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ISOLATION AND IDENTIFICATION OF SOME ENDOPHYTIC FUNGI FROM SOME LOCAL ALGAE

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Abstract

The current search was conducted to isolate and identify endophytic fungi from some filamentous algae that isolate from two different region in Iraq (Radwaniyah and Musayib) during November 2018, with measurements performed of physico-chemical parameters values of the water in both station, for the purpose of investigating the quality of water in which these algae live such as air and water temperature, Water pH , CA^{+2} concentration, Mg^{+2} concentration, T.D.S. (Total Dissolved Solids), Turbidity (NTU), Electrical Conductivity (EC) and Salinity%, Total Hardness and Total Alkalinity. The results show in this study that, algae species most occurrence, namely *Cladophora glumerata, Ulothrix* sp. and *Spirogyra* sp. were 68.43, 13.35. and 13.08 respectively in Radwaniyah site and 62.97, 15.02 and 14.65 respectively in Musayib site. The most endophytic mold occurrence in both site was *Aspergillus niger*, with occurrence % in Radwaniyah site for *Ulothrix* sp. , *Spirogyra* and *Cladophora glumerata* were :82.75, 55.56 and 57.14 respectively. While, in Musayib site were: 68.18, 71.42 and 61.90 respectively.

***Keywords*:** Algae, Endophytic fungi, Physico-chemical parameters.

Introduction

Endophytic fungi (that present inside living tissues and reside in them without causing any visible disease symptoms) .In some cases their presence is beneficial to the host due to the synthesis of bioactive compounds. Substances produced by endophytic fungi may be used in agro industries for the biological control of pests and diseases. Endophytes are also known to produce a variety of biologically active secondary metabolites (Gao *et al*., 2010 and Giménez *et al*., 2007). Endophytes have various role in different approach such as their roles in agriculture and biofuel production (Pochon *et al*., 2015). Inoculating crop plants with certain endophytes may provide increased disease or parasite resistance, thus improving food production to fulfill the needs of increasing populations (Dina *et al*., 2014; Ahmed *et al*., 2016). Some endophytes have been proved to possess metabolic processes that convert cellulose and other carbon sources into "myco-diesel" and other hydrocarbon derivatives(El-Swaify *et al*., 2017; De Paula *et al*., 2015) Recently, various reports attention to the role of endophytes that isolate from macroalgae as an untapped source of biodiversity with potential to yield novel bioactive metabolites(Schulz *et al*., 2002; Debbab *et al*., 2012 Dina & Ahmed, 2016) and their ability to isolates from both tropical (Suryanarayanan *et al*., 2010) and temperate (Schulz *et al*., 2002; Flewelling *et al*., 2013) environments have exhibited significant antibiotic activities. The main aim of this study was to isolate and identification the endophytic mold from some local algae in Degla river.

Materials and Methods

Samples Collection and Isolation

According to (Zhang *et al*., 2009) the fresh filamentous algae having undamaged were collected weekly for two months from two station from Radwaniyah and Musayiband brought to the laboratory in sterile polythene bags and processed within 24 h. Plus bring water sample from the station for purpose Physicochemical Parameters. The algae surface sterilization protocol for isolating endophytes was as follows. The algae were washed in running tap water and dipped in 70% ethanol for 10 seconds followed by immersion in sterile distilled water for 15 seconds and cut into segment (approximants 0.5-1cm), then transport in to PDA media that add Rose Bengal (to prevent rapid growth of some mold) and antibiotic (to prevent growth bacteria).and incubate for 72hr. at 28±2ºC,until endophytic fungi mycelium growth and make subculture for purification.

