# 4. RESULTS AND DISCUSSION

Cyanobacteria are photosynthetic $N_2$- fixing biological system widely used as biofertilizer in wetland rice crop. In this work, cyanobacteria were isolated from different Egyptian rice soils at different Governorates, i.e., Damietta, Kafr EL-Sheikh, EL-Dakahlia and EL-Sharkia. The isolated cyanobacteria isolates were then identified and tested for their biomass production, carbohydrate contents, lipids content and pigment content. As well as the collected soil samples were exposed for chemical analysis to ascertain their chemical properties.

## 4.1. Soil chemical properties - cyanobacteria interrelationship:

Data in Table (1) indicate the chemical and biological analyses for soil samples collected from different locations at different Governorates.

**Table (1): Soil chemical and biological analyses for the studied soils**

| property | Damietta | | Kafr EL-Sheikh | | EL-Dakhlia | | EL-Sharkia | |
|---|---|---|---|---|---|---|---|---|
| | Soil I | Soil II | Soil III | Soil IV | Soil V | Soil VI | Soil VII | Soil VIII |
| pH | 7.93 | 7.78 | 7.69 | 8.01 | 8.16 | 8.05 | 7.30 | 7.45 |
| EC (dS $m^{-1}$ | 0.49 | 0.46 | 0.87 | 0.66 | 0.51 | 0.69 | 0.50 | 0.58 |
| Organic carbon (%) | 1.56 | 1.52 | 0.72 | 0.68 | 0.21 | 0.23 | 0.88 | 0.92 |
| Organic matter (%) | 0.92 | 0.89 | 0.42 | 0.40 | 0.12 | 0.13 | 0.52 | 0.48 |
| Available N mg $kg^{-1}$ | 82 | 90 | 71 | 60 | 10.30 | 8.67 | 85 | 76 |
| Available P mg $kg^{-1}$ | 39.4 | 41.35 | 33.4 | 24.84 | 25.52 | 42.35 | 37 | 31 |
| Available K mg $kg^{-1}$ | 74 | 64 | 110 | 116 | 250.07 | 224.77 | 85 | 72 |
| Dehydrogenase activity μ L $H_2$ $g^{-1}d^{-1}$ | 1026 | 1275 | 1425 | 1920 | 892 | 957 | 630 | 577 |
| $CO_2$ evolution $mgCO_2$/100g soil $d^{-1}$ | 473 | 462 | 451 | 467 | 148 | 252 | 198 | 136 |

Also, data in Table (1) indicate the chemical and biological analyses for soil samples collected from different location at different governorates. The soil characteristic can be described as they degraded from with slightly alkaline like those of EL- Sharkia with pH of 7.30 and 7.45 (soil VII and soil VIII), to those goes towards alkalinity as shown in Table (1) for soils belonged to Damietta, Kafr EL-Sheikh and EL-Dakahlia. For salinity, EC ranged between 0.46 (soil II, Damietta) and 0.87 $dSm^{-1}$(soil III, Kafr EL-Sheikh). Organic matter is poorly appeared in the studied soils; however, the soils due to Damietta have relatively considerable organic matter of 0.92 and 0.89 for soil I and Soil II, respectively, compared to the soils collected from the other tested Governorates.

These aforementioned soil properties are of the reasons that encourage the cyanobacteria to inhabit, dominate and prevail in rice soils. Thus, rice soils can save the environmental conditions required for the growth and proliferation of cyanobacteria **(Roger and Kulasooriya, 1980).**

**4.2. Identification of the cyanobacteria isolates:**

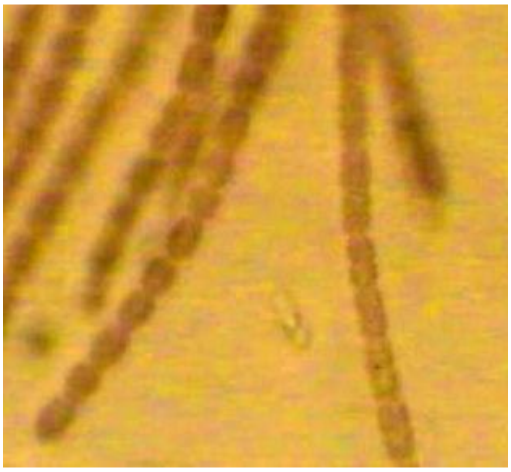
Eight cyanobacteria isolates were isolated from the rice soil at different locations from the aforementioned Governorates and identified according the description of **Desikachary (1959).** Three isolates were belonged to Damietta Governorate, two isolates were belonged to Kafr EL- Sheikh Governorate, two isolates was belonged to EL- Sharkia Governorate and an additional isolate was belonged to EL- Dakahlia Governorate. However, all isolates were examined under the magnification of 1000 x. Table (2) and Plates (1 &2) show the names and locations of the isolated cyanobacteria strains.

**Table (2): Names and locations of the isolated cyanobacteria strains**

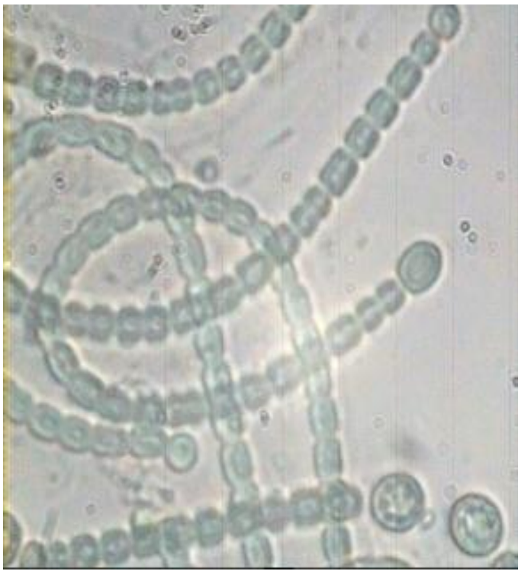
| Cyanobacteria Isolate number | Isolate name | Location |
|---|---|---|
| **1** | ***Nostoc calcicola*** **Brébisson ex. Born. et Flah.** | **EL-Sharkia** |
| **2** | ***Nostoc muscorum*** **Ag. ex. Born. et Flah.** | **Kafr EL-Sheikh** |
| **3** | ***Nostoc humifusum*** **Carmichael ex. Born. et Flah.** | **EL-Dakahlia** |
| **4** | ***Nostoc maculiforme*** **Born. et Flah.** | **Kafr EL-Sheikh** |
| **5** | ***Anabaena flos aquae*** **(Lyngb.) Bréb. ex Born. Et Flah.** | **Damietta** |
| **6** | ***Anabaena laxa*** **(Rabehn)** | **Damietta** |
| **7** | ***Microchate tenra*** **Thuret ex. Born. et Flah.** | **EL-Sharkia** |
| **8** | ***Wollea*** **sp. Born. et Flah.** | **Damietta** |

