



## Assessment of Thermal, Salinity and Heavy Metal Tolerance of *Avicennia marina* Associated Endophytic and Soil Fungi and Evaluation of their Hydrolytic Activity



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**S**TRESS conditions in many unique habitats represent a selecting force for the biological forms found in these niches. Organisms in such habitats cope up with these unfavorable conditions and modify their life machinery to maintain a successful life. Mangrove ecosystem is mainly characterized by high salinity beside the high affinity of its sediments to retain heavy metals. In a trial to explore the microbial life found in such unique ecosystems, the endophytic and soil fungi associated with *Avicennia marina* growing on Red sea coast at Ras-Mohammad area in Egypt have been studied. Such studies not only play a role in understanding how a successful life is maintained in these niches, but also helps in exploring microbial enzymes with promising characters needed potentially in many industrial applications. It has been found that, the investigated isolates are mesophiles with a remarkable halotolerant behavior and a variable tolerance behavior for  $Mn^{++}$ ,  $Zn^{++}$ ,  $Fe^{++}$  and  $Cu^{++}$ . Soil isolates have recorded halotolerance behavior higher than that of endophytic isolates. One endophytic isolate, *Chaetomium globosum*, showed low removal capacity for  $Cu^{++}$  from the broth medium, however its growth was slightly inhibited in presence of this metal (100ppm). Also, a soil isolate, *Aspergillus flavipes*, has recorded enhanced growth in presence of  $Fe^{++}$  (100ppm) although no removal capacity has been detected. That reflects their possible ability to produce extracellular enzymes retaining considerable stability and activity under the presence of these heavy metals. *Aspergillus flavipes* showed a promising hydrolytic profile and the endophytic isolates showed acceptable profiles.

**Keywords:** *Avicennia marina*, Endophytic fungi, Hydrolytic activities, Soil fungi, Tolerance behavior.

### Introduction

There is a great interest in finding life in extreme environments. Microorganisms have received significant interest in this field as indicators of biological activities in such habitats. Moreover, an understanding of the functioning of microorganisms in extreme environments could also provide a wealth of new enzymes and other metabolites which could be exploited biotechnologically (Kubicek & Druzhinina, 2007). One of such unique environments is the mangrove ecosystems.

Mangrove is a halophyte plant that generally confined to the tropical and subtropical regions (Bayen, 2012; Sabri et al., 2018). Mangroves can adapt to extreme and shifting environmental conditions including; salinity, water tide, extreme wind, pH, temperature and nutrients (Saravanakumar et al., 2018). On the other hand, mangrove ecosystem may efficiently retain metals (Bayen, 2012). The biotic components associated with mangroves rapidly cope up with these unfavorable and harsh environmental conditions (Kulkarni et al., 2018) making these sites a reservoir for promising microbial species which can adapt to these shifting and harsh

conditions (Rigonato et al., 2018). Eventually many biological forms found in mangrove ecosystems have been utilized as sources of different biotechnologically important products (Kulkarni et al., 2018). For example; among the great biodiversity of mangrove associated fungi, many isolates were found to produce enzyme with better physiological characteristics in relation to temperature, pressure, pH, and salinity of the medium. Many studies showed that, most fungi isolated from mangrove ecosystem are able to produce enzymes such as cellulase, xylanase, and laccase in media prepared with seawater (Thatoi et al., 2013).

There are many mangrove genera in the world, however mangrove ecosystems are dominated by four: *Rhizophora*, *Bruguiera*, *Sonneratia*, and *Avicennia* (Veetil & Quang, 2019). In Egypt there are two mangrove species, *Avicennia marina* and *Rhizophora mucronate*. *Avicennia marina* grows and predominates along the whole stretch of the Red Sea Coast in two monospecific stands, one at Ras Mohammed national park and the second in Nabq protected area (El-Hussieny & Ismail, 2017). *A. marina* is relatively more tolerant and better adapted to salinity, low rainfall and extreme temperature conditions than *R.* (Sabri et al., 2018), thus it has been selected for this study.

Plants in nature are closely associate with a diverse community of microorganisms termed as plant microbiota (Hardoim et al., 2015; Müller et al., 2016). Plants harbor these microorganisms both inside and outside their tissues, in the endosphere and ectosphere (Vandenkoornhuysen et al., 2015). Endophytes represent one of the most important elements in plant microecosystem. They are colonizing the endophytic compartment of plants without causing apparent harmful effect to their hosts (Hardoim et al., 2015; Jia et al., 2016). Endophytic fungi are one of unexplored and less studied microorganisms (Devaraju & Satish, 2010). Some endophytic fungi have been reported to confer stress tolerance to their host plants, such as heat and salt stress tolerance, and play a significant role in the survival of some plants in high-stress environments (Rodriguez et al., 2008).

On the other hand, soil fungi rank as the most abundant among soil inhibiting microorganisms in terms of biomass and physiological activity. Soil fungi are of interest to ecologists and to

applied researchers because of their importance in decomposition, carbon and nitrogen storage, biogeochemical cycles and soil stabilization (Bills et al., 2004).

