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PLANT-PARASITIC NEMATODES, SOIL FUNGI ASSOCIATED WITH BANANA PLANTATIONS IN EGYPT

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ABSTRACT

Banana (*Musa Spp.*) is considered an economical monocotyledons plant grown in the most governorates of Egypt, Banana suffers from a wide range of plant disease especially root-knot Nematode and root-rot by different soil borne fungi, six to eight genera of nematodes representing ecto and endo-parasitic forms were recorded from the banana orchards grown in four Egypt Governorates with different population densities, frequencies of occurrence and prominence values. The root-knot nematodes, *Meloidogyne spp* and the spiral nematodes, *Helicotylenchus spp.* Are the major nematode pests on banana roots. The incidence of other sixgenera of Nematodes at low population densities, percentage of frequency and prominence values were also reported. Occurrence of the surveyed nematodes associated with banana roots are recorded in relation to different soil fungi isolated from rhizosphere of banana roots. About twenty one soil-borne fungi were isolated from naturally infected banana roots, *Botryodiplodia theobromae* and *Fusarium solani* were the most frequent isolates followed by *Rhizoctonia solani* and *F. equiseti*

Keywords: Banana-Root-Knot Nematode-Soil fungi-Egypt.

INTRODUCTION

In Egypt, the total cultivated area of banana reached 59651 feddans in 2003, but the fruiting area equals 50711 feddans producing 870880 tons with an average 17.45 tons per feddan (Statistics Dept. Reports, 2003 A.R.C., Ministry of Agriculture).

Banana plants are subjected to infection with many diseases by different pathogens, the interaction between root-knot nematode and soil-borne fungi considered the most important disease because of its damge and widespread occurrence in banana plantations. Brun and Sioussaran (1968) demonstrated that species of *Fusarium*, *Rhizoctonia* and *Botryodiplodia*, which were isolated from banana roots, were pathogenic to the same organs from which they were originally isolated. Root weights in inoculated soils were less than those in uninoculated soils. Abdel-Hadi *et al.* (1987) reported that inoculation of banana roots with *F. oxysporum* f.sp. *cubense* and the nematode *Radopholus similes* induced lesions on banana roots after one week. The percentage of rotted roots was 36.5% with the nematode alone, 47.8% with both organisms and 4% with the fungus alone. Ogundero (1987) stated that among fungi which had repeatedly isolated from bananas randomly collected from fields were: *Botryodiplodia theobromae, Colletotrichum musa, Ceratocysits paradoxa, F. solani, F. roseum, F. moniliforme, F. oxysporum* and *verticillium theobromae*. EL-Sheikh (1989) reported that four *Fusarium species* associated with diseased banana plants

were isolated from different parts of naturally infected samples collected from Menoufyia, Qaluobyia and Dakahlyia governorates in Egypt. these fungi were identified as *Fusarium oxysporum* Schlecht, *F. moniliforme* Sheld, *F. semitectum* Berk and Rav. And *F. equiseti*. The pathogenicity test on banana transplants of Williams cv. under greenhouse conditions revealed that all isolated species were pathogenic. However, *F. oxysporum* was the most aggressive fungus which exhibited wilt 2 monthes after inoculation and severe wilt after 6 monthes. *F. moniliforme* and *F. semitectum* caused moderate wilt to banana transplants, while *F. equiseti* was less virulent.

