LEAF SURFACE FUNGI OF *ARGEMONE MEXICANA* GROWING IN SAUDI ARABIA

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SUMMARY. — Forty-two species belonging to 22 genera were collected from leaf surface of *Argemone mexicana* by using the dilution plate method on glucose- and cellulose-Czapek's agar at 28°C. The total counts of phyllosphere fungi fluctuated between 660 and 1540, and between 220 and 800 colonies/g fresh weight of leaves on the two media, respectively. The most common fungi were *Alternaria alternata, Cladosporium herbarum, Ulocladium atrum, U. botrytis, Phoma humicola* and *Aspergillus niger* on glucose; and *A. alternaria, U. botrytis, P. humicola* and *Curvularia ellisii* on cellulose agar plates. Also, there are basic similarities between the phyllosphere and those of Saudi Arabian wheat plant and airborne fungi at Taif city.

RÉSUMÉ. — Quarante deux espèces appartenant à vingt deux genres ont été isolés de la surface de feuilles d'Argemone mexicana par la méthode des dilutions sur milieu Czapeck (glucose ou cellulose), à 28°C. Les nombres de champignons trouvés fluctuent respectivement entre 660 et 1540, et entre 220 et 800 colonies par gramme de poids frais de feuilles, sur les deux milieux. Les champignons les plus communs sont *Alternaria alternata, Cladosporium herbarum, Ulocladium atrum, U. botrytis, Phoma humicola* et *Aspergillus niger* sur glucose; et *A. alternata, U. botrytis, P. humicola* et *Curvularia ellisii* sur cellulose. Il existe des similitudes de base entre cette flore fongique, celle des blés d'Arabie Saoudite, et celle contenue dans l'air prélevé à Taif.

KEY WORDS : *Argemone mexicana*, leaf surface fungi, Saudi Arabia.

I. — INTRODUCTION

In different places of the world and in recent years, several investigations were caried out on the saprophytic activity of micro-organisms on leaf surface of numerous plants (ABDEL-FATTAH & al., 1977; ABDEL GAWAD, 1978, 1984; ABDEL-HAFEZ, 1981, 1984; ABDEL-WAHAB, 1975; DICKINSON, 1965, 1967; DI MENNA & PARLE, 1970; MISHRA & DICKINSON, 1981; SINHA, 1971) which is influenced by the seasonal changes in the meteorological

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factors such as temperature, humidity and rain fall, and by the type and the age of the plant.

In Saudi Arabia, information on leaf surface fungi was very limited. ABDEL-HAFEZ (1981, 1984) studied the composition, density and occurrence of phyllosphere fungi of wheat and four fern plants. The aim of this investigation is to characterize the fungus flora (sugar and cellulose-decomposing) of leaf surfaces of *Argemone mexicana*, a medicinal plant, widespread in Taif region.

II. – MATERIAL AND METHODS

Argemone mexicana L. (Fam. Papaveraceae) is an erect prickly herbaceous plant up to 1 m high with yellow juice, with leaves of blue-green colour, white-veined, clasping spiny pinnatifid, with flowers 5 cm across, of 4-6 petals, capsule prickly with radiating stigmas, very common at south of Hijaz, the southern part of the western region extending south of Jedda till Yemen boundaries. Leaf surface fungi were studied in 10 samples collected from Taif region (this region is mainly a mountanainous area, 5000-7000 feet above sea level, and has a cold winter with a minimum temperature range of 4-10°F, and annual rainfall is between 5-12 ml) during April and May 1983, when the plant was 3-4 months old. The young leaves (3-4 weeks old) were put in sterile polyethylene bags and transferred immediately to biology laboratory (in the Junior College and Center of Science and Mathematics, at Taif in Saudi Arabia) for fungal analysis.