## *Cyanobacteria strains*

*Nostoc calcicola (EL-Sharkia)*



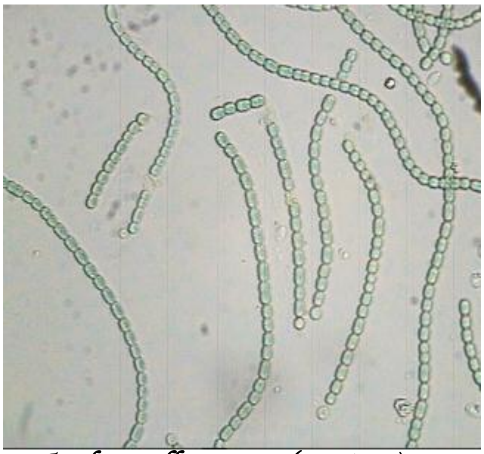
*Nostoc muscorum (Kafr EL-Sheikh)*



*Nostoc humifusum (EL-Dakahlia)*

*Nostoc maculiforme (Kafr EL-Sheikh)*

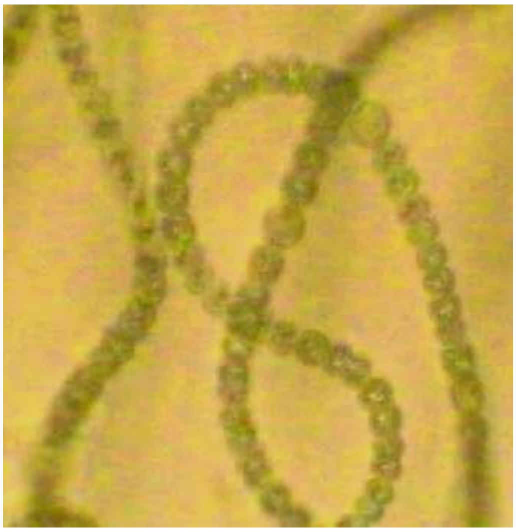
*Plate (1): Photograph of some isolated cyanobacteria strains (Magnification is 1000 x).*



*Anabaena flos aquae (Damietta)*

*Anabaena laxa (Damietta)*

*Microchate tenra (EL-Sharkia)*



*Wollea sp. (Damietta)*

***Plate (2): Photograph of some isolated cyanobacteria strains*** *(Magnification is 1000 x).*

### 4.2.1. Description of the isolated cyanobacteria strains:

The following cyanobacteria isolates description is done according **Desikachary (1959).**

**a) *<u>Nostoc calcicola</u>***

It was isolated from rice soil (VII &VIII) at **EL-Sharkia** Governorate; the soil has pH 7.30 and 7.45, EC 0.50 and 0.58, organic matter 0.52% and 0.48 and available N, P and K at concentrations of 85.00 and 76, 37.00 and 31 and 85, 00 and 72 mg $kg^{-1}$ for both soils, respectively. This isolate was described as the thullus is mucilaginous, slightly diffluent, expanded, olive, grey or blue green, often up to 5cm in diam., filamently loosely; sheath mostly indistinct, or indistinct only at the periphery of the thullus, colorless or yellowish brown; trichome 2.5 µ broad, pale blue-green; cells barrel-shaped, subspherical, rarely longer than broad; heterocyst subspherical, 4 (-5) µ broad; spores subspherical, 4-5 µ broad, with smooth yellowish membrane. The isolate identified as *Nostoc calcicola*, which belongs to order *Nostocales* and family *Nostocaceae.*

**b) *<u>Nostoc muscorum</u>***

It was isolated from rice soil (I) at Kafar El-Sheikh Governorate; the soil has pH 7.69, EC o.87, organic matter % 0.42, available N, P and K at concentrations of 71, 33.4 and 110 mg $kg^{-1}$, respectively. This isolate was described as the thullus is gelatinous-membranous, irregularly expanded, attached by the lower surface, tuberculate, dull olive or brown, 2-5 cm diam.; filaments densely entangled; sheath distinct only at the periphery of the thullus, yellowish brown; trichome 3-4 (-5) µ broad; cells short barrel-shaped to cylindrical, up to twice as long as broad; heterocysts nearly spherical, 6-7 µ broad; spores oblong, many in series, 4-8 µ broad, (7-) 8-12 µ long, epispore smooth and yellowish. The isolate identified as *Nostoc muscorum*, which belongs to order *Nostocales* and family *Nostocaceae.*

**c) *Nostoc humifusum***

It was isolated from rice soil (I) at Dakahlia Governorate, the soil has pH 8.16, EC 0.51, organic matter 0.12 %, and available N, P and K at concentrations of 10.30, 25.52 and 250.07 mg $kg^{-1}$, respectively. The description of this isolate was, a thullus gelatinous or mucilaginous, irregular with variable size, sometimes punctiform, sometimes many smaller ones uniting and expanding, tuberculate; olivaceous or brownish in color, attached by the under surface; filaments twisted and flexuous, densely entangled, sheath mostly indistinct, rarely distinct and yellowish brown; trichomes 2.2-3 µ broad; heterocysts 3 µ broad, subspherical; spores subspherical or oval, 4 µ broad, 6 µ long, epispore smooth and yellowish. The isolate identified as *Nostoc humifusum*, which belongs to order *Nostocales* and family *Nostocaceae.*

