



## OCCURRENCE OF NEMATODE – ANTAGONISTIC FUNGI AND BACTERIA ASSOCIATED WITH PHYTONEMATODES IN THE RHIZOSPHERE OF WHEAT GROWN IN DIFFERENT GOVERNORATES OF EGYPT

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### Abstract

Survey of plant – parasitic nematodes and their fungal and bacterial antagonists in the rhizosphere of wheat was done in eight governorates, Egypt. A total of 467 soil sample were collected from 72 locations during 2017-2018 growing season. Samples contained eleven phytonematodes, four of them were more common in samples namely, *Helicotylenchus* spp., *Heterodera* spp., *Pratylenchus* spp., and *Tylenchorhynchus* spp. Fifteen nematode-antagonistic fungi were isolated/ from the wheat rhizosphere, nine of them were nematophagous fungi viz. *Arthrobotrys conoides*, *A. oligospora*, *Dactylaria brochopaga*, *D. thaumasia* var. *longa*, *Dactylella* spp., *Monacrosporium* spp., *Harposporium anguillulae*, *Meria* spp., *Verticillium* spp., and six of them were fungi producing toxic substances viz. *Alternaria* spp., *Aspergillus* spp., *A. niger*, *Fusarium* spp., *Penicillium* spp. and *Trichoderma* spp. *Penicillium* spp., *A. conoides*, *D. Thaumasia* var *longa*, *Aspergillus* spp., *Verticillium* spp. and *Trichoderma* spp. were the most frequent in samples, their % frequencies were 28.2%, 28.0%, 28.0%, 22.0%, 16.0% and 9.0%, respectively. Six rhizobacteria colonies, *Bacillus* ( $B_{sp1}$ ,  $B_{sp2}$ ,  $B_{sp3}$ ), *Pseudomonas* ( $P_{sp1}$ ,  $P_{sp2}$ ) and *Serratia* sp. were also isolated from wheat rhizosphere.  $P_{sp1}$ ,  $B_{sp2}$  and  $B_{sp1}$  isolates were the most frequent with 100%, 75.5% and 62.5% frequency, respectively.

**Keywords:** Phytonematodes, nematode antagonists, microbial community, occurrence, wheat, Egypt.

### Introduction

Wheat is considered one of the most important source of food, particularly in developing countries, as it is the staple food of about 35% of world population (Oerke *et al.*, 1999; Shewry and Hey, 2015). Wheat also stands the prime position, in terms of production and acreage within the cereal crops in the world (FOASTAT, 2016).

Wheat (*Triticum aestivum* L.) is subject to attack by many phytonematodes reducing its production worldwide (Nicol, 2002; McDonald and Nicol, 2005; Smiley *et al.*, 2005 a; Bockus *et al.*, 2009; Smiley and Nicol, 2009). Nematode pathogens such as the cereal cyst nematodes (*Heterodera* spp.) and the root-lesion nematodes (*Pratylenchus* spp.) were reported to cause a dramatic damage to wheat production causing as much as 50% yield loss (Meagher, 1972; Orion *et al.*, 1984; Taylor *et al.*, 1999; Smiely *et al.*, 2005b; Namouchi-Kachori *et al.*, 2008). So controlling these nematode pathogens is of considerable importance to increase wheat production. Different control options such as chemical, cultural, genetic and biological control are accessible (Nicol and Rivoal, 2008). Chemical nematicides are costly and restricted due to their adverse impact on the environment and human health, as well as cultural control or nematode-resistant host plants are often not practical or not available (Nyczepir and Thomas, 2009). On the other hand, from an ecological perspective, a complete eradication of nematodes from soil is not required, but nematode control objective is how to maintain nematode population densities under damage levels. Biological control of nematodes using potential bioagents offers a promising mean to maintain nematode populations under economic damage thresholds. The fungal antagonists have been reported to be the most promising and practicable bioagents which regulate nematode densities in the soil. They included predacious fungi (Trapping fungi), endo – parasitic of vermiform nematodes, parasites of females and eggs and fungi producing antibiotic substances. Their biological control

against nematodes have extensively been reviewed by many investigators (Morgan – Jones and Rodriguez – Kabana, 1987; Stirling, 1991; Siddiqui and Mahmood, 1996; Walia and Vats, 2000; Chen and Dickson, 2004a; Jansson and Lopez- Llorca, 2004; Hallman *et al.*, 2009; Moosavi and Zare, 2012; Yang and Zhang, 2014; Askary, 2015).