Distribution study of the algae & endophytic fungi

The percentage of occurrence and frequency of each algae and endophytic species isolate were calculated according to the following formula (HEINO, 1987; Sharma. and Raju, 2013).

$$\%\text{Occurrence of species} = \frac{\text{Colonies number of species}}{\text{Total number of species colonies}} \times 100$$

$$\%\ \text{Frequency of species} = \frac{\text{Appearance in the sample}}{\text{Total number of species appearance}} \times 100$$

Results and Discussion

The current results indicate that, different algae have been isolate and identify in Radwaniyah station were: *Chroccous*, *Cladophora glumerata*, *Spirogyra* sp., *Enteromorpha* sp. and *Nitella sp.* while, in Musayib station were: *Cladophora glumerata*, *Spirogyra* sp., and *Ulothrix* sp. as seen in table (1) and figure (1& 2). The results which observed in this study that, the Musayib station have been isolated different species of algae were: *Chara sp.*, *Cladophora glumerata*, *Ulothrix* sp. and *Spirogyra* sp. The highest value of both the percentage of occurrence and frequency was (62.97 and 49.29) respectively in *Cladophora glumerata*. While, the lowest value was (7.36 and 11.11) respectively in *Chara sp.* The results of the current study show that, the Radwaniyah station isolate different algae which were: *Chroccous*, *Cladophora glumerata*, *Nitella sp.* and *Spirogyra* sp. with percentage of occurrence ranged between (1.38-68.43) which was the highest value (68.43) in *Cladophora glumerata*. While, the lowest value was (1.38) in *Enteromorpha* sp. The percentage of frequency in the same station ranged between (6.89-40.65) which was the highest value (40.65) in *Ulothrix* sp. While, the lowest value was (6.89) in *Chroccous sp.*

Fig. 1: Sampling sites

S1 = 33 ^{o}11'17"N44^{O}16'13"E 7Km Baghdad (Radwaniyah).

S2= 32 ^{o}46'30"N44^{O}16'48"E 4Km Babylon (Musayib).

Table 1 : Occurrence and Frequency percentage of the algae that isolate from study stations.

The stations	Algae	Occurrence%	Frequency%
S1 Radwaniyah	*Chroccous sp.*	1.94	6.89
	Cladophora glumerata	68.43	21.75
	Enteromorpha sp.	1.38	7.79
	Nitella sp.	1.82	3.47
	Ulothrix sp.	13.35	40.65
	Spirogyra sp.	13.08	19.45
L.S.D		1.33	2.07
S2 Musayib	*Chara sp.*	7.36	11.11
	Cladophora glumerata	62.97	49.29
	Ulothrix sp.	15.02	26.27
	Spirogyra sp.	14.65	13.33
L.S.D		8.25	2.07

