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Research article

Survey and Identification of Phytonematodes and Their Fungal Antagonists Associated with Soybean in Different Ecological Locations of Egypt

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Abstract

Survey and identification of plant-parasitic nematodes and their fungal antagonists associated with soybean in six locations at four governorates of Egypt i.e. Beni-Suef, Menia, Beheira and Alexandria were conducted during the summer growing season of 2020 year. Results indicated the presence of ten phytonematodes, these were *Helicotylenchus*, *Heterodera*, *Hoplolaimus*, *Longidorus*, *Meloidogyne*, *Pratylenchus*, *Rotylenchulus*, *Trichodorus*, *Tylenchorhynchus* and *Xiphenema*. The stunt nematodes (*Tylenchorhynchus* spp.) was the most frequent (50.4%) followed by the root-knot nematodes (*Meloidogyne* spp.) with 15.6% frequency, the root-lesion nematodes (*Pratylenchus* spp.) with 12.0% and the spiral nematodes (*Helicotylenchus* spp.) with 8.4% frequency. Other nematodes were less frequent. Results also revealed the occurrence of three root-knot nematode species infecting soybean; *Meloidogyne incognita*, *M. javanica* and *M. arenaria*. *M. incognita* was the most prevalent species with 48.8% frequency followed by *M. javanica* (37.5%) and *M. arenaria* (13.7%). Also four nematophagous fungi were isolated from the rhizaosphere of soybean, three of them are predacious fungi; *Arthrobotrys conoides, Dactelaria brochopaga, Monacrosporium* sp. and one is endoparasitic, *Verticillium* sp. *A. conoides* and *D. brochopaga* were more frequent with 80% and 60% frequency, respectively followed by *Verticillium* sp. (40%) and *Monacrosporium* sp. (20%).

Introduction

Soybean (*Glycine max* L. Merril.) is a leguminous crop has known from about seven thousand years Brothwell & Brothwell, [1]. It was first cultivated for food in northern China over 3000 years ago Hymowitz, [2]. Currently it is grown in at least 70 countries located in different ecological regions of the world, especially in South America, Brazil, the Far–East and more recently Africa Hymowitz *et al.*, [3]. Seeds of soybean contain about 30% oil and 40% protein El-Abady *et al.*, [4]. So, it is the most important oilseed crop worldwide extensively used in the manufacture of animal and human foodstuffs, and other purposes such as plastics, lubricants, candles, varnishes, soap, biodiesel and lecithin Fontana, [5].

Plant parasitic nematodes are considered one of the limiting factors for soybean production in the world. Many nematode species have been reported to associate with soybeans in different regions worldwide; some of them are of economic importance Goodey *et al.*, [6]; Good, [7]; Robbins, [8]; Donaled *et al.*, [9]; Schmitt and Noel, [10]; Noel, [11]; Doucet *et al.*, [12]. The soybean cyst nematode (*Heterodera glycines*) and root-knot nematodes (*Meloidogne* species) are the most nematodes responsible for most of the crop loss in soybean yield worldwide Noel, [13]. In the USA, during the years of 2003-2005 soybean yield loss caused by *H. glycines* was estimated by 8.29 million tons Wrather & Koenning, [14], and the combined crop loss due to *Meloidogyne* spp. and all other species ranged from 106 to 139 southand tons Noel, [13]. In Canada (Ontario and USA, over 93000 mt of soybean were lost annually between 1999-2002 due to *Meloidogyne* species Wrather *et al.*, [15]. Other phytonematodes as the reniform nematode (*Rotylenchulus reniformis*), the columbia lance nematode (*Hoplolaimus columbus*), the root-lesion nematodes (*Pratylenchus* spp.) and the sting nematodes *Belonolaimus gracilis* and *B. longicoudatus*) have been reported to attack soybean plants in many regions Noel, [13]. Consequently, controlling of these nematodes should be considered. Sustainable nematode control is a new approach for avoiding of toxic chemical nematicides use. Identification of nematode-antagonistic microorganisms inhabiting plant rhizosphere such as nematophagous fungi and applying practices which activate and increase their abundance in the soil is one of the important elements of sustainable nematode management.