Bacterial antagonists of nematodes have been also studied and appeared to be an important bioagents for regulating nematode population in the arable soils. Different group of bacteria have been reported to have a nematocidal effect, including genera *Acetobacter*, *Agrobacterium*, *Bacillus*, *Burkholderia*, *Chromobacterium*, *Enterobacter*, *Pseudomonas*, *Serratia*, *Stenotrophomonas* and *Streptomyces* (Chen and Dickson, 2004b; Ibrahim 2011). The Nematophagous bacteria have been isolated from soil, plant tissues and nematode cysts and eggs. Their antagonistic activity against plant-parasitic nematodes have been reported by many investigators (Sayre and Starr, 1988; Stirling, 1991; Siddiqui and Mahmood, 1999; Dong and Zhang, 2006; Tian *et al.*, 2007; Hallman *et al.*, 2009; Trivedi and Malhotra, 2013; Eissa and Abd-Elgawad, 2015). Therefore, identification of nematode antagonistic microorganisms and studying their mechanisms that regulate nematode population in soils are of considerable importance. Occurrence and identification of nematode- antagonistic microflora associated with plant rhizosphere of the cereal crops is not well studied in Egypt. The objective of the present study was to survey and identify the nematode-antagonistic fungi and bacteria associated with phytonematodes in the rhizosphere of wheat grown in different locations in Egypt. Total microbial count of fungi and bacteria was also determined.

### Materials and Methods

**Locations and sampling:** The study was conducted during 2017-2018 season in 25 districts belonging to eight governorates located in Northern and Middle Egypt (Table 1). A total of 467 soil samples were taken during the grain

filling period to harvest time by digging the soil to a depth of 15-20 cm, then kept in plastic bags, stored in the refrigerator at 4-5°C until processing for analysis.

Nematode extraction and identification: soil samples were carefully mixed and 250 g from each one were processed for nematode extraction by sieving method (Christe and Perry, 1951). Identification of nematode genera and species was made based on the morphology of adult and larval forms (Oteifa, 1964; Golden, 1971). Frequency of occurrence and population density of nematodes were calculated according to Norton (1978).

**Table 1:** Governorates and districts in which samples were collected

Governorates	Districts
<b>Northern Egypt</b>	
<b>Beheira</b>	Damanhur (9), El-Mahmoudia (21), Kafr-El-Dawwar (10), Rosetta (10)
<b>Dakahlia</b>	Aga (20), Mit – Ghamr (30)
<b>Gharbia</b>	Al-Santa (40), Zefta (10)
<b>Monufia</b>	Ashmoon (25), Menouf (25), Sadat City (56)
<b>Qalyubia</b>	El-Qanater-El-Khayria (6), Shihin- El-Qanater (24), Qalyub (10), Tukh (16)
<b>Middle Egypt</b>	
<b>Giza</b>	Al- Aiat (25), Atfeih (25), El-Manashi (15), Kerdasa (20)
<b>Fayum</b>	Ibsheway (15), Fayum (5), Senuris (25), Tamyia (5)
<b>Minya</b>	Beni-Mazar (15), Maghagha (35)

Figures between brackets are the number of collected samples.

