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Investigating the *in vitro* and *in vivo* nematicidal performance of structurally related macrolides against the root-knot nematode, *Meloidogyne incognita*

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Summary Avermectins and spinosyns are structurally related natural products of microbial origin and belong to a new family of macrolides which are active against a vast array of invertebrate pests. In the present study, the effects of four members of macrolides; abamectin (ABM), emamectin benzoate (EMB), spinosad (SPI) and spinetoram (SPIT), on *Meloidogyne incognita* were investigated under *in vitro* and *in vivo* conditions. All compounds reduced egg hatching and led to high mortality of the nematode second-stage juveniles (J_2). ABM showed the maximum rate of egg hatching inhibition and J_2 mortality while SPIT recorded the minimum. All treatments reduced the number of galls, egg masses, eggs/egg mass in roots and J_2 in the soil when compared to the control. Based on the 10 folds of the 24 h-LC_{50} values of J_2 mortality *in vitro*, EMB and ABM exhibited higher percent reduction in galls (79.68 and 71.45%), egg masses (75.19 and 70.54%), eggs/ egg mass (60.49 and 40.91%) and J_2 in the soil (90.31 and 86.54%), respectively, compared to SPI and SPIT. Significant increase in tomato shoot height occurred in all biopesticides (10 folds) and SPIT (20 folds). SPI at 10 folds of the 24 h-LC_{50} values of J_2 mortality *in vitro,* significantly increased root length while ABM at 50 folds and SPIT at 20 folds decreased root length by 5.15% and 5.88%, respectively, compared to the untreated inoculated plants. In all treatments, the dry shoot and root weights increased, compared to the untreated control. Our findings suggest that these macrolides have the ability to regulate nematode population densities and may be an alternative to classical nematicides.

Additional keywords: avermectins, biopesticides, macrolides, nematicidal activity, root-knot nematodes, spinosyns

Introduction

Tomato (*Solanum lycopersicum* L.) is an important and vastly grown vegetable in Egypt and worldwide. However, its growth, yield and economic productivity are significantly reduced by pests and diseases. Plant parasitic nematodes (PPNs) are found to be the most common and destructive pests causing estimated crop losses of US $ 118 billion each year worldwide (Atkinson *et al.*, 2012). Among PPNs, *Meloidogyne* spp., root-knot nematodes, are harmful agricultural pests causing huge damage around the world (Sikora and Fernandez, 2005).

For sustainable tomato production, effective management of PPNs especially root-knot nematodes is essential. Several approaches are used to minimise PPNs in the field, including synthetic nematicides, resistant plant cultivars, botanical pesticides, antagonistic microorganisms (e.g. fungi and bacteria), beneficial fungi (Mycorrhiza), organic amendments, soil solarization and plant extracts (Collange *et al.*, 2011; D'Addabbo *et al.,* 2011; Radwan *et al.*, 2012; Saad *et al.*, 2017). Farmers rely mainly on the application of synthetic nematicides rather than on other approaches. However, lately many of these chemicals are being withdrawn from the markets due to environmental health and safety concerns (Rich *et al.,* 2004). This highlights the need for devel-

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oping environmentally safer, target-specific ways of controlling these parasites.

To date, there is an increasing interest towards the utilisation of microorganisms as biocontrol agents in sustainable agriculture as an alternative to synthetic pesticides for controlling various crop pests and diseases, as well as improving crop yield. These microorganisms produce a great variety of structurally unique bioactive secondary metabolites. For example, Actinomycetes, which are found in soil and aquatic habitats produce more than 10,000 such active compounds. Among the bacteria used as microbial antagonists, Actinobacteria, especially *Streptomyces* spp., display activity against PPN by generating nematicidal metabolites (Mishra *et al.,* 1987; Sun *et al*., 2006) and chitinolytic enzymes (Barka *et al.,* 2016).

Avermectins, a new class of 16-membered macrocyclic lactones, have four pairs of homologue compounds, *i.e.* four major components $A1_a$, $A2_a$, $B1_a$ and $B2_a$, and four minor components $A1_b$, $A2_b$, $B1_b$ and $B2_b$. Avermectins have been isolated from the crude fermentation product of *Streptomyces avermitilis* (Faske and Starr, 2007), and proved to possess a broad spectrum of pesticidal effects such as insecticidal, acaricidal, nematicidal and anthelmintic activities (Jansson and Dybas, 1998).

ABM, a blend of avermectins $B1_a$ (<80%) and $B1_b$ (>20%) with identical biological and toxicological properties (Pitterna *et al*., 2009), has nematicidal effects against root-knot and other nematode genera against several crops (Faske and Starr, 2007; Saad *et al.,* 2017). On the other hand, EMB, a second generation avermectin derivative, is being developed for control of insect pests on different vegetable crops worldwide (Jansson and Dybas, 1998). It is structurally related to ABM having higher insecticidal action than ABM. It is also effective against root-knot nematodes (Rehman *et al*., 2009).

Spinosyns are novel macrolides, natural metabolites produced under aerial fermentation conditions by the soil actinomycete *Saccharopolyspora spinosa*. This Gram-positive bacterium produces SPI, a natural pesticide which is a mixture of spinosyn A and spinosyn D (85:15), that was reported to be an effective pest control agent with low toxicity to humans and the environment (Sparks *et al.,* 1996).

