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Pathogenicity and control of *Meloidogyne* spp. on some spinach, Swiss chard, and table beet plant cultivars

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Abstract

The pathogenicity of the root-knot nematodes *Meloidogyne arenaria*, *M. incognita* and *M. javanica* on spinach cvs. 'Balady', 'Barkly', 'Orient', 'Pacific' and 'Solanicy', Swiss chard cvs. 'Balady' and 'Ford Hook', and table beet cvs. 'Asgro Wonder', 'Balady', 'Detroit Dark' and 'Detroit Spainal' was determined in several greenhouse tests. The results show that the tested chenopodiaceous plant cultivars were either susceptible or highly susceptible to the tested root-knot nematode species except spinach cv. 'Pacific' and table beet cv. 'Asgro Wonder' which exhibited moderately susceptible reaction to *M. arenaria* and *M. javanica*. In a separate control experiment, the pathogenicity of *M. incognita* on spinach cv. 'Balady' was studied in a greenhouse experiment. Soil treatments with dried plant materials of Monterey cypress, Brazil pepper- tree, lime and China tree induced great reductions (81.4-91.8%) in the numbers of root galls and egg masses of *M. incognita* on infected spinach plants. On the other hand, treatments with plant materials of blue gum tree, California pepper - tree, lantana and pomegranate gave 49.8-69.3% reduction of root galls and egg masses of *M. incognita* on infected spinach plants.

Keywords: control; Egypt; *Meloidogyne arenaria*; *M. incognita* and *M. javanica*; pathogenicity; spinach; Swiss chard; table beet

Introduction

In Egypt, plant-parasitic nematodes are among the most important agricultural pests. Previous survey studies in Egypt indicated the occurrence of about 60 genera and 171 species of phytoparasitic nematodes associated with many crop plants, grasses, and weeds (Ibrahim *et al.*, 2000, 2010, 2016, 2017). Spinach (*Spinacia oleracea* L.), Swiss chard (*Beta vulgaris* var. *cicle* (L). Moq.) and table beet (*Beta vulgaris* L.) are considered among the important vegetable crops for their nutritional and economical values. Survey studies (Basyony *et al.*, 2020) have shown the occurrence of 15 genera of phytoparasitic nematodes associated with spinach, Swiss chard, and table beet crops in northern Egypt. The cyst (*Heterodera schachtii* and *H. trifolii*), lesion (*Pratylenchus* spp.) and root-knot *(Meloidogyne incognita* and *M. javanica*) nematodes were common

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on the surveyed vegetable plants. Several authors (Partyka, 1969; Potter and Olthof, 1974, 1993; Olthof *et al.*, 1974; Knight *et al.*, 1997; Castillo and Jimenez-Diaz, 2003; Manachini *et al.*, 2002; Ibrahim *et al.*, 2013, 2021; Premachandra and Gowen, 2015; Basyony *et al.*, 2020) reported that certain plant parasitic nematodes such as *Helicotylenchus labiatus*, *Heterodera schachtii*, *Meloidogyne hapla, M. incognita, M. javanica* and *Pratylenchus penetrans* may occur and infect spinach, garden beet and other similar vegetable crops.

Spinach, Swiss chard, and table beet are cool season vegetable crops that are widely grown in northern Egypt with some pest and disease problems (Ibrahim *et al.*, 2021). Little information is available on the pathogenicity of the root-knot nematodes (*Meloidogyne* spp.) on spinach and other similar vegetable crops (Castillo and Jimenez- Diaz, 2003; Manachini e*t al.*, 2002; Premachandra and Gowen, 2015; Ibrahim *et al.*, 2021). Therefore, the objectives of the present research were to study the pathogenicity of *M. arenaria*, *M. incognita* and *M. javanica* on some spinach, Swiss chard and table beet cultivars and the control of *M. incognita* on spinach cv. 'Balady' under greenhouse conditions.

Materials and Methods

Inocula of the root-knot nematodes *M. arenaria* originally isolated from peanut and *M. incognita and M. javanica* originally isolated from spinach plants were obtained from infected roots of tomato (*Solanum lycopersicom* L.) cultivar 'Rutgers' grown in the greenhouse. Eggs of these nematode species were extracted from infected tomato roots with sodium hypochloride (NaClO) solution (Hussey and Barker, 1973).