### Total microbial counts

The total counts of spore-forming bacteria, aerobic bacteria and fungi were determined in wheat rhizosphere by using the plate count technique and dilution method on suitable medium (Ghini *et al.*, 2007). Soil Samples were carefully mixed, then 10g soil was separately suspended in 90 ml sterilized distilled water in a 250 ml flask and shaken for 20 min on a shaker to give a dilution of  $10^{-1}$ . For count of total fungi, serial dilutions from  $10^{-3}$  to  $10^{-5}$  were prepared by transfusing 1.0 ml of  $10^{-1}$  dilution to 9 ml sterilized distilled water in test tube under sterile conditions. Then one 1.0 ml of each dilution was transferred onto surface of Martin medium (Glucose 10g, peptone 5g,  $\text{KH}_2\text{PO}_4$  1 g,  $\text{MgSO}_4$  0.5g, Rose Bengal 30  $\mu\text{g}$ , Streptomycin 0.03g, Agar 15g, Distilled Water 1 L) in sterilized Petri plates. Plates were incubated at  $30^\circ\text{C} \pm 2$  for 7 days. The resulted fungi were counted as colony forming unit cfu/10 g soil (Bridson, 1995). For determining the total counts of spore-forming bacteria, the dilution  $10^{-1}$  was pasteurized at  $80^\circ\text{C}$  for 20 min. serial dilutions from  $10^{-3}$  to  $10^{-5}$  were prepared by transferring 1.0 ml of  $10^{-1}$  dilution to 9 ml sterilized distilled water in test tube under sterile conditions. Then one ml of each dilution was transferred onto surface of nutrient glucose 2% agar medium (NA) [Peptone 5g, Beef extract 3g, Agar 15 g, Distilled water 1 L, PH<sub>7</sub>]. Four plates were used as replicates for each dilution, and were incubated at  $28^\circ\text{C}$  for 2 days. Then total spore-forming bacteria were counted as colony forming unit cfu/10 g soil (Bridson, 1995). For count of total aerobic bacteria, serial dilutions from  $10^{-3}$  to  $10^{-7}$  were prepared as mentioned before. Aliquots 1.0 ml of each dilution was transferred onto surface of Martin medium in sterilized Petri plates. Plates were incubated at  $28^\circ\text{C}$  for 2 days. The resulted bacterial

colonies were counted as total aerobic bacteria as cfu/10 g soil.

### Identification of soil fungi

Plates containing fungi were incubated at  $30 \pm 2^\circ\text{C}$  for 7 days. Then fungi that grew out were identified to genus and species according to the morphological and culture characters by the key described by Ellis (1971); Barnett and Hunter (1972). Each detected fungus was counted and its frequency was calculated according to the following equation: % frequency of fungi = (fungus no. / total fungi no.) x 100 .

### Identification of isolated bacteria

Occurrence of bacteria genera in the wheat rhizosphere was determined using NA medium by the pour plate method and dilution technique as mentioned before. Bacteria were primary identified according to the morphological, culture and biochemical characters (Schaad, 1980; Goszezynska *et al.*, 2000).

### Isolation of nematophagous fungi using nematode water agar (NWA) medium

Twenty grams of agar were added to one liter of distilled water in conical flasks and dissolved if need on a water bath. The water agar medium was poured in Petri plates and sterilized in autoclave at 15 lbs pressure for 20 min. nematodes extracted from soil samples collected from different wheat-growing localities were concentrated in 10 ml nematode suspension and sterilized. One ml from each sterilized nematode suspension was added to plates, then plates were incubated at  $25 \pm 2^\circ\text{C}$  for eight days. The idea of using such a poor medium is to cut down the growth of other moulds such as Mucorales and more vigorously growing Hyphomycetes in order to give chance to more delicate growing nematophagous fungi (Commandon and de Fonbrune, 1938). Examination of plates were carried out after 2, 4 and 8 days to follow the course and development of nematophagous fungi. Identification of fungi was made according to Dollfus (1946); Alexopoulos and Mims (1979); Aboul-Eid *et al.* (1997); Yu *et al.* (2014).

### Statistical analysis

The means of microbial count in all soil samples were subjected to ANOVA procedures (Snedecor and Cochran, 1999). Treatment means were compared by using Duncan's Multiple Range Test, at  $P = 0.05$  level of probability, using Computer Statistical Package Co-analysis by log transformation.

## Results

### Phytonematodes associated with wheat

Eleven plant-parasitic nematode genera and species were recovered from the soil samples collected from wheat fields grown in eight governorates (Table 2). These nematodes were *Criconebella* spp., *Helicotylenchus* spp., *Heterodera* spp., *Hirschmanniella oryzae*, *Hoplolaimus* spp., *Longidorus* spp., *Meloidogyne* spp., *Pratylenchus* spp., *Tylenchorhynchus* spp., *Tylenchus* spp., *Xiphinema* spp. The stunt nematodes (*Tylenchorhynchus*), the root-lesion nematodes (*Pratylenchus*), the spiral nematodes (*Helicotylenchus*) and the cyst nematodes (*Heterodera*) were common in collected samples. The stunt nematodes was the most prevalent nematodes with a high FO average (53.1%)

and high PD average (48.1), followed by the root-lesion nematodes with FO(11.3%) and PD (10.5), spiral nematode with FO (5.8%) and (9.9), the cyst nematodes with FO (4.1%) and PD (2.1). It was observed that highest total nematode population density was found in Giza governorate with 168.1 individuals per 250 g soil, followed by Minya governorate (123.3), Monufia (105.3), while lowest total PD was obtained in Beheira samples.

