

MYCOFLORA ASSOCIATED WITH DRY DATES IN UPPER EGYPT I - MESOPHILIC AND THERMOPHILIC FUNGI

By

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ABSTRACT

Twenty-eight species which belong to 16 genera were identified from 30 dry date samples collected from Upper Egypt and plated at 28°C. *Aspergillus*, *Penicillium* and *Fusarium* were the most common genera on the carposphere and the carpoplane. *A. niger*, *p. rugulosum* and *F. solani* were the most common species on the carposphere while *A. niger* and *Alternaria alternata* were prevalent on the carpoplane.

At 45°C, six species, in addition to one variety of *Humicola grisea*, which belong to five genera were collected from the carposphere and carpoplane of 30 date samples and *A. fumigatus* was the most common species.

There is no correlation between the fungal composition, density of populations and the chemical composition of the dates of the five varieties.

INTRODUCTION

The Arab world is known for various environmental and location advantages for the cultivation of palms and production and marketing. We are all familiar with the fact that dates are The Prophet's favourite food. There are many references to dates or palm trees in the Holy Quran.

Upper Egypt is one of the most important regions in Egypt for the production of high quality of dates. Little attention has been given to the study of the fungal ecology of dates in the Arab countries (Abu-Zinada and Ali, 1977 and Elarosi *et al.*, 1983). The limited knowledge on the mycoflora of dates in Egypt, called for an intensive study of these fungi. Thus, the present investigation is one of a series which dealt with the fungal ecology of dates in Upper Egypt.

The term carposphere fungi in this investigation refers to those organisms found on the fruit surface and carpoplane refers to fungi which are attached to the outer skin of the fruit surface. This would be consistent with the use of the term "phyllosphere" and phylloplane" as coined by Last (1955 and 1971).

MATERIALS AND METHODS

I. Collection of dry date samples from the markets

30 date samples representing 5 abundant and popular varieties, namely, Partamoda, Sakkoti, Balady, Dukan and Gendula were collected from Aswan Area, Upper Egypt.

II. Determination of moisture content

The moisture content of the date fruits was determined by the oven method. The date fruits were ground in an electric mill and flour was dried in an oven for one hour at 120°C or for 24 hours at 105°C then cooled in a desiccator and re-weighed to a constant weight. The moisture content was then calculated, on oven-dry basis.

III. Chemical analysis of date fruits

Total sugars, reducing and non-reducing sugars and total nitrogen were determined in the date flour of dry samples. Date flour was prepared by drying the dates at 70°C for a period of 48-56 hours, and pulverizing the samples to pass through 60-80 mesh sieves. Mineral analysis was made by ashing. K, was analysed using a flame-photometer (Beckman, Kline flame). Ca and Mg were determined with an Atomic Absorption Spectrophotometer (Beckman, Model 603). Phosphorus was estimated spectrophotometrically (Watanabe and Olsen, 1965).

IV. Determination of carposphere fungi

This was made by using the dilution-plate method as described by Christensen (1963) but with some modifications (Moubasher *et al.*, 1979) in case of grains and pea-nut seeds, respectively. Five plates poured with glucose-Czapeck's medium + rose bengal (1/15000) were used for each fruit sample. They were incubated at 28°C and 45°C, and the developing colonies were counted and identified.

Colonies of slow-growing fungi were transferred to slants to ensure counting and then to plates for identification. Other media were also employed, Czapeck's + 0.05% yeast extract, malt extract and potato dextrose agar for the purpose of identification of some fungi.

Table 1

Moisture, sugar and mineral contents (expressed as percentage of dry weight) of 30 date samples tested.