SPI is toxicologically classified by the U.S. Environmental Protection Agency as a reduced risk material. SPIT is an analogue to SPI that belongs to spinosyns and it is a mixture of chemically modified spinosyns J and L. These molecules were found to have a wide spectrum of insect control potential on a variety of crops with high residual action (Huang *et al*., 2009).

Although the interest in avermectins, as one class of macrocyclic lactones (MLs) for nematicidal use, is increasing there is scarce information in the literature about the effectiveness of MLs compounds against root-knot nematodes. This encouraged us to continue investigating this group of chemicals for root-knot nematodes management. Therefore, the main goals of the present study were to assess the *in vitro* nematicidal potential of the structurally related macrolides; ABM, EMB, SPI and SPIT against *Meloidogyne incognita*. An *in vivo* pot trial was also undertaken to investigate their efficacy against the nematode on tomato under greenhouse conditions.

Materials and Methods

Macrocyclic lactones and a standard nematicide

ABM (Tervigo® 2% SC) and EMB (Proclaim® 5 % WG) were supplied by Syngenta, Egypt and SPI (Tracer® 24 % SC) and SPIT (Radient® 12 % SC) by Dow AgroSciences, Egypt and the standard nematicide oxamyl (Vydate24% SL) was supplied by E. I. du Pont de Nemours & Company Inc. was used for comparison.

Root-knot nematode inocula

A single egg mass was excised from the roots of an infested eggplant (*Solanum melongena* cv. Black Beauty) and a pure culture of the root knot nematode isolate was propagated on the roots of tomato (*S. lycopersicum*

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cv. Golden Stone) under greenhouse conditions. The population was eventually identified as *Meloidogyne incognita*, according to Taylor and Nelscher (1974) using perineal patterns. During the course of this study, eggs were being extracted from infected roots with sodium hypochlorite (NaOCl) according to Hussey and Barker (1973) and second stage juveniles (J_2) obtained using the Baermann plate technique (Ayoub, 1980).

In vitro assays

The assessment of the effect of ABM, EMB, SPI and SPIT on hatching and mortality of *M. incognita* J_2 was carried out *in vitro*. Preliminary experiments were conducted to establish the effective range of concentrations of the chemicals.

Hatching assays

The concentrations of each chemical tested during this study were as follows: for ABM and EMB, 25, 50, 100, 200 and 400 mg/l; for SPI, 250, 500, 1000, 2000 and 3000 mg/l and for SPIT, 250, 500, 1000, 1500 and 2000 mg/l. Vials (each one ca. 15 ml) containing distilled water served as control. Each treatment was replicated four times and each replicate involved approximately 1200 eggs. The numbers of J_2, hatched from eggs, were recorded at 3 and 7 days after application.

Mortality assays

The concentrations of each chemical tested during this study were as follows: for ABM, 12.5, 25, 50, 75 and 100 mg/l; for EMB, 25, 50, 75, 100 and 200 mg/l; for SPI, 250, 500, 1000, 1500 and 3000 mg/l and for SPIT, 250, 500, 1000, 1500 and 2000 mg/l. Each treatment was replicated four times including distilled water as a control and each replicate involved 200 J_2. The numbers of both dead and alive J_2 were recorded after 24 and 48 h exposure and the mortality percentages was estimated.

Pot assay

The nematicidal performance of ABM, EMB, SPI and SPIT was tested on tomato plants infested with *M. incognita*. Pots with capacity of one kg soil were filled with autoclaved loamy sand soil. ABM and EMB were applied as a soil drench at the rate of 10 and 50 folds of their LC_{50}'s values based on J_2 mortality test after 24 h exposure, while SPI and SPIT were applied at the rate of 10 and 20 folds of their LC_{50}'s values after 24 h exposure. Oxamyl was used as a standard nematicide.

One one-month old tomato seedling cv. HERMIS was transplanted in each pot, and three days later inoculated with 5000 eggs. Untreated uninoculated and untreated inoculated plants served as controls. All treatments were replicated five times and arranged in a complete randomized design on a bench in a greenhouse (28 ± 2°C, 65 ± 2 RH and 12: 12 L:D photoperiod). During the course of the experiment, irrigation and fertilization were applied when appropriate. Fifty days after the inoculation, the plants were removed and washed free of soil. Shoot height and dry weight, root length and dry weight were measured and number of galls/root system, egg-masses/root system, eggs/egg-mass and J_2/250g soil were estimated. J_2s were extracted as previously mentioned and Phloxine B was used to stain the roots to facilitate egg mass counting (Holbrook *et al*., 1983).

Statistical analysis

The statistical analysis of data was carried out using a computer Costat program (2005) version 6.303. Statistically significant differences between the means were compared using analysis of variance (ANOVA) with the least significant differences (LSD) and *P*-values at 0.05 probability. Hatching and J_2 mortality percentages were estimated using the Abbott formula (1925), and Probit analysis was used to calculate LC_{50} for each compound according to Finney (1971).

