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Fungi Associated with *Calotropis procera* and *Capparis spinosa* Leaves

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Abstract. A monthly survey was conducted from 1989 to 1991 to examine the effect of seasons on the inhabited surface fungi on leaves of medicinal plants, *Calotropis procera*, and *Capparis spinosa*. A total number of 46 species of 21 genera of fungi from *C. procera* and 35 species of 19 genera of fungi from *C. spinosa* were isolated. On both plants, a high percent of fungi was obtained during May while the lowest percentage was found in September in the case of *C. procera* and in July in the case of *C. spinosa*. *Alternaria* (7 species) was the most predominant genus on leaves of *C. procera* and *Ulocladium* (4 species) on leaves of *C. spinosa*. *Alternaria alternata, Aspergillus flavus, A. niger* and *Penicillium chrysogenum* were isolated during all seasons on leaves of both plants.

Introduction

After the second world war, synthetic medicines (allopathic), completely outclassed the use of herbal folk medicine but during the last 20 years herbal products have re-emerged as a major competitor of synthetic products, being used in the forms of herbal preparations, herbal teas, confectionery and non-alcoholic beverages [1]. Pharmacologically active substances such as calotropin, calotropagenia, calotoxin and calactin are some important chemicals obtained from leaves and latex of *Calotropis procera* plant [2, p.270]. The leaves and roots of *C. procera* are used for boil dressing, leprosy, venereal diseases, syphilis, elephantiasis, cough expectorants, rheumatic pains, purgative and as antihelminthics. Its flower is digestive and used for asthma [3,4]. *Capparis spinosa* plant contains some important chemicals like flavenoids, glycosides, rutin, rutic acid, pectic acid and saponin [2]. Its leaves and the bark of roots are of medical importance.

These are used for respiratory diseases, liver problem, heart stimulant, rheumatism, paralysis, enlarged spleen, tubercular gland and as analgesic [2,4].

The surfaces of leaves are reservoirs of several types of microorganisms including fungi. Some of these fungi are pathogenic, some are saprophytic which, under favorable conditions may become pathogenic while there are others which live on leaves only as saprophyte and get their nutrition from exudates of leaves tissue, insect excretion or from air-borne organic matters deposited on the surface of leaves [5].

The success of inhabited fungi on leaves depends on the types of plant, location and environmental conditions [6,7, p.319].These inhabited fungi are usually not harmful to the plants but under favorable conditions, not only become pathogenic [8] but also produce mycotoxins which cause diseases in animals too, like geelkop, facial eczema, etc. [9,10].

Since these plants are mostly used as a raw material for the treatment of various diseases, information on fungi that live on the plants surface are very much necessary because these fungi may cause infections which sometime could be fatal if used for elderly patients or patients having sugar diseases or persons with weak immune system.

The study of these fungi are also important because some of them have antagonistic properties [11]. *Botrytis cineria, Candida* sp., *Trichoderma* sp. are antagonistic to several plant pathogenic fungi like *Pillularia, Drechslera, Cladosporium*, and *Cochliobolus* etc. [12]. Some of these fungi are parasitic on plant pathogenic fungi, therefore, could be used as a biological control for plant disease caused by plant pathogenic fungi [13]. These mycoflora also have a role in the biodegradation of decaying plant material [14].

The surface inhabited mycoflora of leaves are extensively studied throughout the world [5-7]. These mycoflora are also studied in Saudi Arabia but to a limited extent [16]. Therefore, this study is focused on the mycoflora and their success on the leaves of medically important plants.

Materials and Methods

Leaves of different ages of the wild plants *Calotropis procera* (Ait) Ait.f. and *Capparis spinosa* L. were collected monthly during 1989-1991 from Diriyah area, Riyadh. Leaves were collected in plastic bags and examined directly for their fungal load.

For determination of leaf surface fungi, 1 cm^2 area from leaves was cut, using a sharp sterile knife. Five grams of leaves cutting was placed in a flask containing 45 ml of presterilized distilled water and shaken mechanically, using a shaker (1000 rpm/h). Serial dilutions of the fluid part were made and 1 ml portion was plated on the surface of prepreparcd Sabouraud Dextrose agar (Oxoid Ltd., London) plates with rose bengal (0.03 g/L) and streptomycin sulphate (0.033 g/L). The plates were incubated in the dark at 25±1°C for seven days. Fungal colonies grown on agar plates were counted. Five

replicates were used from each sample. Fungal colonies were purified using single spore or hyphal tip techniques.