**d) *Nostoc maculiforme***

It was isolated from rice soil (I) at Kafar El-Sheikh Governorate; the soil has pH 7.69, EC 0.87 and organic matter 0.42 %, available N, P and K at concentrations of 71, 33.4 and 110 mg $kg^{-1}$, respectively. The isolate was as a thullus flat attached, forming thin irregular blue-greens spots, flexuous, contorted densely packed into loose net-like masses, sheath distinct, close; trichome 3.5-4 µ broad, cells oblong to depressed spherical; spores (immature) globose, mostly 6 µ rarely 8 µ broad, in long catenate chains, smooth, thick and fused with sheath, heterocysts present intercalary. The isolate identified as *Nostoc maculiforme*, which belongs to order *Nostocales* and family *Nostocaceae.*

**e) *Anabaena flos aquae***

It was isolated from rice soil (1) at Damietta Governorate; the soil has pH 7.93, EC 0.49, organic matter 0.92 %, available N, P and K at concentrations of 82, 39.40 and 74.00 mg $kg^{-1}$, respectively. This isolate was described as the thullus is frothy, gelatinous, lubricous, free floating, bluish in color, trichomes circinate, 4-8 µ (commonly 5.5 µ) broad, without sheath; cells ellipsoidal, seldom spherical, as long as broad or longer, 6-8 µ long, mostly with gas-vacuoles; heterocyst ellipsoidal, 4-9 µ broad and 6-10 µ long; spore prominently bent, on the outside convex, on the inside straight, 7-13 µ broad, mostly 9 µ, 20-35 µ rarely 50 µlong, single near heterocyst or seldom away from it, epi-spore smooth, colorless or yellowish, often surrounded by a wide gelatinous sheath. The isolate is identified as *Anabaena flos aquae*, which belongs to order *Nostocales* and family *Nostocaceae.*

**f) *Anabaena laxa***

It was isolated from rice soil (1) at Damietta Governorate; the soil has pH 7.93, EC 0.49, organic matter 0.92 %, available N, and P & K at concentrations of 82, 39.40 and 74.00 mg $kg^{-1}$, respectively. This isolate was described as a thullus floccose, free floating or attached to other algae, blue-green; trichomes 4-5 µ broad, straight, parallel, sometimes free and sometimes with a mucilaginous sheath; cells spherical or barrel-shaped, 5-6 µ long, apices hardly attenuated, end-cell rounded; heterocyst spherical, 6 µ broad, or elongate and up to 10 µ long; spores single or many away from the heterocyst, 6-8 µ broad and 14-17 (-20) µ long; epispore smooth and yellowish. The isolate identified as *Anabaena laxa*, which belong to order *Nostocales* and family *Nostocaceae.*

g) ***Microchate tenra***

It was isolated from rice soil (1) at EL-Sharkia Governorate; the soil has pH 7.3.16, EC 0.50, organic matter 50 %, and available N, P and K at concentrations of 85.00, 37.00 and 85 mg $kg^{-1}$, respectively. This isolate was described as it has filaments up 1mm long, single or in small stellate clusters, 6-7 (-8.5) µ broad, slightly bent, prostrate at the base; sheath thin, close to trichome, hyaline, unlamellated; trichome 5 µ broad, blue-green; cells at the base twice as long as broad, at the apices as long as broad; heterocyst basal and intercalary, nearly spherical or cylindrical, 6 µ broad, 6-8.5 µ long; spores single or in series, basal or intercalary, cylindrical with brownish wall, 6-7.5 µ broad, 13-17 µ long. The isolate identified as *Microchate tenra*, which belong to order *Nostecales* and family *Nostocaceae.*

**h) *Wollea* sp.**

It was isolated from rice soil (II) at Damietta Governorate; the soil has pH 7.87, EC 0.46, organic matter 0.89, available N, P and K at concentrations of 90, 41.35 and 64.00 mg $kg^{-1}$, respectively. This isolate was described as having filaments generally in definite colony, thullus with finger shaped, attached at first, thullus may tubular, cylindrical, soft, filaments sub erect, parallel, to slightly curved, agglutinated, sheath confluent, heterocyst intercalary, spores in series continuous with the heterocyst or remote from it. The isolate identified as *Wollea* sp., which belongs to order *Nostocales*, family *Nostocaceae.*

**4.2.2. Frequency of cyanobacteria strains isolated from different rice soils:**

Table (3) shows the frequency of the eight isolated cyanobacteria strains from different rice soils of four Governorates (Damietta, Kafr EL- Sheikh, EL-Dakahlia and EL-Sharkia) at two soil samples for each. The frequency of the isolate strains is distributed in the tested Governorates. For instance *Anabaena flos aquae*, *Anabaena laxa*, and *Wollea* sp. are frequent in Damietta, while both of *Nostoc calcicola and Microchate tenra* are frequent in EL-Sharkia Governorate, *Nostoc muscorum* and *Nostoc maculiforme* are frequent in Kafr EL-Sheikh Governorate, *Nostoc humifusum* is frequent in EL-Dakahlia Governorate. This frequency and diversity for the isolated cyanobacteria strains is due to the variance in the ecological conditions commonly prevailed in different tested Governorates (**EL-Gaml, 2006)**. Also in this concern, **Goyal *et al*. (1984)** in India, found that in rice fields, *Nostoc* and *Anabaena* were widely distributed in different regions followed by *Oscillatoria*, *Calothrix* and *Hapalosiphon*. They also added that *Anabaena ambigua*, *Oscillatoria amoena* and *Rivularia globiceps* showed restricted distribution. They explained that there