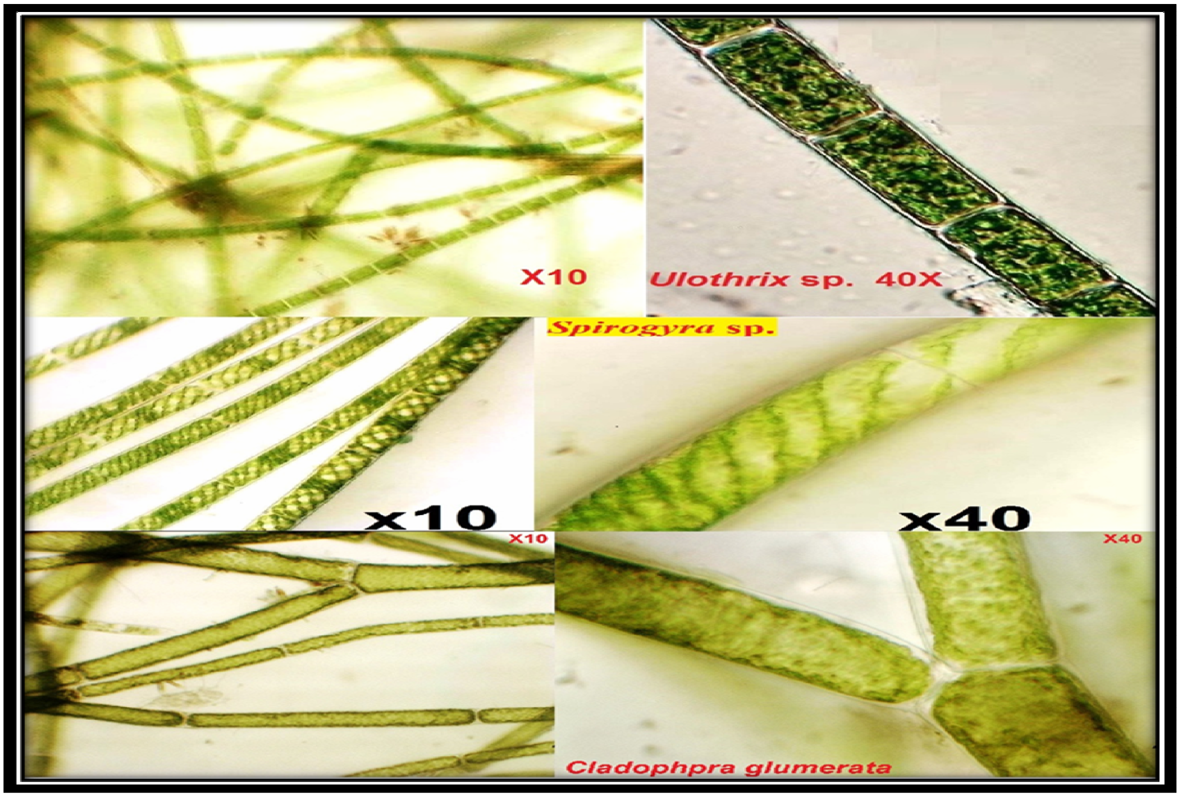


Fig. 2 : Some filamentous algae :*Ulothrix* sp., *Spirogyra* sp. and *Cladophora glumerata,* that isolate from Radwaniyah and Radwaniyah stations.

Water samples Physicochemical Parameters

As seen in Table (2), some physiochemical properties for analysis water at station Baghdad and Babylon during the study period (November 2018). The air temperature was 18 and 17 °C in Radwaniyah and Musayib stations. While 13 and 11 °C for water temperature respectively, and this temperature is good for the growth of most organisms, including algae. Generally, heat consider one of the most important factor that effect directly on the dissolved oxygen ratio in water and that effect on growth of living organisms, In addition to the rate of photosynthesis and metabolic rates of aquatic organisms (Boyd, 2000). That as well as the pH. It should be noted that the decline in temperature because November is the coldest month in Iraq.

The value of pH measured in the study period were 7.4 and 7.8 in Radwaniyah and Musayib stations, which is within the limits of the World Health Organization, which is between (6.5 - 9), Iraqi waters tend to be alkaline more than acidic during the winter due to the lack of biological activities and changes with the temperature of atmospheric and weather condition (Dwaish, 2012).

The results of the Nitrate, Nitrite and Phosphate present in Tables (2) revealed that the mean values concentration were recorded during study period 2.5088, 1.22 and 0.9369 respectively in Radwaniyah station and 5.0724, 0.98 and 0.6795 in Musayib station.

In general, rivers serve as sinks for nitrogen under nutrient rich and non-rich conditions. Nitrogen can be introduced into a river stream in the form of nitrate / nitrate from fertilizers in surface and groundwater, or from air pollution (James *et al*., 2005). The availability of nutrients and the presence of algal biomass are widely based on a range of time scales from days to seasons to years. Nutrient bioassays can be useful indicators for nutrients that have the potential or potential to limit the growth of algae at a time and place (Shinozuka *et al*., 2017). The main cause is ammonia oxidation of ammonia by nitrifying microbial and biological nitrification (Sugimoto *et al*., 2015). The increase in phosphorus and nitrate levels can result from mode of human disturbance that lead to mobilize the nutrient elements through land clearing, production and applications of fertilizer, discharge of human waste. As a result of the death of algae(decompose), the taste and odor of water will change as a result of increased levels of phosphorus and this leads to break down (Deterioration) the quality of the water (Huang *et al*., 2003).

