

# Survey of Plant Parasitic Nematode Associated with Spinach, Swiss Chard and Table Beet in North Egypt

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## ABSTRACT

A nematode survey was conducted in Alexandria, El-Behera, El-Gharbia, El-Monufia and Kafr El-Sheikh governorates in northern Egypt during the 2017 – 2019 cropping seasons to study the occurrence, population density and host association of plant parasitic nematodes associated with spinach, Swiss chard and table beet. A total of 854 soil and root samples were collected from the rhizosphere of the surveyed vegetable crops. The surveyed governorates and their respective numbers of soil samples were as follows: Alexandria 230, El-Behera 206, El-Gharbia 138, El-Monufia 211 and Kafr El-Sheikh 69 samples. Plant parasitic nematodes in the collected soil and root samples were extracted, identified, and counted under the compound light microscope. The collected soil and root samples contained mixed populations of 15 genera and 18 species of phytoparasitic nematodes that are reported for the first time on spinach, Swiss chard and table beet in Egypt. The root-knot nematode (*Meloidogyne* spp.) with 37 – 67 frequency of occurrence (FO) were the most frequently encountered group of nematodes, followed by spiral (*Helicotylenchus* sp.), cyst (*Heterodera* sp.), lesion (*Pratylenchus* sp.) and stunt (*Tylenchorhynchus* sp.) with 15 – 61 FO. The genera *Criconema*, *Criconemella* and *Tylenchus* were quite common. However, the other genera (*Ditylenchus*, *Hoplolaimus*, *Psilenchus*, *Rotylenchulus*, *Rotylenchus*, *Trichodorus*, and *Xiphinema*) were less common. The nematode species *Meloidogyne arenaria*, *M. incognita*, *M. javanica*, *Heterodera schachtii* and *H. trifolii* were identified on spinach, Swiss chard and table beet roots. The occurrence of the cyst nematodes *H. schachtii* and *H. trifolii* on spinach, Swiss chard and table beet are new records in Egypt. Also, survey results revealed new host plant records for most of the identified nematode genera and species in Egypt.

**Keywords:** Nematodes, Survey, Spinach, Swiss chard, Table beet, Egypt.

## INTRODUCTION

In Egypt, plant parasitic nematodes have been recognized as important plant pests since 1901 when Preyer 1901 first reported a nematode disease of banana in Alexandria, Egypt (Ibrahim and El-Sharkawy, 2001; Preyer, 1901). Previous survey studies showed the occurrence of large numbers of genera and species of phytoparasitic nematodes associated with many plant crops, grasses and weeds at different localities in Egypt

(Ibrahim and El-Sharkawy, 2001; Ibrahim *et al.*, 2000; 2010). Many of these nematodes especially *Helicotylenchus* spp., *Hoplolaimus* spp., *Meloidogyne* spp., *Pratylenchus* spp., *Rotylenchulus reniformis*, *Tylenchorhynchus* spp., *Tylenchulus semipenetrans* and *Xiphinema* spp. are considered limiting factors in crop production in Egypt (Adam *et al.*, 2013; Korayem *et al.*, 2014; Ibrahim and Handoo, 2015; Ibrahim *et al.*, 2010). Also, the practice of continuous cropping and use of local plant cultivars favor survival and rapid build-up of nematode populations in the soil. Information concerning the occurrence and distribution of the genera and species of phytoparasitic nematodes in Egypt is very important as several of the serious nematode pathogens, *i.e.* citrus, cyst, lance, lesion, root-knot, spiral and stunt nematodes, may occur in large numbers and cause economic damage to many plant crops. Recent survey studies showed the occurrence of about 59 genera and 170 species of phytoparasitic nematodes associated with many cultivated plants (Ibrahim and Handoo, 2016; Ibrahim *et al.*, 2017). The objective of the present study was to identify plant parasitic nematodes associated with spinach, Swiss chard and table beet plants in north Egypt and provide more extensive information on the distribution of genera and species of plant parasitic nematodes.