Mateille and folkerstsma (1989) indicated that isolation of fungi from surface lesions on the roots of banana cv. Poyo showed *Fusarium oxysporum* of to be the most widespread in Ivory Coast banana plantations. Extraction of nematodes from these lesions confirmed the pairing *Radopholus similis- F. oxysporum* as the frequent (12-25%) followed by *Helicotylenchus multicinctus- F. oxysporum* (6-13%). But these are random associations, dependent neither on nematode species nor soil type (clay, silt, sand and peat). Up to 30-50% of the lesions contained only nematode and fungi may be individually pathogenic. Aboul-Eid and Ameen (1991) showed that *Helicotylenchus exallus*, *H. dihysteroides, Meloidogyne incognita* and *Rotylenchulus reniformis* were the predomiant nematode species associated with six banana cultivars, i.e. Maghrabi, Paradica, Hindi, Cinnari, Moscat and Baladi from Qaluobyia, Giza and Qena Governorates. They reported the occurrence of *Criconemoides spp.*, *Longidorus africanus.*, *L. taniwha*, *Tylenchorhynchus spp.* and *Xiphinema sp.* as well on banana. Khalil *et al.* (1993) reported that the major causals of banana diseases in Libya were *Pseudomonas solanacearum, F. oxysporum* and the root-knot nematode *Meloidogyne javanica*. Mahdy *et al.* (1993) isolated *F. moniliforme*, *Pythium spp.* and *Botryodiplodia spp.* from the external rotted tissues of corms and roots of banana Williams cv.. Abd-Allah (1994) reported that *F. moniliforme, F. solani* and *R. solani* were most frequently isolated from naturally infected banana roots showing rot symptoms and the three fungi were virulent at various degrees, to banana cvs. *F. moniliforme* was the most virulent followed by *R. solani* and *F. solani.* Frisullo *et al.*, (1994) reported that foot rot of banana was observed during 1990-1991 in Crete, Greece. *F. compactum* was mostly isolated from infected root and basal corm tissues, followed by *F. oxysporum* and *F. solani.* Vovlas *et al.*, (1994) reported that the most prevalent nematode species inhabiting the root system of banana in Crete were *Meloidogyne javanica*, which occurred in nearly 95% of the sampled sites. *Helicotylenchus multicinctus* and *Paratylenchus goodeyi,* were found in 28% and 18%, respectively of the banana plantations. Nematode-infected banana roots were frequently in a general state of decay, especially when the roots were also concomitantly infested by soil-borne fungi and/or bacteria. Several species of soil-borne fungi were isolated from necrotic tissues that in order of frequency of occurrence were: *Acremonium spp., Rhizoctonia solani, Fusarium oxysporum, F. solani, pythium spp., F. compactum* and *Cylindrocarpon spp.* EL-Said (1995) indicated that the "exposed plate" method was used to trap fungal spores from the atmosphere of Qena over a period of 1 yr (Jan.-Dec. 1992). A total of 78 species and 2

varieties belonging to 38 genera developed on plates of glucose and cellulose-Czapek's agar at 28 degrees C. Counts of airborne fungi on glucose and cellulose agar plates showed seasonal trends with peaks in Dec. and Nov., respectively. The most common genera were Acremonium, Alternaria, Aspergillus, Cladosporium, Cochliobolus, Curvularia, Fusarium, Gibberella, Memnoniella, Mycosphaerella, Myrothecium, Nectria, Penicillium and Setosphaeria. Best counts of fungi were estimated during different monthes.

Krauss *et al.*, (1998) reported that crown rot of banana was in cited caused by *Colletotrichum musae, Fusarium moniliforme* var. *subglutinans, F. moniliforme* [*Gibberella fujikuroi*] *F. pallidoroseum, Botryodiplodia theobromae* and *Nigrospora sphaerica*. Jones (2000) found that in a healthy root system, root rots only affect a very small proporation of roots. Large extensive and deep root systems were developed in well drained and deep loamy soil. Root rot less likely to occur if nematodes were controlled and if soil structure and moisture content permit plants to grow under optimum conditions. Eissa *et al.*, (2003) mentioned that five to eight genera of nematodes were recovered from the banana orchards grown in four Nile Delta and four Upper Egypt Governorates with different population densities, frequencies of occurrence and prominence values. The root-knot nematode, *Meloidogyne spp.* And the spiral nematode, *Helicotylenchus spp.* are the major nematode pests on banana roots. Manal (2004) reported that survey studies reveal that nine genera of plant parasitic nematodes were found associating with banana orchards collected from five different localities; El Behera, Nobaria, Ismailia, Sadat and Ciro-Alex Desert road, representing sandy soils of some newly reclaimed land, Egypt. the root-knot nematodes, *Meloidogyne spp.* Were the most frequently occurred and highest population density in the five surveyed localities.