The Sulphate (SO_4^{2-}) concentration in the canal's water were very high in study period in Radwaniyah and Musayib station, the mean values of concentration was 964.93 µg/L and 326.44 µg/L respectively as show in the table (2). The natural source of sulphate could probably be from rainfall, groundwater, weathering of sulphide-rich minerals, the mineral rocks anthropogenic ally. In addition, to different human activities (Shanley *et al*., 2005). In many cases SO_2 4 concentrations in stream water increase significantly in the autumn (Mitchell *et al*., 2006). Especially, after periods of extended drought increased Sulphate concentrations and acidity are widely observed phenomena (Clark *et al.,* 2005; Mayer *et al*., 2010). In addition, both station are water drainage, where all nutrients and salts accumulate at high concentrations of excessive use of fertilizers, plant and animal waste.

The results of the Magnesium (Mg^{2+}) and Calcium (Ca^{2+}) revealed that the mean values concentration were recorded during study period 137.26 and 78.92 µg / l respectively in Radwaniyah station, and 41.84 and 68.78 µg/l in Musayib station. In most cases, higher concentrations of calcium levels in winter were due to low solubility of calcium carbonate as the temperature increase, this was mainly by inverse correlation existed between Calcium, water temperature and total hardness during summer season. Magnesium is a lithophile metallic element and essential for all organisms and non-toxic under normal circumstances. Play important role in plant nutrient and forms the active site in the chlorophyll enzyme molecule during photosynthesis process. (Mons *et al*., 2006) The higher Mg^{2+} concentrations in winter in studied period might be derived from the increasing in water flow at this season and leading to increase washing of carbonate rocks and land around river, also many tributes were effected to the increase the Ca^{2+} and Mg^{2+} concentrations in river water (Al-Sarraf, 2006).

Total Dissolved Solids (T.D.S) is a measurement of inorganic salts, organic matter and other dissolved materials in water. TDS concentration in the canal's water were in Radwaniyah and Musayib station, the mean values of concentration was 1637µg/L and 692µg/L respectively as show in the table (2). The natural concentration of TDS water is usually less than 500 mg/L, and water with more than 500 mg/L is undesirable for drinking and many industrial uses. The measurement of TDS combines both anions and cations in the sample and total combinations of ions are substantially more toxic than other ions (Akhtar and Tang, 2013). A species might be more sensitive to TDS toxicity at certain life stages, as many fish are during fertilization. Total soluble solids can cause toxicity by increasing the salinity ratio and achieve changes in ionic water structure and individual ionization. It has been shown that increases in salinity can lead to changes in vital communities, reduce biodiversity, exclude sensitive species and cause acute or chronic effects in certain stages of life Where previous research of TDS toxicity in form $CaSO_4$ was reported on Alaska fish especially at fertilization (Brannock *et al*., 2002 and Stekoll *et al*., 2003).

Result of the present study showed that maximum turbidity was found in Radwaniyah stations(3.3 NTU). While, lowest turbidity was found in Musayib stations (1.1 NTU). At both site, the amount of turbidity found at the two sites are kindly low, perhaps due to the located away from residential complexes, as they discharge their waste and wastewater directly to the drainage. Moreover, the farms adjacent to the Radwaniyah station depend mainly on dig random wells drilling. This study agreed with the results obtained by Al-Tamimi (2006) who was recorded (1-54.5 NTU). While, and Farka (2006) recorded (25-70 NTU), as they reported high turbidity in some water of Iraq. Measuring turbidity in streams and rivers is one of the most important indicators for measuring the concentration of suspended sediments and impurities in water. The presence of sediments is part of streams, rivers and other water. It is natural that, water will become more turbid after rainy seasons.

In the present study the results showed that the electrical conductivity in Radwaniyah stations and Musayib stations were (3.06 and 1298) µS/cm respectively, while, the Salinity were was (0.7 and 0.16)% respectively. Through a measure the ability of the water to pass the electric flow represents the Conductivity. It is direct relationship to the amount of ions present, dissolved salts and inorganic materials such as alkalis, chlorides, sulphates, carbonates, sodium, magnesium, calcium and potassium .The greater the amount of ions in the water the greater the conductivity and vice versa. Generally, freshwater sources have a low conductivity while, seawater has a high conductivity. Water conductivity were observed lower in fall might be due to the decrease in temperatures causes prevent evaporation of the water and so, limited concentrating the ions, conductivity was temperature sensitive and decreases with decreasing temperature. These results agreed with some studies in Iraq water bodies (Al-Obaidi, 2006; Mahmood, 2008). In the rainy season, the amount of salts in the water decreases due to lack of evaporation and increasing water level.

