Journal of Basic & Applied Mycology 3 (2012): 35-40

Fungi from soil of wilted and healthy banana plants with special reference to the associated fusaria

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Received 5/4/2012, Accepted 10/6/2012

Abstract: The dilution-plate method was used to enumerate and isolate fungi in soil samples of both wilted and healthy banana plants. Fungal propagules in the soil of wilted plants were as much as twice of those in healthy ones. *Aspergillus*, *Penicillium* and *Fusarium* were the most common genera in both soils, and also their counts were higher in soil of wilted plants. *Aspergillus flavus*, *A. niger*, *Penicillium chrysogenum* and *Fusarium oxysporum* were the prevalent species in both soils. It is noteworthy that *F. oxysporum*, the causal agent of Panama disease constituted about 4.4% of the total propagules in the soil of wilted plants while it constituted only 1.2% of those of healthy ones. Some other fungi were dominant in either soil. Four species of *Fusarium* were found associated with the diseased plants, and only *F. oxysporum* was isolated from all wilted tissues examined. When the isolated *Fusarium* species were tested for cellulase production, 16 out of 18 isolates were capable of producing cellulase. Of these, 7 isolates were high cellulase producers (4 of *F. oxysporum*, and 1 of each of *F. avenaceum*, *F. solani* and *F. verticillioides*).

Key words: Banana wilt, soil fungi, *Fusarium*, cellulase.

Introduction

Fusarium oxysporum is a common soil fungus, found in almost all parts of the world as a harmless colonizer of root surfaces or weak invader of the root cortex of many plants. However in addition to this background population there are over 80 known strains that show specific pathogenicity to particular crops causing the vascular wilt diseases (Deacon 1990). The strains that specifically affect banana are termed *F. oxysporum* f. sp. *cubense* (Ploetz 1990).

The banana wilt pathogen can be detected in soil by a suitable host test. It often enters the host through living rootlets from which it passes into the vascular strand of the main root and hence into the rhizome; apparently infection does not occur through dead roots (Rishbeth 1955). The spread of wilt through plantations by flooding is probably important in local dispersal of the pathogen, as it is in long-range dispersal (Rishbeth 1955).

Fusarium wilt of banana has been and remains constraint in tropical and subtropical regions (Rutherford and Viljoen 2003).The disease is reported in practically all countries where bananas are grown and it is thought that it had its origin in the India-Malaysia area (Stover 1987).

To the best of our knowledges, there is no record in Assiut area on the incidence of Panama disease. Thus, this study was designed to shed lights on the fungal population in banana field with special attention to *Fusarium* and incidence of banana wilt agent and their producing abilities of cellulase enzyme.

Materials and Methods

Collection of samples

Twenty samples of soil fields with wilted (10 samples) and healthy banana plants (10 samples) were collected from Awlad Ibrahim and El-Moteiah fields at Assiut city, Upper Egypt. At the same time, samples from 10 banana rhizomes appeared wilted were also collected. The samples were put in clean plastic bags and transferred to the mycological laboratory for fungal analysis.

Fungal analysis

The dilution- plate method (Johnson and Curl 1972) was used for enumeration and isolation of fungi from soil samples. 10 g soil from each sample was suspended in 90 ml of sterile water into 250 ml Erlenmeyer flasks. The flasks were mechanically shaken for 30 minutes. 10 ml soil suspension were transferred immediately through successive 90 ml sterile water blanks until the desired final dilution was reached. One ml of the appropriate dilution was transferred aseptically into each 5 Petri dishes and about 20 ml agar medium, cooled to just above the solidifying temperature, were added to each dish. Glucose Czapek-Dox agar (Raper and Thom 1949) supplemented with rose Bengal (5 % w/ v in water, 0.5 ml/l) and chloromphenicol (100 mg/1) as bacteriostatic agents was used as the isolation medium.

For the isolation of the causative organism(s) of Panama disease from the banana rhizome, rhizome was surface sterilized with sodium hypochlorite solution (Pitt and Hocking 1997), cut into small pieces and put on the surface of glucose Czapek-Dox agar plates (3 pieces / plate, 3 plates / sample).

All plates were incubated at 25°C for 7-10 days and the developing colonies were counted, isolated and identified. Identification of fungi to species level were based on their macro-and microscopic features using the methods of Raper and Fennell (1965), Booth (1971), Ellis (1971), Pitt (1979), Domsch *et al*., (2007), Nelson *et al*. (1983), Sivanesan (1987), Moubasher (1993), Pitt and Hocking 1997, Leslie and Summerrell (2006).

