

MYCOFLORA OF COTTON FLOWERS AND FUNGI ASSOCIATED WITH BOLL ROT IN EGYPTIAN COTTON. I-SAPROPHYTIC FUNGI

By

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Key words : Cotton flowers , boll rot , mycoflora .

ABSTRACT

Isolation of fungi was made for nine weeks post flower opening for two consecutive years from young floral segments and capsule components, on glucose and cellulose agar media.

The capsule components possessed fewer fungi than those of young flower segments and this was due to the use of organophosphorus insecticide, Dursban, during this period.

Alternaria alternata followed by *A. niger* were the most common species on young floral segments, but *A. niger* followed by *Rhizopus oryzae* were the most frequent species on capsule component segments.

INTRODUCTION

The production of cotton lint, the crop of first economic importance to Egypt was 410 thousand tons in 1983. This value represents 34% of the total cotton lint production in Africa (FAO 1983).

Saprophytic fungi colonization in cotton bolls or flowers has, in recent years, been described by Mostafa (1959), Balk (1958), Henderson and Christensen (1961), Marsh and Kerr (1961), Stands *et al.* (1962), Pinchard (1964), Marsh (1965), McCarter *et al.* (1970), Abdalla and El-Tayeb (1981), Klich *et al.* (1984), Pizzinatto *et al.* (1984), Sharma and Sandhu (1985) and Mellon *et al.* (1986). Others studied fungal colonization of winged bean flowers and of peach twigs and flowers (Gunasekara *et al.*, 1985 and Melgarejo *et al.*, 1985).

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Marsh and Kerr (1961) found that losses of cotton crop have been attributed to boll rot caused by moulds such as *Diplodia* sp., *Alternaria* sp., *Nigrospora* sp., *Aspergillus niger* and *A. flavus* which occur as contaminants and may subsequently lead to boll rot when high relative humidity prevails at the time of boll opening. Stands *et al.* (1962) confirmed that the decrease of fibre strength as well as length, followed fungal infection of lint.

Abdalla and El-Tayeb (1981), found that fungal colonization of cotton bolls took place between the first and second week following flower opening under Sudanese field conditions. Moulds isolated during the 7 weeks that followed flower opening were *A. flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *Chaetomium* spp., *Curvularia* sp., *Helminthosporium*, *Rhizopus stolonifer* and *Thielavia* sp.

The aim of the present work was to contribute to the knowledge of incidence of fungi associated with boll rot in cotton, record of which is lacking under Assiut field conditions.

MATERIALS AND METHODS

Seeds of *Gossypium barbadense* L.(var. Dandara) were sown between April-September. Random samples were collected once every week during the period 25 June - 27 August in the two consecutive years (1985 and 1986) and this was made one week following flower opening.

Samples were collected from eight different localities (El-Kasser, El-Maasara, Riefa, El-Zawia, Mousha, Awallad Bader, Dair Dronka and Dronka) in Assiut area, and one sample from the Botanical Garden of the Faculty of Science, Assiut University. Samples were classified into young flowers (1-5 weeks post-flower opening), and developing to fully mature bolls (6-9 weeks post-flower opening). Each sample contained three flowers. The young flowers were aseptically dissected into four parts; epicalyx, calyx, corolla and ovary (androecium and gynoecium). The developing and fully mature bolls were segregated into epicalyx, pericarp or ovary wall and locks (locular contents of the capsule consisting of lint and seeds).

The floral segments were thoroughly washed in 20 ml sterile distilled water with 5 to 8 fresh changes. Finally, the segments were drained off and dried between sterilized filter papers. Two pieces of each type were inserted on the surface of the agar medium in each plate, 5 replicates were used for each type. Two media namely modified glucose-Czapek's agar and cellulose-Czapek's agar were used and incubated at 28 °C for 5 days.

RESULTS

a. The fungal flora on young floral segments

Twenty seven genera and 42 species in addition to one variety were isolated at 28 °C on glucose and cellulose agar. The most common genera were *Alternaria*, followed by *Aspergillus*, but *Drechslera*, *Fusarium*, *Rhizopus* and *Circinella* appeared in moderate, low or rare frequency of occurrence.

