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FUNGI ASSOCIATED WITH THE CRAB *Sesarma boulengeri* CALMAN FROM SHATT-AL-ARAB RIVER BASRAH -IRAQ

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Abstract

The fungal species isolated from crab (*Sesarma boulengeri* Calman) and from its environment (mud) with their colony count, frequency, and occurrence were illustrated. The results of cultured samples revealed that out of 50 cultivated crabs, 32 species belong to 15 genera of fungi imperfecti, 2 ascomycetes species, and one yeast like fungi, besides of unidentified yeasts and black, brown, yellow, and white sterile mycelia were isolated on both SDA and PDA. The occurrence ranged between 6-100% on crab samples, while from mud samples only 12 species of fungi imperfecti with several isolates of black, brown, and white mycelia sterilia were isolated The colony forming units (CFU)/ crab reached 8097 while those from mud were 5452/gm. *Aspergillus terreus* and *Fusarium sacchari* showed the higher colony count (1428 and 1376 colonies/ crab, respectively) while from mud samples, these species showed 134 and 1000 colonies/gm respectively. *Acremonium blochii* was isolated from mud only with colony count of 334 colonies/gm. The mostly frequent fungal species on crab was *A. terreus* (100%) followed by *F. sacchari* (96%) and *Curvularia senegalensis* (94%) with frequency of 17.64%, 17.00%, and 8.31%, respectively, while from mud, *F. sacchari* and *Acremonium potroni* were the most frequent species isolated (18.3%). The least occurred species were *Acremonium* sp., *A. strictum*, and *Scytalidium* sp. (6%) with the frequency of 0.09%, 0.09%, and 0.19%, respectively.

Introduction

The crabs *Sesarma boulengeri* Calman is generally regarded as an intertidal zonal species in southern Iraq. It was firstly collected at Basrah, 60 miles up the Euphrates, in perfectly fresh water from burrows in the banks of Shatt Al-Arab River and its canals (Calman, 1920). This species also extends into brackish water of Shatt Al-Arab and its branches from Basrah city to Fao (South of Basrah), in which the salinity increases to about 9% in the surface (Arndt and Al-Saadi, 1975).

After insects, fungi are the second largest group of organisms have unusually wide morphological diversity and many life strategies which explain their enormous importance in evolution, the ecosystem, human progress, and most of the progresses that take place on the Gaia i.e. the Earth considered as a whole, the atmosphere, the oceans, biota and lithosphere (Hawksworth, 1979). Animals are known to play an important role in the epidemiology of both animal and human mycoses. They can act as a reservoirs or vectors for these diseases (Aninsworth and Austwick, 1973; Rippon, 1982; Connole, 1990). Studies on the isolation of dermatophytes, keratinophilic and soil fungi from surface sediments of Shatt Al-Arab River (represents the aquatic habitat of the crabs) has been done by other workers (Abbas, 1993; Abdullah and Hassan, 1995; Al-Dossary *et al.*, 2004). This study was undertaken to investigate the fungal flora of the crustacean aquatic animal, *Sesarma boulengeri*, which may act as a reservoir for potentially pathogenic fungi for human and animals.

Materials and Methods:

Crabs:

One hundred crabs collected from three sites at Shatt Al-Arab River banks during October – November 2006 at high tide (to collect the naturally washed animals), placed in 500 ml sterilized bottles and brought to the laboratory of Biotechnology/ Department of Marine Biology/ Marine Science Centre / University of Basrah / Basrah – Iraq.

Culture:

Fifty adult crabs (males and females) weighed 8-12.5 gm were chosen for culture procedures which has been done as following:

A-Washing method:

Each five crabs were placed in 500 ml sterilized bottles, then 50 ml of sterilized tab water were added, shacked vigorously, then 50 ml of sterilized tap water were added again to obtain a dilution of fungal colony forming units per crab of 1:20, then 1 ml from each wash was transferred in to 25 ml of unsolidified potato dextrose agar (PDA) and sabauroud dextrose agar (SDA) with 250 mg/ L. Chloramphenicol (at about 45̊C) in 9 cm Petri dishes, shaked horizontally and left at room temperature until the

medium is solidified. Plates were transferred to a cooling incubator at 25˚C. and examined at two days intervals for the any growth of fungal species.