## MATERIAL AND METHODS

The present survey was carried out during 2017-2019 cropping seasons. A total of 854 rhizosphere soil samples were randomly collected from spinach, Swiss chard and table beet plants in five Egyptian governorates. The surveyed governorates and their respective numbers of soil samples were as follows: Alexandria 230 (Abbies, El-Amiria, El-Maamoura, Eslah and Khorshid), El-Behera 206 (Abo El-matamir, Bader, Edko, Etay El-Barod, Kafer El-Dawar, Rosetta and Shobrakit), El-Gharbia 138 (Basyoon, Kafer El-Zayat, Qtour and Tanta), El-Monufia 211 (Berkt El-Sabh, El-Shohada, Shepin El-Kom and Tala) and Kafr El-Sheikh (Desuq, Fowh, Metopes and Qalen) 69 samples. Rhizosphere soil samples of about 1 Kg each were collected from the surveyed plants at a depth of 15 – 30 cm and placed in labeled plastic bags. Root samples were washed free of soil and examined for

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cyst and root-knot nematode infections. Root-knot nematodes were isolated from galled roots and identified by the examination of perineal patterns of adult females as well as the characters of the second stage juveniles (Taylor and Sasser, 1978). Cysts, females and juveniles were extracted from soil and root samples and identified by morphological characteristics (Golden, 1986; Mulvey and Golden, 1983; Subbotin *et al.*, 2010). Nematodes from a composite sample of 250 cm<sup>3</sup> soil were extracted by means of Cobb's wet-sieving and centrifugal sugar flotation techniques (Ayoub, 1980). Nematodes were fixed in 2% formaldehyde solution then identified to genus and counted under a bionocular stereomicroscope (Mai and Lyon 1975; Müller *et al.*, 1985; Handoo, 2000). Frequency of occurrence ( $[\text{number of positive samples}/\text{number of total samples}] \times 100$ ) and nematode density (nematodes per 250 cm<sup>3</sup> soil sample) were determined for the identified nematodes in composite samples and recorded.

## RESULTS AND DISCUSSION

Data presented in Table 1 show the frequency of occurrence (FO) and population density (PD) of plant parasitic nematode genera presented in the soil samples collected from spinach, Swiss chard and table beet fields in Alexandria governorate of Egypt. Ten nematode genera were detected in the soil samples of spinach plants while soil samples of Swiss chard and table beet showed the presence of 11 and 8 nematode genera, respectively. The root-knot nematode

(*Meloidogyne* spp.) were the most frequent nematodes with 55 – 64% FO and PD of 234 – 433 J<sub>2</sub>'s /250 g soil. The genera *Tylenchorhynchus*, *Pratylenchus* and *Helicotylenchus* showed 28 – 53 % FO and PD of 134 – 288 nematodes /250 g soil. The cyst nematodes (*Heterodera* spp.) exhibited 13 – 19.5% FO and PD of 190 – 263 J<sub>2</sub>'s /250 g soil. On the other hand, the genera *Criconema*, *Criconemella*, *Ditylenchus*, *Hoplolaimus*, *Tylenchus* and *Xiphinema* showed 4.7 – 25.8% FO and 63 – 398 nematodes /250 g soil.

In El-Behera governorate soil samples, fifteen nematode genera were detected in the soil samples of spinach plants while soil samples of Swiss chard and table beet showed the presence of 13 and 12 nematode genera, respectively. The root-knot nematode (*Meloidogyne* spp.) were the most frequent nematodes with 51.5 – 65.5% FO and PD of 443 – 546 J<sub>2</sub>'s /250 g soil. The genera *Tylenchorhynchus*, *Pratylenchus* and *Helicotylenchus* showed 26.4 – 60.6 % FO and PD of 195.6 – 312.1 nematodes /250 g soil. The cyst nematodes (*Heterodera* spp.) exhibited 16.4 – 31.6% FO and PD of 293 – 391 J<sub>2</sub>'s /250 g soil. On the other hand, the genera *Criconema*, *Criconemella*, *Ditylenchus*, *Hoplolaimus*, *Psilenchus*, *Rotylenchulus*, *Rotylenchus*, *Trichodorus*, *Tylenchus* and *Xiphinema* showed 4.3 – 12.9 % FO and 76 – 395 nematodes /250 g soil (Table 2).