**Table (3): Frequency of cyanobacteria strains isolated from different rice soils of eight stands belong to four Governorates**

| Name of cyanobacteria isolates | Damietta | | Kafr-El-Sheikh | | EL-Dakahlia | | EL-Sharkia | |
|---|---|---|---|---|---|---|---|---|
| | Soil I | Soil II | Soil III | Soil IV | Soil V | Soil VI | Soil VII | Soil VIII |
| ***Nostoc calcicola*** | | | | | | | +++ | +++ |
| ***Nostoc* muscorum** | | | +++ | +++ | | | | |
| ***Nostoc humifusum*** | | | | | +++ | +++ | | |
| ***Nostoc maculiforme*** | | | +++ | +++ | | | | |
| ***Anabaena flos aquae*** | +++ | +++ | | | | | | |
| ***Anabaena laxa*** | +++ | +++ | | | | | | |
| ***Microchate tenra*** | | | | | | | +++ | +++ |
| ***Wollea* sp.** | +++ | +++ | | | | | | |

**+++ = Severe prevailing.**

is a crop-growth cycle dependent variation in the cyanobacteria population in rice fields. **Kolte and Goyal (1985)** reported that in rice fields, a total of 174 cyanobacteria forms belonging to 35 genera of cyanobacteria were isolated from 2013 soil samples. *Anabaena* and *Nostoc* showed a very wide distribution. Soils with pH below 6.5 were found to support very good growth of cyanobacteria. Cyanobacteria are present abundantly in rice fields and are important in helping to maintain rice fields fertility through nitrogen fixation. Many rice fields soil contain a high density of cyanobacteria, and over 50% of cyanobacterial genera that are existence in rice paddy fields are heterocystous filamentous forms **(Dong and Lee, 2006)**. They also explained that the collected cyanobacteria from rice fields were classified into a total of 14 genera including seven genera of filamentous cyanobacteria. In particular, 142 heterocystous cyanobacteria were isolated and classified into six genera, including *Anabaena*, *Nostoc*, *Calothrix, Cylindrospermum, Nodularia, Scytonema and Tolypothrix*. Yet, over 90% of the heterocystous filamentous isolated from rice paddy fields belonged to two genera: *Anabaena* and *Nostoc*. However, it appeared that cyanobacteria grow rapidly in the rice fields that contain ample organic matters in the soil and water as well as pH, temperature, organic sources…..etc. that allowed propagation **(Choudhury and Kennedy, 2004).**

**4.3. Biomass production by the isolated cyanobacteria strains:**

The isolated cyanobacteria strains were grown under continuous illumination (3000 lux) and ambient temperature of 28-32 $^{o}$C at different growth stages of 7, 14, 21and 28 days in the laboratory. Data in Table (4) and Fig. (2) illustrate the patterns of growth of the isolated cyanobacteria strains. However, highest biomass of 97 mg dry weight / 100 ml medium was recorded by *Anabaena flos aquae* after 4 weeks followed by 96 mg/ 100 ml medium for *Nostoc muscorum*. The other tested cyanobacterial strains came in the order of *Nostoc maculiforme, Noctoc calcicola*, *Microchate tenra*, *Anabaena laxa*, *Nostoc humifusum* and finally *Wollea* sp. for biomass. Therefore the first six cyanobacterial strains, which, exhibited the best growth, were chosen to investigate and evaluate their carbohydrate, lipids and pigment contents.

**Table (4): Growth of the cyanobacteria strains grown on BG11 medium at different incubation periods (mg dry weight 100 ml$^{-1}$ medium)**

| Cyanobacteria strains | Incubation period (days) | | | |
|---|---|---|---|---|
| | 7 | 14 | 21 | 28 |
| *Nostoc calcicola* | 21 | 37 | 69 | 90 |
| *Nostoc muscorum* | 33 | 43 | 75 | 96 |
| *Nostoc hnmifusum* | 13 | 16 | 19 | 37 |
| *Nostoc maculiforme* | 22 | 45 | 62 | 93 |
| *Anabaena flos aquae* | 30 | 44 | 82 | 97 |
| *Anabaena laxa* | 19 | 24 | 30 | 39 |
| *Microchate tenra* | 29 | 51 | 69 | 79 |
| *Wollea* sp. | 11 | 19 | 24 | 30 |

**Initial inoculum = 10 ml filtrate cyanobacteria culture containing $10^9$ cfu (Colony formed /unit) hormogonia cyanobacteria cell ml$^{-1}$.**