B-Crabs powder:

Crabs were killed by separating cephalothoraxes from the carapaces, then placed in sterilized plastic Petri dishes, dried in hot air oven at 40˚C for 48 hrs, then each dried animal was crushed in sterilized piston and mortar separately and 0.5 gm of the powder was mixed with unsolidified SDA and PDA with Chloramphenicol (at about 45˚ C), left to solidify at room temperature, then incubated under similar condition as before.

C-Sediment samples:

Six sediment samples were collected from three locations at Shatt Al-Arab River banks (from which crabs were collected) at low tide using scraper, placed in polyethylene bags and brought to the laboratory. From each sample 1 gm of mud was diluted in 99 ml of sterilized tap water to obtain a dilution of 1:100, then 1 ml of the diluted mud were transferred to 9 ml of sterilized tap water to give a dilution of 1:1000. From each dilution 1 ml was transferred to 9 cm Petri dishes to which 25 ml of unsolidified PDA and SDA with Chloramphenicol (at about 45˚ C) was poured and mixed gently then processed as the previous methods.

Total colony count of each species was counted and the isolated fungi were identified by the macroscopic and microscopic characteristics using the criteria of: Ellis, 1971; Ellis , 1976 ; Frey *et al.*,1979 ; Von Arx ,1981; Von Arx *et al.*,1986; de Hoog and Guaro, 1995; Klich, 2002.

Data analysis:

Number of isolates, occurrence and frequency for each crab was calculating as follows:

$$\text{No. of isolates / crab} = \text{No. of isolates in 0.5 gm powder} \times \frac{\text{Average dry weight of crabs (3.8gm)}}{0.5} + [\text{No. of isolates in 1 ml wash} \times \text{dilution (20)}]$$

$$\text{Occurrence \%} = \frac{\text{No. of + ve animals of fungal genera or species}}{\text{Total No. of animals (50)}} \times 100$$

$$\text{Frequency \%} = \frac{\text{No. of colonies for genus or species}}{\text{Total No. of colonies}} \times 100$$

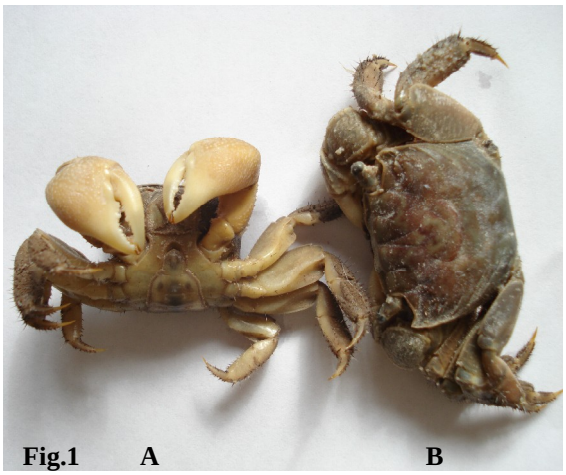
Results:

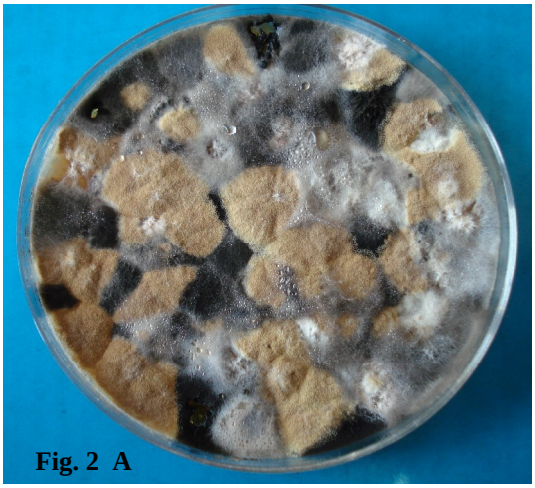
The fungal species isolated from crab (Fig.1:A& B) and from its mud with their colonies count, frequency, and occurrence are illustrated in Table (1-2).

The results of cultured samples revealed that out of 50 crabs cultivated, 32 species belong to 15 genera of fungi imperfecti, 2 ascomycetes species, and one yeast like fungi, besides of unidentified yeasts and black, brown, yellow, and white sterile mycelia were isolated on both SDA and PDA with the occurrence ranged from 6-100% on crab, while from mud only 12 species of fungi imperfecti with several isolates of black, brown, and white mycelia sterilia were cultivated.