Results

Impact of test compounds on egg hatching and J_2 mortality of *M. incognita* under laboratory conditions

The egg hatching inhibition rate (%) un-

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der laboratory conditions, due to exposure to the tested bioproducts after two time intervals is illustrated in Fig. (1). Hatching was inversely proportional to the concentration of the bioproducts. After 3 and 7 days exposure, the most effective compounds causing hatching reduction were ABM (96.32 and 85.41%, respectively) and EMB (88.55% and 71.23%, respectively) at 400 mg/l. At 2000 mg/l, hatching inhibition was 73.83% and 69.40% for SPI and 77.72% and 73.35% for SPIT (Fig. 1). LC_{50} values on hatching inhibition after 3 and 7 days exposure were respectively, for ABM 24.61 mg/l and 46.89 mg/l, for EMB 47.97 mg/l and 83.09 mg/l, for SPI 629.53 mg/l and 781.52 mg/l and for SPIT, 487.46 mg/l and 635.66 mg/l (Table 1).

J_2 mortality increased by increasing compound concentration and exposure time, whereas no mortality occurred in the controls. After 24 and 48 h exposure, J_2 mortality for ABM at 100 mg/l was 73.01% and 86.00%, respectively, and for EMB 51.43% and 63.08%, respectively. SPI at 1500 mg/l caused a 45.22% and 50.66% mortality, while SPIT 32.86% and 42.03%, respectively. This indicates that there is a marked increase in J_2 mortality caused by ABM over EMB and by SPI over SPIT (Fig. 2). Probit analysis of these results indicates that, after 24 h exposure, ABM was the most toxic compound against J_2 (LC_{50} = 36.64 mg/ml) followed by EMB, SPI and SPIT. LC_{50} values after 48 h exposure were 22.89, 79.03, 1611.27 and 2355.52 mg/l for ABM, EMB, SPI and SPIT, respectively. In general, these compounds could be arranged according to their effectiveness on J_2 mortality as follow: ABM > EMB > SPI > SPIT (Table 1).

Effect of test compounds against *M. incognita* at pot assay

All treatments showed differential nematicidal properties when compared to the untreated inoculated control. Gall formation was significantly suppressed by EMB, ABM, SPI and SPIT with reductions of 71.65, 69.46, 64.54 and 64.01%, respectively. However, no significant differences were observed between ABM and EMB and between SPI and SPIT (Table 2). Except for EMB, no significant differences were observed between the lower and the higher rates of ABM, SPI and SPIT. The same trend was exhibited with respect to egg masses/root system. EMB was the most effective followed by ABM, SPI and SPIT, reducing egg masses by 76.28, 74.57, 56.20 and 51.24%, respectively. No significant differences were detected between the lower and higher rates of all treatments. With respect to the number of eggs/egg mass, EMB, SPI, ABM and SPIT recorded reductions of 61.71, 54.08, 52.34 and 45.61%, respectively. The application of EMB, ABM, SPI and SPIT suppressed population density in soil by 91.82, 89.26, 74.33 and 72.64%, respectively, compared to the control. No significant differences were observed between the lower and the higher rates of all applied treatments (Table 2).

The effect of ABM, EMB, SPI and SPIT as a soil drench on the shoots and roots of the tomato seedlings is shown in Table 3. Shoot height increased in all the treated plants by 23.35% to 48.24%. The maximum increase was observed in plants treated with SPIT, followed by SPI, EMB and ABM. No significant differences were observed between the lower and the higher rates of all treatments. Noticeable increases were also recorded in the mean root length of plants treated with SPI, SPIT and EMB, i.e. 19.85%, 8.82% and 4.41%, respectively, whereas ABM reduced root length by 4.78%. Noticeably, the higher rate of SPIT exhibited a root length reduction by 5.88% (Table 3).

Regarding dry shoot weight, data indicate an increase as compared to the control; the highest dry weight was observed with SPI (43.46%), followed by SPIT (34.11%), ABM (16.93%) and EMB (16.54%). ABM at the lower rate (10 folds) decreased dry shoot weight by 8.97%. Plants treated with ABM showed significant differences between the lower and the higher rates, whereas no significant differences were found between the lower and the higher rates of EMB, SPI and SPIT (Table 4). All treatments recorded an increase in dry root weight over the untreated inoculated control. Such increase was min-

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imum (14.26%) in plants treated with ABM, while treatment with SPI induced the maximum increase (74.26%) over the control (Table 4).

Discussion

The present investigation revealed that the tested MLs compounds possess nematicidal properties against *M. incognita* under laboratory and greenhouse conditions with the following descending order ABM >EMB >SPI> SPIT. The findings of the present *in vitro* studies are in conformity with previous studies in which ABM nematode toxicity was higher than that of EMB. ABM was more effective than EMB on hatching inhibition and juveniles mortality of *M. incognita* in laboratory tests (Ullah *et al*., 2015). ABM has been found more toxic than EMB with respect to the number of galls and egg masses in roots, with 61.77 and 78.82%, and 43.75 and 56.41% reductions, respectively (Shahid *et al*., 2009). d'Errico *et al.* (2017) reported that Tervigo® (ABM 2% SC) and two other formulations, CHA 2061-02 EW and CHA 2080 SC, showed a nematostatic activity against *M. incognita* J_2 *in vitro*, where after exposure to these products, J_2 were immobilized and subsequently resumed mobility over time following a recovery test. AVM B_1 when used at 10 and 100 mg/l completely inhibited egg hatchability of *Meloidogyne arenaria* Chitwood *in vitro* (Cayrol *et al.,* 1993). Avicta® containing ABM reduced hatching and increased *M. javanica* J_2 mortality *in vitro*. In addition to the nematostatic effect, Avicta® possessed a nematicidal effect (Almeida *et al.,* 2017). However, while studying the toxicity of EMB and ABM to *M. incognita* juveniles in the laboratory, Ding *et al.* (2009) reported that the toxicity of EMB was found higher than that of ABM, their LC_{50} being 0.1645 and 0.4532 mg/l, respectively. Also, EMB was highly toxic to *M. incognita* juveniles with LC_{50} and LC_{90} values of 3.59 and 18.20 mg/L after 48 h of exposure, respectively (Cheng *et al.,* 2015).

