

MAJOR ORGANISMS CAUSING ROOT-ROT/WILT AND THEIR RELATIVE IMPORTANCE ON FABA BEAN, LENTIL AND CHICKPEA

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Abstract

Intensive systematic survey of root-rot/wilt diseases of faba bean, lentil and chickpea in the two governorates; Beheira (north Egypt) and Assiut (middle Egypt) were done twice in Jan-Feb. and in March - April 1996.

Fungi associated with diseased samples collected from surveyed fields were isolated, identified and kept in stock cultures for further studies. Also, pathogenic potential of the most important isolated fungi were determined in the three crops.

Obtained data indicate that, the major fungal diseases affecting legume crops in Egypt are root-rot caused by *Fusarium solani* in faba bean, vascular wilt caused by *Fusarium oxysporum* in lentil, and *Sclerotinia stem rot* caused by *Sclerotinia sclerotiorum* in chickpea.

INTRODUCTION

Food legume crops (faba bean, lentil and chickpea) are important human food and animal feed in Egypt and in many other countries in the world. Root-rot/wilt complex is considered to be the most important disease affecting these crops productivity in Egypt (Mohamed 1982, El-Awadi 1993 and Yahia *et al.* 1994).

Several fungi were recorded as causal pathogens of root-rot and wilt diseases on these crops; viz., *Rhizoctonia solani*, *Fusarium oxysporum*, *F.solani*, *Fusarium* spp. *F.moniliforme*, *Gliocladium* spp., *Macrophomina phaseolina*, *Verticillium* spp.,

Pythium sp., and *Sclerotium rolfsii* (Abdallah 1969, Nene 1980 B, Mohamed 1982, Abou-Zeid *et al.* 1990, El-Garhy 1994, Abou-Zeid *et al.* 1995).

F.oxysporum has specialized formae that cause wilt disease on legume crops; viz., *F.oxysporum f.sp.fabae* on faba bean, *F.oxysporum f.sp. lentis* on lentil and *F.oxysporum f.sp. ciceri* on chickpea (Booth, 1971).

In spite of the importance of root-rot and wilt diseases in food legume crops as a causal of considerable yield losses to these crops, there are no intensive field surveys for these diseases in Egypt. Therefore, this work aimed to surveying these diseases, intensively, in two governorates representing different growing legume areas in Egypt. Also, to identify the causal organisms of root-rot/wilt diseases and to study their relative importance and pathogenic potential.

MATERIALS AND METHODS

Field Survey :

Survey of root-rot / wilt diseases of faba bean, lentil and chickpea crops was carried out in two governorates; Beheira (north Egypt) and Assiut (middle Egypt), during growing season in Jan. - Feb. and in April 1996. Number of visited fields of faba bean, lentil and chickpea were 166, 86, and 92, respectively; representing the total production acreage of each crop in the two governorates. The fields were chosen randomly at every 5-10 km. At each field the symptoms of root-rot and wilt diseases existed were expressed and the percentage of infected plants was recorded. Twenty random diseased plants were picked diagonally from each field for laboratory isolation .

Isolation and Identification :

Roots and basal stems of diseased plants showing typical symptoms of root-rot and wilt in each sample were washed carefully with running tap water. Plants in the diseased sample were cut into small pieces, separately, surface sterilized by immersing in 2% sodium hypochlorite for 3 min, then washed twice in sterilized distilled water. The sterilized plant pieces were dried between two sterilized filter papers, and directly planted on PDA medium, 5 pieces/Petri dish, then incubated at 25°C for 7 days. Emerged fungi were isolated and purified on PDA plates, then identified according to their morphological characters using compound microscope de-

pending on the descriptions of Smith (1965), Booth (1971), Alexopoulos and Mims (1979), and Nelson *et al.* (1983), Stock cultures were maintained on PDA slants and kept in refrigerator at 5°C for further studies.

