SPM and SPD applications, respectively, whereas SPT treatment resulted in a slight reduction (6.67%). Using of SPD, SPM and SPT increased SH relatively by 32.17, 28.88 and 27.71%, consecutively. In addition, SPM, SPD and SPT markedly promoted RL with relative GMI values of 80.79, 66.10 and 33.90%, respectively. Overall, the highest application rate (4L/feddan) across all tested consistently produced the greatest improvements in all measured growth parameters. However, no statistically significant differences were detected among most of the treatments.

Discussion

The control of plant-parasitic nematodes is challenging and often requires integrated pest management strategies, including the use of synthetic nematicides (Haydock *et al.*, 2013). In the present study, the highest nematicidal efficacy was recorded with fosthiazate, an organophosphrus nematicide. This pronounced effect was attributed to its mode of action as an acetyl cholinesterase inhibitor, which disrupts the central nervous system of plant-parasitic nematodes at all developmental stages (Saad *et al.*, 2017). Certain reports indicated that fosthiazate has moderate to high nematicidal efficacy against root-knot nematodes (Saad *et al.*, 2017; Li *et al.*, 2020; Wang *et al.*, 2023). Attia and Nofel (2023) found that fosthiazate (Melotherin® 90 % EC), Salicylic acid and bacterium, *Serratia marcescens* were effective against *Meloidogyne incognita* infecting cucumber plants. Fosthiazate was the superior treatment which reduced galls, egg masses, soil population (J_2s), females and developmental stage by 86.15, 93.89, 79.49, 67.81 and 73.22%, respectively.

In previous studies, fosthiazate decreased the root-knot disease (*Meloidogyne* spp.) on Melon plants by 57.85 and 31.78% after 31 and 69 days of inoculation, respectively. These results clarified that fosthiazate had better control on short term than long term (Wang *et al.*, 2023). A strong nematicidal action of fosthiazate and fluopyram was recorded against the soil population of *Meloidogyne* spp. on guava trees.

Fluopyram recorded reduction at range of 71.75 to 77.85%, while fosthiazate gave reductions at range of 76.70 to 82.95% during two seasons (Massoud *et al*., 2021).

The derivatives of spirocyclic representing the most recently developed class of insecticides/ acaricides (Tian *et al*., 2021). These compounds act as acetyl-coenzyme A carboxylase inhibitor (IRAC, Group 23), disrupting lipid biosynthesis (Faraji *et al*., 2018; Pastor-Belda *et al*., 2015). Owing to their high efficacy, they have been extensively applied for the control of a broad spectrum of sucking insects in various fruit and vegetable crops such as, red spider, aphid and whitefly (Tian *et al*., 2021).

Spirotetramat, which has been reported in earlier studies to exhibit impacts against certain plant nematodes. It showed nematicidal activity against *Meloidogyne* spp, *Pratylenchus* spp., *Heterodera* spp., *Tylenchulus* spp. and *Rotylenchulus reniformis* (Waisen *et al*., 2019; Smiley *et al*., 2011). Also, it did not suppress root penetration by *R. reniformis*, while egg hatching and soil population (J_2s) were decreased significantly (Waisen *et al*., 2019).

Inside plant system, spirotetramat is hydrolyzed to enol form (spirotetramate-enol) and systemically translocated from leaf to root without causing any phytotoxic effects (Nauen *et al*., 2008). Moreover, the early developmental stages of *Heterodera schachtii* were suppressed on spirotetramat-treated plants significantly, and the produced females were noticeably smaller compared with those developing on untreated-inoculated plants (Gutbrod *et al*., 2020). However, Vang *et al*. (2016) reported that application of spirotetramat either prior or post inoculation with *Meloidogyne* spp. didn't result in a significant reduction in egg masses formation. These previously published findings were consistent with our results, which demonstrated that SPT exhibited limited efficacy outside the plant system. Accordingly, under *in vitro* conditions its nematicidal activity was relatively weak, with mortality percentage not exceeding 30%. The Plant- parasitic nematodes are depending on the stored lipids as a source of energy (Perry *et al*., 2013). Meanwhile, this keto-enol group was depending on inhibition in lipid biosynthesis that reduced lipid content, inhibited ecdysis, and reduced nematode fecundity and fertility (Faraji *et al*., 2018; Pastor-Bida *et al*., 2015; Nauen *et al*., 2008).

Notably, spirodiclofen and spiromesifen have not been previously evaluated for their nematicidal activity, in contrast to spirotetramate. Also, spiromesifen and spirodiclofen are recent commercialized as acaricides with a novel mode of action (lipid synthesis inhibitor), against all developmental stages and adult females of tetranychid mite species. They are showed no cross-resistance in high resistance strains to at least one of organophosphates, mitochondrial electron transport inhibitors (Khamis and Khalil, 2019). Also, spirodiclofen exhibited reductions against the immature stages of the two spotted spider mites *(Tetranychus urticae*) ranged from 61% to 91% and pyridaben decreased the adults by 41% to 64% and the immatures up to 67% on strawberries in the potting trial (Niu *et al*., 2016). Spirodiclofen and pyridaben are effective alternatives beside abamectin in the insecticides resistance management (IRM) program against the two spotted spider mites (Peshin *et al*., 2009). Kaur and Bhullar (2019) reported that spiromesifen was relatively less toxic than fenpyroximate against *Tetranychus urticae* on cucumber plants under protected cultivation in Punjab. Spiromesifen recorded LC_{50} values ranged from 1.88 to 16.05 ppm, while fenpyroximate recorded values ranged from 1.59 to 2.32 ppm. Application of spirodiclofen was succeeded to reduce the *T. urticae* in citrus orchards by more than 50% compared to the untreated plots (Assouguem *et al*., 2022).

Conclusions

The following conclusions can be drawn from this work: The spirotetramat product (Movento®) exhibited nematicidal efficacy and can be used as a nematicide, which could be shared in integrated pest management. According to our results using spirotetramat recorded satisfied results in the pot trial than both spirodiclofen and spiromesifen, however, more trials are needed to support these results.

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Novelty Statement

In the current study the authors tried to provide new members to the family of nematicides. The results exhibited that spirotetramat and spirodiclofen at the high rate of 4 L/feddan could be considered as promising nematicides against the disease of the root-knot nematode (*Meloidogyne incognita*).

Author's Contribution

Aida M. El-khouly: Plan the study conception and design, Data collection, Reviewed the manuscript and approved the final version.
Mohamed S. Khalil: Plan the study conception and design, Data analysis, Written the draft of manuscript, Reviewed the manuscript and approved the final version.
Shady Selim: Plan the study conception and design, Materials preparation, Reviewed the manuscript and approved the final version.

Generative AI or AI-assisted technology statement
The authors declare that no generative AI was used in the creation of this manuscript.

Conflict of interest
The authors have declared no conflict of interest.

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