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Pathogenicity of Fungi Colonizing Some Hard Corals and Invertebrates from the Northern Egyptian Red Sea Coast

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ABSTRACT

Fungi colonizing hard coral species collected from Hurghada, Red Sea were isolated and identified to the species level. A total of 47 fungal isolates (37 isolates from hard corals and 10 isolates from other invertebrates) were collected. Twelve of them are belonging to 4 genera; *Aspergillus, Penicillium, Nigrospora* and *Botrydiploida*. *Aspergillus* and *Penicillium* were represented by 5 species each whereas, *Nigrospora* and *Botrydiploida* were represented by one species each. Pathogenicity of 12 fungal species on *Galaxea fascicularis* and *Stylophora pistillata* corals revealed that that the degree of pathogenicity depend up on the fungal species, hard coral species and the duration of exposure to spores. Colonies of *G. fascicularis* were more susceptible to fungal infection than *S. pistillata* where they were infected in the first week by spores of four fungi compared to one fungal species for *S. pistillata*. *G. Fascicularis* was infected by *Aspergillus niger*, and *A. parasiticus* where black spots appeared on some parts of the colonies and the skeleton started to decay. In general *A. flavus, A. fumigatus, Botryoldiploida sp., P. crustosum*, and *P. echinulatum* had no effect on coral colonies.

INTRODUCTION

Fungal diseases can act as major limiters of natural and cultured populations of marine organisms. Mycopathogens of aquatic animals have become the focus of considerable attention because of the high occurrences of fungal diseases in wild populations and aquaculture (Polglase *et al.,* 1986; Noga, 1990). Most marine fungal infections, once established in an individual, are often fatal and difficult to treat. This indicates that these fungi will continue to be problematic pathogens of marine organisms (Noga, 1990). The phylum Cnidaria, comprised of an approximate 10,000 species (Zhang, 2011) has been the most widely studied with regard to fungal prevalence. While the presence of fungi in coral hosts is acknowledged in the literature (Bentis *et al.,* 2000; Golubic *et al.,* 2005).

A high diversity of bacteria, archaea, viruses, algae, protozoa and fungi comprise complex assemblage that, including the coral animal, has been termed the coral holobiont (Rohwer *et al*., 2002; Knowlton and Rohwer, 2003). These organisms have been found to differ from those in the adjacent water column and represent potentially co-evolved symbionts (Rosenberg *et al*., 2007).Although the association between corals and fungi (henceforth 'coral fungi') has been reported from a wide host, geographic and climatic range (Freiwald *et al*., 1997), very little is known about their identity or the nature of their interaction with the holobiont. Coral fungi were long believed to be parasitic to the coral itself (Kendrick *et al.*, 1982) or to endolithic algae within the coral skeleton (Priess *et al*., 2000).

The most important mitosporic fungal pathogens are *Fusarium* species (e.g. *F. solani*) which have been reported to be associated with shell disease of marine crustaceans (Lightner, 1988), and mycotic infections in hermit crabs (Smolowitz *et al.*, 1992) and lobsters (Stewart 1984). Other mitosporic fungal pathogens include an unnamed Scolecobasidium which causes infection among massive coral species from the Andaman Islands (Raghukumar and Raghukumar, 1991), *Ochroconis humicola* which causes ulcerative lesions in devil stinger (*Inimicus japonicus*) cultured in Japan (Wada *et al.,* 1995), and *Lasiodiplodia theobromae* which was isolated from an infection in a juvenile boring clam (*Tridacna crocea*) cultured in Australia (Norton *et al*., 1994).

Most marine fungal infections, once established in an individual, are often fatal and difficult to treat. This indicates that these fungi will continue to be problematic pathogens of marine animals (Noga, 1990). Therefore, the current study aimed to investigate the distribution and the most common marine-derived fungi from different hard coral species, Hurghada, Egypt with special emphasis on their pathogenicity.

MATRIALS AND METHODS

Sampling methods

Samples were collected from Hurghada on northern Red Sea coast and about 5 km away from Hurghada city centre in January 2016. The area is adjacent to the National Institute of Oceanography and Fisheries, Red Sea branch at latitudes of 27˚ 17' 13" N and longitudes of 33˚ 46' 43" E. SCUBA diving and snorkelling were used to collect hard corals. Samples were rinsed with sterilized distilled water and then kept in sterile plastic bags till they reach the laboratory. For the purpose of the isolation of marine fungi, 45 samples of which 15 healthy samples, 15 bleached and 15 dead corals were examined.

Isolation of fungi from hard coral

For isolation of fungi collected samples were disinfected with 70% ethanol for 30 seconds and then rinsed three times with distilled sterile water to remove any contamination (Toledo-Hernandez *et al*., 2008). Four tissue fragments were taken from each sample and planted on glucose peptones yeast agar GPYA (1.0 gl^{-1} glucose, 1.0 gl^{-1} yeast extract, 0.5 gl^{-1} peptone, 18 gl^{-1} agar, in 50% seawater and 50% distilled water that is a standard medium for isolating marine fungi. Chloramphenicol (0.25 gl^{-}

[1]) was added to the medium in order to inhibit possible bacterial growth. The samples were incubated for 7-15 days at 28ºC.