### Total microbial counts in wheat rhizosphere

The total microbial counts of bacteria and fungi are presented in Table (3). The spore-forming bacteria count ranged between 4.1 to 5.21 log<sub>10</sub> with general average of 4.86. It was highly increased in samples of Fayum (mean = 5.17) followed by Dakahlia (5.16) and Beheria (5.06), while the lowest count was found in Monufia with mean of 4.5. The aerobic bacteria count ranged between 5.92 – 6.65 Log<sub>10</sub> cfu/10g soil in all samples, with general average of 6.22. The highest count mean was found in Monufia (6.47) followed by Fayum (6.24) and Minya (6.23), while lowest count mean was found in Dakahlia (6.09). The total fungi count ranged between 3.59-4.51 log<sub>10</sub> cfu/ 10 g soil with general average of 4.1. It was highly increased in Beheria (mean of 4.28) followed by Monufia (4.22), the lowest count was found in Fayum (3.82).

### Occurrence of soil fungi

Data presented in Table (4) indicated that nine fungi genera and species occurred in wheat rhizosphere. They were *Alternaria* spp., *Aspergillus* spp., *A.niger*, *Fusarium* spp., *Macrophomina* spp., *Rhizoctonia* spp., *Rhizopus nigricans*, *Penicillium* spp. *Trichoderma* spp. Data showed that *Penicillium* spp., was the most frequent in all samples with average of 28.2%, followed by *Aspergillus* spp. with 22.0%, *Aspergillus niger* with 13.6% and *Trichoderma* with 9.0%. Frequency of occurrence of other fungi ranged between 3.6% to 5.7%. *Penicillium* spp. were more common in samples of Minya governorate (33.2% occurrence), followed by Dakahlia (31.2%), and Gharbia (29.3%). *Aspergillus* spp. and *A.niger* were more common in samples of Giza (26.4% and 20.0%) followed by Qalyobia (23.3% and 17.8%) and Beheira (22.8% and 15.7%), respectively. *Trichoderma* spp. were more common in samples of Beheira with 12.6% occurrence followed by Monufia with 11.9% and Qalyobia (10.8%).

### Occurrence of bacteria

Occurrence of rhizobacteria associated with wheat rhizosphere is presented in Table (5). Five bacterial colonies, three (Bsp<sub>1</sub>, Bsp<sub>2</sub> and Bsp<sub>3</sub>) as *Bacillus* spp., two (Psp<sub>1</sub> and Psp<sub>2</sub>) as *pseudomonas* spp. and one as *Serratia* spp. were isolated from soil samples collected from different governorates. The Psp<sub>1</sub> isolate was the most frequent bacteria, as it occurred in all samples of all governorates with 100% occurrence, followed by Bsp<sub>2</sub> which occurred in all governorates except of samples of both Dakahlia and Qalyobia with 75% occurrence, and Bsp<sub>1</sub> with 62.5% occurrence. Bsp<sub>3</sub> and *Serratia* isolates were less common with 37.5% occurrence for each.

### Occurrence of nematophagous fungi

Data presented in Table (6) indicated that nine nematophagous fungi were associated with nematodes in the rhizosphere of wheat grown in 25 districts cited in eight governorates. Six of them are nematode – trapping fungi,

*Arthrobotrys conoides*, *A.oligospora*, *Dactylaria brochopaga*, *D.thaumasia* var. *longa*, *Dactylella* spp. and *Monacrosporium* spp., Three are endo parasitic (*endozoic*) fungi, *Harposporium anguillulae*,

*Meria* spp. and *Verticillium* spp. *Arthrobotrys conoides* and *Dactylaria thaumasia* var. *longa* were the most frequent fungi, as they occurred 7 times with 28% occurrence, followed by *Verticillium* spp. (4 times) with 16% occurrence. Other fungi occurred one time for each with 4% occurrence. *A. conoides* was more frequent in samples of Beheria governorate (4 times), while *D. thaumasia* var. *longa* was more frequent in samples of Fayum (3 times).