Screening of *Fusarium* isolates for cellulase enzyme

Eighteen *Fusarium* isolates either from soil (8) or banana tissue (10) were screened for their capabilities of producing the extracellular cellulase enzyme. These isolates belong to 5 *Fusarium* species and an unidentified species. Cellulase enzyme was tested on the medium described by Brigde (1985). A positive result was indicated by a clear zone around a streak inoculation within 21 days.

Three replicate agar plates for each isolate were inoculated for the enzyme. The diameter of the colony as well as the clear zone was measured (in mm) and the enzyme index was calculated according to the method of Ismail (2001).

Results and Discussion

Fungi in soil of wilted and healthy banana plants

The total fungal propagules recorded from soil of wilted banana plants were more than twice (759200 colonies/ g soil) of that recorded in soil of healthy ones (369200). *Aspergillus*, *Penicillium* and *Fusarium* were the most common genera isolated from soils of both wilted and healthy banana plants. They were isolated in high frequency from both soils, however, their percentage counts were more in soil of wilted plants. *Aspergillus* constituted the highest percentage counts in comparison with other fungi isolated (58.32% and 42.25% of total fungi) from wilted and healthy banana plants respectively). Eleven species of *Aspergillus* were recorded in soil of wilted plants while only six in soil of healthy plants. Of these, *A. flavus* and *A. niger* predominated over the other species in both soils (Table 1). The current results are in harmony with the findings of Madhanraj *et al*. (2010). They studied the population dynamics of fungi in banana field soil and could isolate 22 species of which *Aspergilllus*, *Penicillium*, *Trichoderma* and *Fusarium* were the most frequent genera.

Penicillium constituted 33.11% and 8.01% of the total propagules in both soils, (Table 1). It was represented by 9 species of which *P. chrysogenum* was the commonest in both soils. The genus *Penicillium* was also dominant in banana field soil as reported by Madhanraj *et al*. (2010) who isolated *P. chrysogenum, P. citrinum, P. javanicum* and *P. lanosum* in high frequency of occurrence.

Fusarium (represented by 5 species) constituted 4.72 and 2.71% of the total propagules in both soils of wilted and healthy plants respectively. *F. oxysporum* was the most prevalent species, accounting for 4.37% of the total propagules in the soil of wilted banana while only 1.19% in that of healthy plants. In accordance with the current results, Naiki and Morita (1983) found that the populations of *F. oxysporum* and spinach wilt fungus (*F. oxysporum* f. sp. *spinaciae*) were always higher in naturally infested fields than in noninfested fields and also in rhizosphere soil than in non-rhizosphere soil irrespective of infection of the plant. Also in this respect, Rishbeth (1955) found that the soil population of *F. oxysporum* f. sp. *cubense* increases considerably when wilted banana collapse and declines shortly after their removal. Also, Hamed (2007) isolated pathogenic and non-pathogenic isolates of *F. oxysporum* from rhizosphere soil of tomato. Other two species (*F. solani* and *F. verticillioides*) isolated in the current work were reported from both soils while *F. dimerum* from only rhizosphere soil of wilted banana and an unidentified *Fusarium* species from the soil of healthy plants. Also, *Fusarium* spp. were found in banana as natural endophytes and have been detected in roots of different banana cultivars in several countries (Speijer 1993, Amin 1994, Schuster *et al*. 1995, Pocasangre *et al*. 2000, Madhanraj *et al*. 2010).

Some other fungi predominated in soil of healthy plants such as *Acremonium, Alternaria, Cladosporium, Cochliobolus, Humicola, Macrophomina, Memmnoniella, Mucor, Myrothecium* and *Scolecobasidium*. While others in soil of wilted banana such as *Epicoccum, Fusariella, Stachybotrys, Trichoderma* and *Verticillium* (Table 1). Most species of the above genera were also reported from soil and the air above banana fields (El-Said & Abdel-Hafez 1995; Madhanraj *et al.* 2010)).

Species of *Fusarium* isolated from wilted banana plants

Four species of *Fusarium* were isolated from the tissues of wilted banana. *F. oxysporum* was recorded from all tissue samples. *F. verticillioides* was isolated from 3 out of the 10 samples investigated. The other two species are *F. proliferatum* (from two samples) and *F. avenaceum* (one sample) being recorded along with *F. verticillioides* (Table 2). These results were greatly similar to those obtained by Pocasangre *et al.* (2000). They reported that *Fusarium* spp. were the predominant banana endophytic fungi in all countries surveyed, found in all localities studied. Hamed (2007) isolated some pathogenic isolates of *Fusarium oxysporum*, in addition to *F. solani* and *F. equiseti* from wilted tomato seedlings collected from different localities of Egypt.