Determination of leaf surface (phyllosphere) fungi :

4 g of *Argemone mexicana* leaves were washed in 200 or 400 ml sterile distilled water, which means that the dilution used for the estimation of sugar and cellulose-decomposing fungi in leaves tested were 1/50 and 1/100 respectively. One ml of the wash water was transferred to a sterile petri dish and poured with melted but cooled glucose- (10 g/l) or cellulose- (19 g/l) Czapeck's agar and rose bengal (1/15000) as a bacteriostatic agent (SMITH & DAWSON, 1944). The plates were used for each sample (5 plates for each medium). Plates were incubated at 28°C for 7-10 days and the developing fungi were identified and counted and the numbers were calculated per 1 g fresh weight of leaves. The colonies of slow-growing fungi which were about to be over grown, as well as mycelial fragment of some colonies, were transferred to Czapek's agar and yeast extract or to malt extract agar.

III. – RESULTS AND DISCUSSION

A. On glucose agar

The total count of fungi in samples tested fluctuated between 660 and 1540 colonies/g fresh weight of leaves. DI MENNA (1971) found that the numbers

of moulds and yeasts/g of leaves were greatest in summer and autumn, greater on litter and senescent leaves than on green leaves, and greater on rye grass than on *Clover* and *Fescue*. ABDEL-GAWAD (1978, 1984) observed that the gross total count of phyllosphere fungi of some egyptian plants was markedly affected by the age and the surface area of the plant organ, so that the highest count was regularly estimated on old leaves and followed in a descending manner by young leaves, buds and stems. Similar observation has been reported by DICKINSON (1967), HOGG & HUDSON (1966), KERLING (1958) and LAST (1955). Twenty genera and 39 species were collected from the leaf surface of *Argemone mexicana* as listed in tables 1 and 2. Most of these species were recovered previously from the phyllosphere of wheat and four fern plants growing in Saudi Arabia (ABDEL-HAFEZ, 1981, 1984). The most frequent genera were *Alternaria, Cladosporium, Phoma, Aspergillus* and *Curvularia*. ABDEL-HAFEZ (1981) found that *Cladosporium, Aspergillus, Alternaria* and *Penicillium* followed by *Rhizopus, Drechslera, Fusarium, Epicoccum* and *Curvularia* were the most common genera in the plyllosphere of wheat plant cultivated in Saudi Arabia. Also, the preceding genera were prevalent in some Egyptian plants as observed by ABDEL-FATTAH & al. (1977), ABDEL-GAWAD (1978, 1984) and ABDEL-WAHAB (1975).

Alternaria occurred in 100 % of the samples comprising 12.65 % of total fungi, and was represented by 3 identified species and unidentified species (conidia with long beak). *A. alternata* was the most common and emerged in 100 % of the samples constituting 91.8 % of total *Alternaria* and 11.62 % of total fungi. ABDEL-HAFEZ (1981, 1984) found that *A. alternata* was the most prevalent *Alternaria* species in the phyllosphere of wheat and four fern plants growing in Saudi Arabia. *A. alternata (= A. tenuis)* was among the fungi recovered from the leaf surface of *Fagus sylvatica* (HOGG & HUDSON, 1966), *Typha latifolia* (PUGH & MULDER, 1971), *Capsicum annuum, Solanum melongena, Solanum tuberosum* and *Lycopersicum esculentum* (SINHA, 1971), *Acer pseudoplatanus* and *Typha latifolia* (PUGH & al., 1972), *Citrus* plants (MOUBASHER & al., 1971), on *Prunus, Citrus, Punica, Gossypium* and *Saccharum* (ABDEL-WAHAB, 1975), Broad-bean (ABDEL-FATTAH & al., 1977), some Egyptian summer and winter plants (ABDEL-GAWAD, 1978), and on *Ammi visnaga, Calotropis procera, Capsicum annuum, Datura arburea, Mentha piperita, Origanum majorana* and *Rosmarinus afficinalis* (ABDEL-GAWAD, 1984). Also, it was very common saprophyte found on many kinds of plants and other substrata including foodstuffs, soil and textiles; cosmopolitan. *A. chlamydospora* and *A. tenuissima* were less common. ABDEL-HAFEZ (1984) isolated 5 species of *Alternaria* from the atmosphere of Taif and these were *A. Alternata* (the most common species), *A. chlamydospora, A. grisea, A. solani* and *A. tenuissima*.