MATERIAL AND METHODS

Source of soil and root samples:

Apparently diseased banana plants were randomly collected from banana plantations in four governorates, i.e. El-Behera, El-Giza, El-Menoufyia and El-Qalubyia during the growing seasons of 2002 to 2003. each sample was composed of six sub samples obtained by carefully digging the soil around the plant.

Nematode extraction, numeration and identification:

A total of 180 soil and root samples were collected from rhizosphere of banana plants from four previously mentioned governorates. Each soil sample was thoroughly mixed and an aliquot of 250 g soil was processed for nematode extraction by means of O' ostenbrink's elutriation (Goodey 1957), then sieved through 60 and 350 mesh screens, the resulting suspension was cleared by mean of Bearmann-pan technique for separating active larvae by leaving the suspension for 48 hours to allow all larvae to pass through the

tissue paper down to the pan. Count of nematodes and identification to the genus, were accomplished, using the Hawksely counting slide.

Isolation, purification and identification of fungi associated with banana roots:

Roots of banana plants showing symptoms of root-rot disease, were collected from different four previously mentioned governorates. Diseased samples were thoroughly washed with tap water to remove any adhering soil particles. The samples were cut into small pieces (0.5 x 0.5 cm.) and surface sterilized with 0.01 sodium hypochlorite for 3 min., rinsed three times in sterile distilled water and dried between two sterilized filter papers. Samples were asceptically placed on potato dextrose agar (PDA) medium containing 1000 ppm of streptomycin sulfate and incubated at 25 °C for one week. Observation was daily carried out and any emerged fungus was picked up and cultured on fresh PDA plates. All the isolated fungi were purified using either single spore or hyphal tip techniques (Dhingra and Sinclair, 1985) and subcultured on PDA medium.

Identification of the isolated fungi was carried out at the Department of Fungal Taxonmy, Plant Pathology Institute, A.R.C, Giza and colleoges in the Phytopathology Department, N.R.C. Dokki. according to Drechsler, C. (1937), Gilman (1957), Ram Nath *et al.* (1970), Barnett and Hunter (1972), Chidambaram *et al.* (1973), Domsch *et al.* (1980), Nelson *et al.* (1983).

Stock cultures were maintained on PDA slants and kept in a refrigerator at 5-10°C for further study. Stocks were routinely sub-cultured on fresh slant every three months. The frequency of the isolated fungi were calculated separately for each collected samples.

RESULTS AND DISCUTION

Plant parasitic nematodes are considered the most pathogenic organisms to Banana plantations especially when associated with soil borne fungi which are responsible for considerable banana growth damage and yield losses in terms of quality and quantity [Abdel-Hadi *et al* (1987), Mateille and Flokerstsma (1989), Khalil *et al.* (1993), Vovlas *et al.* (1994) and Jones (2000)]

Nematodes associated with Banana roots:

The present results of the survey study reveal that eight nematode genera ecto and endo-parasitic forms were recovered from the collected samples.

Soil and root samples were collected from various orchards of Banana cultivars, i.e. Grand-Nain, Hindi, Maghrabi, paradica and Williams. From four different governorates: El-Behera, El-Giza, El-Menoufyia and El-Qaluobyia.