Thus this study aims to discover the enzymatic activity and tolerance behavior of endophytic and soil fungi found in the mangrove ecosystem which represent one of the unique ecological niches in the world. Exploring of the stress tolerance behavior and hydrolytic capacity of such isolates can be exploited in the production of economically and biotechnologically significant enzymes which can be employed under the harsh industrial conditions.

## Materials and Methods

### Isolation

#### Investigated plant

Mangrove plant investigated in this study is *Avicennia marina* (Forssk.) Vierh. growing on Red sea coast at Ras-Mohammad - South Sinai - Egypt (27°44'30" N, 34°14'05" E) (Fig. 1).



Fig. 1. *Avicennia marina* in Ras-Mohammad - South Sinai - Egypt

#### Microorganisms

**Endophytes:** Endophytic fungi were isolated from *Avicennia marina* leaf and flower samples by the method described by Garyalis et al. (2013).

**Soil isolates:** Soil fungi were isolated from *Avicennia marina* soil sediment by soil dilution plate method (Waksman, 1922; Kumar et al., 2015).

#### Identification of fungal isolates

The fungal isolates were identified depending on their morphological and microscopical features with the aid of the identification keys provide by

the following references (Gilman, 1957; Raper & Fennell, 1977; Barnett & Barry, 1987). Identification of endophytic isolates was confirmed by the help of Mycological Center, Assiut-University, Egypt also by using the morphological methods.

#### *Evaluation of growth at different temperature values*

Growth temperature range of the purified isolates was estimated by culturing them on Sabouraud agar medium (Glucose; 40g/L, peptone; 10g/L, agar; 20g/L, pH= 6.8-7.0 (Sabouraud, 1892) and incubating them at different temperature values (i.e., 10, 15, 25, 30, 35 and 40°C) for 7 days. The plates were inoculated with 8 mm fungal plug taken from freshly growing cultures (7 days old). The growth was evaluated by measuring the diameter of the developed fungal colony (mm).

#### *Estimation of salinity stress tolerance*

Tolerance of isolates to salinity was screened by growing them on Sabouraud agar medium supplied with different NaCl concentrations (i.e., 0, 2, 6, 10, 14 and 18%). The plates were inoculated as the previous method and incubated at 25°C for 7 days. The growth was evaluated by measuring the diameter of the developed fungal colony (mm).

#### *Estimation of heavy metal stress tolerance*

The isolated fungi were screened for Cu<sup>++</sup>, Fe<sup>++</sup>, Ni<sup>++</sup>, Mn<sup>++</sup> and Zn<sup>++</sup> tolerance by growing them on Sabouraud agar medium supplemented with 1mM of each metal (individually). The stock solutions for these metals were prepared in concentration of 500 mM using their sulphate salts. These stock solutions were sterilized by membrane filtration method using 0.2µm pore-size Millipore membrane filters (Advantec®). In this test, the glassware was washed with 20% HCl to remove the residues of heavy metal which possible to stick on them. The plates were inoculated and incubated as the previous method. The growth was evaluated by measuring the diameter of the developed fungal colony (mm). Metal tolerance was evaluated by calculating the tolerance index of the tested isolates and monitoring its values during the incubation period (from the 4<sup>th</sup> to 7<sup>th</sup> day).

Tolerance index (TI)= Growth of treated colony/ Growth of untreated colony. High Tolerance index (TI) values reflect good adaptive/ tolerance behavior against a particular heavy metal and TI value which equals or greater than one indicates absolute resistance for this metal at the

tested concentration (Chen et al., 2017; Rose & Devi, 2018).

#### *Detection of heavy metal removal capacity and biomass inhibition percent*

The isolate which showed the best Tolerance index for each metal was tested for the removal capacity of such heavy metal from the broth medium. The metal removal capacity was evaluated by inoculating the tested isolate on MGYB broth medium (Malt extract; 3g/L, Yeast extract; 3g/L, Peptone; 5g/L, Glucose; 10g/L: pH= 6.2 (Wickerham, 1951) supplied with the heavy metal in concentration of 100mg/L (for Cu<sup>++</sup>, Fe<sup>++</sup>, Ni<sup>++</sup>) or 500mg/L (for Mn<sup>++</sup> and Zn<sup>++</sup>). Two fungal discs (8mm diameter) were inoculated to 250ml conical flasks containing 50ml of culture medium and incubated at 25°C for 7 days under static conditions. Un-inoculated medium supplied with the heavy metal in the prementioned concentrations was used as control. Also, MGYB flasks without heavy metal addition was inoculated and incubated under the same conditions and used as a control for calculating the biomass inhibition percent. The growing mycelium was separated from the culture medium by filtration using Whatman filter paper no.1. The growth was evaluated by measuring the mycelium dry weight. Mycelium dry weight was estimated by drying the mycelium at 60°C until fixed weight is obtained. The residual heavy metal concentration in the culture filtrate was detected using Microwave Plasma Atomic Emission spectrometer (Agilent® 4210 MP-AES). Tests were performed in duplicated manner and three reads for each sample were taken in case of Microwave Plasma Atomic Emission spectrometer measurement. Metal removal percent was calculated using the following equation (Gururajan & Belur, 2018):

$$\% \text{Removal} = [(C_0 - C) / C_0] * 100$$

where: C<sub>0</sub>= Initial concentration of metal (control); C= Final concentration of metal after fungal growth.