The colony forming units (CFU)/ crab reached 8097 while those from mud were 5452/gm. *Aspergillus terreus* and *Fusarium sacchari* showed the highest colony count (1428 and 1376/ crab respectively) and from mud these species showed 134 and 1000 colonies/gm respectively. *Acremonium blochii* was isolated from mud only with colony count of 334 colonies/gm (Table 1).

Table (2) showed the occurrence and the frequency of the isolated fungi from crab and from mud where it burrows. The mostly frequent fungal species on crabs was *A. terreus* (100%) followed by *F. sacchari* (96%) and *Curvularia senegalensis* (94%) with frequency of 17.64%, 17.00, and 8.31%, respectively, while from mud, *F. sacchari* and *Acremonium potroni* were the most frequent species isolated (18.3%). The least occurred species were *Acremonium* sp., *A. strictum*, and *Scytalidium* sp. (6%) with the frequency of 0.09%, 0.09%, and 0.19%, respectively.





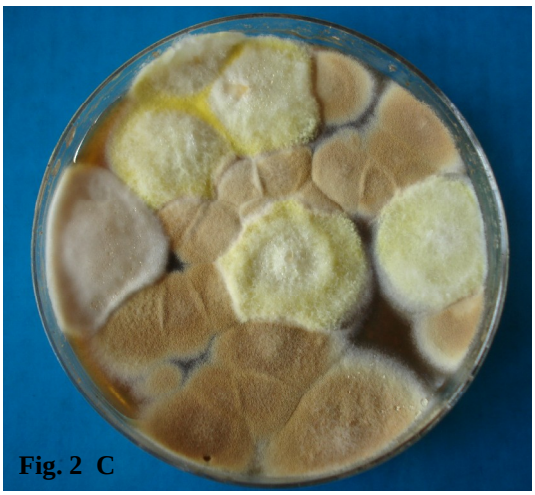


Fig. 1: Adult crab *Sesarma boulengeri* : A- ventral view B- dorsal view

Fig. 2: Fungal colonies from: A. Crab powder B. Crab wash C. Mud

Table (1): Fungal species isolated from the crab and its environment

No.	Fungal species	No. of isolates / crab			No. of + venimals /species	No. of isolates/1 gm mud
		Powder	Wash	Total		
1	*Acremonium blochii* (Matr.) W.Gams	-	-	-	-	334
2	*A. potroni* Vuill.	99	120	219	25	1000
3	*A. strictum* W.Gams	8	-	8	3	-
4	*Acremonium* sp.	8	-	8	3	-
5	*Alternaria alternate* (Fr.) Keissler	54	240	294	39	34
6	*A. chlamydospora* Mouchacca	8	-	8	4	-
7	*A. tennisima* (Kunze ex Pers.) Wiltshire	76	20	96	11	-
8	*Aspergillus alliaceous* Thom & Church	8	-	8	3	-
9	*A. candidus* Link ex Link	23	120	143	32	267
10	*A. flavus* Link ex Gray	137	140	277	40	-
11	*A. fumigatus* Fres.	16	20	36	10	-
12	*A. niger* Van Tieghem	31	120	151	19	334
13	*A. terreus* Thom	1368	60	1428	50	134
14	*Aspergillus* sp.	31	20	51	9	-
15	*Chaetomium* sp.	38	40	78	15	-
16	*Chrysosporum tropicum* Charmichael	8	20	28	6	-
17	*Cladosporium cladosporoides* (Fresen.) de Vires	130	140	270	36	-
18	*C. herbarum* (Pres.) Link ex Gray	137	40	177	33	134
19	*Curvularia clavata* Jain	114	240	354	20	-
20	*C. senegalensis* (Speg.) Subran.	213	460	673	47	300
21	*Curvularia* sp.	23	40	63	8	-
22	*Drechslera austeraliensis* (Bugnicourt) Subram. and Jain ex M.B. Ellis; Subram. and Jain	198	440	638	20	-
23	*D. papendorfi* (van der Aa) M.B. Ellis	168	300	468	26	367
24	*Derchslera* sp.	23	20	43	4	-
25	*Exerohilum rostratum* (Drechsler) Leorand and Suggs	23	60	88	5	-
26	*Fusarium sacchari* (Butler) W. Gams	16	1360	1376	48	1000
27	*Fusarium* sp.	31	160	191	7	434
28	*Geotrichum candidum* Link ex Leman	38	60	98	8	-
29	*Paecilomyces* sp.	16	40	56	6	-
30	*Penicillium* sp.	38	40	78	13	67
31	*Rhizopus* sp.	38	60	98	8	-
32	*Scytalidium lignicola* Pesante	38	20	58	4	-
33	*Scytalidium* sp.	16	-	16	3	-
34	*Ulocladium chartarum* (Preuss.) Simmous	46	20	66	15	-
35	*Wardomyces anomalus* Brooks & Hansf.	61	20	81	16	-
36	Ascomycetes sp.	8	-	8	5	-
37	Yeasts	137	40	177	21	-
38	Black Mycelia Sterilia	54	-	54	9	267
39	Brown Mycelia Sterilia	8	20	28	3	60
40	Yellow Mycelia Sterilia	16	-	16	3	-
41	White Mycelia Sterilia	69	20	89	12	720
	Total	3572	4520	8097	50	5452