Obtained data indicated that: in generally six or eight important plant-parasitic nematodes attacked Banana plants (Tables 1 and 2). These genera were *Criconemoides*, *Helicotylenchus*, *Hoploliamus*, *Meloidogyne*,

Pratylenchus, Rotylenchulus, Tylenchus and *Xiphinema*. On the other hand, nematode frequency of occurrence ranged from 3.9% for the genus *Rotylenchulus* to 85.3% for the genus *Meloidogyne* (Table2 and Fig 1). The root-knot nematodes, *Meloidogyne spp.* and the spiral nematodes, *Helicotylenchus spp.* were the most frequent nematodes and accounted for relatively high population densities, frequencies of occurrence and prominence values in all samples (Table 2 and Fig 1). F.O.% (85.3, 59.1), P.D. (663.3, 378,0), P.V. (614.1, 305.4) respectively.

Table (1): Occurrence of fungi associated with plant-parasitic Nematodes in rhizospheres of banana roots in 4 governorates.

Governorates	Cultivar	Nematode Species	Associated Fungi
Behera	Williams	*Helicotylenchus spp.*	*Fusarium semitectum*
		Meloidogyne spp.	*Fusarium equiseti*
		Xiphenema spp.	*Rhizoctonia solani*
		Hoplolaimus spp.	
Giza	Grand-Nain	*Meloidogyne spp.*	*Acremonium kiliense*
		Helicotylenchus spp.	*Trichoderma virdi*
		Tylenchus spp.	*Penicillium spp.*
	Hindi	*Hoploliamus spp.*	*Arthrobotrys oligospora*
		Helicotylenchus spp.	*Macrophomina spp.*
		Meloidogyne spp.	*Dactylaria thaumasia*
		Tylenchus spp.	*Rhizoctonia solani*
			Aspergillus niger
	Maghrabi	*Helicotylenchus spp.*	*Fusarium solani*
		Meloidogyne spp.	*Penicillium spp.*
		Rotylenchulus spp.	*Pythinum spp.*
		Tylenchus spp.	
	Paradica	*Meloidogyne spp.*	*Alternaria spp.*
		Xiphinema spp.	*Botryodiplodia theobromae*
		Hoploliamus spp.	*Arthrobotrys oligospora*
		Pratylenchus spp.	*Macrophomina phaseolina*
		Helicotylenchus spp.	*Thielaviopsis paradoxa*
			Fusarium monilliform
			Fusarium oxysporum
			Phytophthora spp.
	Williams	*Meloidogyne spp.*	*Acremonium kiliense*
		Helicotylenchus spp.	*Phoma musa*
		Pratylenchus spp.	*Rhizoctonia solani*
		Tylenchus spp.	*Trichoderma virdi*
		Criconemoides spp.	*Aspergillus fluvas*
Menoufyia	Williams	*Helicotylenchus spp.*	*Botryodiplodia theobromae*
		Meloidogyne spp.	*Nigrospora sphaerica*
		Pratylenchus spp.	*Phytophthora spp*
		Tylenchus spp.	
Qalubyia	Maghrabi	*Meloidogyne spp.*	*Fusarium. solani*
			Phytophthora spp.
	Williams	*Helicotylenchus spp.*	*Alternaria spp.*
		Hoploliamus spp.	*Botryodiplodia theobromae*
		Meloidogyne spp.	*Fusarium monilliform*
		Pratylenchus spp.	*Fusarium semitectum*
		Criconemoides spp.	*Fusarium. solani*
			Macrophomina phaseolina
			Rhizoctonia solani

Table (2): Population densities (P.D.), Percentage of Frequency Occurrence (F.O. %) and Prominence Value (P.V.) of plant-parasitic nematode associated with banana cultivars grown in 4 governorates.