Identification of fungal isolates

Fungi were cultured on Czapek`s yeast extract agar and incubated at 28°C for 7 days (Pitt, 1979).). The growing fungal colonies were counted, isolated and stored in agar slopes for further investigation. Identification was done on the basis of macroscopic features of fungi covering growth rate, colony colour as well as production of surface exudates and pigmentation of colony reversed. Wet mounts of fungal strains were prepared in lactophenol cotton blue stain for microscopic examination. Hyphae, conidiophores and conidia were observed and measured using standardized ocular scale. Fungi were then identified commonly used universal keys by Pitt (1979), Moubasher (1993) and Domsch *et al.* (2007). Based up on the frequency of occurrence, Isolated species are classified as very frequent (> 20%) frequent (10-20%) and infrequent (<10%) as adopted by Tan and Leong (1989).

Identification of marine hard corals

Hard corals were identified using Sheppard (1991) and Veron (2000).

RESULTS

Distribution of fungi in different hard coral species

A total of 47 samples from the marine environment were used to isolate fungi of which 37 isolates were taken from hard corals and the other 10 isolates were obtained from other invertebrates. Among the hard coral species used in this study, *Stylophora pistillata* was the most frequently used (20 isolates) followed by, *Acropora tenius* (6 isolates), *Montipora* and *Porites* (4 and 3 isolates, respectively). One isolate was taken from each of *Favia*, *Acropora* sp., *Favites* and *Galaxea* sp.

Identification of coral fungi

The number of fungi varied among the coral species investigated (Fig.1). *Stylophora pistillata* had the highest number of fungal species with 11 species whereas *Favia* sp. had the lowest number of fungal species (2). Fungi were isolated from all of corals, healthy, bleached and dead. However, the occurrence of each fungi in coral species varied coral species *S. pistillata* and *Montipora* were represented in the three whereas *Porites* and *Acropora*were represented in two status *Porites* in healthy and bleached whereas *Acropora* in healthy and dead corals (Fig. 2). *Galaxea* and *Favia* were represented only by healthy status whereas; *Favites* was represented in bleached corals (Fig. 2). Twelve species belonging to four fungal genera were isolated from the different isolates. *Aspergillus* and *Penicillium* were represented by 5 species each whereas, *Nigrospora* and *Botrydiploida* were represented by only one species/ each. In general, species of *Aspergillus* dominated overall the other species and were isolated nearly from all hard coral species. On the other hand species of *Penicillium* were infrequently isolated. The most frequently isolated species were *A. parasiticus* (55% of all isolates) followed by *A. fumigatus* (53% of all isolates) and *A. flavus* (42% of all isolates). The least frequently species were *Botrydiplodia theobroomae* (2%) that was isolated in one case from *S. pistillata* and, *Nigrospora oryzae* that were isolated twice from *Acropora tenius* and *S. pistillata*) (Fig. 3).