Cladosporium emerged in 100 % of the samples contributing 18.67 % of total fungi. From the genus 3 species were identified of which *C. herbarum* was the most prevalent, and was occurred in 100 % of the samples contributing 82.2 % of total *Cladosporium* and 15.35 % of total fungi. *C. herbarum* was also common

in the air at Taif and in the phyllosphere of wheat and 4 fern plants growing in Saudi Arabia (ABDEL-HAFEZ, 1981, 1984), as well as in the leaf surface of several higher plants cultivated in different places of the world, on *Fagus sylvatica* by HOGG & HUDSON (1966) in Cambridge, on *Typha latifolia* by PUGH & MULDER (1971) in Nottingham, on barley leaves by DIEM (1974) in France, on *Acer platanoides* by BREEZE & DIX (1981) in Scotland, and on some Egyptian plants as reported by MOUBASHER et al. (1971) and ABDEL-GAWAD (1978, 1984). *C. macrocarpum* and *C. sphaerospermum* were less frequent. These two species in addition to *C. herbarum* were very common cosmopolitan species, especially abundant in temperate regions on dead herbaceous and woody plants and have been isolated from air, soil, foodstuffs, paint and textiles as observed by numerous workers.

Ulocladium occupied the second place according to the number of cases of isolation. It recovered from 90 % of the samples giving rise to 13.69 % of total fungi, and was represented by 4 species of which *U. atrum* and *U. botrytis* were the most common. They encountered in 80 % and 60 % of the samples comprising 50 % and 21.2 % of total *Ulocladium* and 6.85/ and 2.9 % of total fungi, respectively. These two species were abundant on dead herbaceous plants, rotten wood, paper, textiles and isolated from air and soils in different places of the world as reported by several workers. *U. chartarum* and *U. alternaria* were recovered in moderate and low occurrence, respectively. These species were also recovered, but with variable density and frequency, from the air of Taif and from the phyllosphere of wheat and 4 fern plants growing in Saudi Arabia as reported by ABDEL-HAFEZ (1981, 1984).

Phoma ranked third with regard to the number of cases of isolation (80 % of samples), but its total count (21.16 % of total fungi) put a head of *Cladosporium, Ulocladium* and *Alternaria*. It was represented by 3 species of which *P. humicola* and *P. glomerata* were the most common, and were occurred in 80 % and 40 % of the samples comprising 66.67 % and 27.45 % of total *Phoma* and 14.1 % and 5.81 % of total fungi, respectively. *P. eupyrena* was less frequent (20 % of the samples, 5.88 % of total *Phoma* and 1.24 % of total fungi). ABDEL-HAFEZ (1981, 1984) isolated *P. herbarum, P. humicola, P. hibernica* and *P. glomerata* from the atmosphere of Taif, and from the leaf surface of wheat and 4 fern plants growing in Saudi Arabia, as well as from the phyllosphere of some Egyptian higher plants (ABDEL-GAWAD, 1978, 1984).

Aspergillus occurred in 70 % of the samples constituting 10.16 % of total fungi. *Aspergillus* was also the most frequent genus on the leaf surface of *Zygophyllum coccineum* (EL-MAGRABY, 1980), *Prunus, Citrus, Gossypium, Punica* and *Saccharum* (ABDEL-WAHAB, 1975; MOUBASHER & al., 1971), broad bean (ABDEL-FATTAH & al., 1977), and on eight medicinal Egyptian plants namely, *Ammi visnaga, Calotropis procera,, Capsicum annuum, Datura arburea, Mentha piperita, Mentha pulegium, Origanum majorana* and *Rosmarinus officinalis* (ABDEL-GAWAD, 1984). It was represented by 8 species of which *A. niger, A. tamarii, A. sydowi* and *A. nidulans* were the most prevalent which emerged in 30 to 70 % of the samples contributing 6.12 to 40.8 % of total

Table 1. – Percentage count (calculated per total fungi in every sample) and percentage frequency (calculated per 10 samples) of fungal genera recovered from the leaf surface of *Argemone mexicana* on glucose- and cellulose-Czapeck's agar.