Alternaria, represented by *A. alternata* was identified in high frequency on corolla on both glucose and cellulose agar (48 and 45 of total frequency, respectively), on epicalyx (48) on glucose agar, but absent on cellulose agar. It appeared in moderate incidence on calyx and ovary on the two media, (Fig. 1, A).

Aspergillus was recorded in high frequency of occurrence on the ovary (88 and 77 of total frequency) on glucose and cellulose agar respectively, but it appeared in low frequency on epicalyx (28 and 18 of total frequency) on glucose and cellulose agar respectively. It was recovered in moderate incidence on both calyx and corolla on the two media (Table 1) It was represented by nine species and one variety of which *A. niger* was the most frequent. This species came ahead of all species in frequency of occurrence on the ovary (46–45) on the two media (Fig. 1, A), but it appeared in moderate frequency on calyx and corolla (28–22 and 24–26) on glucose and cellulose agar and in low frequency on epicalyx (18–14) on glucose and cellulose agar, respectively.

From *Drechslera* 2 species were recovered of which *D. spicifera* was the most common. It appeared in moderate frequency on epicalyx and corolla on the two media and on ovary on glucose agar, but it was recovered in low frequency on calyx on the two media and on ovary on cellulose agar, (Fig. 1, A).

Fusarium was represented by 3 species of which *F. moniliforme* was the most prevalent. It appeared in moderate frequency on epicalyx, corolla on both media, but on calyx and ovary on glucose and cellulose agar respectively and in low and rare incidence on calyx and ovary on cellulose and glucose agar respectively.

A single species of *Rhizopus* (*R. oryzae*) was recovered from epicalyx and corolla in moderate frequency on glucose agar. It was recovered almost in low frequency from other segments on both media (Fig. 1, A).

Table 1
Frequency of occurrence and fungi on (epicalyx, calyx, corolla and ovary).

Floral segments Name of fungi	Epicalyx		Calyx		Corolla		Ovary	
	glucose N.C.I.O.R	Cellulose N.C.I.O.R	glucose N.C.I.O.R	Cellulose N.C.I.O.R	glucose N.C.I.O.R	Cellulose N.C.I.O.R	glucose N.C.I.O.R	Cellulose N.C.I.O.R
<i>Aspergillus</i>	28	18	36	26	42	30	88	77
<i>A. niger</i> van Tieghem	18 L	14 L	28 M	22 M	24 M	26 M	46 H	45 H
<i>A. flavus</i> Link	4 R				12 L	2 R	12 L	14 L
<i>A. flavus</i> var. <i>columnaris</i> Raper & Fennell					2 R		16 L	12 L
<i>A. ochraceus</i> Withelm	4 R	4 R	2 R	2 R	2 R	2 R	10 L	6 R
<i>A. chevalieri</i> (Mangin) Thom & Church			2 R					
<i>A. nidulans</i> (Eidam) Wint	2 R		2 R		2 R			
<i>A. candidus</i> Link							2 R	
<i>A. varicolor</i> (Vuill.) Iriaboschi			2 R					
<i>A. fumigatus</i> Fresenius				2 R				
<i>A. sydowi</i> (Bian. & Sart.) Thom & Chuich							2 R	
<i>Penicillium chrysogenum</i> Raper & Thom	4 R						4 R	
<i>Alternaria alternata</i> (Fries) Keissler	48 H		38 M	34 H	48 H	45 H	38 M	38 M
<i>Drechslera</i> (t. f.)	42	42	24	20	56	44	24	22
<i>D. spicifera</i> (Bainier) Van Arx anamorph of <i>Cochliobolus spicifer</i> Nelson	30 M	28 M	14 L	16 L	38 M	30 M	22 M	18 L
<i>D. hawaiiensis</i> (Bugnicourt) Subram & jain ex M.B. Ellis. <i>Exserohilum</i>	12 L	14 L	10 L	4 R	18 L	14 L	2 R	4 R
<i>E. halodes</i> (Derchsler) Leonard & suggs anamorph of <i>Setosphaeria rostrata</i> Leonard		6 R	2 R	4 R	2 R	4 R		6 R
<i>E. rostratum</i> (Drechsler) Leonard & Suggs anamorph of <i>Setosphaeria rostrata</i> Leonard							2 R	

Table 1 (cont.)