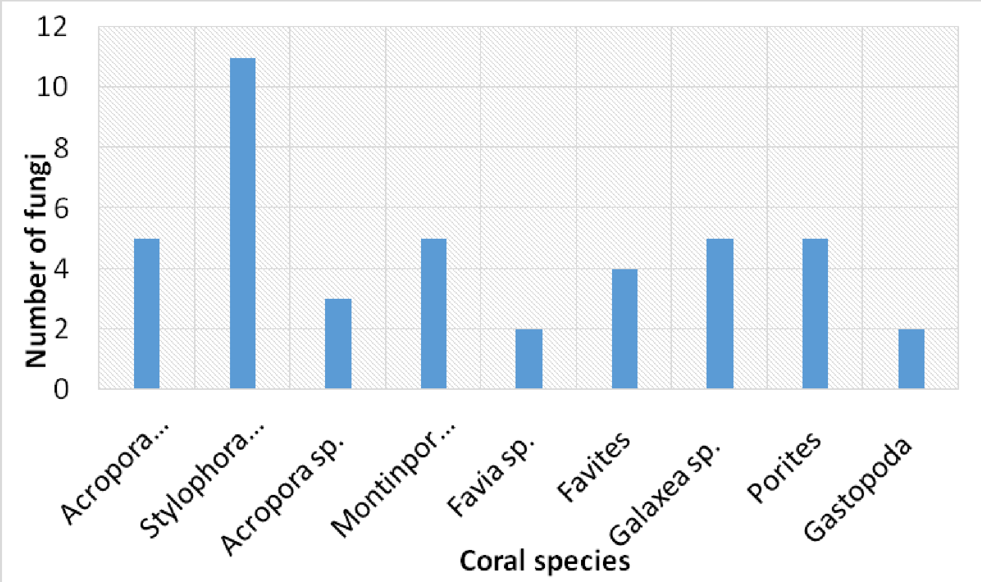


Fig. (1): Number of fungi isolated from each coral species

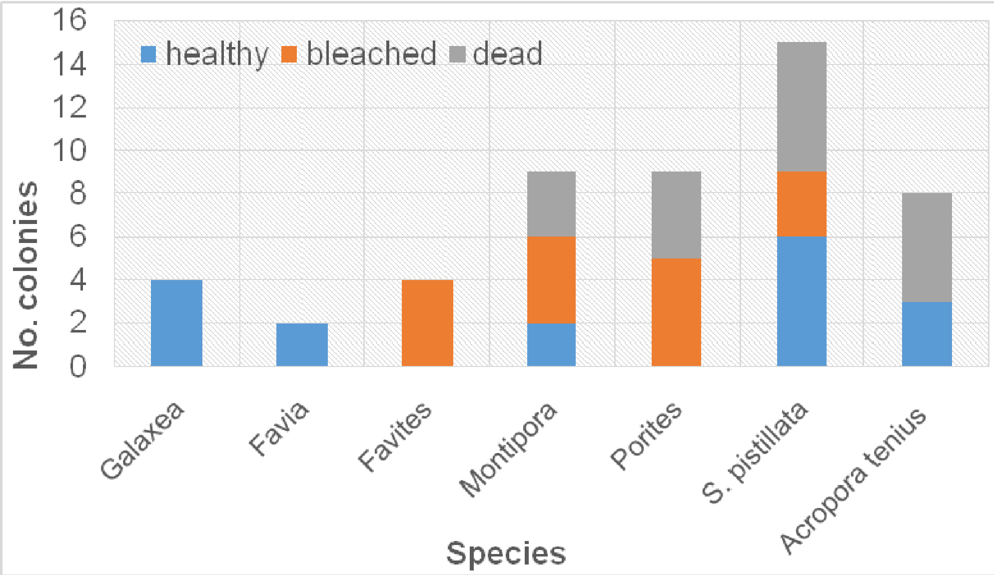


Fig. (2): Number of fungi isolated from different status of coral species

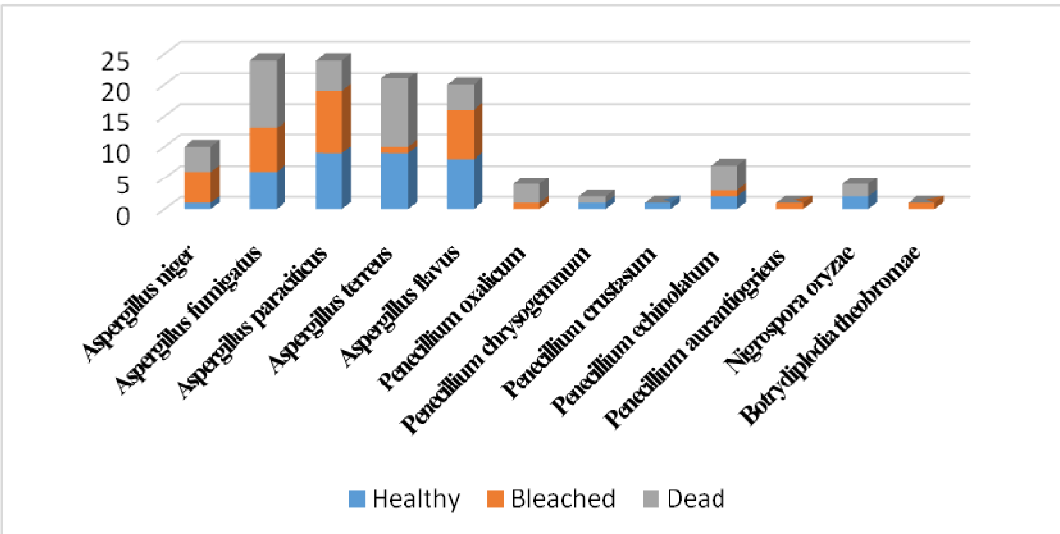


Fig. (3): Percentage of each fungal species in healthy, bleached and dead hard corals

***Aspergillus parasiticus* Speare**

This species was the most frequently recorded among all fungal species. *Aspergillus parasiticus* was isolated from 26 isolates out of 47 isolates (55%) of all isolates. It was most frequently isolated from bleached corals where 42% was taken from bleached corals compared to 37% from healthy corals and 21% from dead corals (Fig. 3). *Aspergillus parasiticus* were found in all coral species but *Galaxea, Favia*, and *Porites*. It was also absent from sponge and other invertebrates.

***Aspergillus fumigatus* Fresenius**

Aspergillus fumigates was the second most frequently isolated species where it was taken from 25 isolates forming 53% of all isolates. It was isolated from all coral species but *Galaxea* and *Favia*. It was frequent and abundant in dead corals with 46%, 25% in healthy corals and 29% in bleached corals (Fig. 3).

Aspergillus flavus Link

This species was the third most frequently recorded species among all fungal species. It was isolated from 20 isolates constituting 42% of all isolates. It was recorded in all coral species except *Favites* and the dead *Acropora tenius*. *A. flavus* along with *A. fumigatus* were the only species to be isolated from gastropod specimen. *A. falvus* was completely absent from sponge and other invertebrate organisms. The species was mostly recorded from healthy and bleached corals and rare in dead corals (Fig. 3).