Confirmation of the identification of some fungi were carried out by Central Bureaue Schimmeculture, Baarn, the Netherlands.

Results and Discussion

Weather data recorded during 1989-1991, as provided by Ministry of Agriculture and Water are summerized in Table 1. The maximum relative humidity was found between December and March while minimum between June to September. The maximum temperatures were recorded between May till August while lower between December and April. Wind velocity was found to be higher during winter (Januray & February) as compared to rest of the months.

A total number of 46 fungal species belonging to 21 genera were isolated from the surface of leaves of *C. procera* (Table 2). The highest number of fungal species were recorded in May (24 species) followed by April (23 species) and March (20 species). In general the number of colonies per gram of leaves, for a particular species was highest in the month of May and April. The number of species seems to decrease at the end of May to November and then start to increase from December until May of next year. *Alternaria alternata, A. chlamydospora, Aspergillus flavus, A. niger, Drechslera australiensis, Fusarium oxysporum, Penicillium chrysogenum* and *Rhizopus stolonifer* were the only species which were found in all the months of the year. While *Absidia corymbifera, Alternaria cucumerina, A. denissi, Aspergillus parasiticus, Botryosphaera rhodina, Circinella simplex, Chrysosporium keratinophilum, Coleophoma* sp., *Curvularia cymbopognis, Drechslera hawaiiensis, D. maydis, D. spicifera, Eupenicillium* sp., *Rhizopus* sp. and *Ulocladium atrum* were restricted to a single month only. Genus *Alternaria* was represented by highest number of species (7 species) followed by *Aspergillus* and *Drechslera* (5 species each).

A total of 35 fungal species of 19 genera were isolated from the surface of leaves of *Capparis spinosa* (Table 3). The highest number of fungal species were isolated in April and May in the case of *C. spinosa. Alternaria alternata, Aspergillus flavus, A. niger* and *Penicillium chrysogenum* which were the only fungal species found in all the months of the year. While *Acroconidiella arecae, Circinella sydowi, Coleophoma* sp., *Curvularia tuberculata, Drechslera spicifera, Fusarium flocciferum, Mucor racemosus,* Mycelia sterilia, *Penicillium notatum, P. verrucossum, Phoma glomerata, Rhizopus* sp., *Ulocladium atrum, U. chartarum* and *U. tuberculatum* were restricted only to a particular month of the year. *Ulocladium* was the predominant genus represented by four species followed by *Aspergillus* and *Penicillium* (three species each). The pattern of seasonal fluctuation of fungal species was almost similar in both plants although the predominant genera were different. *Alternaria* were the predominant genera in the case of *C. procera* while *Ulocladium* was predominant on *C. spinosa* leaves.

Table 1. Weather data recorded during 1989-1991***

Data		Months											
		Jan.	Feb	March	April	May	June	July	August	Sept.	Oct.	Nov.	Dec.
Relative humidity %	Min.	30	28	25	12	8	5	5	5	7	7	15	18
	Max.	73	71	78	42	28	18	15	25	25	35	45	68
Temperature °C	Min.	5	12	12	17	24	24	25	26	23	20	10	7
	Max.	20	22	25	34	38	41	44	43	36	34	27	2
Wind velocity km/h	Min.	7	8	7	6	6	7	7	6	4	7	5	6
	Max.	27	29	18	17	21	20	20	18	15	20	14	18

* Data as provided by Ministry of Agriculture and Water.
** Readings are the means of two years data recorded.