Growth inhibition percent was calculated using the following equation (Nogmaithem et al., 2016):

$$\text{PI} = [(X - Y) / X] * 100$$

where: PI= The percentage of inhibition; X= Biomass in control (zero metal concentration); Y= Biomass in metal containing flask.

### *Evaluation of the basic enzymatic profile of isolates*

Plate assay method was employed to detect the enzymatic activity of the tested isolates by culturing them on the corresponding medium for 6 days at 25°C and testing the hydrolysis of the substrate by the suitable method as following:

#### *Amylase activity*

Screening for amylase production was performed by using modified Czapek-Dox medium supplied with 1% soluble starch as the sole carbon source (Soluble starch; 10g/L, NaNO<sub>3</sub>; 3g/L, KH<sub>2</sub>PO<sub>4</sub>; 1g/L, MgSO<sub>4</sub>·7H<sub>2</sub>O; 0.5g/L, KCl; 0.5g/L, FeSO<sub>4</sub>·7H<sub>2</sub>O; 0.01g/L, Agar; 15g/L). Amylase activity was detected as clear zone formation after flooding the plates with 1% w/v iodine. ((Balkan et al., 2012)).

#### *Cellulase activity*

Cellulose degradation ability was tested by culturing the fungal isolates on CMC agar medium (Carboxymethylcellulose; 10g/L, KH<sub>2</sub>PO<sub>4</sub>; 4g/L, Na<sub>2</sub>HPO<sub>4</sub>; 4g/L, Tryptone; 2g/L, MgSO<sub>4</sub>·7H<sub>2</sub>O; 0.2g/L, CaCl<sub>2</sub>·2H<sub>2</sub>O; 0.001g/L, FeSO<sub>4</sub>·7H<sub>2</sub>O; 0.004g/L, Agar; 15g/L: pH 7.0 (Behera et al., 2017). The medium was supplemented with traces of Rose Bengal as indicator for cellulose decomposition. Cellulose decomposition was detected by observing clear zone formation round the growth (Makeshkumar & Mahalingam, 2011). The visualization of the clear zone can be enhanced by re-incubation of the plates on 50°C for 4-8hrs.

#### *Protease activity*

Proteolytic activity of isolates was demonstrated using gelatin- agar medium described by Ammar et al. (1991) (Gelatin; 10g/L, agar; 20g/L: dissolved by boiling in 0.2M phosphate buffer pH 7.5). Gelatin hydrolysis was detected by clear zone formation after flooding the plates with acidic mercuric chloride solution prepared as described by Cowan (1974) (HgCl<sub>2</sub>; 12ml, Conc. HCl; 16ml, dis. H<sub>2</sub>O; 80ml).

#### *General esterase activity (preliminary lipase screening)*

Fungal isolates were tested for the general esterase activity by testing Tween 20 degradation ability using the following medium (Peptone; 10g/L, NaCl; 5g/L, CaCl<sub>2</sub>·2H<sub>2</sub>O; 0.1g/L, Agar; 20g/L: pH= 6.0, after autoclaving and cooling to 60°C sterilized cooled Tween-20 (10ml/L)

was added and mixed well (Sierra, 1957). Tween 20 degradation by esterases can be detected by observing the formation of visible precipitate due to the formation of calcium laurate salt as a result of the liberation of lauric acid as a product of Tween 20 degradation (Hankin & Anagnostakis, 1975).

#### *Lipase activity*

Screening for true lipase activity is carried out on Rhodamine B-olive oil medium (Nutrient broth; 8g/L, NaCl; 4g/L, Agar; 10g/L: pH= 7.0, after autoclaving and cooling to 60°C sterilized olive oil was added (31.25ml/L) and finally Rhodamine-B solution (0.01% w/v) sterilized by membrane sterilization was added in concentration of 10ml/L and mixed well (Kouker & Jaeger, 1987). Lipase production was detected by exposing the plates to UV light at 350nm, where the fungal colonies that have lipolytic activity show orange fluorescent halo as a result of the interaction of Rhodamine B with the released fatty acids (Savitha et al., 2007).

#### *Statistical analyses*

Standard error for the mean values was estimated. The obtained data was analyzed with Analysis of Variance (ANOVA) and means were compared using Tukey's Honestly Significant Difference test at 95 percent confidence interval. Statistical analysis was carried out using Sigma plot software version 12.5.

## **Results**

### *Isolation and identification*

Two endophytic fungal isolates have been isolated from *Avicennia marina* leaf and flower samples; *Pochonia suclosporia* (from leaf samples) and *Chaetomium globosum* (from flower samples). Additionally, two soil fungal isolates have been isolated from the soil sediment of *Avicennia marina*; *Aspergillus flavipes* and *Aspergillus niger*.

### *Evaluation of growth at different temperature values*

The growth of isolates has been evaluated in the temperature range 10-40°C. All isolates showed similar growth response pattern giving the best growth at 30°C (Fig. 2). However, one soil isolate (*Aspergillus niger*) showed a higher tolerance to relatively elevated temperature where it recorded a growth at 40°C. In contrast, one endophytic

isolate (*Chaetomium globosum*) showed ability to grow at low temperature (10°C).