Name of fungi	Floral segments		Epicalyx		Calyx				Corolla		Ovary	
	glucose	Cellulose	glucose	Cellulose	glucose	Cellulose	glucose	Cellulose	glucose	Cellulose	glucose	Cellulose
	N.C.I.O.R	N.C.I.O.R	N.C.I.O.R	N.C.I.O.R	N.C.I.O.R	N.C.I.O.R	N.C.I.O.R	N.C.I.O.R	N.C.I.O.R	N.C.I.O.R	N.C.I.O.R	N.C.I.O.R
<i>Fusarium</i> (t.f.)	28	26	36	24	26	46	8	22				
<i>F. moniliforme</i> Sheldon	22 M	26 M	26 M	12 L	24 M	36 M	8 R	22 M				
<i>F. oxysporum</i> Schlecht ex. fr.	6 R		6 R	4 R	2 R	10 L						
<i>F. semitectum</i> Berk. & Rav.			4 R	8 R								
<i>Rhizopus oryzae</i> Wen & Prinsen Geerlings	22 M	14 L	16 L	6 R	22 M	12 L	18 L	12 L				
<i>Circinella simplex</i> Van Tieghem <i>Curvularia</i>	14 L	2 R	22 M	6 R	22 M	2 R	22 M					
<i>C. lunata</i> (Wakker) Boedijn anamorph of												
<i>Cochliobolus lunatus</i> Nelson & Haasis	12 L	8 R	6 R	6 R	14 L	12 L	2 R					
<i>Cochliobolus lunatus</i> Nelson & Haasis												
<i>C. oryzae</i> Bugnicourt			2 R			4 R						
<i>Pleospora herbarum</i> (Fr. ex. fr.) Rabenh.	16 L	14 L	14 L	10 L	14 L	10 L	12 L	6 R				
<i>Epicoccum purpurascens</i> Ehrenb. ex Schlecht	4 R		4 R		2 R							
<i>Mucor circinelloides</i> Van Tieghem	4 R	2 R	2 R	4 R	4 R	2 R	4 R	4 R				
<i>Neurospora crassa</i> Shear & Dodge	4 R	6 R	2 R	2 R	6 R	4 R						
<i>Botryotrichum piluliferum</i> Sacc. & Marchal												
<i>Chaetomium</i>			2 R	4 R	4 R	8 R						
<i>C. globosum</i> kunze ex Fries		12 L										
<i>C. spirale</i> Zopf		2 R										
<i>Stachybotrys chartarum</i> Ehrenb. ex link) Hughes		8 R	2 R	8 R	6 R	22 M	2 R	6 R				
<i>Gliocladium roseum</i> Bainier	2 R	4 R		2 R	2 R	4 R	2 R					
<i>Acremonium strictum</i> W. Gams		2 R		2 R								

Table 1 (cont.)

Floral segments Name of fungi	Epicalyx		Calyx		Corolla		Ovary	
	glucose N.C.I.O.R	Cellulose N.C.I.O.R	glucose N.C.I.O.R	Cellulose N.C.I.O.R	glucose N.C.I.O.R	Cellulose N.C.I.O.R	glucose N.C.I.O.R	Cellulose N.C.I.O.R
<i>Humicola grisea</i> Iraaen			2 R					
<i>Trichoderma hamatum</i> (Bon.) Bain.	2 R	4 R				2 R		2 R
<i>Myrcthecium verrucaria</i> (Albertini & Schweinitz Ditmar).					2 R			
<i>Nigrospora State-of Khushia oryzae</i> Hudson					2 R		4 R	2 R
<i>Scopulariopsis brevicaulis</i> (Sacc.) Bainjer	2 R							
<i>Ulocladium atrum</i> Preuss	2 R	4 R			2 R			
<i>Verticillium albo - atrum</i>					2 R			
<i>Syncephalastrum racemosum</i> (Cohn) Schroeter		2 R						
<i>Papulaspora sepedonioides</i> preuss		2 R						
<i>Cladasporium cladosporioides</i> (Fress.) de Vries								2 R
<i>Sterile mycleium</i>							2 R	

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N.C.I. - Number of cases of isolation,
 O.R. - Occurrence remarks,
 H - High occurrence, Between 45 - 90 cases
 M - Moderate occurrence, between 22 - 44 cases.