Table 2. Seasonal variations of fungal species on leaves of *Calotropis procera* (No. of colonies per gram of leaves)

Fungi	No. of colonies per gram (± SD) of leaves/months											
	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Absidia corymbifera (Cohn Lichtheim) Sacc. & Trotter**	-	-	13 ± 3	-	-	-	-	-	-	-	-	-
Acremonium implicatum (Gilman & Abbott) W. Gams	16 ± 2	-	-	-	-	-	-	-	-	-	-	10 ± 3
Alternaria alternata (Fr.:Fr.) Keissler	18 ± 3	9 ± 2	22 ± 4	29 ± 4	20 ± 3	8 ± 2	9 ± 3	10 ± 2	16 ± 2	19 ± 3	16 ± 3	15 ±3
A. cheiranthi (Lib.) Bolle	-	-	10 ±2	-	-	-	-	-	-	-	-	8±2
A. chlamydospora Mouchacca	16 ± 2	15 ± 3	26 ± 3	24 ± 3	29 ± 4	16 ± 2	18 ± 3	8 ± 2	9 ± 2	12 ± 3	16 ± 3	19 ± 3
A. cucumerina (Ellis & Everh) Elliot	-	-	-	16 ±3	-	-	-	-	-	-	-	-
A. denissi M.B. Ellis	-	-	-	-	18 ± 3	-	-	-	-	-	-	-
A. longipes (Ellis & Everh) Mason	-	-	-	-	-	-	-	-	-	12 ± 3	10 ± 2	-
A. tennuissima (Kunze :Fr.) Wiltshire	12 ± 3	10 ± 2	23 ± 2	29 ± 3	21± 2	-	-	-	-	-	-	10 ± 3
Aspergillus candidus Link: Fr.**	-	-	-	18 ± 3	29 ± 3	-	-	-	-	-	-	-
A. flavus Link :Fr.**	30 ± 3	39 ± 2	46 ± 4	59 ± 3	50 ± 4	30 ± 3	30 ± 5	16 ± 2	20 ± 2	24 ± 3	29 ± 3	35 ± 5
A. niger Van Tieghem**	39 ± 3	46 ± 3	45 ± 3	62 ± 4	59 ± 5	46 ± 3	40 ± 4	30 ± 2	25 ± 3	36 ± 4	42 ± 3	49 ± 5

Table 2. (Contd.)

Fungi	No. of colonies per gram (± SD) of leaves/months											
	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
A. parasiticus Speare**	-	-	-	-	-	-	-	-	-	16 ± 4	-	-
A. terreus Thom.	-	-	-	-	-	-	18 ± 3	26 ± 4	-	-	-	-
Botryosphaera rhodina (Berk. & Curt.) V Arx.	16 ± 3	-	-	-	-	-	-	-	-	-	-	-
Capnophialophora sp.	-	-	12 ± 3	18 ± 2	-	-	-	-	-	-	-	-
Circinella rigida G. Smith	-	-	-	-	12 ± 3	-	-	-	-	-	-	-
C. simplex Van Tieghem	-	-	-	-	-	16 ± 3	-	-	-	-	-	-
Chrysosporium keratinophilum D. Frey ex Carmichael**	-	-	18 ±3	-	-	-	-	-	-	-	-	-
Cladosporium sphaerospermum Penzig**	10 ± 2	16 ± 3	24 ± 4	29 ± 2	12 ± 3	-	-	-	-	-	-	-
C. cladosporioides (Fres.) de Varies**	-	-	-	18 ± 3	29 ± 4	16 ± 3	-	-	-	-	-	-
Coleophoma sp	-	-	-	-	-	-	12 ± 3	-	-	-	-	-
Curvularia cymbopogonis (C.W. Dodge) Groves & Skolko	-	16 ± 3	-	-	-	-	-	-	-	-	-	-
C. fallax Boedijn	-	-	16 ± 3	20 ± 2	30 ± 3	-	-	-	-	-	-	-
C. tuberculata Jain	-	-	-	-	-	-	-	-	-	-	10 ± 2	16 ± 3
Drechslera australiensis (Bugnicourt) Sabram. & Jain ex M.B. Ellis	10 ± 2	16 ± 3	29 ± 3	42 ± 3	49 ± 2	40 ± 4	30 ± 3	16 ± 2	12 ± 2	18 ± 3	29 ± 3	20 ± 4

Table 2. (Contd.)