Indeed, avermectins have already been proven nematicidal and effective in reducing nematode populations both in soil and the roots of infested plants. Regardless the method of application, our findings confirmed published reports in which ABM was effective against root-knot nematodes on cotton (Faske and Starr, 2007), tomato (Qiao *et al,* 2012; Ullah *et al.,* 2015; Saad *et al.,* 2017) and cucumber (Huang *et al.,* 2014). Nursery bed soil drenching with EMB 1.9 % WP at 285.0 g a.i./ha before or after sowing, induced high reduction of the J_2 population in the soil as well as of the number of females per g root (Das *et al.,* 2014).

SPIT is often more potent, faster-acting, and longer-lasting than SPI as an insecticide (Sparks *et al.,* 2008; Dripps *et al*., 2011). In the present study, spinosyn compounds displayed satisfactory results regarding the nematicidal activity against *M. incognita*, both under *in vitro* and *in vivo* conditions. However, their nematicidal efficacy was lower than that of the avermectin compounds. To our knowledge, the potency of spinosyn against PPNs has not been reported yet, except for the effect of SPI as a nematicide recorded by Khalil (2013) where Tracer® 24% SC at 0.5 and 0.1% reduced *M. incognita* populations by 70.90 and 62.51%, respectively.

The increase in plant growth parameters, such as shoot height, root length and dry weight of either shoots and roots suggests that the treatments tested during this study have a good potential nematicidal effect on the root knot nematode *M. incognita*, which can result in effective plant protection. The obtained results are consistent with the earlier report by Ding *et al.* (2009) that proved the effectiveness of EMB in improving plant growth of tomato. Such improvement in plant growth is possibly due to the reduction in PPN populations. Our findings are also in agreement with the data of Khalil (2012) and Saad *et al.* (2017), who found that ABM when applied against *M. incognita* infesting tomato plants, increased all plant growth parameters. Moreover, ABM enhanced cucumber plant vigor and fruit yield (Huang *et al.*, 2014). However, Khalil (2013) found that SPI at 0.1% reduced the fresh weight of roots by 20.69% when ap-

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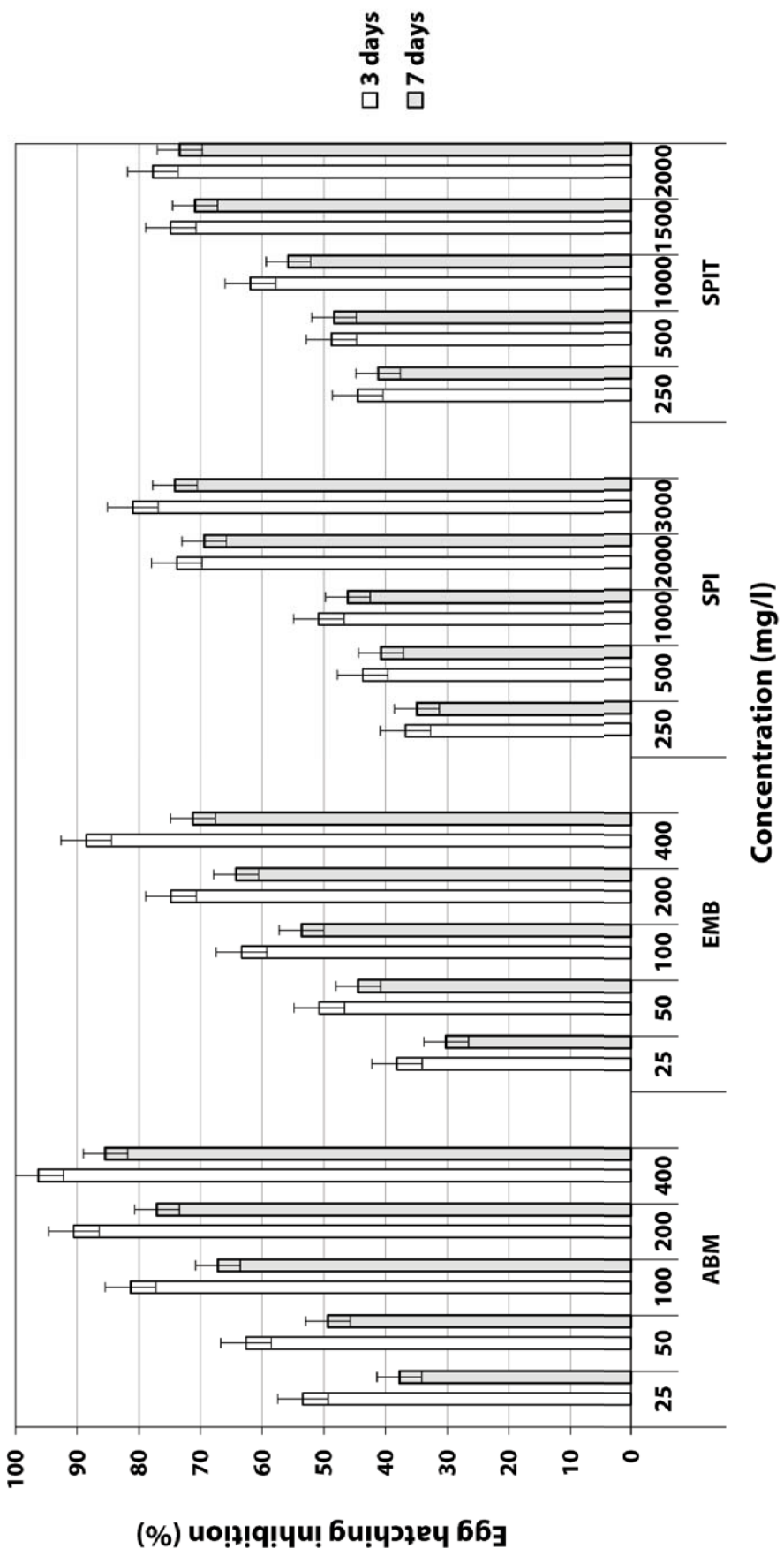