Sample No.	Variety	% M.C.	% Sugars content		Minerals content (%)					
			T.S.	R.S.	S	N	P	K	Ca	Mg
1	Sakkoti	9.51	58.5	26.5	32.0	0.30	0.070	0.66	0.45	0.88
2	Sakkoti	6.31	58.6	25.7	32.9	0.33	0.075	0.70	0.39	0.86
3	Partamoda	24.8	80.9	48.2	32.7	0.45	0.030	0.65	0.22	1.10
4	Sakkoti	8.19	48.6	28.6	20.0	0.35	0.065	0.80	0.38	0.88
5	Sakkoti	9.23	56.8	50.2	6.6	0.38	0.02	0.76	0.58	0.95
6	Sakkoti	8.5	60.2	48.8	11.4	0.36	0.06	0.48	0.46	0.86
7	Balady	11.3	36.8	30.2	6.6	0.42	0.07	0.45	0.25	0.25
8	Balady	11.5	36.0	30.6	5.4	0.26	0.02	0.56	0.56	0.27
9	Partamoda	18.6	78.0	60.8	17.2	0.42	0.05	0.66	0.59	1.15
10	Partamoda	9.8	65.0	60.5	4.5	0.28	0.06	0.68	0.36	1.20
11	Partamoda	7.6	66.7	58.2	8.5	0.36	0.03	0.65	0.57	1.09
12	Balady	8.2	36.5	30.2	6.3	0.35	0.05	0.80	0.35	0.28
13	Balady	9.8	36.2	29.8	6.4	0.32	0.08	0.79	0.27	0.23
14	Balady	12.6	38.4	32.4	6.0	0.29	0.06	0.66	0.55	0.26
15	Dukan	11.5	66.5	59.2	7.3	0.37	0.086	0.60	0.25	0.57
16	Balady	20.2	36.8	28.1	8.7	0.31	0.072	0.71	0.33	0.25
17	Partamoda	16.5	65.1	61.2	3.9	0.40	0.03	0.68	0.36	1.20
18	Balady	16.8	37.5	30.2	7.3	0.41	0.075	0.72	0.45	0.22
19	Sakkoti	9.5	70.0	60.8	9.2	0.35	0.070	0.75	0.28	0.98
20	Balady	16.8	38.2	29.9	8.3	0.28	0.072	0.73	0.39	0.21
21	Balady	8.8	38.0	29.8	8.2	0.35	0.068	0.70	0.35	0.26
22	Balady	8.6	37.7	30.2	7.5	0.41	0.060	0.71	0.55	0.28
23	Partamoda	15.0	59.5	52.6	6.9	0.33	0.035	0.69	0.52	1.10
24	Gendula	12.5	70.2	62.0	8.2	0.32	0.088	0.76	0.37	0.38
25	Balady	10.2	36.8	31.5	5.3	0.36	0.070	0.68	0.38	0.28
26	Sakkoti	11.5	55.1	48.0	2.1	0.30	0.072	0.66	0.39	0.92
27	Partamoda	18.2	57.2	50.0	2.7	0.39	0.033	0.66	0.34	0.99
28	Sakkoti	6.2	55.8	49.2	6.6	0.40	0.030	0.62	0.55	0.98
29	Dukan	7.6	66.7	54.1	12.6	0.35	0.090	0.59	0.56	0.55
30	Gendule	5.4	70.1	59.2	10.9	0.32	0.086	0.72	0.22	0.40

T.S. = Total sugars, R.S. = Reducing sugars, S = Sucrose,
M.C. = Moisture content

Variety: Partamoda 3,9,10,11,17,23,27, Sakkoti 1,2,4,5,6,19,26,28, Dukan 15,
29, Gendula 24, 30, Balady 7,8,12,13,14,16,18,20,21,22, 25

V. Determination of carpoplane fungi

Frutis were thoroughly shaken in a series of sterile distilled water then were removed and thoroughly dried between sterilized filter papers. Four segments of each sample were inserted on the surface of the agar medium in each plate. Ten plates were used for each sample.

RESULTS AND DISCUSSION

The results given in Table 1 show that the moisture content of the date samples ranged from 5.4% - 24.8%, total sugars, from 36 - 80.9%, reducing sugars from 25.7% - 61.2% and sucrose, from 2.1% - 32.9%. Five mineral contents were also determined of which nitrogen fluctuated between 0.28% - 0.45%, phosphorus 0.02% - 0.09%, potassium 0.451 - 0.80%, calcium 0.22% - 0.59% and magnesium 0.21% - 1.20%.

1. Mesophilic fungi (recovered at 28°C)

Twenty-eight species which belong to 16 genera were collected from the 30 dry date samples (Table, 2). The most frequent genera were *Aspergillus*, *Penicillium* and *Fusarium*. All of these genera were isolated previously in this laboratory in high or moderate frequency of occurrence from cultivated soils (Moubasher and El-Dohlob, 1970; Moubasher *et al.*, 1970; Moubasher and Moustafa, 1970 and Moubasher and Mazen, 1971), from grains (Moubasher *et al.*, 1972) and from leaf surfaces (Moubasher *et al.*, 1971; Abdel-Fattah *et al.*, 1977 and Abdel Gawad, 1984), and from different date varieties in Saudi Arabia (Abu-Zinada and Ali, 1977).