***Aspergillus niger* Van Tieghem**

Aspergillus niger was isolated from only 7 isolates constituting about 15% of all isolates. It was common in *Stylophora pistillata* and Montipora but was absent in *Acropora* spp. *Favia* and *Favites*. It was completely absent from sponge and other invertebrate animals. The species was mostly recorded from bleached corals with 50% and dead corals (40%) and rare in healthy corals (10%) (Fig.3).

***Aspergillus terreus* Thom**

Aspergillus terreus occurred in 15 isolates and constituted 32% of all isolates. It was recorded from all coral species but was absent from soft corals, sponges and other invertebrate species. The species was mostly recorded from dead coral (52%) and healthy coral (43%) while it was rare in bleached corals (5%) (Fig. 3).

***Penicillium aurantiogriseum* Dierckx**

This species was one of the least isolated ones as it was recorded only from one isolate. It was taken from bleached *Stylophora pistillata* (Fig. 3).

Penicillium echinulatum

This species was the second most abundant Penicillium species. However, it was very rare where it was isolated only 4 times. It was highly abundant in healthy corals (50%) and less abundant in dead and bleached corals with equal abundance (25%). This species was isolated only from *Stylophora pistillata, Favites* sp. and *Montipora* sp. (Fig. 3).

***Penicillium chrysogenum* Thom**

As other *Pencillium* species, this species was rare in the present survey. It was isolated from healthy *Stylophora pistillata* and *Porites*. This means that the species have equal chances to be present in healthy and dead corals. It was absent from bleached colonies of corals. It was also absent from soft coral, sponges and other invertebrate species (Fig. 3).

***Penicillium oxalicum* Currie & Thom**

Penicillium oxalicum was the most abundant among all Penicillium species. It was isolated from 4 isolates mainly from *Acropora tenius* and *Stylophora pistillata.* This species dominated the dead colonies of hard corals and was less abundant in bleached corals. Whereas, it was totally absent from healthy corals. As all other *Penicillium* species, they are absent from soft corals and other invertebrates (Fig. 3).

***Penicillium crustosum* Thom**

One of the rare species was *P. crustosum* which was taken from one isolate, healthy *Acroporatenius* (Fig. 3).

***Botryodiplodia theobromae* Patouillard**

This species was very rare as it was isolated from one isolate, bleached *Stylophora pistillata.* It occurred in only 2% of all isolates (Fig. 3).

***Nigrospora oryzae* (Berkeley and Broome) Petch**

Nigrospora oryzae was one of the least encountered species during the present survey. It was taken from two isolates only, healthy *Acropora tenius* and healthy *S. pistillata* (Fig. 3).

Pathogenicity of isolated fungi

Pathogenicity test carried out using 12 fungal species on *Galaxea fascicularis* and *Stylophora pistillata* showed that the influence was dependent on: species of fungi, species of hard coral and duration of exposure to the spores. In general, *A. flavus*, *A. fumigatus*, *Botryoldiploida sp.*, *P. crustosum*, and *P. echinulatum* had no effect on the coral colonies. Colonies of *Galaxea fascicularis* were more susceptible to infection by fungal spores than those of *Stylophora*, they were infected during the first week of treatment by spores of four fungi compared with one fungal species for *S. pistillata*. *G. Fascicularis* was infected by *Aspergillus niger*, and *A. Parasiticus* where black spots appeared on some parts of the colonies and the skeleton started to decay (Table 1).

Table (1): Distribution of marine derived-fungi in different hard coral species

Isolate code	Host	Status	Fungi
Isolate1	*Galaxea fascicularis*	Healthy	*Aspergillus niger, Aspergillus terreus, Aspergillus flavus, Penicillium echinulatum*
Isolate 2	*Montipora sp.*	Bleached	*Aspergillus niger, Aspergillus parasiticus, Aspergillus flavus, Aspergillus fumigatus*
Isolate 3	*Acropora tenius*	Healthy	*Aspergillus* terreus, *Nigrosporaoryzae*
Isolate 4	*Porites sp.*	Bleached	*Aspergillus niger, Aspergillus parasiticus, Aspergillus flavus, Aspergillus fumigatus*
Isolate 5	*Favites sp.*	Healthy	*Aspergillus parasiticus, Penicillium echinulatum, Aspergillus fumigatus, Aspergillus terreus*
Isolate 6	*Stylophora pistillata*	Healthy	*Penicillium chrysogenum, Aspergillus parasiticus, Aspergillus flavus, Aspergillus fumigatus, Aspergillus terreus*
Isolate 7	*Montipora sp.*	Bleached	*Aspergillus parasiticus, Aspergillus flavus*
Isolate 8	*Stylophora pistillata*	Bleached	*Aspergillus parasiticus, Aspergillus niger,Botryodiplodia theobromae, Penicillium aurantiogriseum*
Isolate 9	*Favia sp.*	Healthy	*Aspergillus terreus, Aspergillus flavus*
Isolate 10	*Montipora sp.*	Bleached	*Aspergillus parasiticus, Aspergillus terreus,Penicillium echinulatum*
Isolate 11	*Stylophora pistillata*	Bleached	Penicillium oxalicum, *Aspergillus parasiticus, Aspergillus* niger
Isolate 12	*Porites sp.*	Dead	*Aspergillus niger, Aspergillus flavus,Aspergillus fumigatus, Penicillium chrysogenum*
Isolate 13	*Acropora tenius*	Healthy	*Aspergillus parasiticus, Aspergillus terreus*
Isolate 14	*Acropora tenius*	Healthy	*Aspergillus parasiticus*