L - Low occurrence, between 10 - 21 cases.
 R - Rare occurrence, between 1 - 9 cases.
 T.f. - Total frequency.

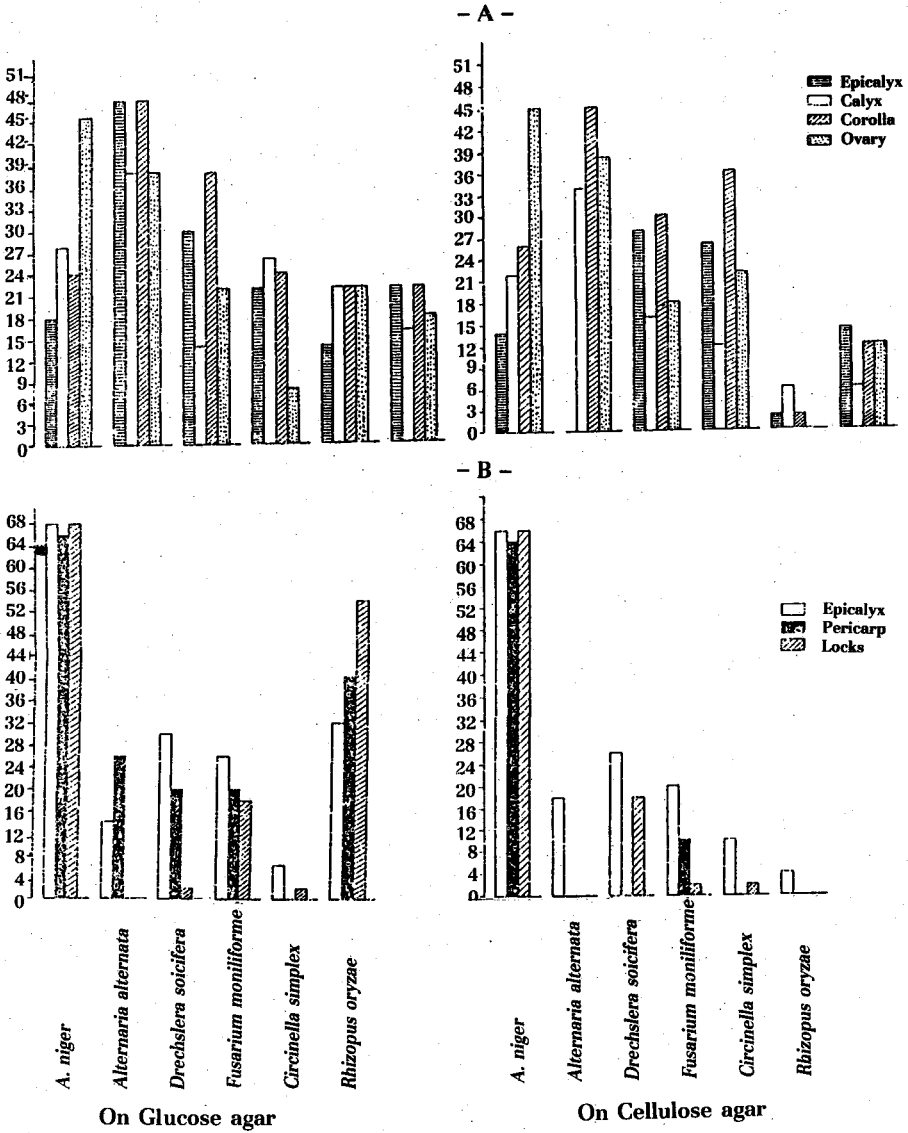


Fig. 1 : Frequency of occurrence of the dominant fungi.