Soybean was introduced to Egypt more than 50 years ago, but its cultivation as economic crop has been begun in 1970 Kella *et al.*, [16]. Now it is grown as a summer crop in many governorates of Egypt. Studies concerning phytonematodes associated with soybean in Egypt are still not sufficient. Some of these studies were conducted in restricted locations indicating many nematodes are in association with soybean Salem *et al.*, [17]; Kella *et al.*, [16].

Information concerning the occurrence and distribution of phytonematodes associated with soybean grown in different regions of Egypt is important to assess their potential to cause economic damage to soybean. The objectives of the present study were (1) to identify phytonematodes associated with soybean in different ecological regions in Egypt for providing more extensive information on their distribution and abundance in each region, (2) to identify nematode – antagonistic fungi inhabiting soybean rhizospheres and assaying their frequency and abundance.

Materials and Methods

Survey study

During the growing season 2020, a total of 250 soil and root samples were collected from six localities of four governorates. i.e. Beni –Suef and Menia representing Middle Egypt and Beheira and Alexandria representing Northern Egypt (Table 1). Samples were collected during flowering and pre-mature stages of soybean plants. Each soil sample was composed of three subsamples obtained from the root zone of growing plants by digging to a depth of about 15-30 cm. Root samples were also taken by uprooting plants carefully with a trowel. All samples were sent directly to the laboratory for nematode and nematophagus fungi analysis.

Nematode extraction and identification

Soil samples were carefully mixed, then a weight of 250 g soil from each one was processed for nematode extraction by sieving and decanting method according to Christie and Perry [18]. Root samples showing lesions were cut into small pieces and incubated in plates containing distilled water for 2-3 days at room temperature to extract the migratory endo-parasitic nematodes Young, [19]. Identification of nematode genera and/or species was based on the morphology of adult and larval forms Oteifa, [20]; Golden, [21]; Mai and Lyon, [22].

Absolute frequency of occurrence, population density (PD) and prominence value (PV) were calculated according to Norton [23] as follows:

$$\text{Absolute frequency} = \frac{no.of\ smaples\ containing\ a\ nematode}{total\ no.of\ collected\ samples}$$

Population density (PD) of a nematode = mean of numbers of a nematode in 250 g soil.

$$\text{Prominence value (PV)} = \text{PD}\ \sqrt{absolute\ frequency}$$

Identification of *Meloidogyne* species

The soybean roots infected with the root-knot nematode (*Meloidogyne* spp.), collected from different localities were washed well from adhering soil, and root galls with mature females were selected and processed for removing adult females, then females were processed for preparing perineal patterns Hartman and Sasser, [24]. Identification of *Meloidogyne species* was done according to the morphological shape of perianal patterns Eisenback *et al.*, [25].

Table 1. Names of localities from which soybean samples were collected.

Governorate	Localities	Number of collected samples
Beni-Suef	Beba	45
	Somsta	45
Menia	Maghagha	100
Beheira	Waid-El-Natoon	20
	Nobaira	20
Alexandria	Maryout	20
Total of samples		250

Isolation & identification of nematophagous fungi

Twenty grams of agar were added to one liter of distilled water in conical flask and dissolved if need on a water bath. The water agar medium was poured in Petri plates and sterilized in autoclave at 15 Ibs for 20 min. Nematode suspension extracted from samples collected from different soybean – growing localities was concentrated in 10ml nematode suspension and sterilized. One ml from each sterilized nematode suspension was added to Petri plates, then plates were incubated at 25±2°C for eight days. Examination of plates was carried out after 2, 4 and 8 days to follow the development of nematophagous fungi. Identification of fungi was made according to Aboul-Eid *et al.*, [26], Yu *et al.*, [27].