Fungi	No. of colonies per gram (± SD) of leaves±months											
	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
D. hawaiiensis (Bugnicourt) Subram. & Jain	-	-	-	-	16 ± 3	-	-	-	-	-	-	-
D. maydis (Nisikodo & Miyake) Subram. & Jain	16 ± 3	-	-	-	-	-	-	-	-	-	-	-
D. sorokiniana (Sacc.) Subram. & Jain	-	-	-	23 ± 4	16 ± 3	-	-	-	-	-	-	-
D. spicifera (Bain) V. Arx	-	-	-	-	-	20 ± 4	-	-	-	-	-	-
Embellisia chlamydospora (Hoes. & al.) Simmons	-	-	-	-	-	20 ± 3	23 ± 4	16 ± 2	-	-	-	-
Eupenicillium sp.	-	-	-	-	-	-	-	16 ± 3	-	-	-	-
Fusarium oxysporum Schlecht	20 ±2	24 ± 3	45 ± 4	53 ± 3	50 ± 4	40 ± 3	35 ± 4	23 ± 3	24 ± 2	18 ± 3	16 ± 3	29 ± 4
F. solani (Mart.) Sacc.	-	-	-	-	25 ±3	29 ± 3	-	-	-	-	-	-
Mucor circinelloides Van Tieghem	-	-	18 ± 3	26 ± 3	20 ± 4	-	-	-	-	-	-	-
M. hiemalis Wehmer	-	-	-	16 ± 3	20 ± 4	-	-	-	-	-	-	-
Penicillium chrysogenum Thom**	8 ±3	12 ± 2	26 ± 2	39 ± 3	46 ± 4	50 ± 4	61 ± 4	69 ± 4	55 ± 4	40 ± 4	36 ± 3	18 ± 3
P. verrucosum Dierckx	-	18 ± 3	20 ± 3	16 ± 3	-	-	-	-	-	-	-	-
Rhizopus sp.	-	-	-	-	-	-	-	-	13 ± 3	-	-	-
R. stolonifer (Ehrenb. :Fr.) Vuill**	10 ± 2	15 ± 3	25 ± 3	36 ± 3	46 ± 4	40 ± 4	30 ± 3	25 ± 3	26 ± 3	33 ± 2	18 ± 3	16 ± 3

Table 2. (Contd.)

Fungi	No. of colonies per gram (± SD) of leaves±months											
	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Sclerotinia sclerotiorum (Lib.) de Bary	-	-	-	-	-	18 ± 3	16 ± 2	10 ± 3	-	-	-	-
Trichoderma harzianum Rifai	16 ± 3	20 ± 4	20 ± 3	20 ± 2	22 ± 2	15 ± 3	-	-	-	-	-	10 ± 3
Ulocladium atrum Preuss	-	-	-	10 ± 3	-	-	-	-	-	-	-	-
U. chlamydosporum Mouchacca	-	-	-	-	16 ± 3	10 ± 2	20 ± 3	10 ± 3	-	-	-	-
U. tuberculatum Simmons	-	-	-	-	-	-	-	-	16 ± 2	10 ± 3	-	-
Total No. of species 46	14	13	18	22	22	16	13	13	10	11	10	13
Total No. of genera 21	10	9	13	13	12	12	10	10	7	7	7	8

** Reported as human or animal pathogen.

Table 3. Seasonal variations of fungal species on leaves of *Capparis spinosa* (No. of colonies per gram of leaves)