Genera	Glucose		Cellulose	
	% Count	% Frequency	% Count	% Frequency
Alternaria	12.66	100	15.88	100
Aspergillus	10.16	70	0.45	10
Cephalosporium	0.41	20		
Chaetomium			5.15	40
Cladosporium	18.67	100	24.6	70
Curvularia	2.7	60	8.5	90
Drechslera	0.83	30	0.45	20
Epicoccum	0.41	20		
Fusarium	0.83	30	0.45	10
Mucor	0.21	10		
Myrothecium	1.24	40	0.89	20
Oedocephalum	2.49	30		
Paecilomyces	0.41	20	0.45	20
Penicillium	0.62	20		
Phoma	21.16	80	27.74	100
Rhizopus	0.21	10	0.22	10
Saccharomyces	8.51	40	2.68	20
Scopulariopsis	0.62	20	0.45	20
Stachybotrys	0.21	10	5.15	40
Torula			0.45	20
Trichoderma	2.9	20		
Ulocladium	13.69	90	10.29	80
Mycelia sterilia	1.04	30	0.89	20

Aspergillus and 0.62 to 4.15 % of total fungi. *Aspergillus flavus, A. ochraceus, A. quadrilineatus* and *A. ustus* were less frequent and accounting collectively 12.24 % of total *Aspergillus* and 1.24 % of total fungi. ABDEL-HAFEZ (1981, 1984) found that *A. niger, A. flavus, A. terreus* and *A. sydowi* were the most common *Aspergillus* species in the atmosphere of Taif and on the leaf surface of wheat plant cultivated in Saudi Arabia. Also, all the preceding *Aspergillus* species were recovered, but with variable frequency and populations, from the phyllosphere of some Egyptian plants (ABDEL-FATTAH & al., 1977; ABDEL-GAWAD, 1978, 1984; ABDEL-WAHAB, 1975).

Curvularia occurred in 60 % of the samples giving rise to 2.7 % of total fungi. It was one of the basic components of the phyllosphere of *Hordeum vulgare, Zea mays, Gossypium barbadense, Hibiscus esculentus, Hibiscus subdariffa* and *Corchorus olitorius* (ABDEL-GAWAD, 1978), and it was also reported in the leaf surface of *Citrus* (MOUBASHER & al., 1971) and broad-bean (ABDEL-FATTAH & al., 1977). From the genus 4 species were collected of which *C.*

Table 2. — Number of cases of isolation (out of 10 samples) and total counts of fungal genera and species of leaf surface (per g fresh weight leaves in every sample) of *Argemone mexicana* on glucose- and cellulose-Czapeck's agar incubated at 28 °C.

Genera and species	Glucose			Cellulose		
	TC	NCI	OR	TC	NCI	OR
Total count	*9640*			*4470*		
Alternaria (total count)	*1220*	10	H	*710*	10	H
A. alternata (Fr. : Fr.) Keissler	1120	10	H	610	10	H
A. chlamydospora Mouchacca	40	2	L	20	2	L
A. tenuissima (Kunze : Fr.) Wiltshire	40	2	L	70	4	M
Alternaria sp. (conidia with long beak)	20	2	L	10	1	R
Cladosporium (total count)	*1800*	10	H	*1100*	7	H
C. herbarum (Pers. : Fr.) Link	1480	10	H	940	7	H
C. sphaerospermum Penzig	20	1	R	60	2	L
C. macrocarpum Preuss	60	3	M			
Cladosporium spp.	240	6	H	100	3	M
Ulocladium (total count)	*1320*	9	H	*460*	8	H
U. atrum Preuss	660	8	H	50	2	L
U. botrytis Preuss	280	6	H	340	8	H
U. chartarum (Pr.) Simmons	320	4	M	70	3	L
U. alternaria (Cke.) Simmons	40	2	L			
Ulocladium sp.	20	1	R			
Phoma (total count)	*2040*	8	H	*1240*	10	H
P. humicola Gilman & Abbott	1360	8	H	1150	9	H
P. glomerata (Corda) Woll. & Hoch	560	4	M	30	2	L
P. eupyrena Saccardo	120	2	L	60	3	L
Aspergillus (total count)	*980*	7	H	*20*	1	R
A. niger V. Tieghem	400	7	H	10	1	R
A. tamarii Kita	300	5	H			
A. sydowi (Bain & Sart.) Thom	100	4	M			
A. nidulans (Eidam) Wint.	60	3	M			
A. flavus Link	40	2	L			
A. ochraceus Wilhelm	40	2	L			
A. quadrillineatus Thom & Raper	20	1	R			
A. ustus (Bain.) Tom. & Church	20	1	R	10	1	R
Curvularia (total count)	*260*	6	H	*380*	9	H
C. ellissi Ahmed & Quraishi	140	4	M	290	9	H
C. pallescens Boedijn	60	2	L	50	4	M
C. brachyspora Boedijn	20	1	R			
C. lunata (Walker) Boedijn	20	1	R	40	2	L
Curvularia sp.	*20*	*1*	*R*			
Drechslera (total count)	*80*	3	M	*20*	2	L
D. spicifera (Bain.) Von Arx	60	3	M	20	2	L
D. halodes (Drech.) Subram. & Jain	20	1	R			
Fusarium (total count)	*80*	3	M	*20*	1	R
F. oxysporum Schlecht.	60	2	L	20	1	R
F. solani (Mart.) Appel & Woll.	20	1	R			