**Table 1. Frequency of occurrence (FO) and population density (PD) of plant parasitic nematode genera present in soil samples of spinach, Swiss chard and table beet plants in Alexandria governorate**

Genus	Spinach (128) <sup>a</sup>		Swiss chard (53)		Table beet (49)	
	FO%	PD	FO%	PD	FO%	PD
<i>Criconema</i>	25.8 <sup>b</sup>	185.1 <sup>c</sup>	16.9	191.1	26.5	210
<i>Criconemella</i>	12.5	146.8	16.9	137.8	6.1	110
<i>Ditylenchus</i>	-----	-----	11.3	200	-----	-----
<i>Helicotylenchus</i>	33.6	288.6	28.3	193.3	28.6	166.4
<i>Heterodera</i>	19.5	262.8	13.2	244.3	14.3	190
<i>Hoplolaimus</i>	4.7	143.7	18.9	200	6.1	320
<i>Meloidogyne</i>	60.2	364	64.2	234.1	55.1	433
<i>Pratylenchus</i>	36.7	209.3	37.7	139	40.8	197
<i>Tylenchorhynchus</i>	42.2	150.4	43.4	190	53.1	134
<i>Tylenchus</i>	14.8	197.4	9.4	398	-----	-----
<i>Xiphinema</i>	5.5	135.7	5.7	63.3	-----	-----

a= Total number of collected soil samples in tested plant.

b= Number of positive samples/ a × 100

c= Average number of nematodes/ 250 g soil.

**Table 2. Frequency of occurrence (FO) and population density (PD) of plant parasitic nematode genera present in soil samples of spanish, Swiss chard and table beet plants in El-Behera governorate**

Genus	Spinach (116) <sup>a</sup>		Swiss chard (33)		Table beet (57)	
	FO%	PD	FO%	PD	FO%	PD
<i>Criconema</i>	12.9 <sup>b</sup>	250 <sup>c</sup>	9.1	223.3	12.3	232.8
<i>Criconemella</i>	12.1	200	12.1	295	8.8	210
<i>Ditylenchus</i>	4.3	124	6.1	150	8.8	180
<i>Helicotylenchus</i>	39.7	312.1	33.3	260.9	26.4	275
<i>Heterodera</i>	16.4	292.6	21.2	348.6	31.6	391.1
<i>Hoplolaimus</i>	12.1	262.1	9.1	280	8.8	256
<i>Meloidogyne</i>	65.5	545.5	51.5	442.9	57.9	482.4
<i>Pratylenchus</i>	35.3	296	59.5	216.8	60.6	280.9
<i>Psilenchus</i>	4.3	177	12.1	262.5	-----	-----
<i>Rotylenchulus</i>	5.2	151.6	-----	-----	-----	-----
<i>Rotylenchus</i>	6.1	101.4	9.1	86.7	5.3	105
<i>Trichodorus</i>	4.3	224	-----	-----	-----	-----
<i>Tylenchorhynchus</i>	33.6	242.8	27.3	195.6	40.4	262.6
<i>Tylenchus</i>	11.2	372.3	6.1	395	7.1	210
<i>Xiphinema</i>	4.3	76	9.1	165	5.3	165.6

a= Total number of collected soil samples in tested plant.

b= Number of positive samples/ a × 100

c= Average number of nematodes/ 250 g soil.

In El-Gharbia governorate soil samples, ten nematode genera were detected in the soil samples of spinach plants while soil samples of Swiss chard and table beet showed the presence of 9 and 8 nematode genera, respectively. The genera *Helicotylenchus* was the most frequent nematodes with 25 – 55% FO and PD of 212 – 307 J<sub>2</sub>'s /250 g soil. The root-knot nematode (*Meloidogyne* spp.) exhibited 40 – 48% FO and PD of

308 – 490 J<sub>2</sub>'s /250 g soil. The genera *Tylenchorhynchus* and *Pratylenchus* showed 20 – 36% FO and PD of 138 – 227 nematodes /250 g soil. The cyst nematodes (*Heterodera* spp.) exhibited 19 – 23% FO and PD of 189 – 317 J<sub>2</sub>'s /250 g soil. On the other hand, the genera *Criconema*, *Criconemella*, *Hoplolaimus*, *Trichodorus*, and *Tylenchus* showed 5 – 35 % FO and 100 – 330 nematodes /250 g soil (Table 3).