Pathogenicity Tests :

This experiment was done to determine the pathogenic potential of the most important fungi isolated from diseased samples of each crop. Sterilized plastic pots, 15 cm in diameter, were used. The pots were potted with sterilized light loam soil, and infested with the tested fungal isolate at the rate of 5% (w/w). Four replicates of each fungal isolate were used. Isolates of six fungi isolated from each crop, i.e. *F. oxysporum*, *F. solani*, *R. solani*, *F. moniliforme*, *S. sclerotiorum* and *V. albo-atrum* were tested. The fungal inoculum were prepared by growing the isolates on autoclaved sorghum grains in 500 ml bottles and incubated at 25°C for 3 weeks. Ten seeds from each crop were sown in each pot, 7 days after soil inoculation. Giza 716 (faba bean), Precoze (lentil), and G 531 (chickpea) cultivars were used for testing fungi isolated from their crops. Data were recorded after 4 weeks for wilt and root-rot diseases.

RESULTS

Field Survey :

Data presented in Table (1) clearly show that, infected plants with root-rot and wilt were found in all inspected fields of the three crops, but the average percentage of infection varied from one field to another. Also, the percentage of infection was slightly higher in April survey than in Jan. - Feb. .

In faba bean, 94 fields in 8 districts in Beheira Governorate and 72 fields in 6 districts in Assiut Governorate were surveyed. Average percentage of infection was higher, in general, in Assiut G, than in Beheira G. It ranged from 1.0 to 7.5% and from 2.1 to 15.0% in Jan and April surveys, respectively in Beheira G. The highest percentage of infection was at Bostan (15.0 %), while the least was recorded at West Noubaria and Mariut (2.1%) in April survey. On the other hand, the average percentage of infection in Assiut G. ranged from 6.7 to 13.0% and from 8.2 to 15.0% in Jan and April surveys, respectively. The highest percentage of infection was recorded at Abou-Teig, while the least was at Abnoub districts, in April survey.

Table 1. Average percentage of infected plants with root-rot/wilt on faba bean, lentil and chickpea in different governorates in the two periods Jan. - Feb. and April 1996. .

Governorates	District	% Infected plants								
		Faba bean			Lentil			Chickpea*		
		NSF ¹	Jan.	April	NSF ¹	Jan.	April	NSF ¹	Jan.	April
Beheira	North	6	1.5	4.1	5	6.2	9.4	3	5.0	7.1
	Tahrir									
	Bangar	23	4.1	7.3	6	7.5	12.3	5	3.5	4.4
	EL-Sukar									
	West Noubaria	19	1.0	2.1	-	-2	-	3	7.5	10.2
	Bostan	15	7.5	15.0	3	5.5	11.4	3	4.0	7.3
	Mariut	10	1.3	2.1		-	-		-	-
	Kafr El-Dawar	11	2.0	3.1	-	-	-	-	-	-
	Delengat	3	1.0	3.5		-	-	2	4.5	6.2
Abouhomos	7	1.5	2.3	-	-	-	-	-	-	
	Subtotal	94			14			16		
Assiut	Manfalout	16	8.8	10.2	16	4.0	7.3	24	4.3	5.4
	Dairout	16	13.0	15.3	16	5.7	7.6	20	4.1	6.0
	El-Kosia	12	6.7	9.9	12	3.2	5.3	12	3.3	4.2
	Assiut	16	8.5	10.1	16	2.0	3.1	-	-	-
	Abouteig	4	11.0	15.0	4	4.0	5.1	8	3.5	5.1
	Abnoub	8	7.0	8.2	8	2.5	3.2	4	5.3	6.1
	Sadfa	-	-	-	-	-	-	8	4.5	4.9
	Subtotal	72			72			76		
	Total	166			86			92		

1. NSF = Number of surveyed fields. 2. The crop is not grown in this area.

* Average percentage of infection included sclerotinia stem rot disease caused by *S.sclerotiorum*

In lentil, 14 fields in 3 districts in Beheira G and 72 fields in 6 districts in Assiut G were surveyed. The average percentage of infection ranged from 5.5 to 7.5 % and from 9.4 to 12.3% in Jan and April surveys, respectively in Beheira G. The highest percentage of infection was at Bangar El-Sukar, while the least was at North Tahrir districts, in April survey. On the other hand, the average percentage of infection in Assiut G. ranged from 2.0 to 5.7 % and from 3.2 to 7.6% in Jan and April surveys, respectively. The highest percentage of infection was recorded at Dairout and the least was at Abnoub districts, in April survey.