Aspergillus occurred in 80% and 100% of samples constituting 28.93% and 50.4% of total fungi on the carposphere and carpoplane of dry dates, respectively. *Aspergillus* was also the most frequent genus on different date varieties in Saudi Arabia (Abu-zinada and Ali, 1977), on the leaf surface of *Zygophyllum coccineum* in Wadi-Bir-El-Ain (El-Magraby, 1980), of *Citrus*, *Prunus*, *Gossypium*, *Punica* and *Saccharum* (Moubasher *et al.*, 1971 and Abdel-Wahab, 1975), of broad bean (Abdel-Fattah *et al.*, 1977) and of eight medicinal plants in Egypt (Abdel-Gawad, 1984). The genus was represented by 5 and 4 species on the carposphere and carpoplane of dry dates, respectively.

A. niger was the most prevalent species and emerged from 80% and 100% of the samples comprising 57.60% and 55.15% of total *Aspergillus* and 16.60% and 27.81% of total fungi on the carposphere and carpoplane, respectively. *A. flavus* was the second most frequent *Aspergillus* species. It was collected from 30.0% and 23.33% of samples giving rise 12.51% and 9.96% of total *Aspergillus* and 3.60%

Table 2

Counts (calculated per gm dry fruit or per 100 segments) and numbers of cases of isolation (out of 30) of carposphere and carpoplane fungi on glucose-Czapck's agar medium at 28°C and 45°C.

Genera and species	Carposphere fungi (Dilution plate-method)				Carpoplane fungi (Segment-plate method)			
	Mesophilic (28°C)		Thermophilic (45°C)		Mesophilic (28°C)		Thermophilic (45°C)	
	T.C.	N.C.I.	T.C.	N.C.I.	T.C.	N.C.I.	T.C.	N.C.I.
<i>Aspergillus</i>	743	24	240	27	582	30	205	17
<i>A. niger</i>	428	23	—	—	321	27	—	—
<i>A. flavus</i>	93	9	22	3	58	7	—	—
<i>A. terreus</i>	70	6	56	11	76	7	25	3
<i>A. ochraceus</i>	91	7	—	—	—	—	—	—
<i>A. versicolor</i>	61	5	—	—	—	—	—	—
<i>A. fumigatus</i>	—	—	162	25	127	16	180	15
<i>Penicillium</i>	482	19	—	—	188	18	—	—
<i>P. rugulosum</i>	282	18	—	—	—	—	—	—
<i>P. corylophilum</i>	87	8	—	—	—	—	—	—
<i>P. chrysogenum</i>	103	8	—	—	73	8	—	—
<i>P. rubrum</i>	10	2	—	—	—	—	—	—
<i>P. funiculosum</i>	—	—	—	—	115	12	—	—
<i>Fusarium</i>	426	18	—	—	123	12	—	—
<i>F. solani</i>	303	17	—	—	—	—	—	—
<i>F. oxysporum</i>	63	6	—	—	95	11	—	—
<i>F. moniliforme</i>	60	4	—	—	28	3	—	—
<i>Alternaria alternata</i>	—	—	—	—	113	15	—	—
<i>Cladosporium</i>	70	6	—	—	66	7	—	—
<i>C. cladosporioides</i>	—	—	—	—	31	4	—	—
<i>Cladosporium sp.</i>	70	6	—	—	35	5	—	—
<i>Drechslera spicifera</i>	230	15	—	—	47	7	—	—
<i>Rhizopus stolonifer</i>	152	10	—	—	35	6	—	—
<i>Trichoderma sp.</i>	196	12	—	—	—	—	—	—
<i>Mucor</i>	119	8	—	—	—	—	—	—
<i>M. racemosus</i>	119	8	—	—	—	—	—	—
<i>M. pusillus</i>	—	—	38	5	—	—	150	12
<i>Myriococcum albonyses</i>	—	—	31	8	—	—	—	—
<i>Myrothecium verrucaria</i>	30	2	—	—	—	—	—	—
<i>Humicola grisea var thermoidea</i>	—	—	82	11	—	—	100	10
<i>Thermoascus aurantiacus</i>	—	—	30	7	—	—	55	6
<i>Acremonium strictum</i>	30	5	—	—	—	—	—	—
<i>Pacilomyces variotii</i>	40	4	—	—	—	—	—	—
<i>Chaetomium globosum</i>	20	3	—	—	—	—	—	—
<i>Curvularia lunata</i>	10	2	—	—	—	—	—	—
<i>Ulocladium atrum</i>	10	2	—	—	—	—	—	—
<i>Phoma humicola</i>	10	2	—	—	—	—	—	—
Total count	2568		421		1154		510	

T.C. = Total count., N.C.I. = Numbers of cases of isolation.

and 5.02% of total fungi, on the carposphere and carpoplane, respectively. *A. ochraceus* and *A. versicolor* were less frequent. Abu-Zinada and Ali (1977) reported that *A. niger* and *A. flavus* were the most dominant species in the different date varieties in Saudi Arabia.