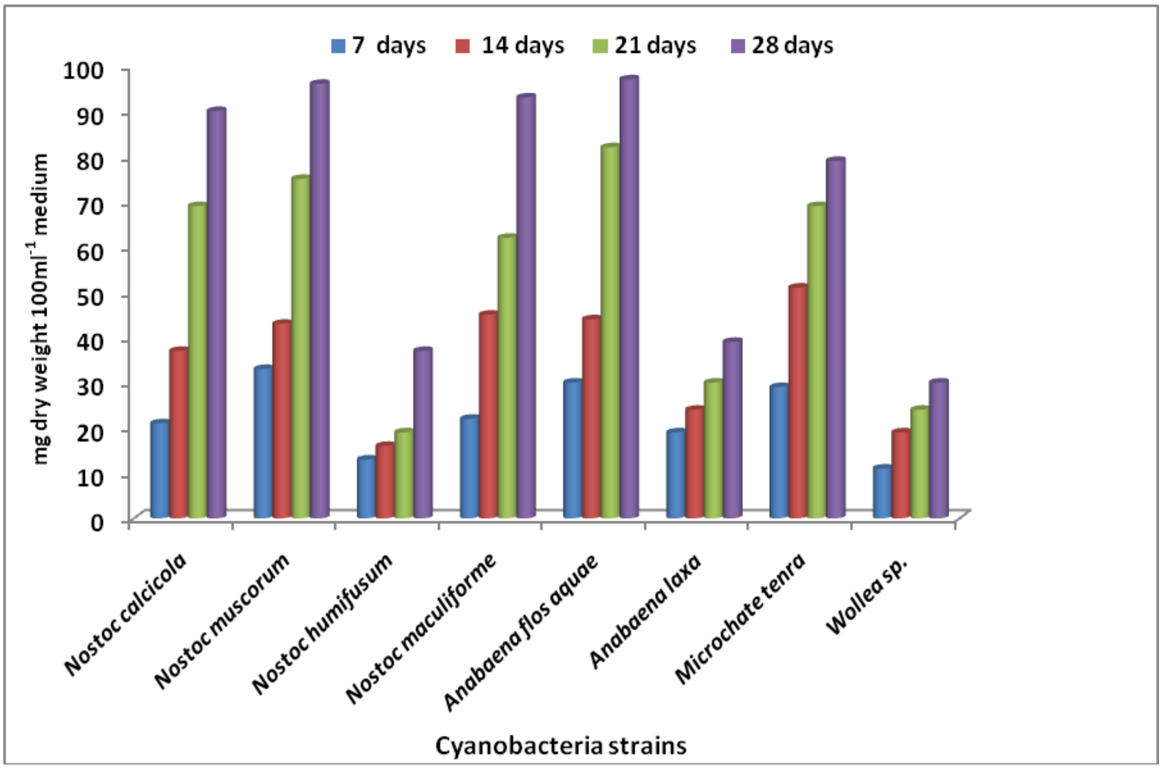



**Figure (2): Growth of cyanobacteria strains on BG11 medium at different incubation periods.**

## 4.4. Natural products contents for the selected isolated cyanobacteria strains:

Cyanobacteria are one of the largest producers of biomass in the marine, fresh water and the terrestrial environments. They produce a wide variety of chemically active metabolites in their surroundings, potentially as an aid to protect themselves against other settling organisms. These active metabolites, also known as bioorganic compounds, produced by several species of cyanobacteria, have antibacterial, antifungal properties and have likely uses, i.e., in therapeutics. The isolated substances with potent pharmacological activity belong to groups of hydrocarbons, fatty acids, lipopeptides, amides, alkaloids, terpenoid, lactones, pigments, nitrogen heterocycles and toxin **(Rezanka and Dembitsky, 2006)**. In the present study the isolated cyanobacteria strains were exposed to check and to evaluate their carbohydrate, lipids and pigments content as natural products can be economically utilized.

### 4.4.1. Total carbohydrate contents:

Data in Table (5) and Figure (3) revealed that, culturing all the tested isolated cyanobacteria strains in BG11 medium for weekly periods of 7 to 28 days led to increase the total carbohydrate contents (TCC) along with increasing the time of incubation. However, the highest TCC of 5.80 % was due to *Nostoc muscorum* followed by 5.70, 5.30, 5.10, 4.60 and 4.20 % for *Nostoc cacicola, Nostoc maculiforme*, *Nostoc humifusum*, *Anabaena flos aquae* and *Microchate tenra*, respectively. While, the least TCC content of 3.70 and 3.85 % was due to both *Anabaena laxa* and *Wollea* sp.

**Table (5): Total carbohydrate contents for the selected isolated cyanobacteria strains measured at different growth stages**

| Cyanobacterial strain | Carbohydrate content (%) Growth stages (days) | | | |
|---|---|---|---|---|
| | 7 | 14 | 21 | 28 |
| *Nostoc calcicola* | 1.50 | 2.10 | 2.80 | 5.70 |
| *Nostoc muscorum* | 0.80 | 1.20 | 2.70 | 5.80 |
| *Nostoc humifusum* | 0.90 | 1.10 | 1.90 | 5.10 |
| *Nostoc maculiforme* | 1.10 | 1.90 | 2.10 | 5.30 |
| *Anabaena flos aquae* | 1.20 | 1.70 | 2.90 | 4.60 |
| *Anabaena laxa* | 0.70 | 0.90 | 1.30 | 3.70 |
| *Microchate tenra* | 1.30 | 1.60 | 2.30 | 4.20 |
| *Wollea* sp. | 0.70 | 0.90 | 1.70 | 3.85 |