Nematode genera	Behera (n=30)			Giza (n=60)			Menoufyia (n=40)			Qaluobyia (n=50)			General average		
	F.O.%	P.D	P.V	F.O.%	P.D	P.V	F.O.%	P.D	P.V	F.O.%	P.D	P.V	F.O.%	P.D	P.V
Criconemoides spp.	0	0	0	5.2	60	13.7	0	0	0	31.5	350	196.4	9.2	102.5	52.5
Helicotylenchus ssp	30.5	150	82.8	75.0	490	424.4	58.3	322	245.9	72.5	550	468.3	59.1	378	305.4
Hoploliamus spp.	0	0	0	25.7	150	76.0	0	0	0	50.8	430	306.5	19.1	145	95.6
Meloidogyne spp.	90.0	820	777.9	87.0	650	606.3	83.8	583	533.7	80.5	600	538.3	85.3	663.3	614.1
Pratylenchus spp.	0	0	0	43.2	306	201.1	30	370	202.7	45.6	380	256.7	29.7	264	165.1
Rotylenchulus spp.	0	0	0	15.5	112	44.1	0	0	0	0	0	0	3 9	28	11.0
Tylenchus spp.	0	0	0	11.0	78	25.9	8.8	81	24.0	0	0	0	5 0	39.8	12 5
Xiphenema spp.	22.8	100	47.6	5.3	60	13.8	0	0	0	0	0	0	7.0	40	15.4

N= Number of collected sample F.O. %= Percentage of frequency occurrence
P.D.= Population densities in 250 gm/soil P.V.= prominence value

Other less occurring nematodes included in a descending order were *Pratylenchus spp., Hoploliamus spp., Criconemoides spp., Xiphinema spp.* and *Tylenchus spp.*

Fungi associated with banana roots:-

Twenty one soil-borne fungi were isolated from naturally infected banana roots showing typical symptoms of root-rot, these fungi were identified as: *Acremonium kiliense* (= *Cephalosporium acremonium*), *Alternaria spp., Arthrobotrys oligospora, Aspergillus fluvas, A. niger, Botryodiplodia theobromae, Dactylaria thaumasia, Fusarium equiseti, F. monilliform, F. oxysporum, F. semitectum, F. solani, Macrophomina phaseolina, Nigrospora sphaerica, Phoma musa, Penicillium spp., Phytophthora spp., Pythinum spp., Rhizoctonia solani, Thieloviopsis paradoxa* and *Trichoderma virdi.*

The Isolated fungi [*Acremonium kiliense* (= *Cephalosporium acremonium*), Domsch K.H. *et al.* 1980], *Botryodiplodia theobromae, Nigrospora sphaerica*, were identified for the first time in Egypt, and the isolation of the fungi : *Arthrobotrys oligospora* (Drchseler. 1937), *Dactylaria thaumasia* (Drchseler 1937, var. longa Dixon (Shepherd, 1955)), *Macrophomina phasiolina, Phoma musa* and *Thieloviopsis paradoxa*. Considered as the first time of these fungi to isolate from banana roots and rhizosphere in Egypt.

Obtained data in Table (3) and illustrated by Fig (2) indicated that *B. theobromae* and *F. solani* were the most frequent isolates (13.9%) followed by *R. solani* and *F. equiseti* (8.9%, 8.3%) while *A. fluvas, Alternaria spp.* and *Penicillium spp.* were the lowest frequent fungi and the rest fungi were intermediate (Table 3 and Fig 2).