The hardness of natural water depends mainly on the presence of dissolved calcium and magnesium salts (Chindah and Braide, 2004). The results showed that total hardness values during the study period 197.3and 121.95mg/ l in Radwaniyah and Musayib stations respectively. While, total alkalinity 165 and 137 mg/ l respectively. As a result of soil erosion plus sweeping the pollutants to the stations water from the hoses wastes close to the stations, as well as the agricultural wastes (drainage, pesticides and fertilizers) from the nearby lands, all of that lead to raising the rates of hardness in the waters. Total alkalinity value increases when nearby farms or population activity is close to the water site leading to release of CO_2 gas as decomposed by microorganisms which dissolved in water (AL-Tweij, 2012).

Table 2: Physico-chemical Parameters Values of the Water

Test	(Mean ± standard deviation)	
	Radwaniyah	Musayib
Air Temperature	18˚C±2	17 ˚C±1
Water Temperature	13˚C±3	11˚C±2
pH	7.4±0.5	7.8±0.4
NO^{-}_{3} ppm	2.5088± 0.2	5.0724± 0.2
NO_2 ppm	1.22±2.5	0.98±2.5
PO_4 ppm	0.9369± 0.1	0.6795± 0.1
SO_4^{-2} ppm	964.93±5.483	326.44±5.483
Mg^+ ppm	137.26± 4	41.84± 4
Ca^+ ppm	78.92± 2	68.78± 2
T.D.S ppm	1637± 7	692± 7
Turb. NTU	3.3±0.4	1.1 ±0.2
E.C µS/ cm	3.06± 0.2	1298± 12
Salinity %	0.07±0.12	0.16±0.39
Total H. mg/l	197.3 ±15	121.95 ± 13
Total AlK. Mg/l	165±17.5	137±15

Endophylic Fungi Isolation

The study concludes that the water channels at Musayib site environmentally fit for growth algae more than Radwaniyah site. However, we have isolated *C. glomerata*, *Ulothrix* sp. and *Spirogyra* sp. from both station with very good quantities. This indicates the susceptibility of this algae to adaptation in this relatively poor and polluted environment. This can be an incentive for algae to manufacture a variety of secondary metabolites to survival inappropriate conditions.

Based on initial screening experiment, twenty three endophytic fungal isolates from algae *Ulothrix* sp., *Spirogyra* sp. and *Cladophora glumerata* in Radwaniyah and Musayib site having maximum growth promotion capacity were selected for further investigations. These fungal isolates were identified depending on (Bessey, 1968; Chaturvedi, & Ren 2007; Bennett, 2010). The current results indicate that, the occurrence% and the Frequency% of the endophytic fungal that isolate from alga *Ulothrix* sp. ranged between (3.44-82.75 %) and (6.06- 63.63%) respectively, which was the highest value (82.75% and 63.63%) in *Aspergillus niger* at Radwaniyah site, while the lowest value was (3.44% and 6.06%) in a cold *Saprolegnia* sp. at the same station.

As seen in table (3) the occurrence% and the Frequency% of the endophytic fungal that isolate from alga *Spirogyra* sp. ranged between (11.10- 71.42%) and (20 – 42.40%) respectively, which was the highest value of was (71.42%) and (42.40%) in *Aspergillus niger* at Musayib site while the lowest value was (11.10%) and (20%) in a *Achlya* sp. and *Aspergillus* sp. respectively at Radwaniyah site.