In chickpea, 16 fields in 5 districts in Beheira G. and 76 fields in 6 districts in Assiut G. were surveyed. The average percentage of infection ranged from 3.5 to 7.5% and from 4.4 to 10.2% in Jan. and April surveys, respectively, in Beheira G. The highest percentage of infection was at West Noubaria, while the least was at Bangar El-Sukar in April survey. In Assiut G., the average percentage of infection with root-rot and wilt diseases ranged from 3.3 to 5.3% and from 4.2 to 6.1 % in Jan. and April surveys, respectively. The highest percentage of infection was at Abnoub and the least was at El-Kosia districts, in April survey.

Fungi Associated with the Diseased Samples and Their Frequency:

Data in Table (2) show that, the most important fungi isolated from diseased faba bean samples were *F.solani*, *F.oxysporum*, *R. solani*, *Verticillium* spp., *F.moniliforme*, and *Fusarium* spp.; and their occurrence frequency were 33.1, 25.0, 16.3, 10.6, 8.1 and 6.9% respectively. Some other fungi were isolated from the diseased samples in lower frequencies, i.e. *Cephalosporium* sp, *Macrophomina phaseolina*, *Sclerotium rolfsii*, and *Pythium* sp. Occurrence and frequency of the root-rot and wilt diseases pathogens associated with the diseased faba bean samples were in the same trend in both Beheira and Assiut G. Also, *F.solani* and *F.oxysporum* were found in all districts in both governorates and *R.solani* was found in all districts in Assiut G., while other fungi were found in most districts of both governorates.

Data in Table (3) clearly show that, the occurrence frequency of the most important pathogens isolated from root-rot/wilt diseased samples of lentil in the two surveyed governorates were *Fusarium solani* (33.0 %), *F.oxysporum* (23.1), *Rhizoctonia solani* (16.5%), *Fusarium* spp. (13.2%), *F.moniliforme* (7.7%) and *Verticillium* spp. (6.5%). The trend of fungi occurrence and frequency was almost the same in the two surveyed governorates. *Fusarium solani* and *F.oxysporum* were found in all surveyed districts, while other fungi were found in some (but not all) districts.

Table 2. Identity, occurrence and frequency of root-rot/wilt fungi isolated from diseased faba bean plants collected from two governorates during 1995/96 season.

Governorates	District	% infection	No. of isolates						Total
			A	B	C	D	E	F	
Beheira	North Tahrir	4.1	0.0	3	2	1	0.0	2	8
	Bangar EL-Sukar	7.3	4	4	6	1	1	2	18
	West Noubaria	2.1	1	2	4	0.0	1	2	10
	Bostan	15.0	2	4	5	2	3	1	17
	Mariut	2.1	2	2	3	0.0	0.0	0.0	7
	Kafr El-Dawar	3.1	2	3	4	1	1	1	12
	Delengat	3.5	0.0	2	2	1	1	2	8
	Abou-homos	2.3	0.0	2	3	0.0	1	1	7
Subtotal			11	22	29	6	11	11	87
Frequency %			12.6	25.3	33.3	6.9	12.6	12.6	100
Assiut	Manfalout	10.2	2	4	5	2	1	1	15
	Dairout	15.3	3	5	5	1	2	2	17
	El-Kosia	9.9	2	3	4	1	1	1	11
	Assiut	10.1	2	2	4	0.0	0.0	0.0	8
	Abou-Teig	15.0	4	3	3	2	1	1	14
	Abnoub	8.2	2	1	3	1	1	1	8
Subtotal			15	18	24	7	6	6	73
Frequency %			20.5	24.7	32.9	9.6	8.2	8.2	100
Frequency %			16.3	25.0	33.1	6.9	10.6	10.6	100

A) *R. R.solani*B) *F.oxysporum*C) *F.solani*D) *F.moniliforme*E) *Fusarium spp.*F) *Verticillium spp.*

Table 3. Identity, occurrence and frequency of root-rot/wilt fungi isolated from diseased lentil plants collected from two governorates during 1995/96 season.