Results

Survey of phytonematodes associated with soybean plants

Plant-parasitic nematodes recovered from soil and root samples collected from the rhizsosphere of soybean are presented in Tables 2, 3, 4, 5 and 6. Data in Table (2) showed the phytonematodes recovered from samples collected from two localities of Beni-Suef governorate.Samples contained seven nematode genera i.e. *Heterodera, Helicotylenchus, Longidorus, Meloidogyne, Pratylenchus, Tylenchorhynchus* and *Xiphinema*. *Tylenchorhynchus* and *Meloidogyne* were attended with samples of the two localities with high average of frequency (52.2% and 13.3%), and population density (26 and 3) respectively. *Heterodera* was also attended with samples of Beba and Somsta, however its frequency was low (8.9%) in average. Lesser FO% and PV were recorded for *Helicotylenchus* (5.6%, 0.24), *pratylenchus* (5.6% of 0.47), *Xiphinema* (5.5% & 0.47), *Longidorus* (5.6% & 0.24), respectively, moreover they were not attended in the two localities of survey.

Data presented in Table (3) showed phytonematodes in samples collected from Maghagha district, Menia governorate. Samples contained seven nematode genera i.e. *Helicotylenchus*, *Hoplolaimus*, *Meloidogyne*, *Pratylenchus*, *Trichodorus*, *Tylenchorhynchus* and *Xiphinema*. *Tylenchorhynchus* was the most frequent, population density and PV with 57% FO, 23 PD and 17.4 PV. Other phytonematodes were less frequent and population density.

Data presented in Table (4) showed phytonematodes associated with soybean grown in two localities; Nobaria and Wadi-El-Natroon of Beheria governorate. Sampels contained four nematode genera; *Helicotylenchus*, *Meloidogyne*, *Pratylenchus*, and *Tylenchorhynchus*. Only *Meloidogyne* was attended with samples of the two localities with high frequent of occurrence (52.5%), population density (185) and PV (134.04) in average, followed by *Tylenchorhynchus* with 25%, 50 and 25 FO%, PD and PV, respectively. other two nematode genera Pratylenchus and Helichotylenchus were attended only in Nobaria locality with low frequent and population density.

Data presented in Table (5) showed phytonematodes associated with soybean collected from Mariout district, Alexandria governorate. Samples contained five nematode genera; *Helicotylenchus*, *Meloidogyne*, *Pratylenchus*, *Rotylenchulus* and *Tylenchorhynchus*. *Pratylenchus* was most frequency (75%), followed by *Helicotylenchus* (65%) and *Tylenchorhynchus* (60%). While, *Tylenchorhynchus* had highest PV (712.6), followed by *Pratylenchus* (519.6) and *Helicotylenchus* (472.4). *Rotylenchulus* and *Meloidogyne* were attended with low frequency, population densities, and PV.

Table 2. Occurrence and population density of phytonematodes associated with soybean in two localities of Beni-Suef governorate.

Phytonematodes	Locality								
	Beba n=45			Somsta n=45			Average n=90		
	FO%	PD	PV	FO%	PD	PV	FO%	PD	PV
Heterodera (J_2) sp.	8.88	1	0.30	8.8	3	0.89	8.88	2.0	0.60
Helicotylenchus sp.	-	-	-	11.1	2	0.67	5.6	1.0	0.24
Longidorus sp.	--	-	-	11.1	2	1.00	5.6	1.0	0.24
Meloidogyne (J_2)	11.11	3	1.00	15.56	3	1.18	13.6	3.0	1.10
Pratylenchus sp.	11.1	4	1.33	-	-	-	5.6	2.0	0.47
Tylenchorhynchus sp.	77.8	42	37.04	26.7	11	5.68	52.2	26.0	18.78
Xiphinema sp.	11.1	4	1.33	-	-	-	5.55	2.0	0.47

FO% = frequency X100, PD = population density in 250 g soil, PV = prominence value = PD $\sqrt{absolute\ frequency}$
n = number of samples.