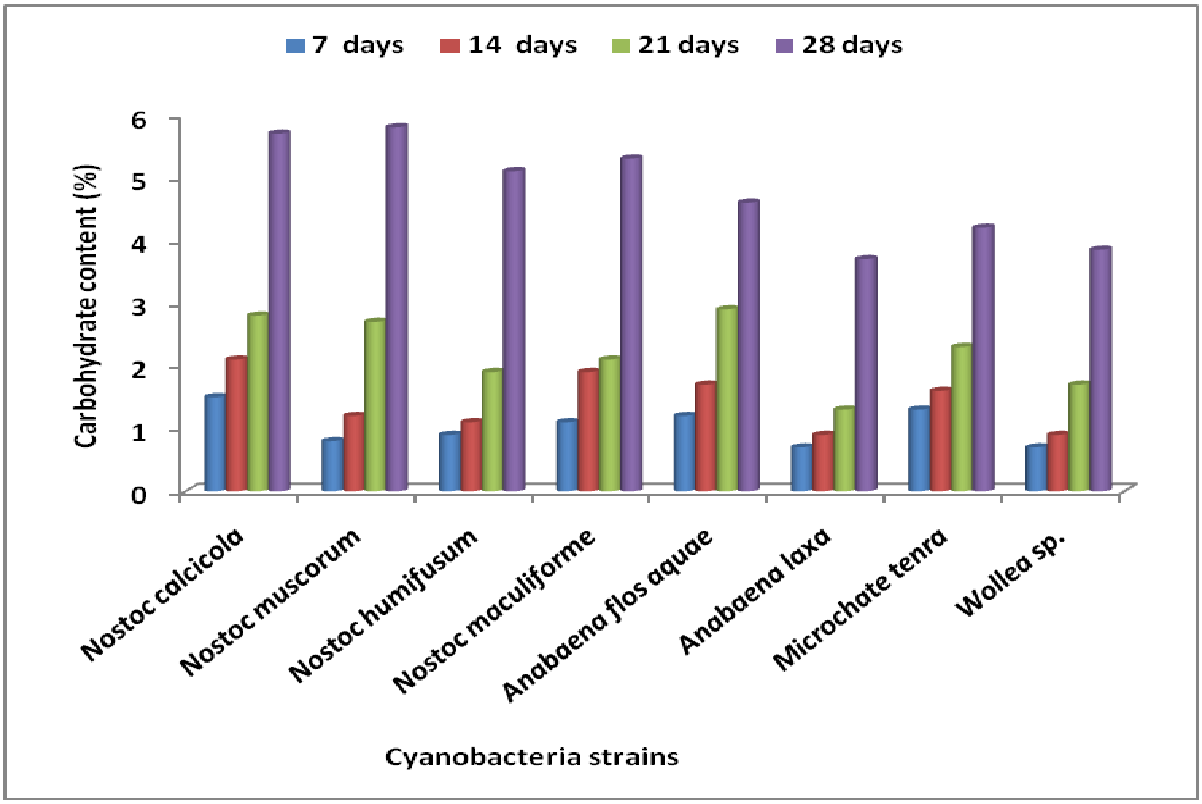



**Fig. (3): Total carbohydrate content of the selected cyanobacteria strains measured at different growth periods (days).**

### 4.4.2. Polysaccharides contents:

Samples of the sixth cyanobacteria isolated strains that containing the highest carbohydrate content were harvested after 28 days, oven dried (70$^{o}$C) up to a constant dry weight and extracted for polysaccharides determination (Table 6 and Fig. 4). Results confirmed the presence of monosaccharide (Fructose, glucose, ribose and xylose), disaccharides (sucrose, sorbitol and maltose) and the trisaccharides (raffinose and galactose). However, *Anabaena flos aquae, Nostoc maculiforme* and *Nostoc humifusum* is lacking of glucose, while any of *Anabaena flos aquae, Nostoc maculiforme* and *Microchate tenra* is lacking of fructose. The amounts of rhaffinose, sorbitol, maltose, and galacturonic recorded by all the tested cyanobacteria strains were quite small compared with those recorded due to sucrose, ribose, xylose, fructose and glucose. However, the highest sugar amount of 994 mg kg$^{-1}$ dry weight was for sucrose scored by *Nostoc cacicola* followed by 925 mg kg$^{-1}$ dry weight for ribose recorded by same cyanobacteria strain. These results demonstrate the presence of polysaccharides in cyanobacteria inhabited the rice paddy soils.

Cyanobacteria are photosynthetic prokaryotic organisms that have been known for long time to produce large amounts of exopolysaccharides **(Drews and Weckesser, 1982**). Different types of cyanobacterial exopolysaccharides are formed: e.g., as mucilaginous external layer around the cell, either being organized into well-defined sheath or a capsule (capsular polysaccharides) that intimately associated with the cell surface, or as slime that is only loosely associated with the cell surface; or as a soluble polysaccharides (released polysaccharides), released into environment (culture medium) during cell growth **(Li *et al*., 2001).** Members of the order *Nostocales* are broadly characterized by unbranched filaments and the production of up to three kinds of differentiated cells. All cyanobacteria are characterized as eubacteria that grow as autotrophs with $CO_2$ as the carbon source, utilizing an oxygen-producing photosynthetic mechanism for the generation of ATP and reductant. **(Castenholz and Waterbury, 1989).** The cyanobacterial members of *Nostocales* have the ability to produce significant amounts of exopolysaccharides into their surrounding environment **(De Philippis *et al*., 2001)**. Such cyanobacterial exopolysaccharides, in particular the capsular polysaccharides (CPS), serve as a boundary to the immediate environment and play a protective role against desiccation or antimicrobial agents **(Volk and Venzke, 2007).** They also added that compared CPS the released polysaccharides are easily extractable from culture media, for instance, by precipitation with alcohol. Consequently, over the past years in particular, this type of cyanobacterial exopolysaccharides has received increasing attention in view of potential industrial application; e.g., as viscosifying or suspending agents, as additives for removal and recovery of dissolved heavy metals, or, in the case of sulfated polysaccharides, as bioactive substances. Soil algae are known to excrete into the surrounding soil a variety of extracellular low molecular and polymeric substances (EPS) like amino acids, polypeptides, amides, proteins, polysaccharides, vitamins, growth regulators and a number of other compounds not yet fully characterized. These EPSs fulfill a variety of different roles. They both protect algae from harmful effects of toxic substances or unfavorable factor (e.g. desiccation, antibiotics, ultraviolet ray etc.) and directly attach to solid surface and other matrix **(Hu *et al*., 2003). (Rezanka and Dembitsky, 2006)** reported that polysaccharides secreted by *Nostoc* strains contain up to 6 sugars (in %): L-rhamnose 3.5, D-xylose 20.9, D-mannose 1.6, D-galactose 21.5, D-glucose 44.0, and 2-O-methyl-D-glucose 8.6.

