

MYCOFLORA ASSOCIATED WITH DRY DATES IN UPPER EGYPT II. OSMOPHILIC FUNGI AND TEST OF OSMOPHILIC ABILITY

By

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ABSTRACT

19 fungal species which belong to 8 genera were collected from 30 dry date samples of which 9 species appeared on all sucrose concentrations (20%, 40% and 60%) namely, *Aspergillus amstelodami*, *A. niger*, *A. ruber*, *A. sydowii*, *Penicillium chevalierii*, *p. brevi-compactum*, *Humicola grisea*, *Alternaria alternata* and *Rhizopus stolonifer*. When 17 species of the collected fungi were tested for their osmophilic abilities, they were classified into 4 groups:

- a) Highly osmophilic: *A. niger*, *A. ruber* and *A. amstelodami*.
- b) Fairly osmophilic: *A. flavus*, *P. funiculosum* and *F. oxysporum*.
- c) Weakly osmophilic: *A. terreus*, *P. citrinum*, *F. solani* and *Paecilomyces variotii*.
- d) Osmotolarent: *F. moniliforme*, *F. equiseti*, *Ulocladium botrytis*, *Rhizopus stolonifer*, *Chaetomium globosum*, *Alternaria alternata* and *Drechslera biseptata*.

INTRODUCTION

The term osmophilic fungi is used in a general sense referring to fungi growing better on media containing high sugar concentration. In general, most of information has been focused on osmophilic fungi isolated from soils and grains (Hudson, 1972; Abdel-Fattah, 1973; Moustafa, 1975 and Moubasher *et al.*, 1979). The osmophilic ability of some fungal species recovered from soil or from other sources was tested by Raper and Fennell (1965), Fulik and Hanlin (1968), Abdel-Fattah (1973), Moustafa and Al-Musallam (1975) Mazen *et al.* (1977) and Rao and Kalyanasundaram (1983).

In Egypt, there are no available records on osmophilic fungi associated with dates. This investigation aimed at studying this group of fungi on dry date samples in Upper Egypt.

MATERIALS AND METHODS

1 Collection of date samples

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

2. Determination of osmophilic fungi

They were determined by using dilution-plate method on Czapek's agar media supplemented with 20%, 40% and 60% sucrose. Rose bengal (1/15000) was used as a bacteriostatic agent (Smith and Dawson, 1944). Six plates were used for each sample. They were incubated at 28°C for 4-10 days and examined daily.

3. Determination of osmophilic ability

Osmophilic abilities were assessed by growing 17 species of those recovered in the present investigation on Czapek's liquid and solid medium containing 20%, 40% and 60% sucrose.

50ml. from each liquid medium were administered in 250ml. flask and were inoculated with one ml. spore suspension obtained from seven-day old culture of the test organism. Flasks were incubated at 28°C for ten days after which the fungal mat was removed, rinsed with distilled water and dried at 105°C. Two replicates were used for each concentration. Also after 2 days-intervals colony diameter, on solid medium was measured.

RESULTS AND DISCUSSION

The results of Table 1, reveal that: On 20% sucrose-Czapek's agar 19 species and eight genera were collected from the carposphere of 30 dry date samples, of which *Aspergillus* (8 species), *Penicillium* (4 species) and *Fusarium* (2 species) were the most frequent genera. *A. amstelodami* and *A. niger* were the most frequent species (recovered from all samples) followed by *P. funiculosum* (14 samples) and *F. oxysporum* (13 samples). Most of the fungal species recovered on 20% sucrose were isolated previously on osmophilic media in this laboratory by Moubasher and his collaborators. Moubasher *et al.* (1979) reported that *A. niger*, *A. amstelodami*, *A. flavus*, *A. repens*, *P. notatum* and *Rhizopus stolonifer* were the most prevalent osmophilic species in barley grains. Moustafa (1975) isolated 101 osmophilic species which belong to 46 genera from Kuwait salt marshes, of which *A.*

Table 1

Total counts of osmophilic fungi (calculated per gm dry fruit in every sample), and their numbers of cases of isolation (out of 30) on 20%: 40% and 60% sucrose-Czapeck's agar media at 28°C.