#### Estimation of salinity stress tolerance

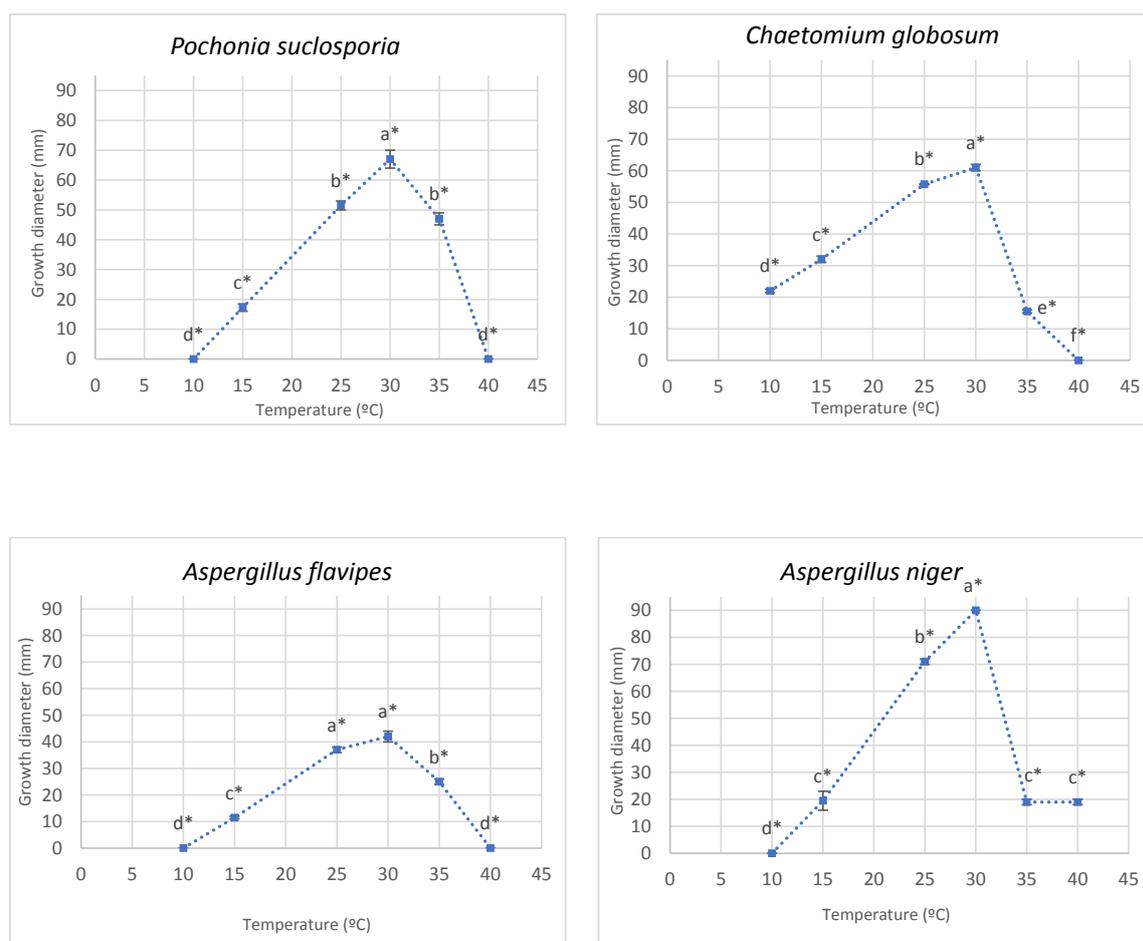
Tolerance of isolates to salinity stress (halotolerance) has been estimated by growing them at different NaCl concentrations up to 18%. All isolates showed remarkable halotolerance behavior (Fig. 3). Their growth didn't affected by NaCl addition in concentration of 2%. *Avicennia marina* associated soil isolates have recorded higher halotolerant behavior than that recorded for the endophytic isolates. Growth of endophytes was inhibited above 10% salt concentration but soil isolates have recorded a growth up to 18% concentration.

#### Estimation of heavy metal stress tolerance

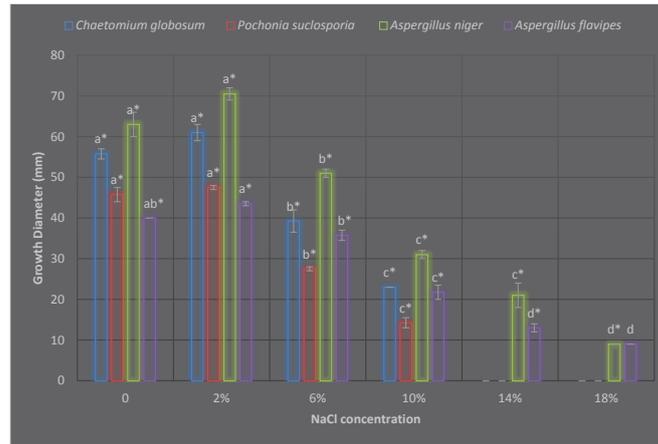
Tolerance of *Avicennia marina* associated endophytic and soil fungi to Fe<sup>++</sup>, Cu<sup>++</sup>, Mn<sup>++</sup>, Zn<sup>++</sup> and Ni<sup>++</sup> stress was estimated by culturing

them on Sabouraud agar medium supplied with these metals in 1mM concentration. Tolerance Index was calculated during the incubation period (from the 4<sup>th</sup> to the 7<sup>th</sup> day).