Cont. Table (1): Distribution of marine derived-fungi in different hard coral species

Isolate code	Host	Status	Fungi
Isolate 16	*Stylophora pistillata*	Dead	*Aspergillus parasiticus, Aspergillus fumigatus, Aspergillus flavus*
Isolate 17	*Stylophora pistillata*	Healthy	*Aspergillus parasiticus, Nigrosporaoryzae, Aspergillus flavus, Aspergillus fumigatus*
Isolate 18	*Stylophora pistillata*	Healthy	*Aspergillus flavus, Aspergillus parasiticus*
Isolate 19	*Stylophora pistillata*	Dead	*Aspergillus parasiticus, Aspergillus terreus*
Isolate 20	*Stylophora pistillata*	Healthy	*Aspergillus flavus, Aspergillus fumigatus, Aspergillus terreus*
Isolate 21	*Acropora tenius*	Healthy	*Aspergillus parasiticus, Aspergillus terreus, Penicillium crustosum*
Isolate 22	*Porites sp.*	Bleached	*Aspergillus fumigatus, Aspergillus parasiticus, Aspergillus flavus*
Isolate 23	*Montipora sp.*	Healthy	*Aspergillus fumigatus, Aspergillus parasiticus*
Isolate 24	*Acropora tenius*	Dead	*Aspergillus fumigatus, Aspergillus parasiticus, Penicillium oxalicum*
Isolate 25	*Stylophora pistillata*	Dead	*Aspergillus fumigatus, Aspergillus parasiticus, Aspergillus terreus*
Isolate 26	*Stylophora pistillata*	Dead	*Aspergillus fumigatus, Aspergillus terreus, Penicillium oxalicum*
Isolate 27	*Acropora tenius*	Dead	*Aspergillus fumigatus, Aspergillus terreus, Aspergillus flavus*
Isolate 28	*Acropora sp.*	Healthy	*Aspergillus fumigatus, Aspergillus terreus, Aspergillus flavus*
Isolate 29	*Stylophora pistillata*	Dead	*Aspergillus fumigatus, Aspergillus parasiticus*
Isolate 30	*Stylophora pistillata*	Dead	*Aspergillus fumigatus, Aspergillus parasiticus, Penicillium echinulatum*
Isolate 31	*Stylophora pistillata*	Dead	*Aspergillus fumigatus, Aspergillus parasiticus, Aspergillus flavus*
Isolate 32	*Stylophora pistillata*	Bleached	*Aspergillus fumigatus, Aspergillus flavus*
Isolate 33	*Stylophora pistillata*	Bleached	*Aspergillus fumigatus, Aspergillus parasiticus, Aspergillus flavus*
Isolate 34	*Stylophora pistillata*	Bleached	*Aspergillus fumigatus, Aspergillus parasiticus, Aspergillus flavus*
Isolate 37	*Stylophora pistillata*	Bleached	*Aspergillus parasiticus, Aspergillus flavus, Aspergillus niger*

The growth of *Nigrospora oryze* formed Black spots and caused the decay of the skeleton whereas, *Penicillium chrysogenum* had no black spots but only growth of fungi that caused the decay of the skeleton of Galaxea *fascicularis* (Table 2).

Table (2): Number of spores in the spore solution of fungal species

Fungal species	Spore count in 1 ml	Spore count in 100 ml
Aspergillus niger	$14.5X10^{6}$	$14.5X10^{8}$
Aspergillus flavus	$15.5X10^{6}$	$15.5X10^{8}$
Aspergillus terreus	$14.6X10^{6}$	$14.6X10^{8}$
Aspergillus fumigatus	$14X10^{6}$	$14X10^{8}$
Aspergillus parasiticus	$14.5X10^{6}$	$14.5X10^{8}$
Nigrosporaoryze	$23X10^{6}$	$23X10^{8}$
Botryodiplodiatheobromae	$110.4X10^{6}$	$110.4X10^{8}$
Penicillium aurantiogriseum	$85.7X10^{6}$	$85.7X10^{8}$
Penicillium chrysogenum	$58X10^{6}$	$58X10^{8}$
Penicillium echinulatum	$45.5X10^{6}$	$45.5X10^{8}$
Penicillium oxalicum	$51.5X10^{6}$	$51.5X10^{8}$
Penicillium crustosum	$53.5X10^{6}$	$53.5X10^{8}$

On the other hand, *S. pistillata* was infected only by *A. Parasiticus* that caused a partial bleaching on some parts of the colony that leads to decay of the skeleton. In the second week, more three fungi started to infect the coral; *A. terreus, Penicillium aurantiogriseum* and *P. oxalicum* causing partial bleaching of both hard coral species. By the end of the third week, hard coral with black spots died and those with partial bleaching converted to complete bleaching (Table 3) and (Fig. 4).