Penicillium was also common on the carposphere and carpoplane of dry dates yielding 18.76% and 16.29% of total fungi in 19 and 18 samples, respectively. Abu-Zinada and Ali (1977) reported that, *Penicillium* was regularly recovered from different date varieties in Saudi Arabia. This genus was represented by five species of which *P. rugulosum* was the most frequent on the carposphere donating 58.5% of total *Penicillium* count and 10.98% of total fungi. *P. funiculosum* was the only species of *Penicillium* recovered from the carpoplane giving rise to 61.17% of total *Penicillium* count and 9.96% of total fungi.

Fusarium was also one of the common fungi on the surface of dry dates. It emerged in 18 and 12 samples accounting for 16.58% and 10.65% of total fungi on the carposphere and the carpoplane, respectively. Three species of *Fusarium* were collected, of which *F. solani* was the most prevalent on the carposphere and *F. oxysporum* on the carpoplane. *Fusarium* was frequent in the different date varieties in Saudi Arabia as reported by Abu-Zinada and Ali (1977), and was recorded in the phyllosphere of some plants cultivated in Egypt (Moubasher *et al.*, 1971; Abdel-Fattah *et al.*, 1977 and El-Magraby 1980).

Alternaria represented by *A. alternata* was also one of the common fungi on the carpoplane and was completely missed on the carposphere. It was collected from 15 samples donating 9.79% of total fungi. *Alternaria alternata* was among the fungi recovered from the surfaces of *Typha latifolia* (Pugh and Mulder, 1971) and Citrus (Moubasher *et al.*, 1971) leaves.

Cladosporium, represented by *C. cladosporioides* and *Cladosporium* sp., was one of the basic components of the fungus flora of the carposphere and the carpoplane of dry dates. *Cladosporium herbarum* appeared in dates in Saudi Arabia (Abu-Zinada and Ali, 1977). This genus was also common on the leaf surface of some Egyptian plants as reported by Moubasher *et al.* (1971) and Abdel-Gawad (1978 & 1984). The remaining genera and species were less frequent than the preceding ones.

2. Thermophilic fungi (recovered at 45°C)

Six species, in addition to one variety of *Humicola grisea*, which belong to five genera were collected from the carposphere and the carpoplane of the 30 dry date samples tested (Table, 2). All of these fungi were isolated previously from Egyptian soils (Mazen, 1973; Abdel-Fattah, 1973 and Abdel-Fattah *et al.*, 1977), peanut seeds (Moubasher *et al.*, 1980) and from wheat and broad bean straw composts (Moubasher *et al.*, 1982).

Table 3

Numbers of species recovered at 28°C or 45°C from the carposphere (using the dilution-plate method. DPM) and carpoplane (using the segment-plate method SPM) of thirty dry date samples.

Sample No.	28°C		45°C	
	Carposphere DPM	Carpoplane SPM	Carposphere DPM	Carpoplane SPM
1	7.0	5.0	3.0	2.0
2	5.0	4.0	2.0	2.0
3	12.0	5.0	3.0	3.0
4	7.0	3.0	3.0	3.0
5	4.0	3.0	3.0	2.0
6	3.0	8.0	3.0	1.0
7	4.0	4.0	3.0	1.0
8	5.0	5.0	4.0	1.0
9	14.0	6.0	2.0	1.0
10	5.0	6.0	1.0	0.0
11	7.0	4.0	1.0	0.0
12	6.0	6.0	1.0	2.0
13	5.0	4.0	3.0	2.0
14	4.0	5.0	1.0	3.0
15	5.0	3.0	2.0	1.0
16	6.0	4.0	2.0	3.0
17	4.0	5.0	2.0	2.0
18	5.0	5.0	2.0	2.0
19	6.0	2.0	1.0	0.0
20	12.0	3.0	3.0	1.0
21	11.0	3.0	2.0	2.0
22	5.0	2.0	2.0	1.0
23	11.0	7.0	3.0	2.0
24	5.0	6.0	2.0	2.0
25	4.0	3.0	2.0	1.0
26	2.0	5.0	1.0	1.0
27	8.0	3.0	5.0	2.0
28	4.0	4.0	1.0	0.0
29	5.0	1.0	2.0	1.0
30	2.0	4.0	3.0	2.0

The mean number of species of fungi recovered in 30 dry dates:

Carposphere fungi at 28°C = 6.1., Carposphere fungi at 45°C = 2.26

Carpoplane fungi at 28°C = 4.26, Carpoplane fungi at 45°C = 1.53

Aspergillus was the most dominant genus, and was recovered from 90% and 56.66% of samples on the carposphere and carpoplane, respectively. It was represented by *A. fumigatus* (the most common fungal species), *A. flavus* and *A. terreus*. These species were the most common *Aspergillus* species in Egyptian peanut seeds (Moubashere *et al.*, 1979 and 1980).