Genera and species	Glucose			Cellulose		
	TC	NCI	OR	TC	NCI	OR
Paecilomyces (total count)	*40*	2	L	*20*	2	L
P. variotii Bainier	20	1	R	10	1	R
P. terricola (Miller & al.) Onions & Barron	20	1	R	10	1	R
Scopulariopsis (total count)	*60*	2	L	*20*	2	L
S. candida (Gueg.) Vuill.	20	1	R			
S. brevicaulis (Sacc.) Bainier	40	1	R	20	2	L
Chaetomium (total count)				*20*	2	L
C. globosum Kunze ex Fr.				10	1	R
C. spirale Zopf				10	1	R
Cephalosporium roseo-griseum Saksena	40	2	L			
Epicoccum purpurascens Ehrenb.	40	2	L			
Mucor racemosus Fresenius	20	1	R			
Myrothecium verrucaria (Alb. & Sch. : Fr.) Ditmar	120	4	M	40	3	M
Oedocephalum sp.	240	3	M			
Penicillium spp.	60	2	L			
Rhizopus stolonifer (Ehrenb. : Fr.) Vuill.	20	1	R	10	1	R
Saccharomyces spp.	820	4	M	120	2	L
Stachybotrys chartarum (Ehrenb.) Hughes	20	1	R	230	4	M
Torula herbarum (Pers.) Link : Fr.				20	1	L
Trichoderma viride Pers.	280	2	L			
Mycelia sterilia (White & dark colour)	100	3	M	40	2	L

TC = total count.
NCI = number of cases of isolation (out of 10 samples).
OR = occurrence remark.
H = high occurrence, from 5-10 cases.
M = moderate occurrence, 3 or 4 cases.
L = low occurrence, 2 cases.
R = rare occurrence, 1 case.

ellisii was the most prevalent (40 % of the samples, 53.85 % of total *Curvularia* and 1.45 % of total fungi). *C. pallescens, C. brachyspora* and *C. lunata* were less common. ABDEL-HAFEZ (1981) isolated *C. lunata, C. pallescens, C. intermedia, C. brachyspora, C. inaequalis* and *C. tuberculata* from the phyllosphere of Saudi Arabian wheat plant.

Myrothecium (1 sp.), *Saccharomyces* spp., *Drechslera* (3 spp.), *Fusarium* (2 spp.) and *Oedocephalum* sp. were isolated in moderate occurrence and were emerged in 30-40 % of the samples comprising 0.83-8.51 % of total fungi (Table 2). The remaining genera and species were less frequent and accounting collectively 7.05 % of total fungi.

B. On cellulose agar

The total counts of cellulose-decomposing fungi in the leaf surface of *Argemone mexicana* ranged between 220 and 800 colonies/g fresh weight of leaves. 16 genera and 26 species were collected on cellulose agar at 28°C (Tables 1 and 2). Most of these species were isolated previously from Saudi Arabian desert soils and from the atmosphere of Taif on cellulose agar (ABDEL-HAFEZ, 1982, 1984).