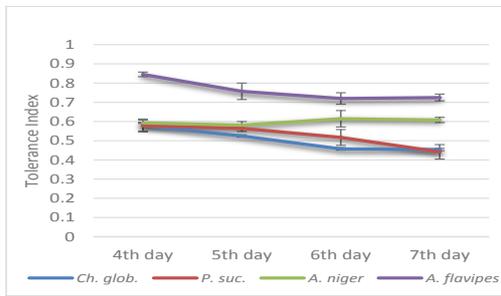
These isolates showed a variable tolerance behavior for the tested metals (Fig. 4). One soil isolate, *A. flavipes*, has recorded the best tolerance for Mn<sup>++</sup>, Zn<sup>++</sup> and Fe<sup>++</sup>. It showed a resistance behavior for Mn<sup>++</sup> and Zn<sup>++</sup> (where the recorded TI values were around 1) and a relative adaptive behavior for Fe<sup>++</sup> (where the recorded TI values ranged between 0.8- 0.7 during the investigated period). In contrast, this isolate showed the lowest adaptive behavior for Cu<sup>++</sup> and the best tolerance for this metal was recorded by *Chaetomium globosum* and *A. niger*. On the other hand, it has been found that the investigated *Avicennia marina* associated endophytic and soil fungi are highly sensitive/susceptible for Ni<sup>++</sup> stress.



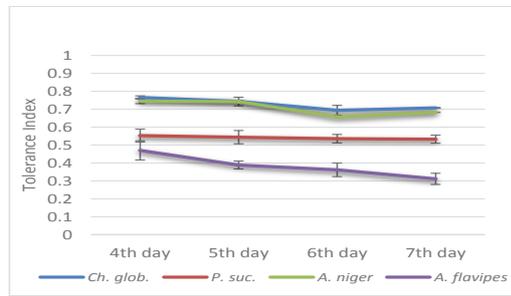
**Fig. 2.** Growth temperature range for endophytic and soil fungal isolates of *Avicennia marina* [\*: Means with different letters have significant differences between each other at P< 0.05; ANOVA test]



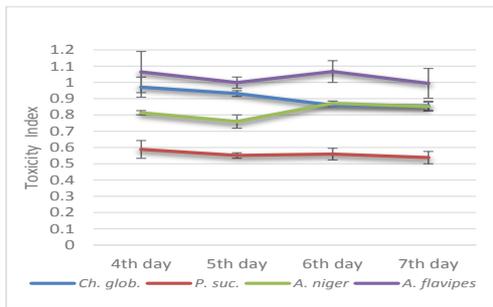
**Fig. 3. Effect of salinity stress on microbial growth [\*: Means with different letters have significant differences between each other at P< 0.05; ANOVA test]**



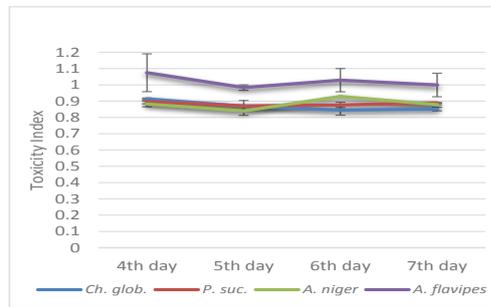
(a) Tolerance to Fe<sup>++</sup> stress



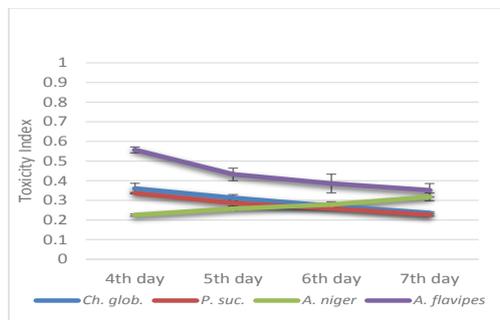
(b) Tolerance to Cu<sup>++</sup> stress



(c) Tolerance to Zn<sup>++</sup> stress



(d) Tolerance to Mn<sup>++</sup> stress



(e) Tolerance to Ni<sup>++</sup> stress

**Fig. 4. Tolerance to heavy metals stress (1mM concentration).**

### Detection of heavy metal removal capacity and biomass inhibition percent

The isolate which showed the best Tolerance Index for each metal was tested for the removal capacity of such heavy metal from the broth medium. *Aspergillus flavipes* was tested for removal of  $Mn^{++}$ ,  $Zn^{++}$ ,  $Fe^{++}$  and  $Ni^{++}$  and *Chaetomium globosum* was tested for removal of  $Cu^{++}$ . The isolates showed low removal capacity for the tested metals (Table 1). The growth of *A. flavipes* was greatly inhibited by  $Ni^{++}$  followed by  $Mn^{++}$  and less inhibited by  $Zn^{++}$ . In contrast the growth of this isolate is enhanced in presence of  $Fe^{++}$ . Growth of *Chaetomium globosum* is slightly inhibited by  $Cu^{++}$ .