A. On the young floral segments.

B. On the old capsule component segments on both glucose and cellulose agar media.

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Circinella simplex also appeared in moderate frequency on calyx, corolla and ovary, but it was recovered in low frequency on epicalyx on glucose agar and in rare frequency on cellulose agar of all floral segments and it was completely absent on ovary on cellulose medium (Fig. 1,A).

The rest of fungal species were recorded in low and rare frequency as shown in Table 1.

b. The fungal flora of damaged capsule components (epicalyx, pericarp and locks)

Nine genera and 16 species with the addition of one variety were recovered on the capsule components on both media at 28 °C. *Aspergillus* followed by *Rhizopus* were the most common genera (Fig. 1,B). From *Aspergillus* 5 species and one variety were recovered of which *A. niger* was of high frequency of occurrence on the three floral segments on the two media (Fig. 1, B). *A. flavus* also appeared in high frequency on glucose agar of locks, but it appeared in moderate, low and rare frequency on the other segments. The remaining species of *Aspergillus* were of moderate, low and rare frequency as shown in Table 2.

Rhizopus oryzae appeared in high incidence on pericarp and locks on glucose agar and disappeared on cellulose agar (Fig. 1,B), but on epicalyx it was recovered in moderate frequency on glucose and in low frequency on cellulose agar.

Alternaria alternata, *Drechslera spicifera* and *Fusarium moniliforme* appeared in moderate, low and rare frequency of occurrence on the two media (Fig. 1,B). The remaining species were less frequent as shown in Table 2.

DISCUSSION

Fourty two species and one variety which belong to 27 genera of fungi were collected on young floral segments. The results show that *Alternaria alternata* was the most frequent species on the young segments except ovary on glucose or cellulose agar. *Alternaria* sp. was previously isolated from rotted boll of cotton by Marsh and Kerr (1961) and *Alternaria tenuissima* was isolated from the flowers of peach by Melgarejo *et al.* (1985).

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Table 2
Frequency of occurrence of fungi on (Epicalyx, Pericarp & Locks).

Capsule segments Name of fungi	Epicalyx				Pericarp				Locks			
	glucose		Cellulose		glucose		Cellulose		glucose		Cellulose	
	N.C.I	O.R	N.C.I	O.R	N.C.I	O.R	N.C.I	O.R	N.C.I	O.R	N.C.I	O.R
<i>Aspergillus</i> (t.f.)	114		96		112		78		122		86	
<i>A. niger</i>	68	H	66	H	66	H	64	H	68	H	66	H
<i>A. flavus</i>	22	M	6	R	26	M			42	H	2	R
<i>A. flavus</i> var. <i>columnaris</i>	14	L	12	L	20	M	14	L			16	L
<i>A. nidulans</i>	10	L	8	L					2	R		
<i>A. niveo-glaucus</i> Thom & Raper			2	R								
<i>A. sulphureus</i> (Fres.) Thom & Chruch			2	R					10	L	2	R
<i>Alternaria alternata</i>	14	L	18	M	26	M						
<i>Drechslera</i> (t.f.)	30		36		20		16		2		18	
<i>D. spicifera</i>	30	M	26	M	20	M			2	R	18	M
<i>D. hawaiiensis</i>			10	L			16	L				
<i>Exserohilum halodes</i>			4	R								
<i>Fusarium</i> (t.f.)	32		20		20		10		18		2	
<i>F. moniliforme</i>	26	M	20	M	20	M	10	L	18	M	2	R
<i>F. oxysporum</i>	6	R										
<i>Rhizopus oryzae</i>	32	M	4	R	40	H			54	H		
<i>Circinella simplex</i>	6	R	10	L					2	R	2	R
<i>Curvularia lunata</i>	14	L	4	R	8	L						
<i>Neurospora crassa</i>			4	R			2	R			6	R
<i>Trichoderma hamatum</i>			4	R			18	M			24	M

N.C.I. – Number of cases of isolation
H. – High occurrence, between 36 - 72 cases
L. – Low occurrence, between 8 - 16 cases
(t.f.) – Total frequency.
O.R. – Occurrence remarks
M. – Moderate occurrence, between 17 - 35 cases
R. – Rare occurrence, between 1 - 7 cases