Table (3): Pathogenicity test of fungal species on hard coral species

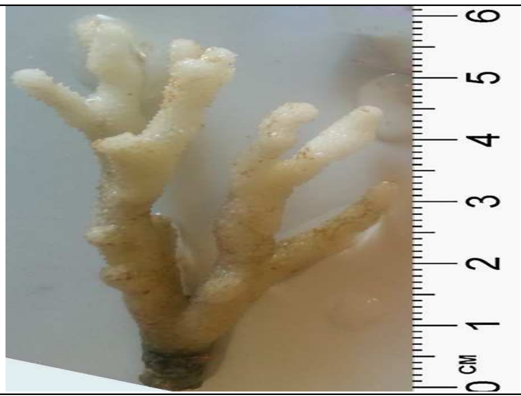
Tank No.	Fungal species	Hard coral species	Symptoms		
			After 7days	**After 14 days**	**after 21 days**
1	*A. niger*	*Galaxea fascicularis*	Black spots on some parts of the colony lead to decay of the skeleton	Black spots and partial bleaching	Death
		Stylophora pistilata	-----------	Partial Bleaching	Complete bleaching
2	*A. flavus*	*Galaxea fascicularis*	-----------	-----------	-----------
		Stylophora pistilata	-----------	-----------	-----------
3	*A. terreus*	*Galaxea fascicularis*	-----------	Partial Bleaching	Complete bleaching
		Stylophora pistilata	-----------	Partial Bleaching	Complete bleaching
4	*A. fumigatus*	*Galaxea fascicularis*	-----------	-----------	-----------
		Stylophora pistilata	-----------	-----------	-----------
5	*A. parasiticus*	*Galaxea fascicularis*	Black spots on some parts of the colony lead to decay of the skeleton	Black spots and bleaching on some parts	death
		Stylophora pistilata	Bleaching on some parts of the colony lead to decay of the skeleton	Partial Bleaching spreads to more parts	Complete bleaching
6	*Nigrosporaoryze*	*Galaxea fascicularis*	Black spots and fungal growth lead to decay of the skeleton	Black spots and bleaching	Death
		Stylophora pistilata	----------	Bleaching on some parts	Complete bleaching
7	*Botryodiplodia theobromae*	*Galaxea fascicularis*	-----------	-----------	-----------
		Stylophora pistilata	-----------	-----------	-----------
8	*P. aurantiogriseum*	*Galaxea fascicularis*	-----------	Bleaching on some parts	Complete bleaching
		Stylophora pistilata	-----------	Bleaching on some parts	Complete bleaching
9	*P. chrysogenum*	*Galaxea fascicularis*	Fungal growth lead to decay of skeleton	Fungal growth lead to decay of the skeleton and bleaching on other parts	Death
		Stylophora pistilata	-----------	Partial Bleaching	Complete bleaching
10	*P. echinolatum*	*Galaxea fascicularis*	-----------	----------	-----------
		Stylophora pistilata	-----------	----------	-----------
11	*P. oxalicum*	*Galaxea fascicularis*	-----------	Partial Bleaching	Complete bleaching
		Stylophora pistilata	-----------	Partial bleaching	Complete bleaching
12	*P. crustosum*	*Galaxea fascicularis*	------------	-----------	-----------
		Stylophora pistilata	-----------	------------	-----------

Stylophora **sp. before injection**

Galaxea **sp. before injection**

Stylophora **sp. after 7 days of injection with** ***A. niger***

Galaxea **sp. after 7 days of injection of** ***A. niger***



Stylophora **sp. after 14 days of injection with** ***A. niger***

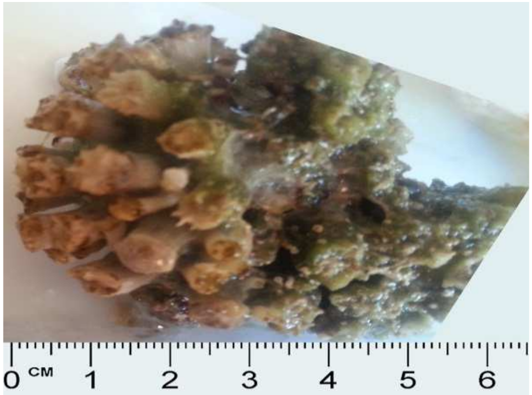
Galaxea **sp. after 7 days of injection of** ***N. oryze***

Stylophora **sp. after 21 days of injection with** ***A. parasiticus***

Galaxea **sp. after 7 days of injection of** ***P. chrysogenum***

Fig. (4): Features of pathogenicity caused by fungal species on selected hard coral species

Galaxea* sp. after 14 days of injection of *P. aurantiogriseum



***Galaxea* sp. after 14 days of injection of *P. chrysogenum* 14 days**

Galaxea* sp. after 7 days of injection of *A. terreus

Cont. Fig. (4): Features of pathogenicity caused by fungal species on selected hard coral species

DISCUSSION

A major environmental problem in the ocean is the alarming increase in diseases affecting diverse marine organisms including corals. Environmental factors such as the rising seawater temperatures and terrestrial microbial input to the ocean have contributed to the increase in diseased organisms (Barrero-Canosa *et al.*, 2013).