Table 3. Occurrence and population density of phytonematodes associated with soybean in Maghagha district, Menia governorate.

Phytonematodes	Mean of 100 samples		
	FO%	PD	PV
Helicotylenchus sp.	1	1	0.1
Hoplolaimus sp.	5	4	0.89
Meloidogyne (J_2) sp.	3	3	0.52
Pratylenchus sp.	9	4	1.2
Trichodorus sp.	1	1	0.1
Tylenchorhynchus sp.	57	23	17.36
Xiphinema sp.	1	1	0.1

FO% = frequency X100, PD = population density in 250 g soil, PV= prominence value = PD $\sqrt{absolute\ frequency}$.

Table 4. Occurrence and population density of phytonematodes associated with soybean in two localities of Beheira governorate.

Phytonematodes	Locality								
	Nobaria n=20			Wadi El-Natroon n=20			Average n=40		
	FO%	PD	PV	FO%	PD	PV	FO%	PD	PV
Helicotylenchus sp.	10	30	9.49	-	-	-	5.0	15	3.35
Meloidogyne (J_2)	50	120	84.85	55	250	185.4	52.5	185	134.04
Pratylenchus sp.	5	60	13.42	-	-	-	2.5	30	4.74
Tylenchorhynchus sp.	50	100	70.7	-	-	-	25.0	50	25.0

FO% = frequency X100, PD = population density in 250 g soil, PV = prominence value = PD $\sqrt{absolute\ frequency}$
n = number of samples.

Table 5. Occurrence, population density and prominence value of phytonematodes associated with soybean in Mariout district, Alexandria governorate.

Phytonematodes	Mean of 100 samples		
	FO%	PD	PV
Helicotylenchus sp.	65	586	472.4
Meloidogyne (J_2) sp.	15	120	46.48
Pratylenchus sp.	75	600	519.6
Rotylenchulus reniformis	25	180	90.0
Tylenchorhynchus sp.	60	920	712.6

FO% = frequency X100, PD = population density in 250 g soil, PV = prominence value = PD $\sqrt{absolute\ frequency}$.

Data presented in table (6) showed phytonematodes recovered from samples collected from all localities of four governorates. Samples contained ten nematode genera, *Heterodera*, *Helicotylenchus*, *Hoplolaimus*, *Longidorus*, *Meloidogyne*, *Pratylenchus*, *Rotylenchulus*, *Trichodorus*, *Tylenchorhynchus* and *Xiphinema*. Four of them, were attended in the four governorates i.e. *Helicotylenchus*, *Meloidogyne*, *Pratylenchus* and *Tylenchorhynchus*. Last nematode was more frequent in samples of Beni-Suef and Menia governorates, while *Meloidogyne* was more frequent in samples of Beheira governorate; *Pratylenchus* was dominant in samples of Alexandria. Over all governorates, *Tylenchorhynchus* was the most frequent (50.4%) as general average, followed by *Meloidogyne* (15.6%), *Pratylenchus* (12.0%) and *Helicotylenchus* (8.4%).

Identification of root-knot nematode *Meloidogyne* species infecting root of soybean collected from different localities showed the occurrence of three *Meloidogyne* species; *M.incognita, M. javanica* and *M. arenaria.* The former species was the most prevalent with 48.75% occurrence as well it was found in samples of all surveyed localities, followed by *M. javanica* with 37.5%, which was found in samples of Somsta, Nobaria and Mariout, and *M. arenaria* with 13.75% occurrence was isolated from samples of Nobaria and Mariout (Table 7).