### Evaluation of the basic enzymatic profile of isolates

Amylase, cellulase, protease, esterase and lipase activities of the isolated *Avicennia marina* endophytic and soil fungi were assayed to evaluate their hydrolytic behavior. One soil isolate, *Aspergillus flavipes*, showed promising profile for the tested hydrolytic enzymes. It showed strong proteolytic activity, good esterase, lipolytic and amylolytic activities and acceptable cellulolytic activity. The another soil isolate, *Aspergillus niger*, has recorded promising lipolytic activity. Endophytic isolates showed acceptable hydrolytic profile (Table 2, Fig. 5).

TABLE 1. Metal removal capacity by *A. flavipes* and *Chaetomium globosum*.

Isolate	<i>Aspergillus flavipes</i>				<i>Chaetomium globosum</i>
	Mn <sup>++</sup> (500ppm)	Zn <sup>++</sup> (500ppm)	Fe <sup>++</sup> (100ppm)	Ni <sup>++</sup> (100ppm)	Cu <sup>++</sup> (100ppm)
% Removal	13.9 ±0.4	11.9 ±4	0 ±0	1.2 ±0.2	29.3 ±1.7
% Inhibition	64.2 ±8.8	30.7 ±9	-36.5 ±10 <sup>#1</sup>	90.4 ±1	16.7 ±5.7

<sup>#1</sup>:The growth was induced in presence of metal .

TABLE 2. Enzymatic profile for *Avicennia marina* associated endophytic and soil fungi.

Isolate/enzyme		Amylase	Cellulase	Protease	Esterase	Lipase
Endophytic	<i>Pochonia suclosporia</i>	±	++	+	++	+++
	<i>Chaetomium globosum</i>	+	+	++	++	±
Soil	<i>Aspergillus flavipes</i>	++	+	++++	+++	++
	<i>Aspergillus niger</i>	+	±	+	-	++++

Activity; -: Not detected, ±: Very low, +: Low moderate, ++: Moderate, +++: High and ++++: Very high

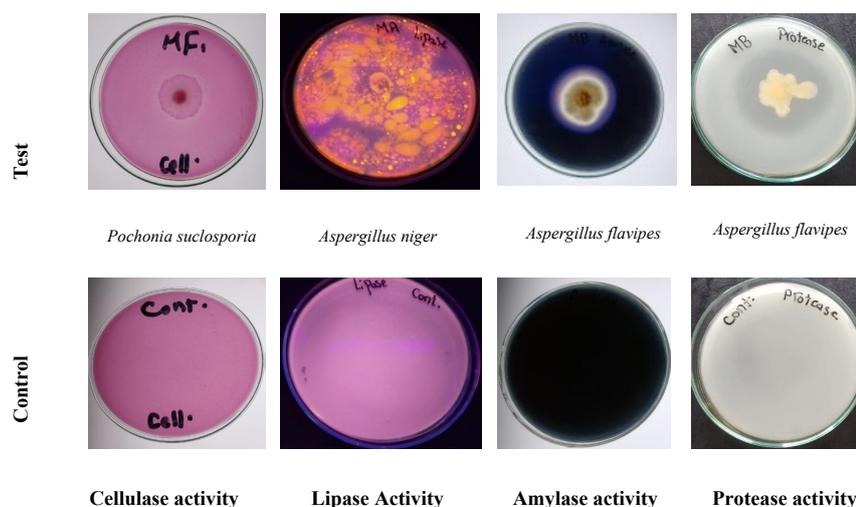


Fig. 5. Hydrolytic activities of some selected isolates

## Discussion

In a trail to explore the tolerance behavior and biological activities of microorganisms inhabiting unique habitats, i.e. mangrove ecosystems, endophytic and soil fungi associated with *Avicennia marina* growing on Red sea coast at Ras-Mohammad area have been investigated. Such studies not only play a role in understanding how the life machinery is operated under stress conditions, but also helps in finding promising sources of biotechnologically and industrially significant metabolic products.

Monitoring of growth temperature range of these isolates showed that, they are mesophiles. Their growth has been detected in 10:40°C range and the maximum growth was obtained at 30°C. The growth of all isolates are highly reduced above 35°C and completely inhibited at 40°C except one isolate, *A. niger*, which recoded a sparse growth at 40°C. The behavior of these isolates resembles that reported for the majority of fungi; where the majority of fungi are mesophiles growing at temperature range of 5-35°C with optimum temperature for growth between 25 and 30°C (Dix & Webster, 1995).

Although there is no significant thermal tolerance have been reported, all isolates have recorded a remarkable halotolerant behavior.