The reactions of five spinach ('Balady', 'Barkly', 'Orient', 'Pacific', 'Salonicy'), two Swiss chard ('Balady', 'Ford Hook') and four table beet ('Asgro Wonder', 'Balady', 'Detroit Dark Red', 'Detroit Spainal') cultivars to *M. arenaria*, *M. incognita* and *M. javanica* were determined in several greenhouse tests. Seeds of the tested plant cultivars were sown in 15 cm diameter clay pots filled with a mixture of equal volumes of steam sterilized sand and clay soil. After emergence, seedlings were thinned to one seedling/pot. Two weeks after emergence, soil of treated pots was infested by creating holes near the plant roots and then adding an initial population (Pi) of 4000 eggs/pot of *M. arenaria*, *M. incognita* or *M. javanica.* Non-treated pots served as control. All treatments were replicated five times. Pots were arranged in a randomized complete block design in a greenhouse maintained at 20-26°C. The experiments were conducted in 2020 and 2021.

Sixty days after inoculation, experiments were terminated, and roots were washed free of soil. Roots infected with root-knot nematodes were immersed in an aqueous solution of phloxine B (0.15 g/L water) for 15 minutes to stain the nematode egg masses. Plants were rated on a 0-5 scale according to the numbers of egg masses (Taylor and Sasser, 1978). Plants with 0-2 egg masses/plant were considered resistant; 3-10 egg masses /plant, moderately resistant; 11-30 egg masses/plant, moderately susceptible; 31-100 egg masses/plant, susceptible; and >100 egg masses/plant highly susceptible (Taylor and Sasser, 1978; Ibrahim *et al.*, 2019).

Soil amendments with dried plant materials were examined in the greenhouse for their effects on the reproduction of *M. incognita* on spinach cv. 'Balady'. These included dried leaves of Monterey cypress (*Cupressus macrocarpa* Hartw.), blue gum (*Eucalyptus globules* Labill.), Brazil peppertree (*Schinus terebinthifolia* Raddi), California peppertree (*Schinus molle* L.), China tree (*Melia azedarach* L.), lantana (*Lantana nana* L.), and peels of both lime fruits (*Citrus aurantifolia* (Christm.) Swingle) and pomegranate fruits (*Punica granatum* L.). The tested plant materials were obtained from the Agricultural Experiment Station of Alexandria University, Abees, Alexandria, Egypt, oven dried at 60° C for 48 hrs, and ground into a fine powder by an electric grinder.

Seeds of spinach cv. 'Balady' were sown in 15 cm diameter clay pots filled with a mixture of equal portions of autoclave sterilized sand and clay soil. After emergence, seedlings were thinned to one seedling/pot and ten days later the tested plant materials were incorporated into the upper part of the soil of treated pots at the rate of 30 g/pot. Two weeks after emergence, the soil was inoculated with the tested nematode at the rate of 5000 eggs/pot. Treatments were replicated five times. Pots were arranged in a randomized complete block design in a greenhouse at 20-26 °C. The experiment was conducted in 2020 and repeated in 2021.

The experiment was terminated 60 days after soil inoculation. Roots were washed free of soil. The numbers of *M. incognita* galls and egg masses were determined along with the dry weights of the shoots and roots.

Data of the dry weights of the shoots and roots of the tested spinach plants were analyzed with analysis of variance (ANOVA) and means separated with least significant differences tests (LSD, SAS Institute, 1997).

Results

Results obtained from experiments of 2020 were almost like those in 2021 (Tables 1-3). The reactions of the tested spinach cultivars to infection by *M. arenaria*, *M. incognita* and *M. javanica* are presented in Table 1.

Table 1. Reactions of five spinach cultivars to infection with the root-knot nematodes *Meloidogyne arenaria*, *M. incognita* and *M. javanica*

Cultivar (Treatment)		No. of galls/ plant[w]	No. of egg masses/ plant	Host reaction[z]	No. of galls/ plant[w]	No. of egg masses/ plant	Host reaction[z]
		Test 1 (2020)			Test 2 (2021)		
'Balady'	*M. arenaria*	402	356	HS	411	382	HS
	M. incognita	540	480	HS	528	471	HS
	M. javanica	484	432	HS	470	418	HS
'Barkly'	*M. arenaria*	310	258	HS	325	269	HS
	M. incognita	527	475	HS	490	452	HS
	M. javanica	483	426	HS	450	411	HS
'Orient'	*M. arenaria*	381	303	HS	378	310	HS
	M. incognita	496	446	HS	511	468	HS
	M. javanica	428	372	HS	412	362	HS
'Pacific'	*M. arenaria*	36	24	MS	40	26	MS
	M. incognita	75	61	S	82	74	S
	M. javanica	38	27	MS	42	28	MS
'Salonicy'	*M. arenaria*	134	121	HS	148	131	HS
	M. incognita	184	166	HS	192	172	HS
	M. javanica	148	134	HS	156	142	HS

[w]Means are the average of five replicates.
[z]HS = Highly susceptible, MS= Moderately susceptible, S = Susceptible.