Fungi	No. of colonies per gram (± SD) of leaves/months											
	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Acrocomidiella arecae (Berk. & Br.) M.B. Ellis	-	-	-	18 ± 3	-	-	-	-	-	-	-	-
*Alternaria alternata***	16 ± 2	22 ± 3	29± 4	46 ± 5	53 ± 3	45 ± 3	20 ± 3	12 ± 2	14 ± 2	18± 3	25 ± 2	27 ± 3
A. chalamydospora	-	-	-	25 ± 2	27 ± 3	-	-	-	-	-	-	-
*Aspergillus flavus***	29 ± 4	25 ± 3	36 ± 4	45 ± 3	52 ± 3	44 ± 3	40 ± 4	16 ± 3	20 ± 3	29 ±	30 ± 5	35 ± 3
*A. fumigatus***	-	-	16 ± 2	26 ± 4	30 ± 3	40 ± 4	35± 3	45 ± 4	40 ± 4	30 ± 3	20 ± 3	-
*A. niger***	39 ± 3	32 ± 4	45 ± 3	55 ± 5	69 ± 5	52 ± 2	43 ± 3	29 ± 2	20 ± 3	16 ± 2	25± 3	32 ± 3
Circinella circinellodies	23 ± 4	36 ± 3	20 ± 2	-	-	-	-	-	-	-	-	-
C. sydowi	-	-	-	-	16 ± 3	-	-	-	-	-	-	-
*Cladosporium sphaerospermum***	-	-	-	15 ± 3	19 ± 2	-	-	-	-	-	-	-
C. cladosporidies	-	-	-	-	29 ± 2	20 ± 2	-	-	-	-	-	-
Coleophoma sp.	-	-	-	-	-	12 ± 2	-	-	-	-	-	-
Curvularia lnmata (Wakker) Boedijn	-	-	-	12 ± 2	19 ± 3	29 ± 4	-	-	-	-	-	-
C. tuberculata	-	-	-	-	13 ± 3	-	-	-	-	-	-	-
Drechslera hawaiiensis	-	-	15 ± 3	20 ± 2	32 ± 4	14 ± 2	-	-	-	-	-	-
D. spicifera	-	-	-	16 ± 3	-	-	-	-	-	-	-	-
Embellisia chlamydospora	-	-	-	-	-	-	20 ± 2	16 ± 2	10 ± 3	-	-	-
E. conodies Simmons	16 ± 2	-	-	-	-	-	-	-	-	-	-	20 ± 4
Fusarium flocciferum Corda**	-	-	-	20 ± 3	-	-	-	-	-	-	-	-
Mucor circinelloides	-	-	16 ± 3	29 ± 4	-	-	-	-	-	-	-	-
M. racemosus Fres.	-	-	-	16 ± 3	-	-	-	-	-	-	-	-
Mycelia sterilia	16 ± 3	-	-	-	-	-	-	-	-	-	-	-
*Penicillium chrysogenum***	22 ± 2	30 ± 3	39 ± 4	46 ± 3	40 ± 2	32 ± 3	30 ± 2	53 ± 4	40 ± 4	35 ± 3	34 ± 4	29 ± 5

Table 3. (Contd.)

Fungi	No. of colonies per gram (± SD) of leaves/months											
	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Acrocomidiella arecae (Berk. & Br.) M.B. Ellis	-	-	-	18 / 3	-	-	-	-	-	-	-	-
P. notatum Westling	-	18 ± 4	-	-	-	-	-	-	-	-	-	-
P. verrucosum	-	-	19 ± 3	-	-	-	-	-	-	-	-	-
Phoma glomerata (Corda) Wollenw. & Hochapf.	-	-	-	29 ± 3	-	-	-	-	-	-	-	-
Rhizophus sp.	-	-	-	-	-	-	-	18 ± 2	-	-	-	-
R. stolonifer	-	23 ± 3	12 ± 2	23 ± 3	39 ± 4	30 ± 2	25 ± 2	26 ± 3	31 ± 3	18 ± 2	16 ± 2	-
Scolecobasidium variable Barron & Busch.	-	-	18 ± 2	25 ± 2	-	-	-	-	-	-	-	-
Syncephalestrum racemosum Cohn ex Schroet**	-	-	-	16 ± 2	23 ± 3	39 ± 3	-	-	-	-	-	-
Trichoderma harziamm Rifai	-	16 ± 2	22 ± 2	26 ± 3	24 ± 2	-	-	-	-	-	-	-
Ulocladium atrum	-	-	-	-	16 ± 3	-	-	-	-	-	-	-
U. chartarum (Preuss) Simmon	-	-	15 ± 2	-	-	-	-	-	-	-	-	-
U. chlamydosporum	-	-	-	23 ± 3	23 ± 3	25 ± 3	16 ± 2	-	-	-	-	-
U. tuberculatum	-	-	-	-	-	-	-	18 ± 3	-	-	-	-
Total No. of species 35	7	8	13	20	17	13	8	9	7	6	6	5
Total No. of genera 19	5	6	10	15	11	12	6	6	5	4	4	4

** Reported as human or animal pathogen.

To our knowledge this is the first record of surface mycoflora on leaves of wild medicinal plants from Saudi Arabia. Earlier works on leaves surface mycoflora were restricted to other economically important plants [15,18]. Most of the isolated fungi in this study were earlier recorded, from leaves surface [15], as plant pathogen [17] and also as inhabited mycoflora of household environment [18]. The following fungal species were not recorded earlier as leaves surface mycoflora from Saudi Arabia; *Acroconidiella arecae, Alternaria denissi, Capnophialophora, Coleophoma, Embellisia conoidea, Fusarium flocciferum* and *Scolicobasidum variable. Alternaria, Aspergillus, Drechslera* and *Ulocaldium* were the predominant genera found here in contrast to *Cladosporium* reported earlier [15].