The investigated mangrove plant, *Avicennia marina*, grows on Red sea coast at Ras-Mohammad area. The mean salinity of seawater along the Red Sea coast of Egypt is >4‰ and this value is higher than the salinity in Southeast Asia where there is more rain (Matsuo et al., 2016). Thus, the probability for finding salt-adapted microorganisms in this niche is quite high.

Non-halotolerant/Non-halophilic organism can tolerate only a small concentration of salt (about 1% w/v). The microorganism can be considered as halotolerant if becomes able to survive at high salt concentrations, higher than those required for growth, but do not require these conditions for growth (Larsen, 1986; Anton, 2014).

Accordingly, the isolated fungal species can be considered as halotolerant isolates rather than halophiles where they can tolerate the elevated NaCl concentrations but the best growth was

maintained in absence of salt. Addition of NaCl in concentration of 2‰ did not inhibited the growth at all, moreover all isolates have tolerated the increasing NaCl concentration up to 10‰. Soil isolates, *A. niger* and *A. flavipes*, showed more halotolerance behavior where they also have recorded a growth in 14‰ and 18‰ concentration.

*A. flavipes* isolates can adapt to reduced water activity conditions and can tolerate relatively high concentrations of osmotically active solutes. *A. flavipes* was isolated from natural habitats with high NaCl concentration such as salterns, brackish water and coastal sand of dead sea (Arzanlou et al., 2016).

Salt-adapted organisms can successfully survive in dehydrated conditions and that increases their potential in many biotechnological applications (Musa et al., 2018). Normally, under elevated salt conditions the cell loses water from the cytoplasmic fluid and undergo plasmolysis (Gunde-Cimerman et al., 2005; Gostinčar et al., 2011). Also, high salinity causes ion toxicity and nutrient deficiency which can lead to molecular damage, growth arrest, and even death of organisms (Liang et al., 2007). Nonetheless, salt-adapted microorganisms evolve adaptive mechanisms enabling them to survive in such elevated salt conditions. One of such mechanisms is the accumulation of inorganic salts (e.g. sodium, chlorine and potassium ions) or other small molecular weight organic solutes (compatible solutes) in cytoplasm to balance the osmotic pressure as much as possible. Other adaptive behaviors can be maintained by developing salt stable membranes and cell surface or by modifying the physiology of the cell (Vreeland, 1987; Gostinčar et al., 2011; Musa et al., 2018).

Regarding to heavy metals tolerance, the investigated isolates showed variable tolerance behavior. The best tolerance was recorded for Mn<sup>++</sup> and Zn<sup>++</sup> followed by Fe<sup>++</sup> and Cu<sup>++</sup>. All isolates were highly sensitive to Ni<sup>++</sup>. The soil isolate; *A. flavipes* has recorded a resistance behavior for Mn<sup>++</sup> and Zn<sup>++</sup>; where the recorded TI value was around 1, also it recorded the best adaptive behavior for Fe<sup>++</sup>. *Chaetomium globosum* and *A. niger* showed the best tolerance for Cu<sup>++</sup>.

Heavy metals represent a highly abundant group of toxic compounds in the environment (Torres-Cruz et al., 2018), thus they are continuing

to be of great concern in the environmental arena (Gururajan & Belur, 2018). Fungi can resist heavy metals' stress using various mechanisms, i.e. production of metal chelation compounds (Shivakumar et al., 2014), creating vacuoles for gathering and immobilization of metal ions in the form of phosphates and production of specific metal binding compounds in the cell. Moreover, active transport of metallic ions outside the cell may contribute to heavy metal resistance by fungi (Akhtar et al., 2013).

According to the TI results, *A. flavipes* was selected for the demonstration of the removal capacity for  $Mn^{++}$ ,  $Zn^{++}$ ,  $Fe^{++}$  and  $Ni^{++}$  and *Chaetomium globosum* was tested to evaluate  $Cu^{++}$  removal capacity from the broth media as they have recorded the best tolerance for these metals. *A. flavipes* showed low removal capacity for the tested metals, but its growth was interestingly enhanced in presence of  $Fe^{++}$  (100ppm). Also, *Chaetomium globosum* showed low removal capacity  $Cu^{++}$  but the growth is slightly inhibited by it.

As indicated from the previous test, there was no sharp decrease in heavy metal concentration as a result of fungal growth, especially for  $Fe^{++}$  in case of *A. flavipes* and  $Cu^{++}$  in case of *Chaetomium globosum*. That reflects the possible ability of these isolates to produce extracellular enzymes which retain a considerable stability and activity under the stress of such metals. On the other hand, as all the investigated isolates showed halotolerant behavior, they are also more likely to produce salt-stabilized enzymes working under low water activity to assist their survival under high salinity conditions. Therefore, it was an essential step to assess their extracellular hydrolytic activities to evaluate their possible use as a potential source of industrially and biotechnologically promising enzymes.

All the investigated *Avicennia marina* associated fungal isolates showed acceptable hydrolytic activities. *Aspergillus flavipes* isolate, which recorded good halotolerant behavior and enhanced growth in presence of iron, showed a promising extracellular enzymatic profile. It recorded promising proteolytic activity and good esterase, lipolytic and amylolytic activity beside acceptable cellulolytic activity. As a result, this isolate can be recommended as a promising source for many industrially and biotechnologically significant hydrolytic enzymes.