**Table (6)**

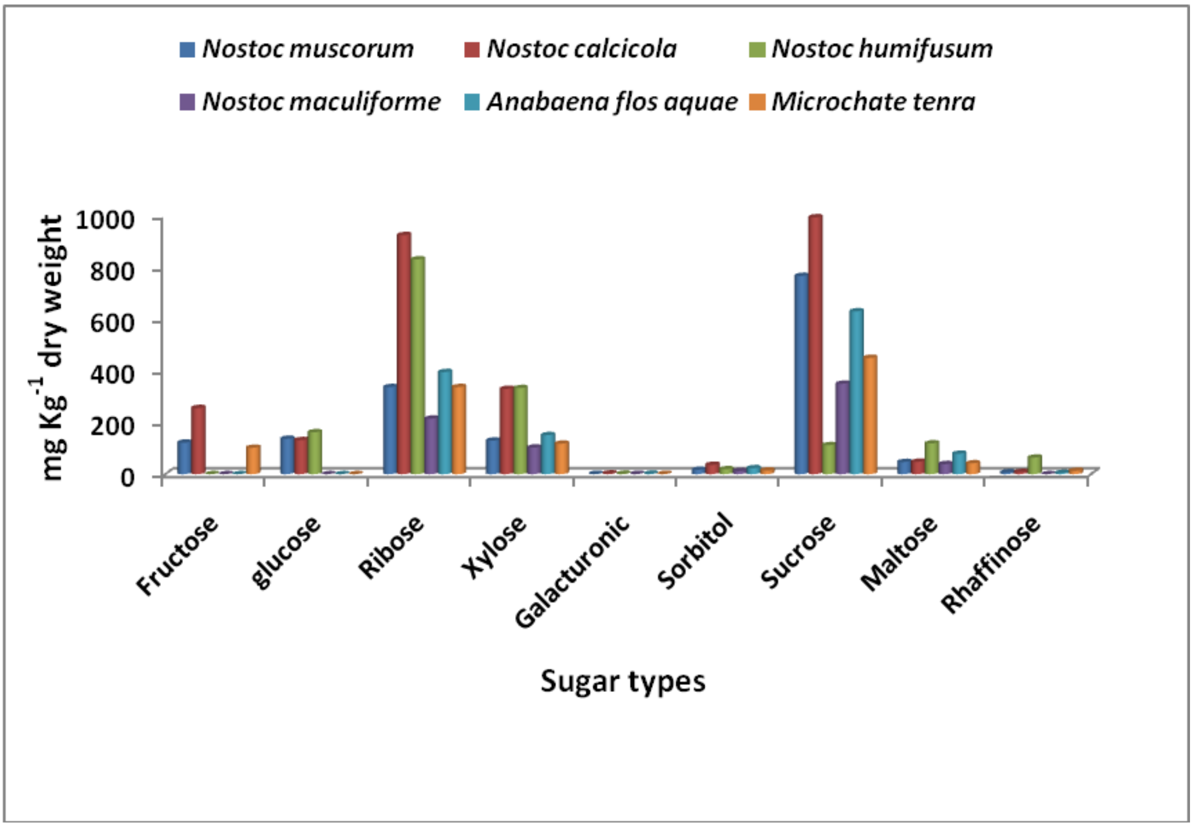
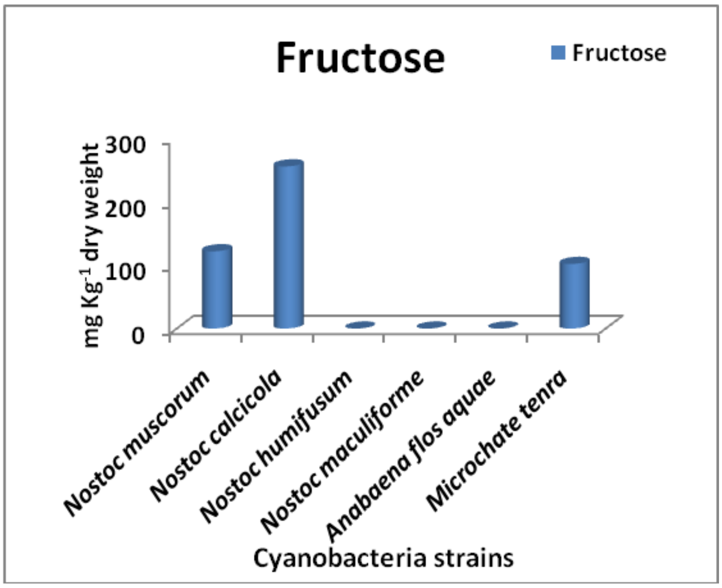
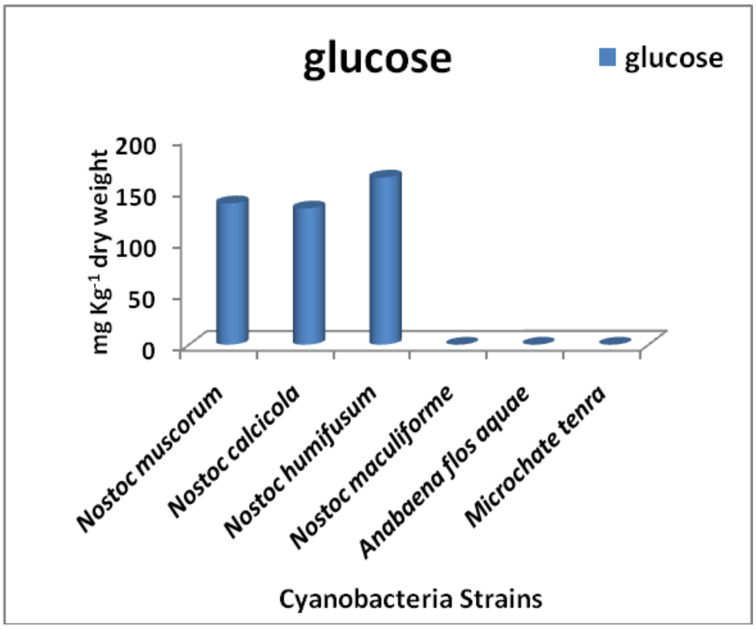