Fungal genera and species	Total count			N.C.I.		
	Sucrose concentrations					
	20%	40%	60%	20%	40%	60%
Total count	854	287	110			
<i>Aspergillus</i>	398	100	85	24	10	6
<i>A. amstelodami</i>	154	75	30	20	6	3
<i>A. niger</i>	100	60	40	20	4	8
<i>A. flavus</i>	42	0	0	7	0	0
<i>A. ruber</i>	20	15	5	4	2	1
<i>A. sydowii</i>	15	5	5	3	1	1
<i>A. chevalieri</i>	20	5	5	4	1	1
<i>A. rugulosus</i>	31	0	0	4	0	0
<i>A. candidus</i>	16	0	0	2	0	0
<i>Penicillium</i>	182	24	5	18	5	1
<i>P. funiculosum</i>	93	10	0	14	2	0
<i>P. corylophilum</i>	60	0	0	8	0	0
<i>P. brevi-compactum</i>	9	9	5	2	2	1
<i>P. citrinum</i>	20	5	0	2	1	0
<i>Fusarium</i>	100	35	0	15	4	0
<i>F. oxysporum</i>	85	35	0	13	3	0
<i>F. solani</i>	15	0	0	3	0	0
<i>Paecilomyces variotii</i>	40	10	0	8	1	0
<i>Humicola grisea</i>	25	6	4	5	1	1
<i>Alternaria alternata</i>	20	16	5	5	3	1
<i>Trichoderma sp.</i>	24	16	0	5	3	0
<i>Rhizopus stolonifer</i>	65	20	11	9	3	1
Number of species	19	14	9			

N.C.I. = Numbers of cases of isolation.

ochraceous, *P. chrysogenum*, *P. notatum*, *Curvularia spicifera* and *Fusarium equiseti* were the most prevalent.

On 40% sucrose, 14 species and 8 genera were collected (Table 1). Also, *Aspergillus* was the most common genus and *A. amstelodami* was the most prevalent species. Raper and Fennell (1965), in their treatise of the genus *Aspergillus*, reported that *A. glaucus* group (to which belong *A. amstelodami* and *A. chevalierii*) and most species of *A. restrictus* group are osmophilic fungi.

On 60% sucrose, the spectrum of fungal genera and species was considerably lowered (5 genera and 9 species). Most probably, this medium becomes very selective to those fungi which could tolerate this high sugar concentration beside those which are real osmophilic. The total fungal count also sharply decreased on 60% sucrose (110 colonies per gm dry fruit) below that on 40% sucrose (287 colonies) and on 20% sucrose (854 colonies). *Aspergillus* was represented by *A. amstelodami*, *A. ruber*, *A. sydowii* and *A. chevalieri*. It was collected in six date samples out of 30 accounting for 77.3% of total fungi. The other genera namely, *Penicillium*, *Humicola*, *Alternaria* and *Rhizopus* were of rare occurrence. Abdel-Hafez *et al.*, (1977) reported that *Aspergillus* followed by *Cladosporium* and *Penicillium* were the most frequent osmophilic genera of Egyptian salt marshes recovered on 60% sucrose.

Test of osmophilic ability of the fungal species

The osmophilic ability of 17 species of those fungi which emerged on the three types of osmophilic media were tested by growing them for 14 days at 28°C on both solid and liquid Czapeck's media supplemented with 20%, 40% and 60% sucrose, in addition to 1% glucose-Czapeck's which was considered as control.

The results after 10 day-incubation on liquid medium at 28°C (Table 2 and Fig. 1) reveal that the test fungi could be classified into four groups:

- 1) Highly osmophilic, which gained their best growth at 60% sucrose. This group included three species namely, *A. niger*, *A. ruber* and *A. amstelodami*. Moustafa and Al-Musallam (1975) reported that *A. repens*, *A. amstelodami* and *A. restrictus* were highly osmophilic. Abdel-Fattah (1973) listed 4 species of *Aspergillus* (*A. amstelodami*, *A. niger*, *A. sydowii* and *A. egyptiacus*), and 2 species of *Penicillium* (*P. asperum* and *P. avellaneum*) as highly osmophilic (best growth on 40% or 60% sucrose).
- 2) Fairly osmophilic; which showed their best growth at 40% sucrose. This group was represented by *A. flavus*, *P. funiculosum* and *F. oxysporum*. These species were recovered on 40% sucrose from Syrian soils (Abdel-Hafez *et al.*, 1983).
- 3) Weakly osmophilic which showed their best growth at 20%. These were *A. terreus*, *P. citrinum*, *F. solani* and *Paecilomyces variotti*.

- 4) Osmotolerant fungi; which showed nearly equal growth at 20% and 1% glucose and could also grow at 40% and 60% sucrose. This group included *F. moniliforme*, *F. equiseti*, *Ulocladium botrytis*, *Rhizopus stolonifer*, *Chaetomium globosum*, *Alternaria alternata* and *Drechslera biseptata*.