Figure 1. Egg hatching inhibition (mean of 4 replications) of *Meloidogyne incognita* exposed to different concentrations of abamectin (ABM), emamectin (EMB), spinosad (SPI) and spinetoram (SPIT) after 3 and 7 days of exposure under laboratory conditions.

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Table 1. Effect of abamectin (ABM), emamectin (EMB), spinosad (SPI) and spinetoram (SPIT) on egg hatching inhibition and juvenile mortality of the root-knot nematode *Meloidogyne incognita* at two time intervals.

Treatments	Exposure times							
	Egg hatching inhibition (days)				J_2 Juvenile mortality (hrs)			
	3 days				24 hrs			
	LC_{50} (mg/l)	Fiducial Limits (Lower – Upper)	Slop ± variance	Regression equation	LC_{50} (mg/l)	Fiducial Limits (Lower – Upper)	Slop ± variance	Regression equation
ABM	24.61	(21.18 - 28.57)	1.43 ± 0.006	Y = -1.99 + 143 X	36.64	(31.50 - 42.60)	1.28 ± 0.01	Y = -2.00 + 1.28 X
EMB	47.97	(41.31- 55.67)	1.19 ± 0.006	Y = - 2.00 + 1.19 X	111.62	(94.98 - 131.28)	1.41 ± 0.02	Y = -2.88 + 1.41X
SPI	487.46	(425.95 - 557.70)	1.23 ± 0.008	Y = -3.40 + 1.22 X	2558.07	(1966.31 - 3331.8)	1.12 ± 0.01	Y = -3.82 + 1.12 X
SPIT	629.53	(563.36 - 703.37)	1.14 ± 0.004	Y = -3.19 + 1.14 X	3077.10	(2099.84 - 4518.96)	1.02 ± 0.01	Y = -3.57 + 1.02 X
	7 days				48 hrs			
ABM	46.89	(41.77 - 52.62)	1.17 ± 0.003	Y = -1.95 + 1.17 X	22.89	(19.21 - 27.22)	1.36 ± 0.01	Y = -1.85 + 1.36 X
EMB	83.09	(72.16 - 95.65)	0.88 ± 0.004	Y = - 1.70 + 0.88 X	79.03	(69.13 - 90.35)	1.5 ± 0.02	Y = -2.84 + 1.50 X
SPI	635.66	(566.50 - 713.17)	1.17 ± 0.007	Y = -2.91 + 1.01 X	1611.27	(1296.81 -2003.44)	1.04 ± 0.01	Y = -3.33 + 1.04 X
SPIT	781.52	(700.45 - 871.89)	1.01 ± 0.003	Y = -2.88 + 1.08 X	2355.52	(1738.75 - 3195.16)	1.09 ± 0.01	Y = -3.69 + 1.09 X