Spinach cvs. 'Balady', 'Barkly', 'Orient' and 'Salonicy' were highly susceptible to the tested root-knot nematode species as great numbers (121-480) of nematode egg masses were observed on infected roots. On the other hand, spinach cv. 'Pacific' was susceptible to *M. incognita* with 61-74 egg masses/plant and moderately susceptible to *M. arenaria* and *M. javanica* with 24-28 egg masses/plant. (Table1).

The reactions of two Swiss chard and four table beet cultivars to infection with *M. arenaria, M. incognita* and *M. javanica* are presented in Table 2. Swiss chard cvs. 'Balady' and 'Ford Hook' were highly susceptible to the tested root-knot nematode species which developed 110-465 egg masses/plant on infected plants. Table beet cvs. 'Balady', 'Detroit Dark Red' and 'Detroit Spainal' were susceptible or highly susceptible to the tested nematode species with 80-454 egg masses/plant on infected plants. On the other hand, table beet cv. 'Asgro Wonder' showed a susceptible reaction to *M. incognita* with 70-78 egg masses/plant and a moderately susceptible reaction to *M. arenaria* and *M. javanica* with 26-29 egg masses/plant on infected plants (Table 2).

Table 2. Reaction of two Swiss chard and four table beet cultivars to infection with *Meloidogyne arenaria*, *M. incognita* and *M. javanica*

Cultivar (Treatment)		No. of galls/ plant[w]	No. of egg masses/ plant	Host reaction[z]	No. of galls/ plant[w]	No. of egg masses/ plant	Host reaction[z]
		Test 1 (2020)			Test 2 (2021)		
Swiss chard							
'Balady'	*M. arenaria*	303	236	HS	310	248	HS
	M. incognita	554	432	HS	572	456	HS
	M. javanica	405	331	HS	418	345	HS
'Ford Hook'	*M. arenaria*	137	110	HS	144	118	HS
	M. incognita	326.	234	HS	346	263	HS
	M. javanica	219	186	HS	236	193	HS
Table beet							
'Asgro Wonder'	*M. arenaria*	46	28	MS	44	26	MS
	M. incognita	96	78	S	88	70	S
	M. javanica	48	29	MS	44	27	MS
'Balady'	*M. arenaria*	266	162	HS	254	186	HS
	M. incognita	471	454	HS	482	452	HS
	M. javanica	413	353	HS	404	361	HS
'Detroit Dark Red'	*M. arenaria*	193	152	HS	186	147	HS
	M. incognita	441	324	HS	458	346	HS
	M. javanica	255	195	HS	272	206	HS
'Detroit Spainal'	*M. arenaria*	128	85	S	114	80	S
	M. incognita	181	137	HS	198	152	HS
	M. javanica	165	103	HS	175	110	HS

[w]Means are the average of five replicates.
[z]HS = Highly susceptible, MS= Moderately susceptible, S = Susceptible.

The effects of soil amendments on reproduction of *M. incognita* on spinach cv. 'Balady' are presented in Table 3. The applied soil treatments suppressed nematode infection and greatly reduced the numbers of *M. incognita* galls and egg masses on infected spinach roots compared to the control. The highest reductions (81.4-91.2%) of nematode egg masses were recorded with treatments of Brazil peppertree (*S. terebinthifolia*), China tree (*M. azedarach*), lime (*C. aurantifolia*) and Monterey cypress (*C. macrocarpa*) plant materials. Treatments with California peppertree (*S. molle*), and pomegranate (*P. granatum*) plant materials gave 64.1 - 69.3% reduction in nematode egg masses. On the other hand, treatments with lantana (*L. nana*) and gum tree (*E. globules*) leaves resulted in only 48.7 - 58.8% reduction in *M. incognita* egg masses. Treatments with the tested plant materials caused significant increases in shoot and root dry weights of treated spinach plants compared to the control (Table 3).

Discussion

This research demonstrated that most of the tested chenopodiaceous plant cultivars were susceptible and good hosts for *M. arenaria*, *M. incognita* and *M. javanica* as these nematode species infected and reproduced successfully on the tested plant cultivars. The results support earlier studies (Ibrahim *et al.*, 2013; 2019) indicating that *M. arenaria*, *M. incognita* and *M. javanica* can infect and reproduce on some cabbage, cauliflower, cotton, flax and turnip cultivars. Also, Ibrahim *et al.* (2019) showed that some species and cultivars of banana, bitter almond, palm trees and peach were susceptible and good hosts for *M. incognita* and *M. javanica*.