The results on agar media (Table 3 and Fig. 2) were similar to those on liquid media except for the following observations:

- 1) *A. amstelodami* was the best highly osmophilic fungus on solid medium (180% of the control) and *A. niger* (133% of the control) came second.

Table 2

Effect of various concentrations of sucrose on growth of some fungal species (Results calculated as percentages of growth on 1% glucose-Czapeck's liquid medium after 10 day-incubation at 28°C).

Group	Species	20%	40%	60%
Highly	<i>A. niger</i>	150	290	380
Osmophilic	<i>A. ruber</i>	160	155	320
	<i>A. amstelodami</i>	175	285	360
Fairly	<i>A. flavus</i>	160	260	50
Osmophilic	<i>P. funiculosum</i>	90	180	50
	<i>F. oxysporum</i>	30	250	120
Weakly	<i>A. terreus</i>	410	290	50
Osmophilic	<i>P. citrinum</i>	315	170	80
	<i>F. solani</i>	260	210	65
	<i>Paecilomyces variotii</i>	350	260	155
	<i>F. moniliforme</i>	85	60	30
	<i>F. equiseti</i>	110	40	20
	<i>Ulocladium botrytis</i>	90	50	35
Osmotolerant	<i>Rhizopus stolonifer</i>	95	40	25
	<i>Chaetomium globosum</i>	120	30	35
	<i>Alternaria alternata</i>	115	55	36
	<i>Drechslera biseptata</i>	88	35	22

- 2) *Paecilomyces variotii* was the best colonizer of 20% sucrose (235% of the control) but *A. terreus* was the best on liquid medium.

Comparison between the present results and those reported from soil (Abdel-Hafez *et al.*, 1977 and El-Magraby, 1980) and grains (Sheila *et al.*, 1978 and Moubasher *et al.*, 1980) reveal that there is no osmophilic fungi characteristic to dry