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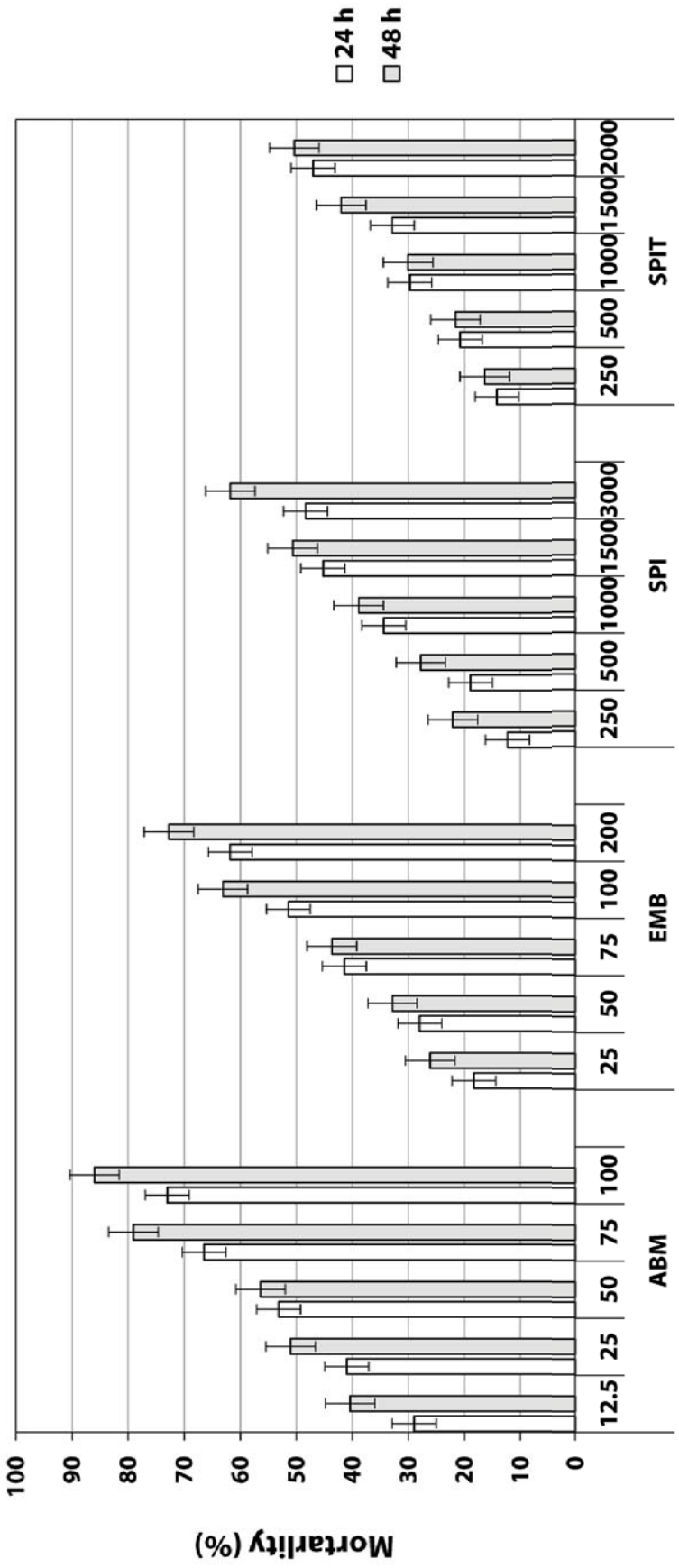

Figure 2. Mortality percentage (mean of 4 replications) of *Meloidogyne incognita* J_2 exposed to different concentrations of abamectin (ABM), emamectin (EMB), spinosad (SPI) and spinetoram (SPIT) at two time intervals under laboratory conditions.

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Table 2. Effect of abamectin (ABM), emamectin (EMB), spinosad (SPI) and spinetoram (SPIT) on galls, egg masses, eggs/mass and juveniles of the root-knot nematode *Meloidogyne incognita,* on tomato.

Treatments 24 h-LC_{50} (mg/l) of J_2	Rate/kg soil	Galls			Egg masses			Eggs/ Mass			Juveniles / 250g soil		
		Mean ± SE	[b]R (%)	[c]MR (%)	Mean± SE	R (%)	MR (%)	Mean ± SE	R (%)	MR (%)	Mean ± SE	R (%)	MR (%)
Untreated control	-	150.60 ± 6.75 [a]	-	-	129.00 ± 7.24[a]	-	-	676.77± 17.99[a]	-	-	897.80 ± 26.31[a]	-	-
ABM (36.64 mg/l)	[a]10 fold	43.00 ±5.08[cd]	71.45	69.46	38.00 ±4.16[c]	70.54	74.57	399.90± 15.45[b]	40.91	52.34	120.80± 9.07[d]	86.54	89.26
	50 fold	49.00 ± 4.70[bc]	67.46		27.60± 3.31[c]	78.60		245.23 ± 11.69[g]	63.76		72.00± 9.56[de]	91.98	
EMB (111.62 mg/l)	10 fold	30.60± 3.43[de]	79.68	71.65	32.00 ± 3.01[c]	75.19	76.28	267.40 ± 9.66[efg]	60.49	61.71	87.00 ±9.05[de]	90.31	91.81
	50 fold	54.80 ± 5.08[bc]	63.61		29.20±3.50[c]	77.36		250.93 ± 8.45[fg]	62.92		60.00 ±6.27[e]	93.32	
SPI (2558.07 mg/l)	10 fold	59.20 ± 5.62 [bc]	60.69	64.54	57.60 ±5.45[b]	55.35	56.20	327.20 ± 11.38[cd]	51.65	54.07	244.60± 10.65[b]	72.76	74.33
	20 fold	47.60 ± 4.02[bcd]	68.39		55.40 ±4.83[b]	57.05		294.42 ± 9.48[def]	56.50		216.40± 10.07[bc]	75.90	
SPIT (3077.10 mg/l)	10 fold	61.80 ± 3.79[b]	58.96	64.01	70.00 ±5.30[b]	45.74	51.24	437.20 ± 11.31[b]	35.40	45.61	256.20 ±10.96[b]	71.46	72.64
	(20 fold	46.60 ± 3.58[bcd]	69.06		55.80 ±5.81[b]	56.74		298.97 ± 12.11[de]	55.82		235.00 ± 8.20[bc]	73.82	
Oxamyl	0.05 ml	23.60 ± 2.87[e]	84.33	84.33	25.40± 3.60[c]	80.31	80.31	347.63 ± 14.67[c]	48.63	48.63	180.60 ±15.00[c]	79.88	79.88
$LSD_{0.05}$		17.22	-	-	15.35	-	-	44.64	-	-	58.83	-	-