Table (1): Percentage count (C %), frequency (F) and occurrence remark (OR) of fungi recovered from soils of wilted and healthy banana plants on glucose Czapek Dox agar medium at 25 ±2˚C.

Soil type / Fungi	Soil of wilted plants		Soil of healthy plants	
	C%	F& OR	C %	F& OR
Acremonium Fr.	0.18	3M	1.84	8H
A. roseum Petch.			0.11	1L
A. strictum W. Gams	0.18	3M	1.73	8H
Alternaria alternate (Fries) Keissler	0.05	1L	0.65	4 M
Aspergillus Micheli	58.32	10H	42.25	10H
A. clavatus Desmazieres			0.11	1L
A. aegyptiacus Moubasher & Moustafa	0.05	1L		
A. flavus Link	26.21	10H	30.44	10H
A. flavus var. *columnaris* Raper & Fennell	0.90	5M	0.11	1L
A. fumigatus Fresenius	22.26	7H	1.08	3M
A. niger van Tieghem	7.59	10H	4.33	10H
A. ochraceus Wilhelm	0.32	3M		
A. oryzae (Ahlburg) Cohn	0.53	4M		
A. parasiticus Speare	0.05	1L		
A. tamarii Kita	0.03	1L		
A. terreus Thom	0.34	5M	6.18	5M
A. ustus (Bainier) Thom & Church	0.05	1L		
Basipetospora variabilis Matsushima			0.22	1L
Botryotrichum atrogriseum J.F.H. Beyma			0.11	1L
Chaetomium sp.	0.03	1L	0.22	1L
Cladosporium Link: Fr.	0.13	3M	3.68	7H
C. cladosporioides (Fresenius) de Vries	0.05	1L	3.57	7H
C. sphaerospermum Penzig			0.11	1L
Cladosporium sp.	0.08	2L		
Cochliobolus Drechsler	0.05	2L	0.76	5M
C. hawaiiensis Alcorn			0.22	1L
C. lunatus Nelson & Haasis			0.54	5M
C. spicifer Nelson	0.05	2L		
Epicoccum nigrum Link	0.21	2L		
Fusariella aegyptiaca Mouchacca	0.21	3M		
Fusarium Link	4.72	9H	2.71	8H
F. dimerum Penzig	0.16	2L		
F. oxysporum Schlechtendal	4.37	8H	1.19	5M
F. solani (Martius) Saccardo	0.12	1L	0.98	1L
F. verticillioides (Sacc.) Nirenberg	0.08	1L	0.43	3M
Fusarium sp.			0.11	1L
Gliocladium roseum Bainier			0.11	1L
Humicola grisea Traaen	0.21	1L	0.43	3M
Macrophomina phaseolina (Tassi) Goidanch			0.33	3M
Memnoniella echinata (Riv.) Galloway			7.04	4M
Mucor circinelloides van Tieghem			1.95	4M
Myrothecium Tode ex Fr.	0.79	3M	25.89	10H
M. roridum Tode			4.66	3M
M. verrucaria (Albertini & Schweinitz) Ditmar	0.79	3M	21.23	9H
Nectria inventa Pethybr.			0.43	2L
Neosartorya fischerii (Wehmer) Malloch & Cain	0.05	1L		
Paecilomyces Bainier	0.05	1L	0.33	2L
P. variotii (Thom) Samson	0.05	1L		
Paecilomyces sp.			0.33	2L
Penicillium Link	33.11	10H	8.01	9H
P. albidum Sopp	0.21	1L		
P. chrysogenum Thom	18.86	7H	6.28	8H