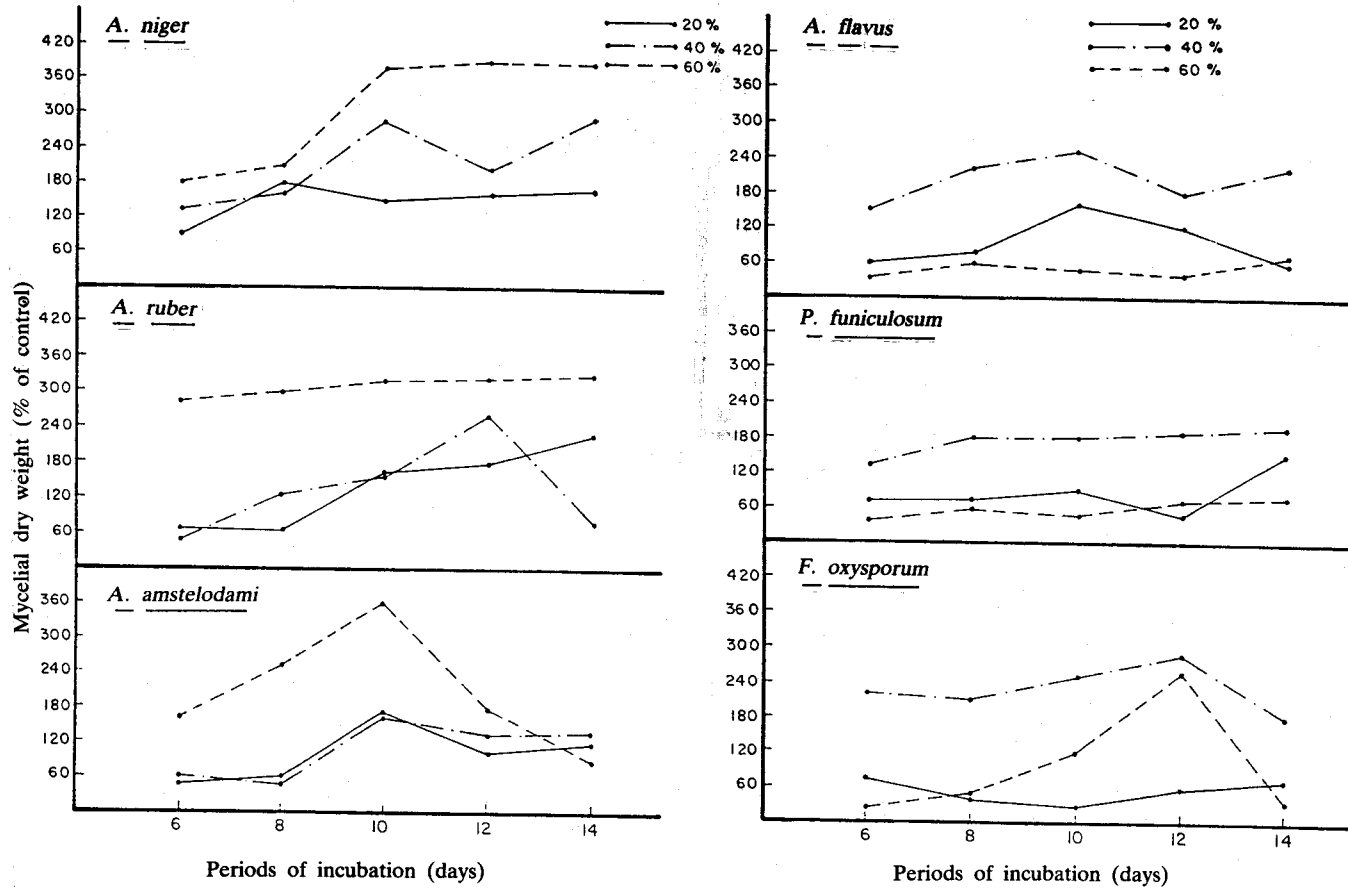


Fig. 1: Effect of various sucrose concentrations on the mycelial growth of some test fungi after various experimental periods on czapek's liquid medium.

dates in Upper Egypt, but the density and occurrence of some fungal genera and species were promoted or decreased.

Table 3

Effect of various concentrations of sucrose on the growth of some fungal species (Results calculated as percentages of linear growth on 1% glucose-Czepeck's agar medium) after 10 day incubation at 28°C.

Group	Species	20%	40%	60%
Highly	<i>A. niger</i>	33	75	133
Osmophilic	<i>A. ruber</i>	36	57	57
	<i>A. amstelodami</i>	60	90	180
Fairly	<i>A. flavus</i>	150	250	200
Osmophilic	<i>P. funiculosum</i>	111	244	89
	<i>F. oxysporum</i>	180	230	200
Weakly	<i>A. terreus</i>	220	120	55
	<i>P. citrinum</i>	215	105	30
Osmophilic	<i>F. solani</i>	235	95	25
	<i>Paecilomyces variotii</i>	255	110	12
Osmotolerant	<i>F. moniliforme</i>	102	30	32
	<i>F. equiseti</i>	90	25	25
	<i>Ulocladium botrytis</i>	86	32	28
	<i>Rhizopus stolonifer</i>	88	15	36
	<i>Chaetomium globosum</i>	110	22	30
Osmotolerant	<i>Alternaria alternata</i>	95	40	25
	<i>Drechslera biseptata</i>	96	35	20