**Table 3. Frequency of occurrence (FO) and population density (PD) of plant parasitic nematode genera present in soil samples of spanish, Swiss chard and table beet plants in El-Gharbia governorate**

Genus	Spinach (67) <sup>a</sup>		Swiss chard (31)		Table beet (40)	
	FO%	PD	FO%	PD	FO%	PD
<i>Criconema</i>	17.9 <sup>b</sup>	196.6 <sup>c</sup>	19.4	169.2	35	111.4
<i>Criconemella</i>	4.5	123	16.1	170	15	166.6
<i>Helicotylenchus</i>	25.4	306.5	54.8	211.5	52.5	251.9
<i>Heterodera</i>	19.4	260	22.6	188.7	22.5	316.6
<i>Hoplolaimus</i>	10.4	235.7	-----	-----	12.5	148
<i>Meloidogyne</i>	46.2	490.3	48.4	307.9	40	407.5
<i>Pratylenchus</i>	32.8	138.6	27.6	227.1	20	157.5
<i>Trichodorus</i>	13.4	155.6	19.4	216.7	-----	-----
<i>Tylenchorhynchus</i>	29.9	167.5	35.5	137.9	30	167.5
<i>Tylenchus</i>	11.9	330	12.9	100	-----	-----

a= Total number of collected soil samples in tested plant.

b= Number of positive samples/ a × 100

c= Average number of nematodes/ 250 g soil.

In El-Monufia governorate soil samples, fourteen nematode genera were detected in the soil samples of spinach plants while soil samples of Swiss chard and table beet showed the presence of 11 and 8 nematode genera, respectively. The root-knot nematode was the most frequent nematodes with 56 – 72 % FO and PD of 301 – 515 J<sub>2</sub>'s /250 g soil. The genera *Tylenchorhynchus*, *Pratylenchus* and *Helicotylenchus* showed 25 – 56 % FO and PD of 148 – 313 nematodes /250 g soil. The cyst nematodes (*Heterodera* spp.) exhibited 21 – 61% FO and PD of 181 – 498 J<sub>2</sub>'s /250 g soil. On the other hand, the genera *Criconema*, *Criconemella*, *Ditylenchus*, *Hoplolaimus*, *Psilenchus*, *Rotylenchulus*, *Trichodorus*, *Tylenchus* and *Xiphinema* showed 2 – 27.8 % FO and 60 – 268 nematodes /250 g soil (Table 4).

In Kafr El-Sheikh governorate soil samples, twelve nematode genera were detected in the soil samples of spinach, Swiss chard and table beet plants. The genera *Meloidogyne* was the most frequent nematodes with 37 – 67% FO and PD of 252 – 295 J<sub>2</sub>'s /250 g soil. The genera *Tylenchorhynchus*, *Pratylenchus* and *Helicotylenchus* showed 22 – 48 % FO and PD of 66 – 236 nematodes /250 g soil. The cyst nematodes (*Heterodera* spp.) exhibited 15 – 40% FO and PD of 200 – 229 J<sub>2</sub>'s /250 g soil. On the other hand, the genera *Criconema*, *Criconemella*, *Hoplolaimus*, *Psilenchus*, *Rotylenchus*, *Tylenchus* and *Xiphinema* showed 4 – 27 % FO and 55 – 205 nematodes /250 g soil (Table 5).

Data in Table 6 indicated that the results of the morphological study show that only three species of the root-knot nematodes namely *M. arenaria*, *M. incognita* and *M. javanica* were identified in the collected galled root samples from spinach, Swiss chard, and table beet plants. It is evident that *M. incognita* was very common and widely distributed in north Egypt with frequency of occurrence (FO) of 57 – 73%, followed by *M. javanica* with FO of 20 – 34%, whereas *M. arenaria* had the lowest FO of 7 – 9%. These results are in agreement with those of other workers (Ibrahim and Mokbel, 2009; Ibrahim and Handoo, 2015; 2016; Ibrahim *et al.*, 2017).

In the present study, soil and root samples collected from the different surveyed districts in five governorates of spinach, table beet, and Swiss chard clarified the presence of 15 genera of plant parasitic nematodes. Some of plant parasitic nematodes cause dangerous quantity and quality losses to various plants in Egypt (Ibrahim and El- Sharkawy, 2001). Root knot nematode (*Meloidogyne* spp.) acts as one of the most pathogenic nematodes, as it distributes in the most Egyptian soils (Korayem *et al.*, 2014; Ibrahim *et al.*, 2000, 2010). Although some researches in Egypt were carried out to study the relationship between *H. schachtii* and certain crops (Ibrahim *et al.*, 2016, 2017).