### Discussion

Biological analysis of soil samples collected from the rhizosphere of wheat grown in different locations in Egypt showed that samples contained many phytonematodes in association with different types of nematode-antagonistic fungi and bacteria. The root-lesion nematodes (*Pratylenchus* spp.) and the cyst nematodes (*Heterodera* spp.) were common in the samples. Some of these nematode species are reported as serious nematode pathogens to wheat causing economic damage (Nicol and Rival, 2008; Smiley and Nicol, 2009). The cereal cyst nematode, *Heterodera avenae* was detected in some wheat growing regions in Egypt causing considerable yield loss (Baklawa *et al.*, 2012; Korayem and Mohamed, 2015 and 2018).

Total microbial counts viz. spore-forming bacteria, aerobic bacteria and fungi were determined in the collected samples as log<sub>10</sub> cfu/10 g soil, the total count ranged between 5.92 to 6.65 log<sub>10</sub> cfu/10 g soil for bacteria and between 3.59 to 4.51 log<sub>10</sub> cfu/10g soil for fungi. Bacteria and fungi have been recognized as the most numerically abundant numbers of soil biota (Buée *et al.*, 2009; Coleman, 2008). So they constitute the biggest biomass of soil microbial communities. These microbial communities are profitable for plant health by improving plant physiology and development (Berendsen *et al.*, 2012; Mendes *et al.*, 2013) and by antagonizing many plant-pathogens living in soil (Patkowska, 2002).

Identification of soil microflora associated with wheat rhizosphere revealed that fungi genera of *Alternaria*, *Aspergillus*, *Fusarium*, *Penicillium* and *Trichoderma* were common in samples. All of these fungi are reported as nematode antagonists by producing different substances toxic to nematodes (Chen and Dickson 2004a). Also six rhizobacteria isolates, three *Bacillus* spp. (Bsp<sub>1</sub>, Bsp<sub>2</sub> and Bsp<sub>3</sub>), two *pseudomonas* spp. (Psp<sub>1</sub> and Psp<sub>2</sub>) and one *Serratia* spp. were also isolated from the wheat rhizosphere. All of these bacteria had antagonistic effect against phytonematodes (Ehteshamul *et al.*, 1997, Siddiqui and Mahmood, 1999; Chen and Dickson, 2004 b; Canbolat *et al.*, 2006; Tian *et al.*, 2007; Eissa and Abd-Elgawad, 2015).

Results also revealed that nine nematophagous fungi were associated with nematode in the rhizosphere of wheat viz., *Arthrobotrys conoides*, *A.oligospora*, *Dactylaria brochopaga*, *D.thaumasia* var. *longa*, *Dactylella* spp. and *Monacrosporium* spp., *Harposporium anguillulae*, *Meria* spp. and *Verticillium* spp. All of these fungi are reported as nematode- trapping and/or endo-parasitic fungi, which are an important, fascinating group of soil microbial community that can suppress phytonematodes populations (Yang and Zhang, 2014; Askary, 2015).

The obtained results suggested that occurrence and population densities of phytonematodes associated with wheat were different in the surveyed localities. These difference in nematode densities and distribution may be due to the differences in the indigenous microflora (fungi and bacteria). These indigenous microflora is the reason that plants are effectively protected from the soil pathogens, a phenomena that is known as disease suppression (Stirling, 2011). Indigenous microflora of arable soils were occasionally reported to suppress plant-parasitic nematodes (Kerry and Crump, 1998; Bent et al., 2008; Korayem et al., 2016/2017). Thus the indigenous microflora that suppress plant-parasitic nematodes may be a good solution for nematode management. Studying the nature of soil environment and understanding the ecological factors that

enable these antagonists to persist compete and function may improve the basis for biological control strategies.

In summary, our results suggested that wheat rhizosphere contained many plant-parasitic nematodes like the root-lesion nematodes and the cyst nematode, that are serious nematode pathogens to wheat. Soil samples also contained different kinds of microflora have been reported as nematode antagonists, these were nematophagous fungi, endozoic fungi, toxin-producing fungi and rhizobacteria. All of these antagonists could be used as alternative methods for nematode management, especially chemical control are costly and restricted due to their harmful effect on the environment and human health as well as cultural control and wheat resistant varieties are often not practical or not available.