Governorates	District	% infection	No. of isolates						
			A	B	C	D	E	F	Total
Beheira	North Tahrir	7.1	1	0.0	0.0	2	0.0	4	7
	Bangar EL-Sukar	4.4	0.0	0.0	1	1	1	3	6
	West Noubaria	10.2	1	1	3	2	0.0	5	12
	Bostan	7.3	0.0	1	2	0.0	1	4	8
	Delengat	6.2	0.0	1	1	1	0.0	2	5
Subtotal			2	3	7	6	2	18	38
Frequency %			5.3	7.8	18.4	15.8	5.3	47.4	100
Assiut	Manfalout	5.4	1	1	1	1	0.0	3	7
	Dairout	6.0	0.0	1	2	1	1	2	7
	El-Kosia	4.2	0.0	0.0	1	2	0.0	2	5
	Sadfa	4.9	0.0	0.0	1	1	1	2	5
	Abou-Teig	5.1	1	0.0	2	0.0	0.0	3	6
	Abnoub	6.1	1	1	2	0.0	1	4	9
Subtotal			3	3	9	5	3	16	39
Subfrequency %			7.7	7.7	23.1	12.8	7.7	41.0	100
Total			5	6	16	11	5	34	77
Frequency %			6.5	7.7	20.8	14.3	6.5	44.2	100

A) *R. R.solani* B) *F.oxysporum* C) *F.solani*
 D) *F.moniliforme* E) *Fusarium spp.* F) *Verticillium spp.*

Data in Table (4) show that, the most important fungi isolated from root and basal stem of chickpea diseased samples collected from Beheira and Assiut governorates were *Sclerotinia sclerotiorum* (44.2%), *F.solani* (20.8%), *F.moniliforme* (14.3%), *F.oxysporum* (7.7%), *Fusarium* spp. (6.5%) and *R.solani* (6.5%). Data clearly show that, *Sclerotinia sclerotiorum*, the causal pathogen of sclerotinia stem rot of chickpea, was found in all surveyed districts with high frequency level compared with other fungi. Also, data show that *F.oxysporum*, the causal pathogen of wilt disease of chickpea was one of the fungi that has low frequency (7.7%) among the isolated fungi.

Pathogenic Potential of the Most Important Isolated Fungi :

Isolates of the most important pathogens isolated from different legume crops were tested to determine their pathogenic potential using cultivar Giza 3 for testing faba bean isolates, Precoze for testing lentil isolates, and Cultivar Giza 531 for testing chickpea isolates.

F.oxysporum In faba bean, four isolates were tested. Two of them caused 100% infection with wilt disease and the other two did not cause any disease symptoms. In lentil, 6 isolates were tested. All of them caused 100% mortality of lentil seedlings after 4 weeks from sowing. In chickpea, six isolates were tested. Obtained data proved that *F.oxysporum* is considered a moderate pathogen on chickpea. Average percentage of wilt infection among the tested isolates was 42.5%.

Isolate No. 174 gave the highest percentage of wilt (55%), while isolates No. 22 and 55 gave the least percentage of wilt disease (35%) (Table 5).

F.solani: Two isolates were tested in each crop. Data in Table (5) indicate that average percentages of infection with root-rot disease caused by *F.solani* were 40 and 50% in faba bean, 30 and 20% in lentil, and 60 and 65 % in chickpea. It seems that the disease is important in faba bean and chickpea crops, and has less importance in lentil crop.

R.solani : As shown in Table (5), *R.solani* the causal pathogen of root-rot disease is considered one of the most important pathogens infecting legume crops. Average percentages of infection ranged from 50 to 70%. *R.solani*, usually infected legume plants in the early growth stage causing damping-off and seedling rot.

F.Moniliforme and *V.albo-atrum* : Two isolates from each pathogen were test-

ed in each crop. Data indicate that, these pathogens are weak pathogens on the three legume crops. Average percentage of infection ranged from zero to 15% only.

S.sclerotiorum : Two isolates were tested on chickpea. Average percentages of infection were 100 and 95% (Table 5).

Table 5. Percentage of infection with the most important pathogens of legume crops in pathogenicity tests.