Values are means of five replicates ± SE. Values in each column followed by the same letter(s) are not significantly different according to LSD (p = 0.05)
[a]10, 20 and 50 fold were calculated based on the 24 h-LC_{50}value (mg/l) of J_2 juvenile mortality *in vitro*, [b]R(%): Reduction percentage, [c]MR(%): The average reduction percentage.

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Table 3. Impact of abamectin (ABM), emamectin (EMB), spinosad (SPI) and spinetoram (SPIT) on shoot height and root length of tomato plants infected with the root-knot nematode *Meloidogyne incognita*.

Treatments 24 h-LC_{50} (mg/l) of J_2	Rate/kg soil	Shoot height (cm)			Root length (cm)		
		Mean ± SE	Increase (%)	[b]MI (%)	Mean ± SE	Increase (%)	MI (%)
Untreated inoculated plants	-	22.70± 0.95^{d}	-	-	13.60± 0.82^{de}	-	-
Untreated uninoculated plants	-	30.10±1.30^{abc}	32.60	32.60	23.20±1.31^{a}	70.59	70.59
ABM (36.64 mg/l)	[a]10 fold	31.50± 1.23^{abc}	38.77	23.35	13.00± 0.95^{e}	-4.41	-4.78
	50 fold	24.50± 1.20^{cd}	7.93		12.90±1.18^{e}	-5.15	
EMB (111.62 mg/l)	10 fold	31.50±1.31^{abc}	38.77	29.74	13.60± 0.99^{de}	0.00	4.41
	50 fold	27.40± 1.26^{bcd}	20.70		14.80±1.00^{cde}	8.82	
SPI (2558.07 mg/l)	10 fold	32.70± 1.29^{ab}	44.05	37.22	17.70±0.95^{bc}	30.15	19.85
	20 fold	29.60± 1.08^{abcd}	30.40		14.90± 0.70^{cde}	9.56	
SPIT (3077.10 mg/l)	10 fold	36.30± 1.28^{a}	59.91	48.24	16.80± 0.73^{bcd}	23.53	8.82
	20 fold	31.00±0.98^{abc}	36.56		12.80± 0.54^{e}	-5.88	
Oxamyl	0.05 ml	29.70±1.29^{abcd}	30.84	30.84	20.00± 1.32^{ab}	47.06	47.06
$LSD_{0.05}$		7.00	-	-	3.45	-	-

Values are means of five replicates ± SE. Values in each column followed by the same letter(s) are not significantly different according to LSD (p = 0.05)
[a]10, 20 and 50 fold were calculated based on the 24 h-LC_{50} value (mg/l) of J_2 juvenile mortality *in vitro*, [b]MI(%)= average increase percentage.

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Table 4. Dry weight of shoot and root system of tomato plants infected with the root-knot nematode *Meloidogyne incognita*, after treatment with abamectin (ABM), emamectin (EMB), spinosad (SPI) and spinetoram (SPIT).

Treatments 24 h-LC_{50} (mg/l) of J_2	Rate/kg soil	Shoot dry weight (g)			Root dry weight (g)		
		Mean ± SE	Increase (%)	[b]MI (%)	Mean ± SE	Increase (%)	MI (%)
Untreated inoculated plants	-	0.78± 0.07^{cd}	-	-	0.47±0.03^{d}	-	-
Untreated uninoculated plants	-	1.02±0.04^{abc}	30.77	30.77	0.57± 0.08^{bcd}	20.43	20.43
ABM (36.64 mg/l)	[a]10 fold	0.71±0.05^{d}	-8.97	16.92	0.48± 0.04^{d}	1.28	14.26
	50 fold	1.11±0.09^{ab}	42.82		0.60± 0.05^{bcd}	27.23	
EMB (111.62 mg/l)	10 fold	0.84±0.07^{cd}	7.18	16.54	0.74± 0.10^{abc}	58.30	66.38
	50 fold	0.98±0.06^{abc}	25.90		0.82±0.08^{a}	74.47	
SPI (2558.07 mg/l)	10 fold	1.14±0.15^{ab}	45.64	43.46	0.72±0.08^{abc}	54.04	74.26
	20 fold	1.10±0.05^{ab}	41.28		0.91±0.05^{a}	94.47	
SPIT (3077.10 mg/l)	10 fold	1.17± 0.12^{ab}	50.26	34.10	0.78±0.03^{ab}	65.11	40.21
	20 fold	0.92± 0.05^{bcd}	17.95		0.54±0.06^{cd}	15.32	
Oxamyl	0.05 ml	1.21±0.07^{a}	54.62	54.62	0.76±0.08^{ab}	61.70	61.70
$LSD_{0.05}$		0.26		-	0.21	-	-

Values are means of five replicates ± SE. Values in each column followed by the same letter(s) are not significantly different according to LSD (p = 0.05).
[a]10, 20 and 50 fold were calculated based on the 24 h-LC_{50} value (mg/l) of J_2 juvenile mortality *in vitro*, [b]MI(%)= average increase percentage.