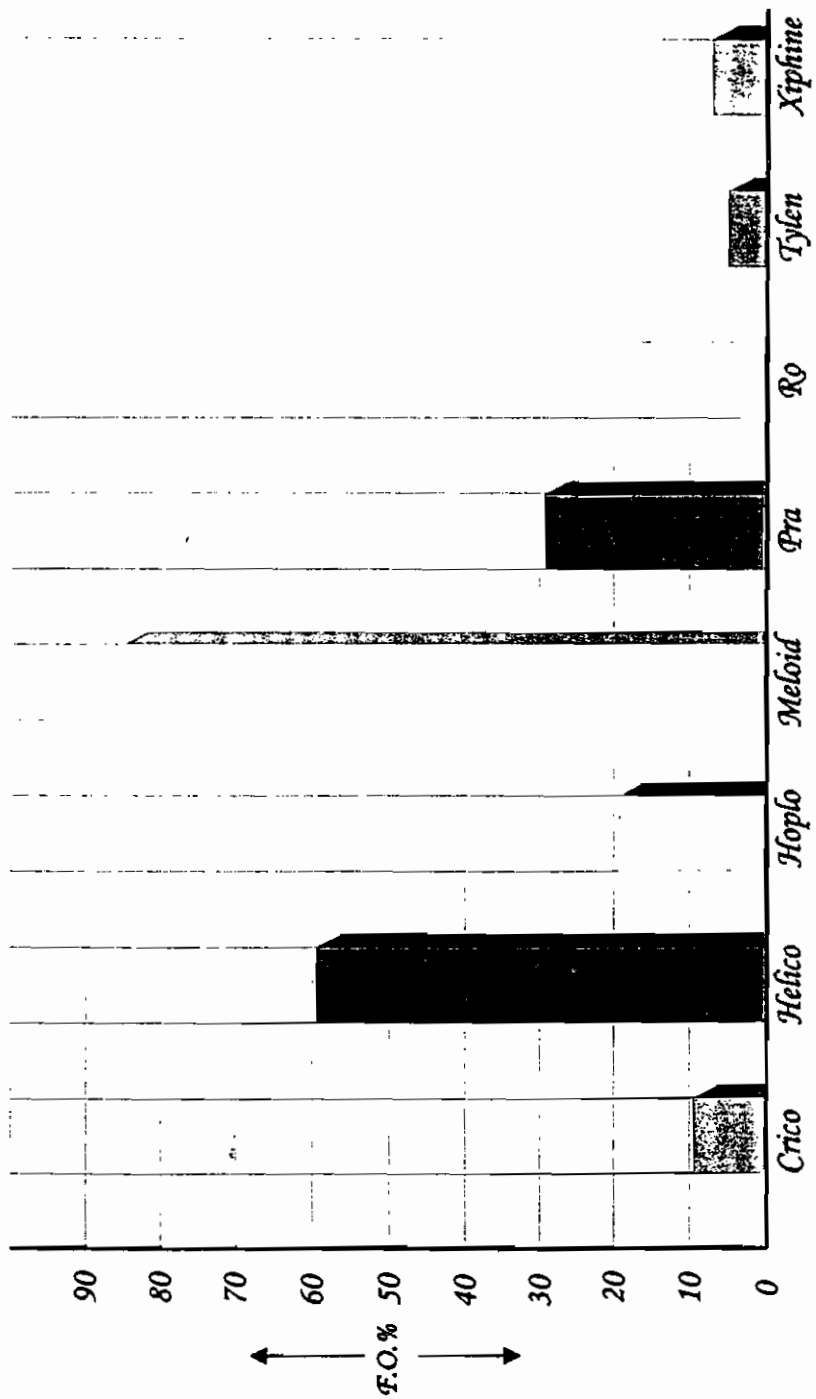

Fig (1): Frequency occurrence (F.O.%) of plant parasitic nematode genera associated with banana cultivars , in Egypt during 2002 - 2003 growing season .

Table (3): Frequency occurrence of different isolated fungi from naturally infected banana roots of different varieties collected from 4 governorates during 2002-2003 growing seasons.