Soil type / Fungi	Soil of wilted plants		Soil of healthy plants	
	C%	F& OR	C %	F& OR
P. corylophilum Dierckx	1.42	2L	1.08	5M
P. funiculosum Thom			0.11	1L
P. oxalicum Currie & Thom	0.21	2L		
P. pinophilum Hedgcock	0.90	1L	0.33	3M
P. purpurogenum Stoll			0.11	1L
P. vinaceum Gilman & Abbott			0.11	1L
Penicillium sp.	11.51	4M		
Rhizopus stolonifer (Ehrenberg) Vuillemin			0.11	1L
Scolecobasidium variabile Barron & Busch			1.30	3M
Scopulariopsis brevicaulis (Saccardo) Bainier	0.58	1L		
Stachybotrys chartarum (Ehrenberg) Hughes	0.87	4M	1.52	2L
Stemphylium botryosum Wallroth			0.11	1L
Trichoderma viride Pers.	0.32	2L		
Verticillium sp.	0.12	2L		
% Total counts	100	10H	100	10H
Total propagules / g	759200		369200	
Mean of total propagules ± SD	75920± 59032.67		36920±20325.40	
Number of genera	20		23	
Number of species	39		42	

*C %: Percentage count, calculated per total fungi in 10 soil samples
F: Frequency of fungi; calculated out of 10 soil samples.
OR: Occurrence remark: H (high) = 6-10 cases; M (moderate) = 3-5 cases, L (low) = 1 or 2 cases.

Table (2): *Fusarium* species isolated from 10 samples of wilted banana plants.

Species \ Sample No.	1	2	3	4	5	6	7	8	9	10	*NCI	F%
F. oxysporum	+	+	+	+	+	+	+	+	+	+	10	100
F. verticillioides				+					+	+	3	30
F. proliferatum									+	+	2	20
F. avenaceum										+	1	10

*NCI: Number of cases of isolation (out of 10 samples), F%: Percent frequency calculated per 10 samples investigated.

Cellulase production by isolates of *Fusarium* isolated from soil and wilted banana tissues

Eighteen isolates representing 6 species of *Fusarium* were screened for their capabilities of producing cellulase. All isolates except two were capable of cellulase production but with variable degrees. Seven isolates belonging to *F. avenaceum* (1), *F. oxysporum* (4), *F. solani* (1) and *F. verticillioides* (1) were rated as high producers (cellulase index ≥ 0.1) while the other 9 were rated as low producers (cellulase index < 0.1). The two non-producing isolates belong one to *F. oxysporum* and the other to *F. proliferatum* (Table 3). In agreement with the current results, all isolates tested of *F. oxysporum* (10 isolates), *F. verticillioides* (1) and *F. avenaceum* (2) were capable of producing C_x and C_1 cellulases (Svaloova *et al*. 1980). Also, from the results obtained, there was no correlation between the source of isolates, whether it was soil or host plant, and their producing abilities of the enzyme.

Table (3): Cellulase enzymes produced by different *Fusarium* species isolated from soil and banana tissue.

Isolate	Source	Cellulase			
		MCD±SD	MDCZ±SD	Ce I	AR
F. avenaceum	Tissue	51.00±2.58	56.00±2.31	1.10	H
F. oxysporum	Tissue	58.25±3.30	61.00±1.15	1.05	L
F. oxysporum	Tissue	61.50±5.00	66.50±5.00	1.08	L
F. oxysporum	Tissue	57.50±1.73	65.50±1.29	1.14	H
F. oxysporum	Tissue	53.75±3.50	59.50±3.70	1.11	H
F. oxysporum	Tissue	56.25±0.96	62.00±1.63	1.10	H
F. oxysporum	Soil	51.75±4.50	52.00±2.31	1.01	L
F. oxysporum	Soil	53.00±2.94	58.25±4.03	1.10	H
F. oxysporum	Soil	51.00±5.48	00.00	0.00	-ve
F. proliferatum	Tissue	40.50±1.00	43.50±1.00	1.07	L
F. proliferatum	Tissue	49.25±3.77	00.00	0.00	-ve
F. solani	Soil	62.00±2.83	67.00±3.46	1.08	L
F. solani	Soil	57.00±2.58	66.50±2.65	1.17	H
F. verticillioides	Tissue	47.25±4.57	51.00±4.76	1.08	L
F. verticillioides	Tissue	49.25±3.40	53.50±3.70	1.09	L
F. verticillioides	Soil	46.5±7.72	52.00±8.49	1.12	H
F. verticillioides	Soil	54.00±7.66	58.50±8.54	1.08	L
Fusarium sp.	Soil	44.25±4.35	48.00±2.16	1.09	L

- MCD±SD: Mean colony diameter (in mm) ± standard deviation.
- MDCZ±SD: Mean diameter of clear zone (in mm) ± Standard deviation.
- Ce I: Cellulase index.
- AR: Activity remark: -ve, non-producer with enzyme index = 0; L, low producer with enzyme index <1.10; H, high producer with enzyme index ≥1.10.

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