A. niger came ahead of all species on ovary on the two media tested. *A. niger* was also isolated by Marsh and Kerr (1961) from rotted boll of cotton. *A. niger* followed by *A. nidulans* and *Rhizopus stolonifer* were also recorded as the principal pioneer contaminants of the floral sigments of cotten flowers in Sudan (Abdalla and El-Tayeb, 1981). *A. niger* was frequently isolated from flowers of peach by Melgarejo *et al.* (1985). *Drechslera spicifera*, *Fusarium moniliforme*, *Rhizopus oryzae* and *Circinella simplex* were moderately recovered.

Nine genera, 16 species and one variety were collected from the floral segments of the capsule components (epicalyx, pericarp and locks) on glucose and cellulose agar. The capsule components supported relatively fewer fungi than other young flower segments. due to the use of the organophosphorus insecticide (Dursban) which used to prevent cotton worm infestation at this period (5 weeks post flower opening). Tu (1970) found that the

organophosphorus insecticides (Bayer 37289, Dizinon Dursban and Zinphos) when added to sandy loam soil at concentrations of 10 and 100 p.p.m. showed a toxic effect on fungi and bacteria for the first and second weeks of incubation, followed by a recovery to levels similar to those of controls. El-Hissy and Abdel-Kader (1980) reported that Dursban and Dipterex were significantly toxic to the mycelial growth of *Sclerotium cepivorum*, *A. fumigatus*, *F. moniliforme* and *P. italicum*. Abdel-Mallek (1981) found that the organophosphorus insecticide phosphamidon was generally toxic to the total count of fungi and most of the fungal genera and species in the phylloplane and the toxicity persisted in one sowing till after 40 days.

Abdalla and El-Tayeb (1981) found that the accessory floral parts, namely the pedicel and epicalyx supported relatively fewer fungi than either the pericarp or locks.

A. niger was the principal pioneer contaminant on the all floral segments on capsule components on glucose and cellulose agar. It was found that *A. niger* colonization was associated with the ovary on the young flowers until the developing and fully mature bolls, which appeared in high frequency of occurrence on epicalyx, pericarp and locks on the two media. Also *A. flavus* appeared in low frequency on ovary on the two media and in high frequency on locks on glucose agar only. Klich *et al.* (1984), reported that entry of *A. flavus* into cotton seeds, was introduced into several natural openings in cotton plants at or before anthesis. Klich *et al.* (1986) found that *A. flavus* was isolated from flower buds as well as from developing bolls.

R. oryzae appeared in high frequency on pericarp and locks on glucose agar as shown in Fig. 1 .

From the observation in this investigation, it was found that the principal saprophytic fungi isolated from rotted boll were *A. niger* followed by *A. flavus* and *R. oryzae*. Mostafa (1959) reported that boll rot is due to *R. nigricans* (syn. *R. stolonifer*) invasion subsequent to worm damage in Egypt. Also, *R. stolonifer* colonization was especially associated with boll-worm damaged by Abdalla and El-Tayeb (1981).

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الفلورا الفطرية لأزهار القطن والفطريات المرتبطة بتعطن اللوزة في القطن المصري

خيرية محمد عبد الجواد

تم عزل الفطريات خلال التسعة أسابيع بعد تفتح الأزهار خلال عامين متعاقبين للأجزاء الزهرية الصغرى والأجزاء التي تحتويها العلبة (الكبسولة) على الأوساط الغذائية جلوكوز وسليلوز آجار .

١ - شملت محتويات العلبة فطريات ضئيلة عن الأجزاء الزهرية للأزهار الصغرى وهذا يرجع إلى إستخدام المبيد الحشري العضوي الفسفوري دورسبان خلال هذه الفترة .

٢ - فطرة الترناريا الترنااتا يتبعها فطرة أسبرجلس نيجر كانا الشائعان على الأجزاء الزهرية الصغرى . ولكن فطرة أسبرجلس نيجر يتبعها فطرة ريزوبس اوريزي كانا السائدان على الأجزاء التي تحتويها العلبة .