Nematophagous fungi associated with nematodes in soybean rhizsosphere

Data presented in Table (8) indicated that four nematophagous fungi were associated with samples i.e. *Arthrobotrys conoides*, *Dactelaria brochopaga*, *Monacrosporium* spp., and *Verticillium* spp. *A.conodies* was the most frequent fungus with 80% frequency. Followed by *D. brochopaga* with 60% frequency. *Verticillium*, sp. and *Monacrosporium* sp. were less frequent with 40% and 20% frequency, respectively.

Table 6. Frequency, Population density and prominence value of nematodes associated with soybean in four governorates of Egypt.

Nematodes	Beni-suef n=90			Menia n=100			Beheira n=40			Alexandria n=40			General average n=250		
	FO%	PD	PV	FO%	PD	PV	FO%	PD	PV	FO%	PD	PV	FO%	PD	PV
Heterodera	7.7	2	0.56	-	-	-	-	-	-	-	-	-	3.2	0.75	0.13
Helicotylenchus	5.6	1	0.24	1	1	0.1	5.0	15	3.35	65	586	472.4	8.4	50.0	14.5
Hoplolaimus	-	-	-	5	4	0.89	-	-	-	-	-	-	2.0	1.6	0.33
Longidorus	5.6	1	0.24	-	-	-	-	-	-	-	-	-	2.0	0.36	0.5
Meloidogyne	13.3	3	1.1	3	3	0.52	52.5	185	134.04	15	120	46.48	15.6	41.5	16.4
Pratylenchus	5.6	2	47.0	9	4	1.20	2.5	30	4.79	75	600	519.6	12.0	55.1	19.1
Rotylenchulus reniformis	-	-	-	-	-	-	12.5	90	31.8	25	180	90.0	4.0	28.8	5.8
Trichodorus	-	-	-	1	1	0.1	-	-	-	-	-	-	0.4	0.4	0.03
Tylenchorhynchus	52.2	26.0	18.78	57.0	23	17.96	25.0	50	25.0	60	920	712.6	50.4	100.2	71.1
Xiphinema	5.55	2	0.47	1	1	0.1	-	-	-	-	-	-	2.4	1.12	0.17

Table 7. Species of Meloidogyne infecting soybean roots collected from different localities of Egypt.*

Phytonematodes	(%) Frequency of occurrence		
	M. arenaria	***M. incognita***	***M. javanica***
Somsta (Brni-Suef)	-	55	45
Nobaria (Beheira)	30	40	30
Wadi-El-Natroon (Beheira)	-	50	-
Mariout (Alexandria)	25	50	50
Average	13.75	48.75	37.5

* Roots of soybean collected from locations of Beba (Beni-Suef) and Maghagha (Menia) were not infected with the root-knot nematodes.

Table 8. Occurrence of nematophagous fungi associated with nematodes recovered from soybean rhizsosphere in different localities.

Governorate	Locality	Associated fungi			
		Arthrobotrys conodies	***Dactelaria brochopaga***	***Monacrosporium* sp.**	***Verticillium* sp.**
Beni-Suef	Somsta	+	-	+	+
	Beba	-	+	-	-
Alexandria	Amria	+	-	-	-
El-Beheria	Nobaria	+	+	-	-
Menia	Maghaga	+	+	-	+
% Occurrence		80%	60%	20%	40%