Faba bean (G3)			Lentil (Precoze)			Chickpea (G531)		
Pathogen	Isolate No	Infection %	Pathogen	Isolate No	Infection %	Pathogen	Isolate No	Infection %
<i>F.oxysporum</i>	53	100	<i>F.oxysporum</i>	151	100	<i>F.oxysporum</i>	22	35
	63	100		155	100		23	50
	67	0		159	100		55	35
	67	0		160	100		173	40
	-	-		167	100		174	55
	-	-		169	100		179	45
<i>F.solani</i>	52	40	<i>F.solani</i>	153	30	<i>F.solani</i>	175	60
	81	50		166	20		129	65
<i>R.solani</i>	60	50	<i>R.solani</i>	156	60	<i>R.solani</i>	180	70
	96	50		168	70		181	65
<i>F.moniliforme</i>	62	0	<i>F.moniliforme</i>	154	5	<i>F.moniliforme</i>	185	10
	106	10		161	0		187	15
<i>V.alboatrum</i>	84	15	<i>V.alboatrum</i>	162	10	<i>V.alboatrum</i>	172	100
	101	15		163	15		184	95

DISCUSSION

Field inspection and laboratory isolation indicated that, root-rot was the most widespread and damaging disease of faba bean in Egypt. Several fungi were involved in this disease, especially *F.oxysporum*, *F.solani* and *R.solani*. *R.solani* is a temperate pathogen, so losses from *R.solani* infection are severe in the early growth but not in the late season (Mohamed 1982). Therefore, it causes damping off and seedling rot in the early growth season.

Researchers, usually deal with root-rot in faba bean as a complex disease caused by several fungi, including *F.oxysporum*, *F.solani*, *F.avenaceum*, *R.solani*, *Pythium* sp., and *S.rolfsii* (Abdallah 1969, Mohamed 1982, Salt 1982 and Abou-Zeid *et al.* 1995). Hence, breeding and screening programs for resistant faba bean varieties to root-rot disease become unreliable and confusing since we are using a mixture of these fungi. This work proved that, the main causal pathogen of root-rot and collar rot of faba bean in Egypt is *F.solani*. This conclusion may help in breeding and

screening programs for this disease or in developing control strategy.

Vascular wilt is usually caused by *F.oxysporum f.sp. fabae*, a form specially pathogenic to faba bean. Fortunately, field inspection proved that the disease was not widespread in faba bean fields in Egypt. *F.oxysporum* isolated from diseased samples was less frequent than *F.solani*.

In pathogenicity test, 50% only of *F.oxysporum* isolates produced wilt disease. It is well known that *F.oxysporum* is an abundant facultative saprophyte in soil on organic matter. Some strains have specific pathogenic activity causing vascular wilt, and some are poorly specialized and cause seedling blight, necrosis or root-rot (Diekmann 1994). This means that, a number of pathogenic isolates of *F.oxysporum* is less than what is usually isolated, and wilt disease is less important than root-rot disease in faba bean in Egypt. In this respect, Salt (1982) mentioned that, wilt is unlike root-rot, it is not usually a problem in crops grown in good rotations and individual infected plants scattered throughout the crop, because of the high specificity of the wilt pathogen.

Lentil : Although the frequency of *F.solani* isolates were higher than *F.oxysporum*, field observation and laboratory tests indicated that, vascular wilt caused by *F.oxysporum* still is the most important disease affecting this crop in Egypt. All tested *F.oxysporum* isolates produced 100% mortality of lentil seedlings, while *F.solani* produced only 25% infected plants with root-rot. So far, no sources of resistance to wilt disease are known all over the world. Hence, development of tolerant lentil lines to wilt disease is important. The local climatic conditions including temperature, relative humidity and precipitation may favour infection with wilt disease caused by *F.oxysporum* but not infection with root-rot pathogens such as *F.solani* and *R.solani*. These results are in agreement with those obtained by Yahia *et al* (1994) and El-Garhy (1994).