Finally, the occurrence% and the Frequency% of the endophytic fungal that isolate from alga *Cladophora glumerata* ranged between (07.14- 61.90%) and (10.86 - 33.33%) respectively, which was the highest value of occurrence and Frequency was (61.90%) and (33.33%) in *Aspergillus niger* and *Pencillium sp.* respectively at Musayib site, while the lowest value was (07.14%) in a *Pencillium* sp. and *Saprolegnia* sp. at Radwaniyah site and lowest value of Frequency was (10.86 %) in *Aspergillus* sp. at Radwaniyah site as seen in table (3), figure (3) and Plate (1). *Penicillium* spp. and *Aspergillus* spp. are common endophytes of marine macroalgae (Jones *et al.*, 2012; Flewelling *et al.*, 2013).

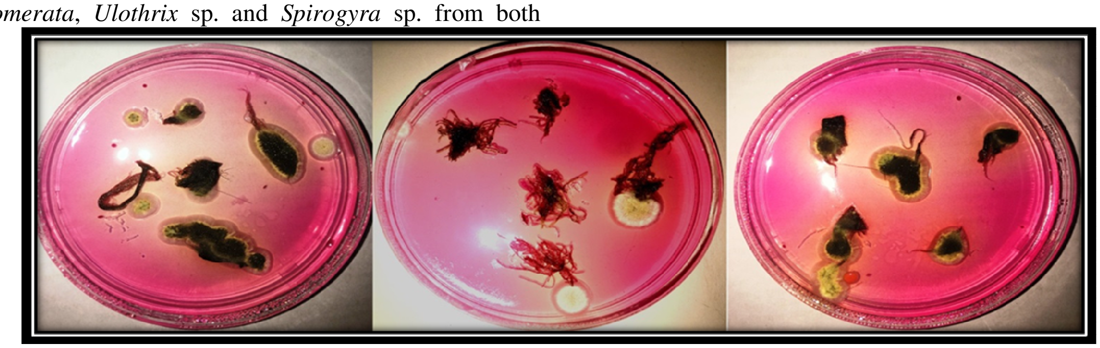
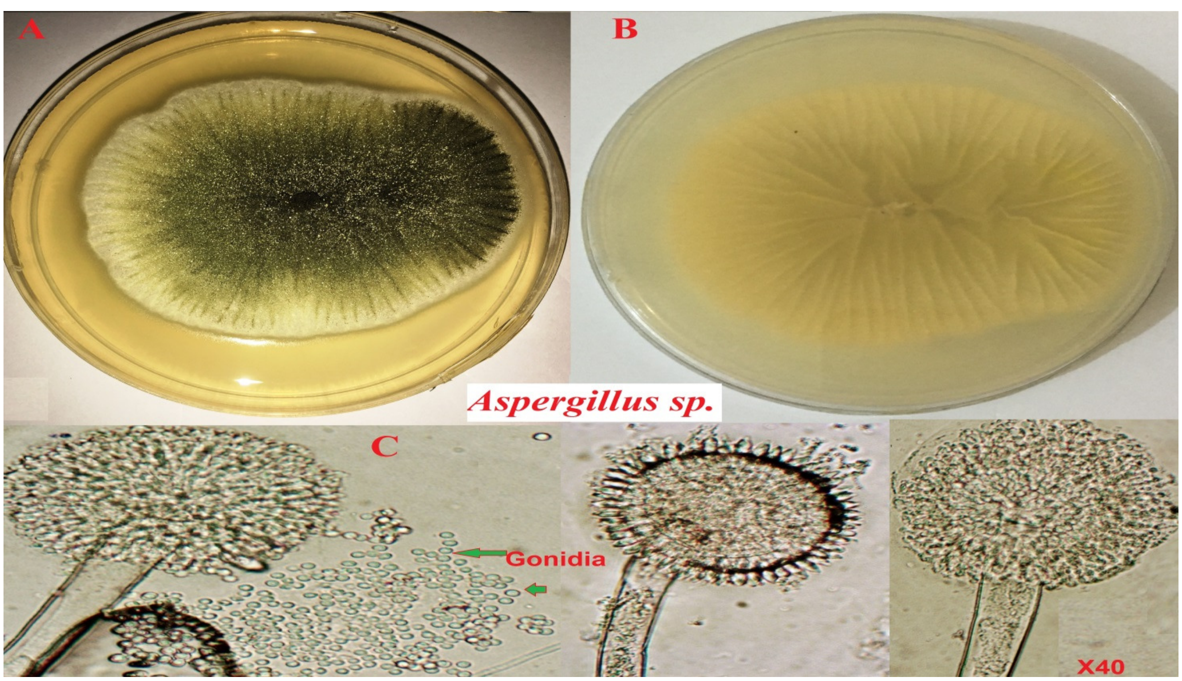