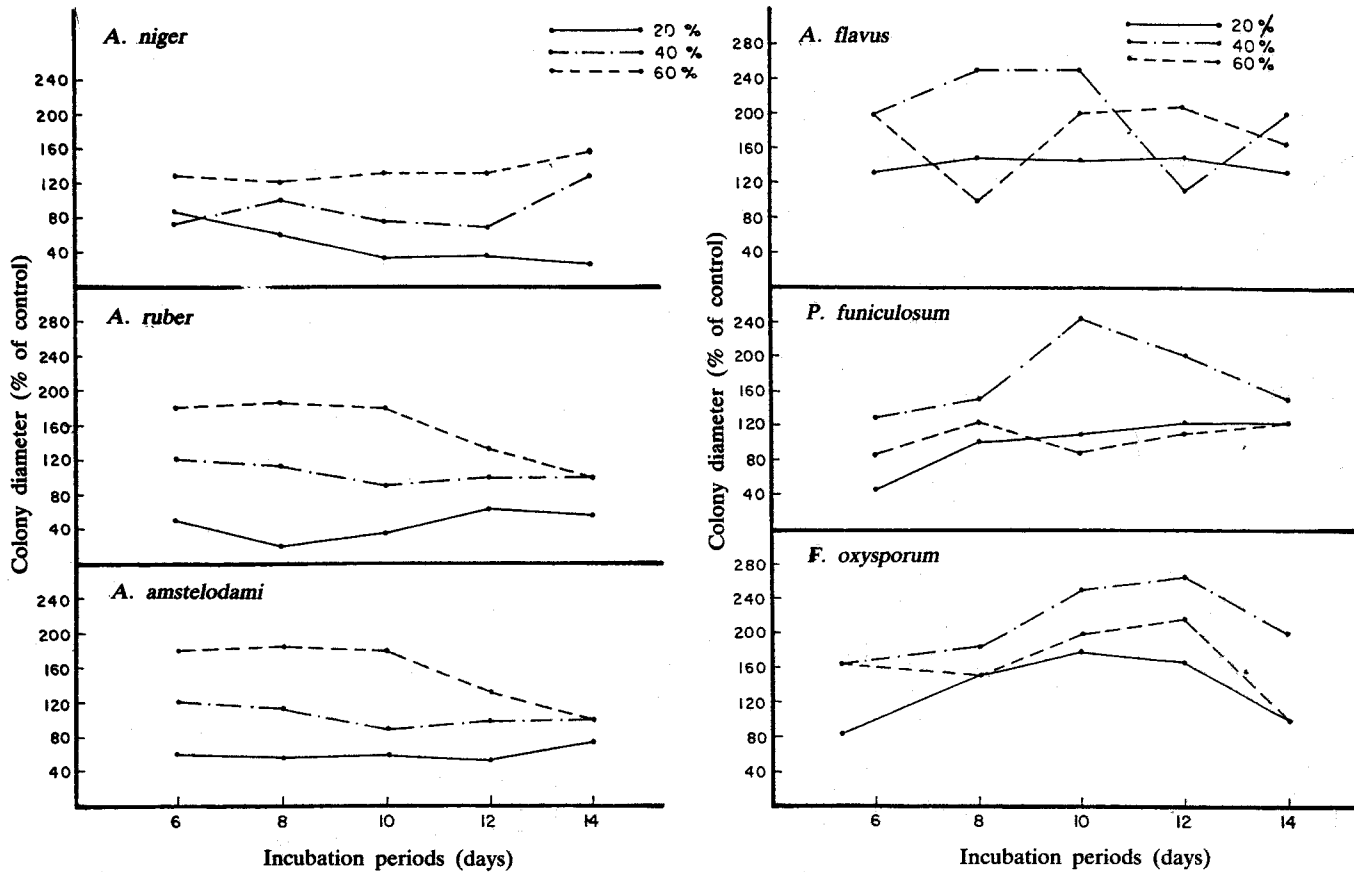


Fig. 2: Effect of various sucrose concentrations on the colony diameter of some test fungi after various experimental periods on czapek's agar medium.

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الفورا الفطرية للبلح الجاف في صعيد جمهورية مصر العربية

٢ - الفطريات المحبة للأسموزية

حسن موسى الشاروني - عبد العال حسن مباشر
و مرتضى شاكر نصلر

الهدف من هذا البحث هو عزل وتعريف الفطريات المحبة للأسموزية من ٣٠ عينة من البلح الجاف تم جمعها من بعض المناطق في صعيد مصر ، وكذلك تضمن البحث دراسة قدرة بعض الفطريات المعزولة على تحمل درجات مختلفة من الأسموزية وذلك عند زراعتها على أوساط غذائية تحتوي على تركيزات ٢٠٪ ، ٤٠٪ ، ٦٠٪ من السكروز . ولقد أمكن التوصل إلى بعض النتائج نوجزها فيما يلي :

١ - تم عزل وتعريف ١٩ نوعا تنتمي إلى ٨ أجناس من الفطريات المحبة للأسموزية . وقد أمكن التعرف على تسعة أنواع منها كانت لها القدرة على النمو عند جميع تركيزات السكروز .

٢ - عن دراسة مدى تحمل ١٧ نوعاً من الفطريات على النمو عند جميع التركيزات أمكن تقسيمها إلى ٤ مجموعات رئيسية هي كما يلي :

(١) فطريات عالية الأسموزية (٣ أنواع) وتتم بصورة أفضل عند تركيز ٦٠٪
سكروز .

(ب) فطريات متوسطة الأسموزية (٣ أنواع) وتتم بصورة أفضل عند تركيز ٤٠٪
سكروز .

(جـ) فطريات ضعيفة الأسموزية (هـ أنواع) وهي التي تنمو بصورة أفضل عند تركيز ٢٠٪
سكروز .

(د) فطريات تتحمل الأسموزية (٧ أنواع) وهي التي تستطيع النمو بصورة واحدة عند جميع التركيزات .