**Table 2 :** Occurrence % (FO) and population density (PD) of phytonematodes associated with wheat in different governorates of Northern and Middle Egypt.

Phytonematodes	Beheira		Dakahlia		Gharbia		Monufia		Qalyobia		Giza		Fayum		Minya		General average	
	FO	PD	FO	PD	FO	PD	FO	PD	FO	PD	FO	PD	FO	PD	FO	PD	FO	PD
<i>Criconebella</i> spp.	-	-	-	-	-	-	1.3	1	1.8	0.7	4.7	3.8	-	-	-	-	0.9	0.7
<i>Helicotylenchus</i> spp.	-	-	-	-	-	-	5.3	1.7	-	-	23.5	50.1	6.0	2.0	-	-	5.8	9.9
<i>Heterodera</i> spp.*	-	-	-	-	2.0	1.8	7.9	2.3	-	-	9.4	6.2	2.0	0.8	6.0	2.4	4.1	2.1
<i>Hirschmanniella oryzae</i>	2.0	1.3	6.0	2.9	-	-	-	-	-	-	-	-	-	-	-	-	0.9	0.6
<i>Hoplolaimus</i> spp.	-	-	-	-	2.0	0.2	-	-	-	-	-	-	-	-	-	-	0.4	0.1
<i>Longidorus</i> spp.	-	-	-	-	-	-	-	-	-	-	1.2	0.5	-	-	-	-	0.2	0.1
<i>Meloidogyne</i> spp.*	-	-	-	-	-	-	1.3	0.3	-	-	-	-	-	-	-	-	0.2	0.04
<i>Pratylenchus</i> spp.	2.0	0.8	2.0	1.2	6.0	1.4	26.3	19.2	5.4	3.3	17.6	28.1	12.0	7.6	8.0	6.2	11.3	10.5
<i>Tylenchorhynchus</i> spp.	20.0	10.5	30.0	13.9	40.0	29.1	76.3	79.3	57.1	61.4	50.6	79.1	70.0	74.1	70.0	114.7	53.1	48.1
<i>Tylenchus</i> spp.	-	-	2.0	1.7	2.0	2.8	2.0	1.5	-	-	-	-	1.0	1.8	-	-	0.8	0.9
<i>Xiphinema</i> spp.	-	-	-	-	-	-	-	-	-	-	1.2	0.3	-	-	-	-	0.2	0.06
Total PD	-	12.6	-	19.7	-	35.3	-	105.3	-	65.4	-	168.1	-	86.3	-	123.3		

FO = (Number of samples containing a nematode/ total number of samples) X 100

PD = Number of nematodes in 250 g soil.

\* = Larval forms.

**Table 3:** Total counts of spore-forming bacteria, aerobic bacteria and fungi in wheat rhizosphere in different governorates, Egypt

Governorates	Total microbial count (log10 cfu/10g soil)			
		Spore-forming Bacteria	Aerobic bacteria	Fungi
Beheira	Range	5.01 – 5.12	5.99-6.30	4.11-4.51
	Mean	5.06 a	6.17d	4.28a
Dakahlia	Range	5.10-5.21	5.92-6.26	3.91-4.39
	Mean	5.16a	6.09f	4.15d
Fayum	Range	5.14-5.19	6.18-8.30	3.59-4.4
	Mean	5.17a	6.24b	3.82h
Gharbia	Range	4.26-5.15	6.20-6.21	4.13-4.20
	Mean	4.71c	6.21c	4.17c
Giza	Range	4.46-4.94	6.11-6.32	4.00-4.32
	Mean	4.63d	6.21c	4.10e
Qalyobia	Range	4.56-5.04	5.92-6.30	3.59-4.17
	Mean	4.87b	6.15e	3.98g
Minia	Range	4.43-5.11	5.95-6.41	3.97-4.13
	Mean	4.77e	6.23b	4.05f
Monufia	Range	4.10-4.94	6.30-6.65	4.03-4.35
	Mean	4.50e	6.47a	4.22b
General average		4.86	6.22	4.10

Mean in each column followed by the same letter are not significantly different according to Duncan's Multiple Rang Test (P=0.05).

**Table 4:** Occurrence % of common fungi associated with wheat rhizosphere in different governorates of Northern and Middle Egypt.