**Table 4. Frequency of occurrence (FO) and population density (PD) of plant parasitic nematode genera present in soil samples of spanish, Swiss chard and table beet plants in El-Monufia governorate**

Genus	Spinach (154) <sup>a</sup>		Swiss chard (39)		Table beet (18)	
	FO%	PD	FO%	PD	FO%	PD
<i>Criconema</i>	10.4 <sup>b</sup>	220 <sup>c</sup>	10.3	75	16.7	220
<i>Criconemella</i>	8.4	268.4	17.9	90	-----	-----
<i>Ditylenchus</i>	1.9	101.7	12.8	60	-----	-----
<i>Helicotylenchus</i>	42.2	250.7	43.6	147.6	44.4	160
<i>Heterodera</i>	20.8	497.7	38.5	289.1	61.1	180.9
<i>Hoplolaimus</i>	5.2	256.2	15.4	105	27.8	166
<i>Meloidogyne</i>	55.8	515.3	64.1	300.6	72.2	306.2
<i>Pratylenchus</i>	40.9	294.6	43.6	224.7	55.6	154
<i>Psilenchus</i>	1.9	136.6	-----	-----	-----	-----
<i>Rotylenchulus</i>	8.4	217.9	-----	-----	-----	-----
<i>Trichodorus</i>	4.5	224.3	-----	-----	-----	-----
<i>Tylenchorhynchus</i>	25.3	296.2	30.8	256.7	38.8	312.9
<i>Tylenchus</i>	7.8	210.1	15.4	150	-----	-----
<i>Xiphinema</i>	4.5	85.7	12.8	130	16.7	140

a= Total number of collected soil samples in tested plant.

b= Number of positive samples/ a × 100

c= Average number of nematodes/ 250 g soil.

**Table 5. Frequency of occurrence (FO) and population density (PD) of plant parasitic nematode genera present in soil samples of spanish, Swiss chard and table beet plants in Kafr El-Sheikh governorate**

Genus	Spinach (27) <sup>a</sup>		Swiss chard (27)		Table beet (15)	
	FO%	PD	FO%	PD	FO%	PD
<i>Criconema</i>	18.5 <sup>b</sup>	112 <sup>c</sup>	14.8	90	20	120
<i>Criconemella</i>	-----	-----	-----	-----	13.3	75
<i>Helicotylenchus</i>	22.2	65.8	25.9	82.9	30.1	88
<i>Heterodera</i>	33.3	218.9	14.8	200	40	228.6
<i>Hoplolaimus</i>	7.4	100	14.8	132.5	-----	-----
<i>Meloidogyne</i>	66.6	252	37	254.8	60	294.6
<i>Pratylenchus</i>	48.1	235.8	33.3	165.6	46.7	211
<i>Psilenchus</i>	14.8	205	11.1	166.6	-----	-----
<i>Rotylenchus</i>	-----	-----	14.8	120	26.7	152.5
<i>Tylenchorhynchus</i>	40.7	117.2	40.7	124	33.3	214
<i>Tylenchus</i>	7.4	90	11.1	105	13.3	80
<i>Xiphinema</i>	3.7	55	-----	-----	13.3	65

a= Total number of collected soil samples in tested plant.

b= Number of positive samples/ a × 100

c= Average number of nematodes/ 250 g soil.

**Table 6. Frequency of occurrence of the root-knot nematodes *Meloidogyne arenaria*, *M. incognita* and *M. javanica* associated with host plants in Alexandria, El-Behera, El-Gharbia, El-Monufia and Kafr El-Sheikh governorates**

Host plant	No. of collected soil samples infested with <i>Meloidogyne</i> spp.	Frequency of occurrence % <sup>a</sup>		
		<i>M. arenaria</i>	<i>M. incognita</i>	<i>M. javanica</i>
Spinach	289	7.3	72.7	20.0
Swiss chard	101	8.9	60.4	30.7
Table beet	97	9.3	56.7	34.0

a= Number of positive samples/ Number of collected soil samples infested with *Meloidogyne* spp. x 100.