Fig. 3: Representative plates showing emergence of endophytic fungal mycelia from surface-sterilized algae on PDA media amended with Rose Bengal and antibiotic (Amoxicillin, 500 mg/L)

Table 3: The occurrence and frequency percentage of endophytic fungi that isolated from algae *Ulothrix* sp, *Spirogyra* sp. *and Cladophora glumerata* in two different site.

Algae	Station	Endophytic fungi	Occurrence%	Frequency%
*Ulothrix*sp.	S1 Radwaniyah	*Aspergillus niger*	82.75	63.63
		Aspergillus sp.	6.89	24.24
		Mycelia sterilia (Hyaline)	6.89	6.06
		Saprolegnia sp.	3.44	6.06
	S2 Musayib	*Aspergillus* sp.	68.18	45.45
		*Pencillium sp.*1	18.18	22.72
		*Pencillium sp*2	13.63	31.81
L.S.D			3.12	14.78
Spirogyra sp.	S1 Radwaniyah	*Aspergillus niger*	55.56	20.00
		Aspergillus sp.	16.67	26.27
		Saprolegnia sp.	16.67	23.73
		Achlya sp.	11.10	30.00
	S2 Musayib	*Aspergillus* sp.	71.42	42.40
		Saprolegnia sp.	14.29	27.27
		Mycelia sterilia (Hyaline)	14.29	30.33
L.S.D			4.87	12.04
Cladophora glumerata	S1Radwaniyah	*Aspergillus niger*	57.14	20.00
		Aspergillus sp.	21.43	10.86
		Pencillium sp.1	07.15	13.11
		Pencillium sp.2	07.14	24.10
		Saprolegnia sp.	07.14	33.33
	S2 Musayib	*Aspergillus niger*	61.90	18.50
		Aspergillus sp	14.29	27.27
		Pencillium sp	14.29	33.33
		Saprolegnia sp.	09.52	20.00
L.S.D			3.11	11.61