Mucor pusillus came second in frequency of occurrence (5 and 12 samples of the carposphere and carpoplane, respectively) after *A. fumigatus*. *Mucor pusillus* was recovered from wheat and broad bean straw composts by Moubasher *et al.*, (1982).

Humicola grisea var thermoidea was also one of the main constituents of the carposphere and carpoplane fungi. They were respectively collected from 11 and 10 samples out of 30 giving rise to 19.47% and 19.60% of total fungi. *Thermoascus aurantiacus* was recovered from 7 and 6 samples accounting for 7.12% and 10.78% of total fungi on the carposphere and carpoplane, respectively. *Myriococcum albomyces* was recovered from 8 samples accounting for 7.36% of the carposphere fungi but was completely absent on the carpoplane. These species were isolated from wheat and broad bean straw composts using either the dilution-plate or the segment-plate method when the isolation plates were incubated at 45°C (Moubasher *et al.*, 1982).

3. Range of fungal species in the thirty dry date samples

a. Species recovered from the carposphere

The results in Table (3) show that at 28°C the average number of species per sample was 6.1. The highest number was fourteen which was recorded in sample No. 9. This sample was a Partamoda variety and contained 18.6% moisture content and 78% total sugars.

The least No. of species was 2.0 which was collected from samples No. 26 (Sakkoti) and 30 (Gendula) with 11.5% and 5.4% moisture content and 55.1% and 70.1% total sugars, respectively.

At 45°C the average number of species was sharply reduced than at 28°C (2.26 and 6.1, respectively). The highest number of species was 5, recorded in sample No. 27 which was also a Partamoda variety with 18.2% moisture content and 57.2% total sugars. One species only was collected from seven samples, No. 10,11,12,14,19,26 and 28.

b. Species recovered from the carpoplane (Table 2)

At 28°C the average number of species was considerably reduced than in the carposphere (4.26 and 6.1, respectively).

The highest number of species was eight, recorded in sample No. 6. It was a Sakkoti variety and contained 8.5% moisture content and 60.2% total sugars. The least number of species was one and emerged from sample No. 29 which was Dukan variety.

At 45°C the range of fungal species was also drastically reduced and the average number of species was 1.53 (it was 5.26 at 28°C). The number of species fluctuated between one to three species in all the test samples.

The preceding results reveal that

1. There was no basic difference in the composition of fungal floras of the dates of the five varieties recovered at 28°C or 45°C.
2. There was no correlation between the density of populations and number of genera and species, as well as the chemical composition of the thirty date samples.

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الفلورا الفطرية للبلح الجاف في صعيد جمهورية مصر العربية

١ - الفطريات الوسطية والمحتملة للحرارة

عبد العال حسن مباشر - حسن موسى الشاروني
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الهدف من هذا البحث هو دراسة تصنيفية شاملة للفطريات التي تنمو على أسطح (الكاربوسفير) وبشرة (الكاربويلين) شاملة عينة من البلح الجاف تم جمعها من مناطق مختلفة من صعيد مصر هذا بالإضافة إلى المحتوى الكيميائي لهذه العينات وقد أمكن التوصل إلى بعض النتائج الهامة نوجزها فيما يلي :

- ١ - عند ٢٨°م تم عزل وتعريف ٢٨ نوعاً تنتمي إلى ١٦ جنساً من الفطريات كان أكثرها شيوعاً هي اسبرجيلس نيجر ، بنسليوم ريجيولوزم وفيوزاريوم سولاني على الكاربوسفير بينما كانت فطريات اسبرجلس نيجر ، الترناريا الترنااتا هي أكثرها شيوعاً على الكاربويلين .
- ٢ - عند ٤٥°م تم عزل وتعريف ستة أنواع بالإضافة إلى صنف واحد من الفطريات المحتملة للحرارة وكان أكثرها شيوعاً هو فطر الاسبرجلس فيوميجاتس .
- ٣ - أظهرت النتائج عدم وجود علاقة بين كل من التركيب الكيميائي للبلح الجاف والمحتوى الفطري لهذه العينات .