Nematode species associated with spinach include cyst (*Heterodera schachtii*), lesion nematode (*Pratylenchus penetrans*) and root-knot nematodes (*Meloidogyne arenaria*, *M. hapla*, *M. incognita*, *M. javanica*) (Olthof and Potter, 1973; Olthof *et al.*, 1974; Potter and Olthof, 1974; Manachini *et al.*, 2003). Recently, severe infection of spinach by *M. incognita* was found in commercial fields of Andalusia, southern Spain (Castillo and Jiménez-Díaz, 2003). *Meloidogyne* spp. are the most common and damaging nematodes of spinach in several countries (Potter and Olthof, 1993). Previous investigations reported that *M. incognita* is potential pathogens for spinach and table beet (Anamika, 2015; Dammini Premachandra and Gowen, 2015). Lance nematode *Hoplolaimus indicus* has also been reported associated with table beet in India (Singh and Kumar, 2013). Moreover, Spinach, table beet, and Swiss chard were efficient hosts for *H. schachtii* and *H. trifolii* in the Netherlands (Steele *et al.*, 1983).

In brief, the collected soil samples from certain districts in the five surveyed governorates clarified the

distribution of plant parasitic nematodes under the predominant environmental conditions and soil types in the surveyed fields. More research is needed to further identify other genera and species of phytoparasitic nematodes that might occur in Egypt. This helps in developing appropriate necessary plans for managing these nematode pests by eco-friendly methods leading to an increase in an economic production and safe agricultural byproducts.

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## الملخص العربي

### حصر النيماتودا المتطفلة المصاحبة لنباتات السبانخ والسلق وبنجر المائدة في شمال

### جمهورية مصر العربية

أيمن بسيوني عبده بسيوني، ابراهيم خيرى عتريس ابراهيم، صابرين محمد على زيادة، مها عادل ابراهيم كونه

(*Helicotylenchus*)، نيماتودا الحوصلات (*Heterodera*)، نيماتودا التقرح (*Pratylenchus*)، ونيماتودا التقزم (*Tylenchorhynchus*). وكانت أجناس النيماتودا (*Criconeema*, *Criconemella*, *Tylenchus* شائعة بينما كانت الأجناس الأخرى التي تم التعرف عليها وهى *Ditylenchus*, *Hoplolaimus*, *Psilenchus*, *Rotylenchulus*, *Rotylenchus*, *Trichodorus*, *Xiphinema* أقل شيوعاً. وقد تم التعرف على أنواع نيماتودا تعقد الجذور *Meloidogyne arenaria*, *M. incognita*, *M. javanica* ونوعى نيماتودا الحوصلات *Heterodera schachtii*, *H. trifolii* على جذور السبانخ والسلق وبنجر المائدة ويعتبر ذلك أول تسجيل لهذه النيماتودا على هذه النباتات في مصر. وقد أظهرت نتائج الحصر أن نباتات السبانخ والسلق وبنجر المائدة قد تعتبر عوائل نباتية جديدة لمعظم أجناس النيماتودا التي تم التعرف عليها في هذه الدراسة.

أجريت دراسة لحصر النيماتودا المتطفلة المصاحبة لنباتات السبانخ والسلق وبنجر المائدة في خمسة محافظات في شمال جمهورية مصر العربية خلال المواسم الزراعية ٢٠١٧-٢٠١٩. حيث تم جمع عدد ٨٥٤ عينة تربة وجذور من محاصيل الخضر التي تمت دراستها. وكانت المحافظات التي تم مسحها وعدد عينات التربة الخاصة بكل منها كالتالى: الاسكندرية ٢٣٠، البحيرة ٢٠٦، الغربية ١٣٨، المنوفية ٢١١، كفر الشيخ ٦٩ عينة تربة وجذور. وقد تم استخلاص النيماتودا الموجودة في عينات التربة وتعريفها وتسجيل عددها تحت الميكروسكوب الضوئى المركب. وقد احتوت عينات التربة والجذور التي تم جمعها على عدد ١٥ جنساً و ١٨ نوعاً من النيماتودا المتطفلة على النباتات والتي تم تسجيلها لأول مرة على نباتات السبانخ والسلق وبنجر المائدة في جمهورية مصر العربية. وقد كانت نيماتودا تعقد الجذور (*Meloidogyne*) هى الأكثر شيوعاً في عينات التربة والجذور المختبرة، تليها كل من النيماتودا الحلزونية