Corals, hexacorals, black corals, and octocorals are the frameworks of the most important marine ecosystems in the Red Sea due to the high diversity, three dimensional structures, and associated fauna and flora. Anthropogenic activities cause degradation to corals along the Red Sea coast. Global climate change also has contributed to a decline of marine ecosystems greater than any seen in the last millennium (McCallum *et al*., 2003; Harvell *et al*., 2007; 2009). Most studies performed on coral-associated fungi have focused on either parasitic or opportunistic interactions. Even though the majority of these describe either the fungal species associated with coral or potential detrimental outcomes of fungal presence within the animals, the importance of another key form of interactions – mutualism, has yet to be probed in detail, even though support for this notion has been clearly discussed (Wegley *et al*., 2007). The occurrence and severity of marine diseases have dramatically increased during the last two decades (Altizer *et al*., 2003; Harvell *et al*., 2009). Emerging coral diseases could be caused by environmental conditions, including high UV light, increasing water temperature, changes in nutrient availability, pollution, ocean acidification, and changes in salinity (Rosenberg and Ben-Haim 2002). For a clear understanding on marine diseases, information on host range, in conjunction with information on the environmental drivers leading to disease, is urgently needed to comprehend the emerging appearance of diseases worldwide (Harvell *et al*., 2004; 2007).

This work aimed to study the prevalence of fungi in hard coral species, sea cucumbers, gastropods and sediments from the Northern Red Sea. Twelve species of fungi from nine hard coral species were obtained. The isolated fungi are belonging to four genera; *Apsergillus, Penicillium, Nigrospora* and *Botrydiplodia.*

Fungi of the genus *Aspergillus* were isolated from coral colonies in all status (bleached, healthy and dead) but in different percentages,and they were collected also from gastropods. Five species were identified as *A. flavus, A. fumigatus, A. terreus*, *A. parasiticus*, and *A. niger*. *Aspergillus parasiticus* was the most frequently isolated (55%) followed by *A. fumigatus* that was isolated from 53% of colonies. It is worthy to mention that *Aspergillus fumigatus* and *A. terreus* were more abundant in the dead colonies compared with bleached and healthy corals, whereas, *A. niger* and *A. parasiticus* were abundant in bleached colonies (42%)compared with 37% from healthy corals and 21% from dead corals. *Aspergillus flavus* had almost similar abundance both in healthy and bleached colonies but was found less abundance in dead colonies. Barrero-Canosa *et al*. (2013) isolated *A. flavus* and *A. sclerotum* from diseased colonies of *P. eximia*, and *A. terreus* and *A. fumigatus*from healthy ones.

On the other hand, fungi under the genus *Penicillium* were isolated from healthy, bleached and dead coral colonies. They were taken from healthy colonies of *Galaxea fascularis, Favites*, and *Stylophora pistillata* and from bleached *Montipora* and *S. pistillata*in addition to dead *Porites*, *S. pistillata*, and *Acropora* tenius.

Of the four species that belong to *Penicillium, P. oxalicum* was the most abundant where it was isolated from 7 colonies, it was found mainly in the dead colonies (75%) of *Acropora tenius*, *S. parasiticus* and *S. pistillata* and bleached colonies (25%) of *S. pistillata*.

Many diseases of fish and shellfish have also been observed in studies at monitoring sites in the oceans around the world. The impact of these diseases on population sizes in marine ecosystems in general is poorly understood. Less information is available on identity, diversity and ecological roles of fungi in coral disease progress. Most reports on coral diseases (e.g. Antonius, 1981; Goldberg and Makemson, 1981; Rutzler *et al*. 1983; Goldberg *et al*., 1984) do not include fungi among coral pathogens. It is assumed that their effect on the host is negligible, or that the fungi in corals are many saprophytes that exploit dead organic matter incorporated in coral skeletons by the coral or produced by endolithic algae and cyanobacteria (Kendrick *et al*., 1982).

A successful isolation and culturing of higher, ascomycotic and basidiomycotic fungi from the skeletons of Atlantic and Pacific hermatypic corals (Kendrick et al., 1982) allowed the first identification of fungal genera and species that were likely candidates for the known and widespread coral borers. Kendrick et al. (1982) cultured 20 fungal taxa isolated from coral reefs, documenting the presence and viability of fungal propagules in this marine environment. A similar approach was achieved by Raghukumar and Raghukumar (1991), who reported fungi associated with coral necrosis. Both studies covered only distribution of fungi inside coral skeleton but did not establish their pathogenicity.

Bak and Laane (1987) were the first authors reporting dark discoloration and banding inside coral skeleton caused by fungi, they also called attention to a possible active interaction between corals and fungi.