Discussion

Survey of phytonematodes found in rhizsosphere of soybean grown in different locations of middle and northern Egypt indicated the presence of ten nematode genera. Five of them viz. *Tylenchorhynchus*, *Meloidogyne*, *Pratylenchus*, *Helicotylenchus*, and *Rotylenchulus* were found with considerable frequencies of occurrence (FO %), and prominence value (PV). The stunt nematodes, *Tylenchorhynchus* spp. were the most frequent nematodes with 50.4 FO%. These nematodes were also commonly found associated with legumes in *Mediterranean* countries Greco *et al.*, [28]; Di Vito *et al.*, [29]; Castillo *et al.*, [30]; Salem *et al.*, [17], however their pathogenicity on soybean has not been demonstrated yet.
The root-knot nematode, *Meloidogyne* spp. occurred in all locations with 15.6% frequency, as well as their species infecting soybean included *M. incognita, M. javanica* and *M. arenaria*. *M. incognita* was the most prevalent (48.75% frequency), followed by *M. javanica* (37.5%) and *M.arenaria* (13.75%). Similarly, these Meloidogyne species were found to infect soybean in different regions in USA, and *M. incognita* was the most prevalent (70%) followed by *M. javanica* (24%) and *M. arenaria* (6%) Garcia and Rich, [31]. Also, *M. incognita* and *M. javanica* were the most species frequently detected in soybean fields in Argentina and South Africa Doucet and Pinochet, [32]; Fourie *et al.*, [33]. All of these species were reported to cause damage to soybean grown in Alabama/USA Sikora *et al.*, [34], in North Central Florida/USA Garcia and Rich, [31], in South Africa Fourie *et al.*, [33], in Brazil Lima et al., [35] and in Egypt Korayem and Mohamed, [36]. So, a good strategy for their management should be considered.
The root-lesion nematodes, *Pratylenchus* spp. were found to attack soybean throughout the world Noel, [37]. Important lesion nematodes species reported in soybean include fifteen species, *Pratylenchus brachyurous* and *P.zeae* is dominant in tropical and subtropical regions Sikora *et al.*, [38]. *P. brachyurus* is considered the most important nematode in soybean in Brazil, reducing yield by 30-50% Rodrigues *et al.*, [39]. Effect of the root-lesion nematodes on soybean yield is still not studied under Egyptian conditions. So many studies upon relation between the root-lesion nematode populations and soybean yield are necessary.
The reniform nematode *Rotylenchulus reniformis* was also found in rhizsosphere of soybean. It was first identified as a pathogen of soybean in West Africa in 1956 McGawley and Overstreet, [40]. Yield reductions in soybeans of over 30% have been attributed to reniform nematode infestation in the southern USA Lawrence and Mclean, [41]. *R. reniformis* is also widely distributed infecting soybean in Brazil causing uneven growth Dias *et al.*, [42]. In a previous survey study on plant-parasitic nematodes associated with soybean in Upper Egypt *R. reniformis* were frequent Salem *et al.*, [17], however its real impact on soybean yield is still not clear, so estimation of its economic threshold on soybean under Egyptian conditions is wanted.
The spiral nematodes *Helicotylenchus* spp. was also associated with soybean plants having 8.4%, FO% and 14.5 PV. These nematodes were found to be associated with different leguminous crops Sikora *et al.*, [38]. However, effect of these nematodes on soybean production is not clear either in Egypt or in the world. Thus they are considered of limited economic importance on soybean worldwide.
Data also revealed the presence of four nematophagous fungi in the rhizsosphere of soybean plant. *Arthrobotrys conoides* and *Dactelaria brochopaga* were most frequency of occurrence. These trapping fungi were reported to suppress nematode pathogens Tian *et al.*, [43]; Hallman *et al.*, [44]; Korayem *et al.*, [45]. Suppressiveness depends on their abundance in the soil. Activation and increasing population of these bioagents in the soil be possible by adding specific organic amendments to soil. Using sustainable control of pathogenic nematodes is new approach for avoiding use of toxic chemical nematicides polluting environment. Using nematode-antagonistics microorganisms for controlling nematodes is one of the important element of the sustainable nematode management.

Conclusion

In general, our data showed that there are a significant number of phytonematodes negatively impact soybean yield in worldwide and Egypt. Due to the intensified use of agricultural land and applying unsustainable control practices, nematode problem on soybean may come to be more seriousness. Information concerning the occurrence and distribution of phytonematodes associated with soybean grown in different regions of Egypt is important to assess their potential to cause economic damage. Growers should believe that nematode pests negatively affect soybean yields, and sustainable control practices should be considered for decreasing nematode population under economic levels to have a good yield performance.

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