Five genera were isolated in high occurrence and these were *Alternaria*, *Phoma*, *Curvularia*, *Ulocladium* and *Cladosporium* and were encountered in 100 %, 100 %, 90 %, 80 % and 70 % of the samples comprising 15.88 %, 27.74 %, 8,5 %, 10.29 % and 24.6 % of total fungi, respectively. From the preceding genera 5 species were recovered in high frequency namely, *Alternaria alternata*, *Curvularia ellisii*, *Phoma humicola*, *Ulocladium botrytis* and *Cladosporium herbarum*. They emerged in 100 %, 90 %, 90 %, 80 % and 70 % of the samples constituting 15.88 %, 6.49 %, 25.73 %, 7.6 % and 21.03 % of total fungi, respectively. *Alternaria tenuissima*, *Curvularia pallescens*, *Myrothecium verrucaria* and *Stachybotrys chartarum* were recovered in moderate occurrence and were occurred in 30 or 40 % of the samples giving rise to 0.89-5.15 % of total fungi. The remaining genera and species were less frequent and listed in tables 1 and 2. The preceding fungi are well known as cellulose decomposer, but with different degrees, as reported by several workers (ABDEL-HAFEZ, 1982; ABDEL-HAFEZ & ABDEL-KADER, 1980; MAZEN & al., 1980; PUGH, 1964; RAPER & FENNEL, 1965; STEWART & WALSH, 1972; TRIBE, 1957, 1966; and several others). Also, MAZEN (1973) made an extensive survey of cellulolytic activity among Egyptian soil fungi (95 species) and he classified these test fungi into five groups:

– High cellulolytic activity, demonstrated by 17 species, of which *Penicillium corylophilum*, *Fusarium solani*, *Gliocladium catenulatum*, *Myrothecium verrucaria*, *Stachybotrys atra* var. *microspora* and *A. niger*:

– Moderate cellulolytic activity, observed by 23 species, including *Mucor racemosus*, *Chaetomium globosum*, *Rhizopus nigricans* (= *R. stolonifer*), *Trichoderma viride*, *Cephalosporium curtipes* and *A. sydowi*.

– Low cellulolytic activity, demonstrated by 19 species, including *Alternaria alternata*, *Fusarium oxysporum*, *Epicoccum purpurascens*, *Curvularia spicifera*, *C. lunata*, *Ulocladium botrytis* and *Cladosporium herbarum*.

– Weak cellulolytic activity, demonstrated by 25 species, of which *Aspergillus egyptiacus*, *Aspergillus nidulans* var. *latus*, *Penicillium funiculosum*, *P. italicum*, and *P. rubrum*.

– No cellulolytic activity, shown by 11 species which did not show any growth on cellulose, including *Aspergillus candidus*, *A. clavato-nanica*, *A. rugulosus* and *A. caesiellus*.

Most of the fungi recovered in the present investigation are among the cellulolytic fungi tested by MAZEN (1973).

It is worth mentioning that, the population density of leaf surface fungi and the number of genera and species collected on glucose agar plates were more than on cellulose, some fungi were more frequently on cellulose than glucose such as *Alternaria tenuissima*, *Curvularia ellisii*, *C. pallescens* and *Stachybrotys charbarum*, and some species were recovered on cellulose and not on glucose and vice versa (Table 2).

When comparing between the present result and those of Saudi Arabian wheat plant (ABDEL-HAFEZ, 1981) and the atmosphere of Taif (ABDEL-HAFEZ, 1984), as well as of air and some Egyptian higher plants (ABDEL-GAWAD, 1978, 1984) the following observations were drawn; there is a great similarity between the phyllosphere and air-borne fungi; dematiaceous hyphomycetes such as *Cladosporium*, *Ulocladium*, *Alternaria*, *Phoma*, *Drechslera* and *Curvularia* were prevalent in both phyllosphere and atmosphere; several fungi were more frequently recovered in the atmosphere than in the phyllosphere such as *Cephalosporium*, *Epicoccum*, *Paecilomyces* and *Penicillium*; and some fungi isolated from the leaf surface and not from the air and vice versa.

ACKNOWLEDGEMENT

The author is very indebted to S.O. Al-Otabi (Head of Biology Department, Junior college and Centre of Science and Mathematics, Taif) for the excellent research facilities he provided.

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