The highest number of fungal species found in some months of the year may be due to increased glutation fluid excreted by the plants during those months which become the nutrient food for the inhabited fungi [19, p. 213] and the lowest number in some months may be attributed to the increased excretion of antifungal substances by the plants [20, p. 245]. These inhibitory substances may be monoterpenes or essential oils [21], diterpenes [22], and phenolic compounds [23, p. 343]. The fluctuation of mycoflora during different months also depends upon temperature, humidity and other environmental conditions [24]. In arid climate like Saudi Arabia, the velocity of wind are generally higher during winter when rainfall also occurs giving a favorable condition for a distribution and germination of spores [25], hence, the number of fungal species were found higher during the months of March, April and May.

The common molds like *Absidia, Alternaria, Aspergillus, Penicillium* which are usually saprophyte in nature but could cause infection in human and animal under favorable conditions [26]. Recently *Aspergillus,* a common and usually harmless mould found in most households was reported to be a big killer of patients with AIDS, those having organ transplant and others who had weakened immune system [27]. Since different parts of these medicinal plants are used in the folk medicine for the treatment of several diseases [1-4] including wounds. Therefore, it is recommended that a thorough washing of the plant specimen with sterilized water should be done prior to using any part of the plant. Since these fungi are mostly saprophytic they could be eradicated by thorough washing.

In addition, plant parts should be selected carefully to avoid these fungi because under favorable condition these fungi could penetrate deep into the plant and cause infection. Any infected parts should not be used or if necessary, should be used after boiling in water for 15-20 minutes to kill inside fungus but this boiling might alter the activity of effective components in the plant parts. So it will be better not to use the infected parts.

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الفطريات المصاحبة لأوراق *كالوتروبس بروسيرا* و *كاباريس سبينوزا*

حسن بخاري، صالح السحياني، قتيبه السعدون(*) وثروت برويز

قسم النبات والأحياء الدقيقة، كلية العلوم، جامعة الملك سعود، ص. ب ٢٤٥٥، الرياض ١١٤٥١

و (*) الهيئة الوطنية لحماية الحياة الفطرية وإنمائها، الرياض؛ المملكة العربية السعودية

(استلم للنشر في ١٥/ ١٠/ ١٤١٩هـ؛ وقبل للنشر في ٨/ ٦/ ١٤٢٠هـ)

ملخص البحث. تم القيام بمسح شهري من عام ١٩٨٩م إلى ١٩٩١م لدراسة تأثير الفصول على الفطريات قاطنة سطوح أوراق النباتات الطبية *كالوتروبس بروسيرا Calotropis procera* و *كاباريس سبينوزا Capparis spinosa*. كان العدد الكلي ٤٦ نوع تابعة لـ ٢١ جنس من الفطريات من نبات *C. procera*. و ٣٥ نوع تابعة لـ ١٩ جنس من الفطريات من نبات *C. spinosa*. تم الحصول على نسبة عالية من الفطريات أثناء شهر مايو على كلا النباتين بينما وجدت أقل نسبة في شهر سبتمبر في حالة *C.procera* وفي شهر يوليو في حالة *C. procera*. كان جنس *الترناريا Alternaria* (٧ أنواع) أكثر سيادة على أوراق نبات *C. procera* وجنس *يولوكلاديوم Ulocladium* (٤ أنواع) أكثر سيادة على أوراق نبات *C. spinosa* وتم عزل كل من *الترناريا التيرناتا Alternaria alternata*، *أسبرجيلس فلافس Aspergillus flavus*، *أسبرجيلس نيجر A. niger* و بنسيليوم كريزوجينم *Penicillium chrysogenum* في كل الفصول من على أوراق كلا النباتين.