The isolated fungi	Behera	Giza					Menoufyia	Qaluobyia		Total	F.O. %
	Williams cv.	Grand-Nain cv.	Hindi cv.	Maghrabi cv.	Paradica cv.	Williams cv.	Williams cv.	Maghrabi cv.	Williams cv.		
Acremonium kiliense	0	5	0	0	0	5	0	0	0	10	5.6
Alternaria spp.	0	0	0	0	2	0	0	0	1	3	1.7
Arthrobotrys oligospora	0	0	3	0	2	0	0	0	0	5	2.8
Aspergillus fluvas	0	0	0	0	0	1	0	0	0	1	0.6
A. niger	0	0	5	0	0	0	0	0	0	5	2.8
Botryodiplodia theobromae	0	0	0	0	10	0	8	0	7	**25**	**13.9**
Dactylaria thaumasia	0	0	2	0	0	0	0	0	0	2	1.1
Fusarium equiseti	15	0	0	0	0	0	0	0	0	15	8.3
F. monilliform	0	0	0	0	2	2	0	0	3	7	3.9
F. oxysporum	0	0	0	0	3	0	0	0	0	3	1.7
F. semitectum	5	0	0	0	0	0	0	0	5	10	5.6
F. solani	0	0	0	10	0	0	0	12	3	**25**	**13.9**
Macrophomina phaseolina	0	0	1	0	3	0	0	0	5	9	5.0
Nigrospora sphaerica	0	0	0	0	0	0	7	0	0	7	3.9
Phoma musae	0	0	0	0	0	3	0	0	0	3	1.7
Penicillium spp.	0	2	0	1	0	0	0	0	4	7	3.9
Phytophthora spp.	0	0	0	0	1	0	3	3	0	7	3.9
Pythium spp	0	0	0	4	0	0	0	0	0	4	2.2
Rhizoctonia solani	5	0	4	0	0	5	0	0	2	16	8.9
Thielaviopsis paradoxa	0	0	0	0	7	2	0	0	0	9	5.0
Trichoderma virdi	0	5	0	0	0	2	0	0	0	7	3.9
Total	25	12	15	15	30	20	18	15	30	180	