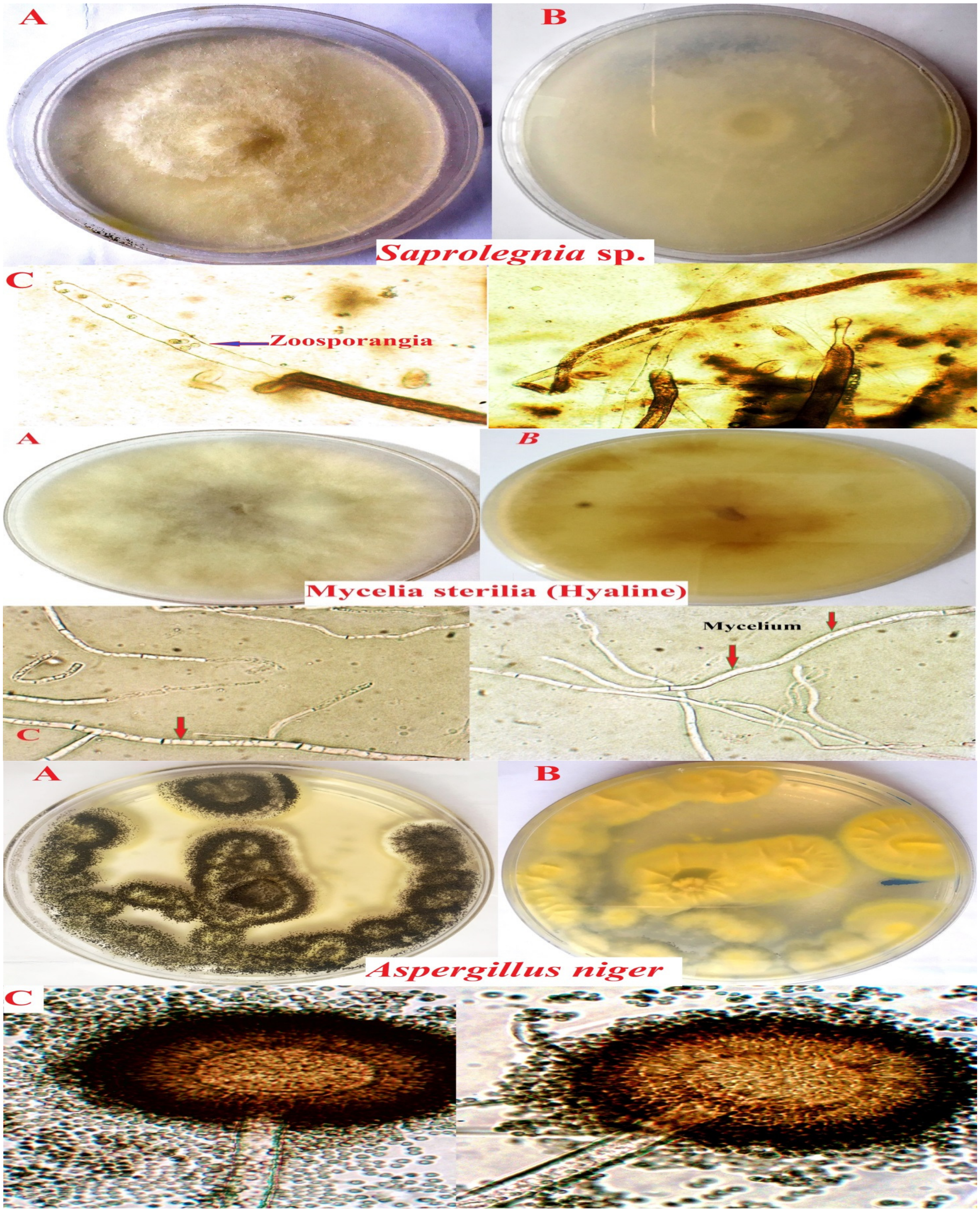


Plate 1: Cultural and microscopic features of some endophytic fungal radically on PDA media at 28°±2°C. A= colonies on PDA after 7 days. B= underside myceliaC= Microscopic features at 40 X

Algae endophytic mold are molds that come from inside of algae. The results shows that the identification of *Aspergillus* is widely available in endophytic algae. In addition, *Penicilium* sp. was identified as isolate from *Ulothrix* sp. and *Cladophora glumerata* .There are two types of unidentified isolates because of the unknown morphology. Due to the urgent need for new sources of pharmaceutical compounds, endophytic mold plays an important role through the production of many compounds that compete with artificial pharmaceutics' compounds isolated in addition to being fungal endophytes have been known to release various effective secondary metabolites (Gao *et al*., 2010 and Giménez *et al*., 2007). The fungal endophytes possess a synergistic effect of promoting plant growth and improving biotic and abiotic stress resistance as well (Tanaka *et al*., 2005; Vega *et al*., 2008). They also induce host plant defences against phytopathogenic organisms through regulating plant physiological responses (Giménez *et al., 2007*). Previous studies have revealed that plants infected with endophytes have a unique cost benefit plant-microbe association offering synergistic effects in growth promotion (Khastini *et al*., 2014). Based on recent reports, endophytes use to inhibit plant pathogens, production phytohormones are well known for their plant growth promotion. The enhancement of plant growth may be influenced by the ability of endophytes to produce a wide array of bioactive chemical constituents like plant growth regulating hormones. Besides that, endophytes exhibits Eco-friendly strategies.

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