Generally there is an increasing demand for fungal extracellular enzymes that are able to work under harsh conditions. Such enzymes are of great interest in industrial field which requires enzymes showing a higher tolerance and stability in the presence of inhibitors and metal ions present massively in industrial processes (Mtibaa et al., 2017).

Anilkumar & Pradeep (2017) have isolated five halotolerant fungal isolates from mangrove sites in Kerala which are potent protease producers (Anilkumar & Pradeep, 2017). Furthermore, *Aspergillus caesiellus* which showed a moderate halotolerant behavior was isolated by Batista-García et al. (2014). This isolate recorded combined xyylanolytic, estereolytic and cellulolytic activity. Also was found to be halothermostable and have the potential to degrade lignocellulosic materials for biotechnological applications (Musa et al., 2018 ).

## Conclusion

Mangrove ecosystems represent one of the unique habitats around the world. Stress conditions in such niches act as a selecting force for the microbial forms found there. The microorganisms in these habitats must cope up with its harsh conditions and modify their life machinery to maintain a successful life making these niches a reservoir for many unique and characteristic microorganisms. *Avicennia marina* growing on Red sea coast at Ras-Mohammad area- South Sinai is one of the less studied mangrove plants in Egypt specially concerning its microbial flora. In this study the endophytic and soil fungi associated with this plant have been studied and screened for their stress tolerance behavior. Although there is no significant thermal tolerance has been detected for these isolates, they showed remarkable halotolerant behavior. All isolates were sensitive for  $Ni^{++}$  stress but most of them showed high tolerance behavior for  $Mn^{++}$  and  $Zn^{++}$  and relative adaptive behavior for  $Fe^{++}$  and  $Cu^{++}$ . One isolate, *Aspergillus flavipes*, showed good halotolerant behavior where it recorded a growth up to 18% NaCl concentration and its growth is enhanced in presence of  $Fe^{++}$  (100 ppm concentration) although it didn't record any removal capacity for this metal. Moreover this isolate showed a promising enzymatic profile. This isolate can be considered as a promising source for industrially significant enzymes in terms of the stability and

activity under iron and salinity stress.

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## تقييم مقاومة فطريات التربة والفطريات الداخلية لنبات المانجروف (*Avicennia marina*) للحرارة والملوحة والمعادن الثقيلة وتقييم نشاطهم التحليلي

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تمثل الظروف البيئية القاسية فى بعض البيئات الفريدة عامل إنتقائي هام يتحكم فى نوع وطبيعة الكائنات الحية المتواجدة فى مثل هذه البيئات حيث تتميز الكائنات الحية الموجودة بتلك البيئات بقدرة عالية للتكيف مع هذه الظروف القاسية بواسطة تعديل أنظمتها الحيوية والبيولوجية. تعتبر بيئات نمو نبات المانجروف إحدى هذه البيئات الفريدة حيث تتميز بنسبة ملوحة عالية بالإضافة لقابلية التربة العالية للأحتفاظ بالمعادن الثقيلة. ولذا تم الأهتمام بأستكشاف طبيعة الكائنات الدقيقة المتواجدة بمثل هذه البيئات الفريدة. تناول البحث دراسة الفطريات الداخلية وكذلك فطريات التربة لنبات المانجروف النامي بساحل البحر الأحمر بمنطقة رأس محمد بجمهورية مصر العربية وذلك لفهم طرق التكيف و طبيعة الحياة فى مثل هذه البيئات الفريدة وأيضاً لأستكشاف قدرتهم التحليلية وإنتاجهم الإنزيمي كخطوة هامة للبحث عن إنزيمات ميكروبية واعدة ذات صفات فريدة تلائم بعض التطبيقات الهامة بالمجال الصناعي. وجدت الدراسة أن الفطريات المعزولة محبة لدرجات الحرارة المعتدلة وأنها ذات قدرة واضحة لتحمل الملوحة وتم ملاحظة أن قدرة تحمل الملوحة للفطريات المعزولة من التربة أعلى من الفطريات الداخلية لنبات المانجروف. هذا بالإضافة إلى تعيين قدرة متباينة لتحمل تركيزات مرتفعة من معادن المنجنيز والزنك والحديد والنحاس لهذه العزلات فقد تم تعيين قدرة تحمل جيدة لمعدن النحاس لإحدى الفطريات الداخلية "*Chaetomium globosum*" كما سجلت إحدى الفطريات المعزولة من التربة "*Aspergillus flavipes*" تحسن بالنمو فى وجود تركيز مرتفع من معدن الحديد على الرغم من تسجيل قدرة محدودة لكلا العزلتين على إزالة هذين المعدنين من وسط النمو مما يعكس قدرة محتملة لهذه العزلات على إنتاج إنزيمات خارجية ذات قدرة ثبات وتحمل لهذين المعدنين. وبمسح النشاط التحليلي الإنزيمي للعزلات تم تعيين نشاط إنزيمي جيد للفطريات الداخلية و نشاط إنزيمي واعد لأحدى الفطريات المعزولة من التربة "*Aspergillus flavipes*".