F.O. %= Percentage frequency of occurrence

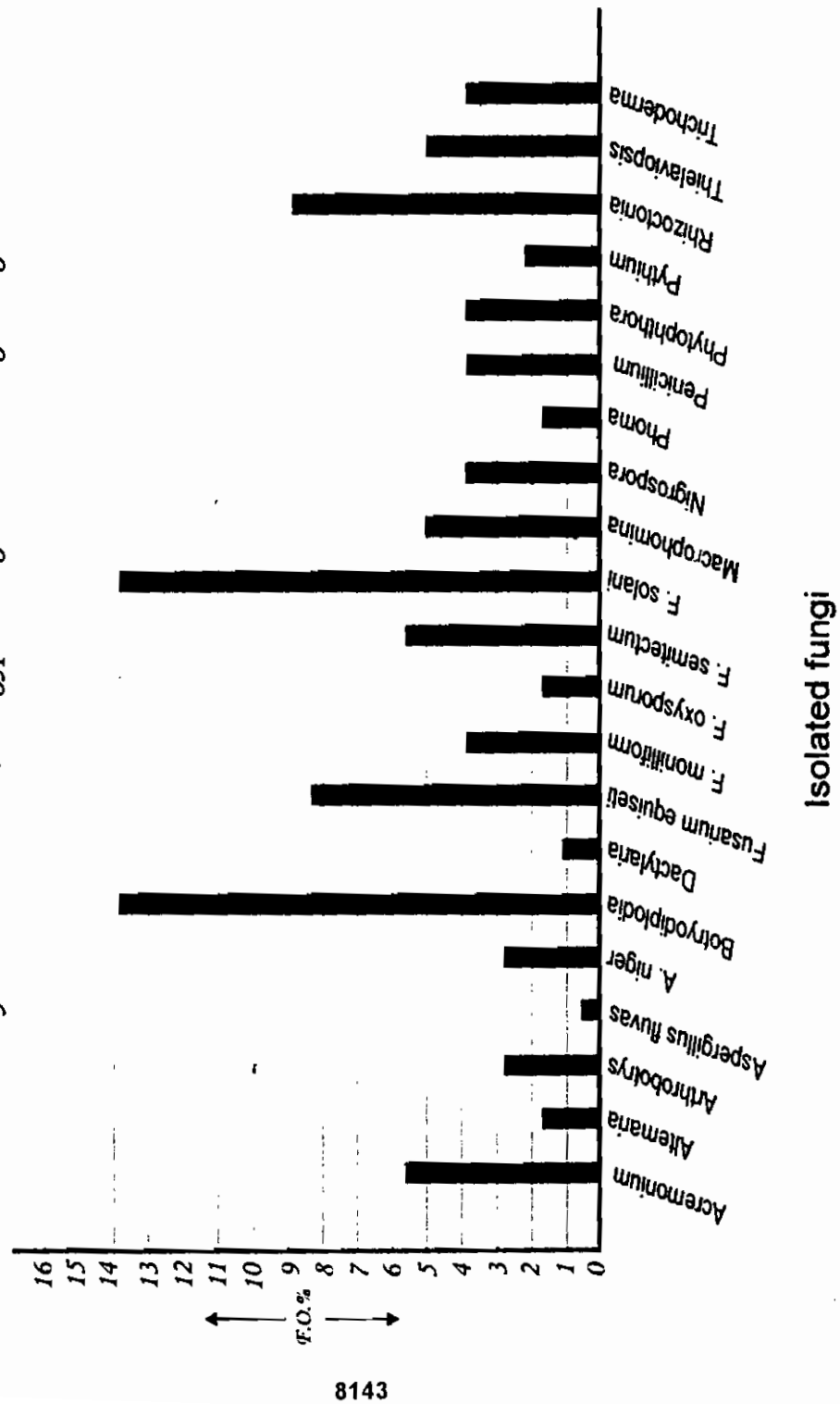

Fig (2): Frequency occurrence (F.O.%) of different isolated fungi from naturally infected roots of banana cultivars , in Egypt during 2002 - 2003 growing season .

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النيماتودا المتطفلة نباتيا وفطريات التربة المصاحبة لمحصول الموز فى مصر

عزة محمد كامل , عبد المنعم ياسين الجندى *, مدحت يوسف مراد , أشرف إسماعيل عافية*

قسم بحوث أمراض الفاكهة والأشجار الخشبية معهد بحوث أمراض النبات

مركز البحوث الزراعيـــة – جيزة – مصر

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يعتبر الموز من أهـــم النباتات الأقتصاديـــة أحادية الفلقه النامية فى معظم محافظات مصـــر والموز يعانى من الأصابـــة بكم كبير من الأمراض النباتيـــه خاصـــة الإصابـــة بنيماتودا تعقد الجـــذور وأعفـــان الجـــذور المتسببـــة عـــن مختلـــف الفطريـــات الكامنـــة فى التربـــــة .

وقد أسفرت هذه الدراسة عن وجود ستة الى ثمانية أجناس نيماتودية مختلفة خارجية وداخلية التطفل سجلت فى بساتين الموز النامية فى أربع محافظات مصرية هى البحيرة – الجيزة– القليوبية – المنوفية بمعدلات مختلفة للكثافة العددية والنسبة المئوية لتكرار الظهور وقيمة التميز ,فكانت نيماتودا التعقد الجذرى (ميليودوجين) والنيماتودا الحلزونية (هليكوتيلنكس) هى الأفات النيماتودية الرئسية التى تصيب المـــوز . اما باقى الأجناس النيماتودية المسجلة فكانت ذات كثافة عددية منخفضـــة وكـــذلك نســـبة مئويـــة منخفضـــة للتكـــرار مـــع أقـــل قيـــم للتميـــــز .

وأيضـــاً سجل معدل تواجد النيماتودا المصاحبة لجذور الموز وعلاقتها بمختلف فطريـــات التربة المصاحبـــة لريزوسفيـــر جـــذور المـــــوز .

حوالى واحد وعشرون فطر من الفطريات الكامنة فى التربة عزلت من جذور الموز المصابة طبيعياً وكانت الفطريـــات البتروذبلوديـــا ثيوربرومى والفيوزاريـــوم سولانى أكثر الفطريات المعزولة تكـــراراً تبعهـــا فطـــر الريزوكتونيـــا ســـولانى ثم فيوزاريـــوم اكوســـــتى .