Table (2): Occurrence and frequency of fungal species isolated from crab and mud

No.	Fungal species	Crab		mud
		Occurrence	Frequency	Frequency
1	*Acremonium blochii*	-	-	6.12
2	*A. potroni*	50	2.71	18.3
3	*A. strictum*	6	0.09	-
4	*Acremonium* sp.	6	0.09	-
5	*Alternaria alternata*	78	3.63	0.62
6	*A. chlamydospora*	8	0.09	-
7	*A. tennisima*	22	1.19	-
8	*Aspergillus alliaceous*	6	0.09	-
9	*A. candidus*	64	1.77	4.9
10	*A. flavus*	80	3.42	-
11	*A. fumigatus*	20	0.45	-
12	*A. niger*	38	1.86	6.12
13	*Aspergillus terreus*	100	17.64	2.5
14	*Aspergillus* sp.	18	0.63	-
15	*Chaetomium* sp.	30	0.96	-
16	*Chrysosporum tropicum*	12	0.35	-
17	*Cladosporium cladosporoides*	72	3.34	-
18	*C. herbarum*	66	2.19	2.5
19	*Curvilaria clavata*	40	4.37	-
20	*C. senegalensis*	94	8.31	5.5
21	*Curvilaria* sp.	16	0.78	-
22	*Drechslera austeraliensis*	40	7.88	-
23	*D. papendorfi*	52	5.78	6.7
24	*Derchslera* sp.	8	0.53	-
25	*Exerohilum rostratum*	10	1.09	-
26	*Fusarium sacchari*	96	17.00	18.3
27	*Fusarium* sp.	14	2.36	7.9
28	*Geotrichum candidum*	16	1.21	-
29	*Paecilomyces* sp.	12	0.69	-
30	*Penicillium* sp.	26	0.96	1.3
31	*Rhizopus* sp.	16	1.21	-
32	*Scytalidium lignicola*	8	0.72	-
33	*Scytalidium* sp.	6	0.19	-
34	*Ulocladium chartarum*	30	0.82	-
35	*Wardomyces anomalus*	32	1.00	-
36	Ascomycetes sp.	10	0.09	-
37	Yeasts	42	2.19	-
38	Black Mycelia Sterilia	18	0.67	4.9
39	Brown Mycelia Sterilia	6	0.35	1.1
40	Yellow Mycelia Sterilia	6	0.19	-
41	White Mycelia Sterilia	24	1.09	13.2

Discussion

The crabs *Sesarma boulengeri* Calman is an intertidal species of arthropods in Iraq, and its microbial association contents has not been studied. Therefore, this study is the first approach has been conducted for isolation and identification of mycoflora associated with the crab. Since crabs live in burrows in moist environment and are covered by a keratinized tissues and mucus that contains suitable carbon and nitrogen source that maintain fungal growth, moreover its limbs are covered by setae, these encourage the fungi to colonize its surface and may parasitize its body. However, by comparison of the density of the colony forming units (8097/ crab) and the diversity of fungal species isolated from crabs by those isolated from mud indicates that the crabs are good substrate for fungi and some of the isolated species were appeared from crab powder only or isolated in larger numbers of colonies than from its wash which indicates that these species either ingested by the animal or infect its structures or firmly adhered to its surface. Moreover, many of the species isolated were keratinophilic or opportunistic human or animals pathogens (Emmons, 1970; Frey *et al*.,1979; de Hoog and Gaurro,1995).