Table 3. Effects of soil amendment with dried plant materials of *Citrus aurantifolia*, *Cupressus macrocarpa*, *Eucalyptus globules*, *Lantana nana*, *Melia azedarach*, *Punica granatum*, *Schinus molle* and *S. terebinthifolia* on *Meloidogyne incognita* reproduction on spinach cv. 'Balady'

Cultivar (Treatment)	No. of galls/plant[w]	No. of egg masses/plant[x]	Reduction%		Dry weight (g)	
			Galls	Egg masses	Shoot	Root
	Test 1 (2020)					
C. aurantifolia	110	89	84.8	85.5	12.01^{a}	3.51^{b}
C. macrocarpa	70	60	90.4	90.2	12.21^{a}	3.83^{a}
E. globules	290	253	60.0	58.8	11.30^{b}	3.20^{b}
L. nana	337	315	53.6	48.7	10.71^{c}	2.90^{c}
M. azederach	132	114	81.8	81.4	11.87^{ab}	3.49^{b}
P. granatum	226	195	68.9	68.2	11.01^{c}	3.05^{c}
S. molle	245	213	66.3	65.3	11.53^{b}	3.28^{b}
S. terebinthifolia	89	80	87.8	87.0	12.15^{a}	3.61^{a}
M. incognita	726	614	-	-	6.49^{e}	1.69^{e}
	Test 2 (2021)					
C. aurantifolia	102	78	85.4	86.5	12.16^{a}	3.60^{a}
C. macrocarpa	64	51	91.0	91.2	12.40^{a}	3.88^{a}
E. globules	276	240	59.6	58.3	11.26^{b}	3.34^{b}
L. nana	320	291	54.2	49.8	10.87^{c}	2.98^{c}
M. azederach	122	102	82.5	82.4	11.76^{b}	3.42^{b}
P. granatum	210	178	69.9	69.3	11.14^{b}	3.18^{b}
S. molle	221	208	68.3	64.1	11.42^{b}	3.36^{b}
S. terebinthifolia	82	72	88.2	87.6	12.26^{a}	3.72^{a}
M. incognita	698	580	-	-	5.87^{d}	1.82^{d}

[w]Means are the average of five replicates.

[x]Means with the same letter within a column are not significantly different at P=0.05.

A recent study by Ibrahim *et al.* (2021) showed that some spinach, Swiss chard, and table beet cultivars were susceptible to the cyst nematode *Hererodera schachtii*. The tested root-knot nematode species significantly decreased shoot and root dry weights of the susceptible and highly susceptible spinach, Swiss chard, and table beet cultivars. Similar results were obtained by Ibrahim *et al.* (2013, 2019) who found that infection with *M. arenaria*, *M. incognita* and *M. javanica* reduced growth of some cabbage, cauliflower, cotton, flax and turnip cultivars. It is evident that infections with *M. incognita* or *M. javanica* induced more root galls and egg masses and growth damage on infected spinach, Swiss chard and table beet plant cultivars as compared with *M. arenaria* infection. Previous studies in Egypt (Ibrahim *et al.*, 2010, 2013, 2019) showed that *M. arenaria* is of low occurrence and less pathogenic compared to *M. incognita* and *M. javanica*.

The results showed that soil treatments with dried plant materials of lime, Monterey cypress, China tree and Brazil peppertree greatly reduced the numbers of galls and egg masses of *M. incognita* on infected spinach plants. In similar study, Tasi (2008) reported that peels of some citrus fruits were effective against *M. incognita*. Also, Ibrahim *et al.* (2013) indicated that soil treatments with dried plant materials of castor bean, goosefoot and lantana greatly reduced infection of *M. incognita* on infected cabbage plants. Moreover, the present results are in agreement with those of other authors who described the effective use of organic soil amendments to control root-knot and cyst nematodes (Saifullah *et al.*, 1990; Radwan *et al.*, 2004; Ibrahim *et al.*, 2013, 2014).

Conclusions

It is concluded from the study of determining the host status of certain spinach, Swiss chard, and table beet cultivars to *M. arenaria*, *M. incognita* and *M. javanica* that resistance to these nematode species is of significance and can be useful to incorporate in breeding programs during planning control measures for root-knot nematodes. More research is needed for the development of resistant or tolerant cultivars of these vegetable crops to root-knot nematodes.

Authors' Contributions

Conceptualization (IKAI, ZAH, SMAZ, MK, MAIK); Data curation (IKAI, SMAZ, MAIK); Formal analysis (IKAI, ZAH, SMAZ, MK, MAIK); Investigation (IKAI, SMAZ, MAIK); Methodology (IKAI, SMAZ, MAIK); Project administration (IKAI, SMAZ, MAIK); Resources (IKAI, ZAH, SMAZ, MK, MAIK); Software (IKAI, ZAH, SMAZ, MK, MAIK); Supervision (IKAI, ZAH, SMAZ, MAIK); Validation(IKAI, ZAH, SMAZ, MK, MAIK); Visualization (IKAI, ZAH, SMAZ, MK, MAIK); Writing (IKAI, ZAH, SMAZ, MK, MAIK).

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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