A coral reef disease with characteristic purple blotches on several species of sea fans was found to be caused by *Aspergillus sydowii*, a member of a large group of terrestrial fungi that also trigger mold allergies and other infections in humans (Geisner *et al*., 1998).

According to a study by El- Hady *et al*. (2015), the fungus *Aspergillus unguis* RSPG-204 associated with the marine Sponge (Agelas sp., Red Sea, Egypt) was investigated. The supernatant and mycelial extracts from static culture supported a previous one by Rypien *et al.* (2008) had the highest free radical scavenging activity against superoxide anion radical. The fungus Aspergillus unguis RSPG-204 2ry metabolites showed significant acetyl cholinesterase and high α-glucosidase inhibition, beside its high antioxidant activities. For the first time it was evidenced that these secondary metabolites *in vivo* studies could play an important role as acetyl cholinesterase and α-glucosidase inhibitors, besides their antioxidant activities.

In general, most studies conducted on fungi in corals focused on parasitic or opportunistic interactions. Even though, the majority of these describe either the fungal species or potential detrimental outcomes of the interaction (Wegley *et al*., 2007).

Rypien *et al*., (2008) examined whether sea fans are locally adopted to pathogens by asking whether the geographically varying isolated of *A. sydowii* induced differential response in sea fans hosts from a single location. Arumugam *et al*. (2015) successfully isolated a piezotolerant fungus *Nigrospora* sp. from deep sea environment and cultured it under submerged fermentation.

The tested colonies of *Galaxea* and *Stylophora* were subjected to 12 species of fungi. The results of this injection started to appear in a week where Galaxea showed signs of sickness in the first week by *Aspergillus niger*, *A. parasiticus* and *Nigrospora* sp. whereas, *A. parasiticus* started to infect *Stylophora pistillata* in the first week with signs of bleaching. *P. chrysogenum* start to form fungal growth on *Galaxea* sp. in the first week whereas *P. aurantiogriseum*, and *P. oxalicum* start their infecting activity in the second week.The test of pathogenicity carried out during this study may be the first on the hard corals of the Red Sea. More studies are urgently needed on the coral diseases and impacts on the survival and mortality of coral reefs in the Red Sea as one of the important sources on national income.

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ARABIC SUMMARY

النشاط الإمراضى للفطريات المعزولة من المرجانيات الصلبة واللافقاريات بالغردقة على الساحل المصرى الشمالى للبحر الأحمر

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تم عزل الفطريات من بعض الشعاب المرجانية الصلبة من منطقة الغردقة على الساحل المصرى للبحر الأحمر حيث تم أخذ ٤٧ عزلة منها ٣٧ عزلة من المرجان الصلب و١٠ عزلات من اللافقاريات الأخرى.

أسفرت عملية العزل عن فصل ١٢ من نوع من الفطريات تنتمى لأربعة أجناس هى أسبرجيلاس وبينيسيليوم ونيجروسبورا و بوترديبلويدا. احتوى كل من أسبرجيلاس وبينيسيليوم على خمسة أنواع من الفطريات بينما مثل كل من نيجروسبورا و بوترديبلويدا بنوع واحد فقط من الفطريات. وجد أن جنس أسبرجيلاس يسود جميع الفطريات التى تم عزلها حيث وجدت فى جميع أنواع الشعاب الصلبة. من ناحية أخرى وجد أن الجنس الآخر وهو بينيسيليوم اقل ظهوراً حيث تم عزله من عدد قليل من العزلات.

أوضحت دراسة خاصية الإمراض أن الإصابة ترتبط بثلاثة عوامل هى نوع الشعاب المرجانية ونوع الفطر وطول فترة التعرض للجراثيم. وبصفة عامة ظهر أن بعض أنواع الشعاب لم تتأثر بالفطريات خلال فترة التجربة والتى شارفت على الثلاثة أسابيع فى حين أن بعض الأنواع تأثرت بسرعة كبيرة وظهرت عليها أعراض الاصابة خلال الأسبوع الأول من التعرض للفطر. كما ظهر أن بعض الفطريات لم تظهر أية تأثيرات مرضية على الشعاب المرجانية الصلبة وهى اسبرجيلاس فلافوس واسبرجيلاس فوميجاتوس وبوتريديبلويدا ثيوبروم وبينيسيليوم كرستوسوم وبينيسيليوم إكينولاتوم. اتضح ايضاً أن المرجان الصلب جالاكسيا فاسيكولاريس كان أكثر وأسرع تأثرا بالفطريات من المرجان الصلب ستايلوفورا بيتسلاتا حيث بدأت أعراض الإصابة فى الظهور خلال الأسبوع الأول حيث تأثر النوع الأول بأربعة أنواع من الفطريات بينما تأثر النوع الثانى بنوع واحد من الفطريات. خلال الأسبوع الأول يتسبب اسبرجلاس فى بقع سوداء على المستعمرة ويبدأ الهيكل فى التحلل. بينما يتسبب بينيسيليوم فى ظهور نمو فطرى يؤدى أيضاً إلى تحلل الهيكل.