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plied against *M. incognita* on tomatoes. The decrease in some plant growth parameters in the present study may be attributed to phytotoxicity.

Overall, the tested avermectins and spinosins can be considered as interesting alternative tools for the management of the root-knot nematode, *M. incognita*, being compounds with a good nematicidal potential, which have different mode of action to the available nematicides (Salgado, 1998; Bloomquist, 2003; Watson *et al*., 2010).

Conclusions

The current study provides evidence that the structurally related macrocyclic lactone compounds ABM, EMB, SPI and SPIT have a good potential to control the population of the root-knot nematode, *M. incognita*, by reducing hatching and increasing J_2 mortality *in vitro*. Also, soil drenching with these compounds significantly reduced the reproduction of *M. incognita* and consequently enhanced tomato growth characters. ABM and EMB as members of avermectins had greater efficacy on the *M. incognita* than SPI and SPIT as members of spinosyns. In general, the tested compounds are promising alternatives (bionematicides) to the classical nematicides for the control of root-knot nematodes in tomato production. Nevertheless, further research is required to assess the nematicidal properties of these compounds under field conditions. Furthermore, future research can extend to designing new controlled release formulations based on a nano-delivery system, which would enhance their efficacy and expand their use in the area of PPN management.

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Διερεύνηση της *in vitro* και *in vivo* νηματωδοκτόνου δράσης δομικά συγγενών δραστικών ουσιών της Ομάδας των μακρολιδίων έναντι του κομβονηματώδη *Meloidogyne incognita*

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Περίληψη Οι αβερμεκτίνες και οι σπινοσίνες είναι δομικά συγγενή φυσικά προϊόντα μικροβιακής προέλευσης και ανήκουν σε μια νέα οικογένεια μακρολιδίων με δράση έναντι ενός μεγάλου εύρους ασπόνδυλων φυτοπαρασίτων. Στην παρούσα μελέτη διερευνήθηκε, σε συνθήκες *in vitro* και *in vivo*, η επίδραση τεσσάρων τέτοιων δραστικών, της αβαμεκτίνης (ABM), της βενζοϊκής εμαμεκτίνης (EMB), του spinosad (SPI) και του spinetoram (SPIT), στον κομβονηματώδη *Meloidogyne incognita*. Όλες οι ενώσεις μείωσαν την εκκόλαψη ωών και οδήγησαν σε υψηλή θνησιμότητα των προνυμφών 2ου σταδίου (J_2) του νηματώδη. Η αβαμεκτίνη κατέδειξε τα μεγαλύτερα ποσοστά αναστολής εκκόλαψης ωών και θνησιμότητας προνυμφών J_2, ενώ το SPIT κατέγραψε το μικρότερο ποσοστό. Όλες οι επεμβάσεις μείωσαν τον αριθμό όγκων (κόμβων), ωών, ωών/ ωόσακους στις ρίζες και προνυμφών J_2 στο έδαφος, σε σύγκριση με το μάρτυρα. Η βενζοϊκή εμαμεκτίνη και η αβαμεκτίνη, σε συγκέντρωση δεκαπλάσια της τιμής LC_{50} για τη θνησιμότητα των προνυμφών J_2 *in vitro* στις 24 ώρες, εμφάνισαν υψηλότερη ποσοστιαία μείωση κόμβων (79,68 και 71,45%), ωόσακων (75,19 και 70,54%), ωών/ ωόσακους (40,91%) και προνυμφών J_2 στο έδαφος (90,31 και 86,54%), αντίστοιχα, σε σύγκριση με το SPI και το SPIT. Επίσης, παρατηρήθηκε σημαντική αύξηση στο ύψος των βλαστών της τομάτας σε όλες τις δραστικές ουσίες (Χ 10 φορές) και στο SPIT (Χ 20 φορές). Το μήκος της ρίζας αυξήθηκε σημαντικά από το SPI σε 10 πλάσια συγκέντρωση της τιμής LC_{50} για τη θνησιμότητα των προνυμφών J_2 *in vitro* στις 24 ώρες ενώ μειώθηκε από την αβαμεκτίνη σε 50 πλάσια συγκέντρωση και το SPIT σε 20 πλάσια συγκέντρωση, κατά 5,15% και 5,88% αντίστοιχα, σε σχέση με τα φυτά του μάρτυρα (χωρίς επέμβαση). Το ξηρό βάρος βλαστών και ριζών αυξήθηκε σε όλες τις επεμβάσεις σε σύγκριση με το μάρτυρα. Τα ευρήματα υποδεικνύουν ότι οι ενώσεις αυτές έχουν την ικανότητα να ρυθμίζουν την πληθυσμιακή πυκνότητα των νηματωδών και μπορεί να αποτελέσουν μια εναλλακτική λύση έναντι των κλασικών νηματοδοκτόνων.

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