There is no any sign of infection on the crabs examined during this investigation and it may not have been infected, but serve as carriers of spores or other fungal elements picked up from the environment. The most frequent genera isolated from crab, in this work, were *Aspergillus, Fusarium, Drechsleria* and *Curvularia* and the mostly occurred species belong to these genera were, *A. terreus* (100%), *A. flavus* (80%), *C. senegalensis* (94%), *D. papendorfi* (52%), and *F. sacchari* (96%), while from the mud, *Fusarium* was the most frequent genus (26.2%) followed by *Aspergillus* (14.52%) and *Drechslera* (6.7%). Some species were found constantly and frequently associated with crab and have not been isolated from mud or isolated in low frequency (Table 1 & 2). Many of these fungi were previously isolated from Shatt Al-Arab River water and sediments by Abbas (1993) and from surface sediments of Shatt Al-Arab by Abdulla and Hassan (1995).

The occurrence of these opportunistic pathogenic fungi on the crab illustrates that this aquatic animal may act as a reservoir for potentially pathogenic fungi for human and animals, and creates a risk for infection, since it is used by the fisheries in catching fish, meantime, Shatt Al-Arab River itself is custimized by swimmers during Summer days.

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الفطريات المرافقة للسرطان النهري *Sesarma boulengeri* Calman من نهر شط العرب / البصرة- العراق

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الخلاصة

تم خلال هذه الدراسة عزل الفطريات المصاحبة بالسرطان النهري (أبو الجنيب) *Sesarma boulengeri* Calman ومن البيئة التي يعيش فيها، وكذلك تم حساب أعداد المستعمرات والتردد والظهور للفطريات المعزولة. حيث بينت نتائج زرع ٥٠ حيواناً، عزل ٣٢ نوعاً من الفطريات الخيطية الناقصة ونوعان من الفطريات الكيسية ونوع واحد من الفطريات الشبيه بالخمائر، إضافة إلى العديد من الخمائر غير المشخصة وكذلك الخيوط الفطرية العقيمة السوداء والبنية والصفراء والبيضاء اللون على الوسطين الزرعيين SDA و PDA، مع مدى ظهور تراوح ما بين ٦-١٠٠% على الحيوان الواحد، بينما من الطين تم عزل ١٢ نوعاً من الفطريات الناقصة مع العديد من المستعمرات السوداء والبنية والبيضاء للخيوط الفطرية العقيمة.

كما أظهرت الدراسة أن الحيوان الواحد أعطى ٨٠٩٧ وحدة مكونة للمستعمرات (CFU)، بينما من الطين كان عدد الوحدات المكونة للمستعمرات ٥٤٥٢ للغرام الواحد. كما أظهر الفطرين *Aspergillus terreus* و*Fusarium sacchari* أكبر عدداً من المستعمرات المعزولة من كل حيوان (١٤٢٨ و ١٣٧٦ على التوالي)، بينما من الطين أعطت هذه الأنواع ١٣٤ و ١٠٠٠ مستعمرة للغرام الواحد على التوالي، كما تم عزل الفطر *Acremonium blochii* من الطين فقط وبمعدل ٣٣٤ مستعمرة للغرام الواحد. كان أكثر الأنواع الفطرية ظهوراً على السرطان النهري هو الفطر *Aspergillus terreus* (١٠٠%)، تلاه الفطر *Fusarium sacchari* (٩٦%) و *Curvularia senegalensis* (٩٤%) بمعدل تردد ١٧,٦٤% و ١٧,٠٠ و ٨,٣١% على التوالي. بينما من الطين أظهر الفطرين *Fusarium sacchari* و *Acremonium potroni* أعلى تردد (١٨,٣%). كانت أقل الفطريات ظهوراً *Acremonium* sp. و *Acremonium stritcum* و *Scytalidium* sp. (٦%) مع تردد مقداره ٠,٠٩%و ٠,٠٩% و ٠,١٩% على التوالي.