Fungi	% Occurrence of fungi								
	Beh	Dak	Fay	Giz	Gha	Qal	Min	Mon	Average
<i>Alternaria</i> spp.	2.3	7.7	5.2	3.8	4.6	2.1	5.0	1.6	4.1
<i>Aspergillus</i> spp.	22.8	21.6	18.0	26.4	24.9	23.3	20.0	19.1	22.0
<i>Aspergillus niger</i>	15.7	8.8	12.7	20.0	14.4	17.8	8.4	10.6	13.6
<i>Fusarium</i> spp.	5.5	2.9	6.5	4.9	5.5	4.9	5.0	10.7	5.7
<i>Rhizopus nigricans</i>	3.1	2.2	6.7	1.3	6.3	5.2	5.0	6.3	4.5
<i>Rhizoctonia</i> spp.	1.4	6.0	4.3	0.9	1.1	4.8	5.0	5.1	3.6
<i>Macrophomina</i> spp.	3.3	3.5	3.3	3.2	3.2	4.0	5.0	3.9	3.7
<i>Penicillium</i> spp.	28.5	31.2	28.8	28.8	29.3	21.1	33.2	24.4	28.2
<i>Trichoderma</i> spp.	12.6	10.0	7.3	5.8	5.3	10.8	8.4	11.9	9.0
Others	4.6	6.1	7.2	4.9	4.4	6.0	5.0	6.4	5.6

Beh = Beheria, Dak = Dakahlia, Fay = Fayum, Giz = Giza, Gha= Gharbia, Qal = Qalyobia, Min = Minya , Mon= Monufia,

**Table 5:** Occurrence of common bacteria associated with wheat rhizosphere in different governorates of Northern and Middle Egypt.

Governorates	bacterial isolates					
	<i>Pseudomonas</i> spp.		<i>Bacillus</i> spp.			<i>Serratia</i> spp.
	Psp <sub>1</sub>	Psp <sub>2</sub>	Bsp <sub>1</sub>	Bsp <sub>2</sub>	Bsp <sub>3</sub>	
Beheira	+	-	+	+	-	+
Dakahlia	+	+	-	-	+	-
Fayum	+	+	-	+	+	+
Giza	+	-	+	+	-	-
Gharbia	+	-	+	+	-	-
Qalyobia	+	+	+	-	+	-
Minya	+	-	+	+	-	-
Monufia	+	+	+	+	-	+
%Occurrence	100	50	62.5	75.0	37.5	37.5

**Table 6:** Occurrence of nematophagous fungi associated with nematodes in rhizosphere of wheat in different districts of Northern and Middle Egypt.

Governorate	District	Associated fungi
Beheira	Kafr-El-Dwwar	<i>Arthrobotrys conoides</i> , <i>Verticillium</i> spp. <i>Monacrosporium</i> spp.
	Damanhur	<i>A.conoides</i>
	El-Mahmoudia	<i>A.conoides</i>
	Rosetta	<i>A.conoides</i>
Dakahlia	Aga	-
	Mit Ghamr	-
Fayum	Tamyia	<i>A.conoides</i> , <i>Verticillium</i> spp., <i>Dactylaria thaumasia</i> var. <i>longa</i>
	Senuris	<i>D.thaumasia</i> var. <i>longa</i>
	Ibsheway	<i>D.thaumasia</i> var. <i>longa</i>
	Fayum	-
Giza	Kerdasa	-
	Al-Aiat	-
	Atfeih	-
	El-Manashi	-
Gharbia	Al-Santa	-
	Zifta	<i>D.thaumasia</i> var. <i>longa</i>
Qalyubia	Tukh	-
	Shibin El-Qanater	-
	Qalyub	-
	El-Qanater El-Khayria	<i>Verticillium</i> sp.
Minia	Beni-Mazar	<i>D.thaumasia</i> var. <i>longa</i> , <i>Dactylella</i> spp.
	Maghagha	<i>A.concoides</i> , <i>D.thaumasia</i> var. <i>longa</i> , <i>Verticillium</i> spp.
Monufia	Ashmoon	<i>Dactelaria brochopaga</i> , <i>D.thaumasia</i> var. <i>longa</i>
	Menouf	<i>A.oligospora</i> , <i>Harposporium</i> <i>anguillulae</i> , <i>Meria</i> spp.
	Sadat City	<i>A.conoides</i>

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