Chickpea : Several diseases have been reported on chickpea from different parts of the world including Egypt. Few of them affecting roots, and collar of the plants i.e., fusarium wilt (*F.oxysporum f.sp. ciceri*), black root-rot (*F.solani*), collar rot (*S.rolfsii*), root-rot (*R.solani*), and stem rot (*S.sclerotiorum*) (Nene 1980 B, and El-Awadi 1993). The most important chickpea disease on a world-wide basis is fusarium wilt, caused by *F.oxysporum f.sp. ciceri* (Nene and Sheila 1992). Field inspection and laboratory isolation proved that percentage of wilt disease and frequency of *F.oxysporum* isolates were very low compared with other diseases and

isolated fungi. It seems that disease is not important on chickpea in Egypt. This may be due to two reasons. The first one is, the climatic condition, especially temperature in early growing season (usually less than 20°C) unfavourable for growth of the fungus and development of the disease. Chauhan (1963) reported that the optimum temperature for growth of the fungus and wilt development in chickpea is about 25°C. The second reason is, the race of *F.oxysporum f.sp. ciceri* found in Egypt might be less virulent having weak pathogenic potential. Jimenez-Diaz *et al.* (1993) mentioned that, seven pathogenic races of *F.oxysporum f.sp. ciceri* and at least two pathogenic groups have been identified. The least virulent race (race 0) appears to occur in countries in the Mediterranean region and in California, USA.

Field observation and laboratory isolation indicated that, incidence of black root-rot disease caused by *F.solani* under field conditions was higher and more common than vascular wilt caused by *F.oxysporum f.sp. ciceri*. Also, pathogenicity test proved that black root-rot was more important than vascular wilt in chickpea in Egypt. These results were previously reported by so many investigators. Grewal *et al.* (1974) in India and Westerlund *et al.* (1974) in the USA reported that, *F.solani* was found to be more common than *F.oxysporum f.sp. ciceri*, which caused vascular wilt in chickpea.

Obtained data indicate that wet root-rot caused by *R.solani* is present on chickpea in Egypt, but it is not considered a serious disease. Most of the infection occurred in the seedling stage when temperature and soil moisture content favour the disease. Optimum temperature and soil moisture content for the disease occurrence are 23°C and 55%. In this connection, Nene (1980 A) reported that, most of the incidence is in the seedling stage when soil moisture content is usually high. In India, it is more frequent in chickpea planted after rice harvest when the soil is wet.

Although Sclerotinia stem rot disease caused by *S.sclerotiorum* is not a root disease we included it in this work because of its importance. The disease has been found in all investigated fields with high incidence. Also, the pathogen isolates were encountered at the highest frequency among isolated fungi. Pathogenicity test proved that this disease is the most important infecting chickpea in Egypt. Nene (1980a) reported that, the disease is favoured by a combination of cool and wet weather, excessive vegetative growth and heavy dew.

Generally, it could be concluded that, the most important diseases infecting roots of cool season food legumes in Egypt are root-rot (*F.solani*) in chickpea. In ad-

dition, sclerotinia stem rot disease (aerial plant disease) is considered the most destructive disease infecting chickpea in Egypt. Breeding and screening programs should be conducted for producing resistant cultivars against these diseases.

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حصر وتعريف أهم الكائنات المسببة لأمراض أعفان الجذور والذبول فى محاصيل الفول البلدى والعدس والحمص ودراسة قدرتها المرضية

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تم عمل حصر مقنن ومكثف لأمراض أعفان الجذور والذبول فى محاصيل الفول البلدى والعدس والحمص فى محافظتى البحيرة (شمال مصر) وأسيوط (مصر الوسطى) وذلك فى مرحلتين من عمر النباتات. الأولى فى يناير وفبراير والثانية فى مارس وأبريل موسم ١٩٩٦ .

تم عزل وتعريف الفطريات الموجودة فى العينات المصابة والتي تم جمعها من الحقول التى تم فحصها. ايضا تم دراسة القدرة المرضية لأهم الفطريات التى تم عزلها من المحاصيل الثلاثة. أوضحت الدراسة أن أهم الأمراض التى تصيب المجموع الجذرى فى المحاصيل البقولية هى عفن الجذور المتسبب عن الفطر فيوزاريوم أوكسيسبورم فى العدس، ومرض عفن قاعدة الساق المتسبب عن الفطر سكليروتينيا سكليروتيورم فى الحمص.