**Fig. (4): Polysaccharides contents of the selected cyanobacteria strains.**

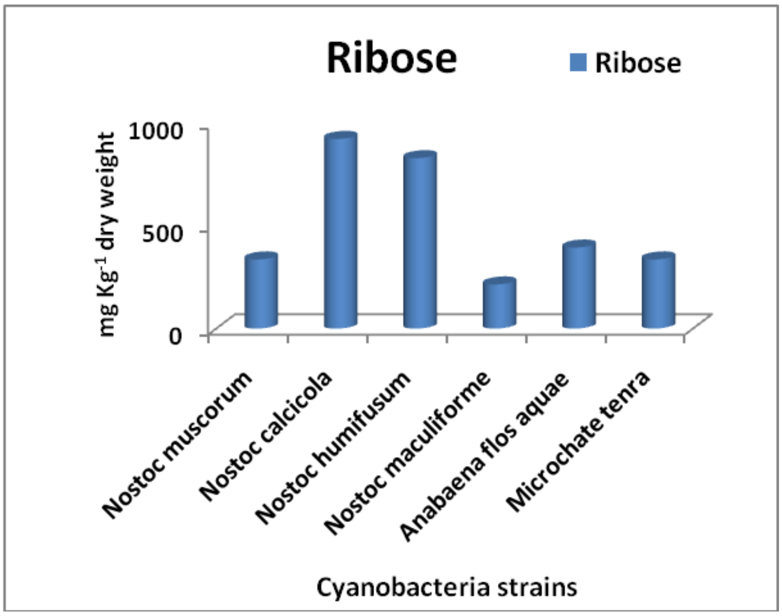
## Sugar fractions content of the polysaccharides for isolated cyanobacteria strains



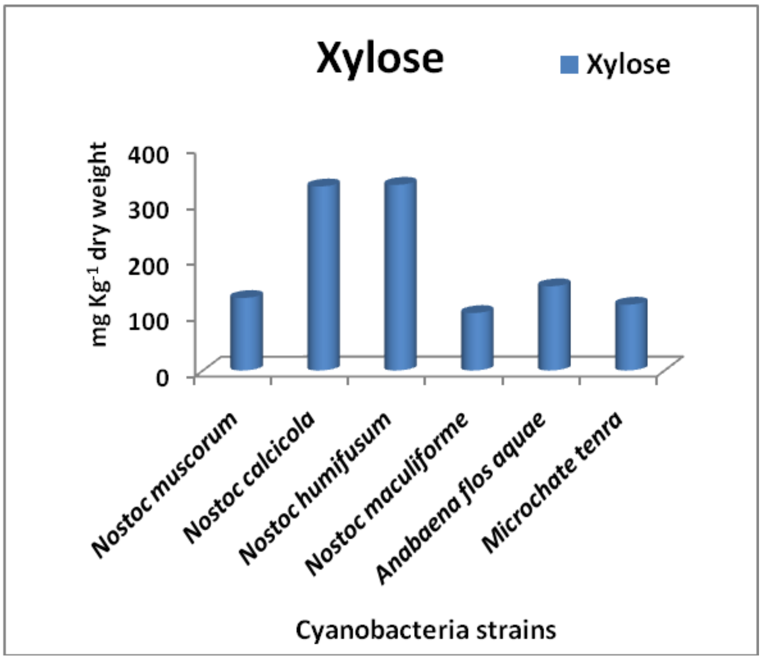

**(1) Fructose**



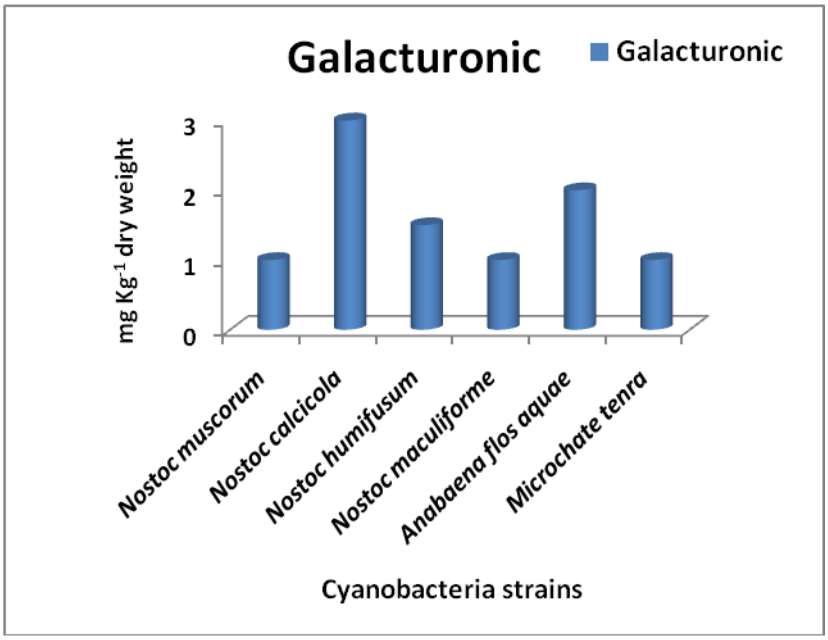

**(2) Glucose**



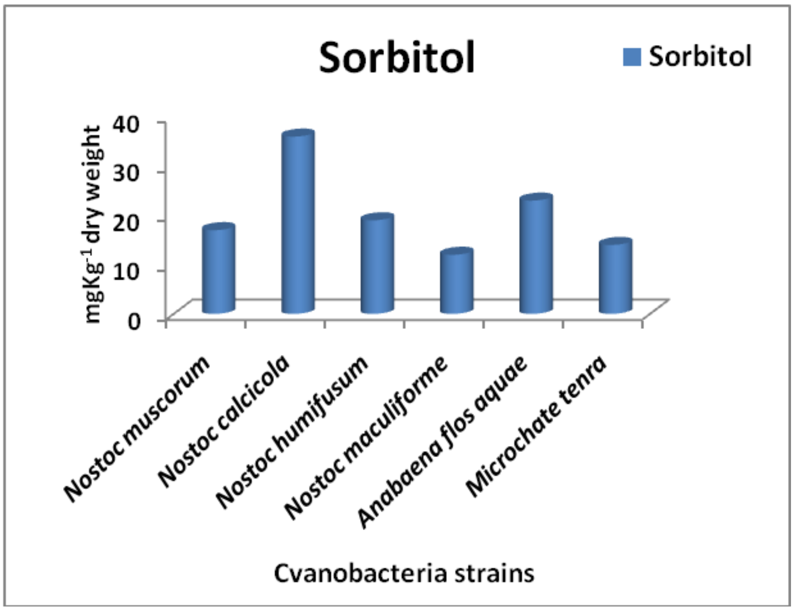

**(3) Ribose**



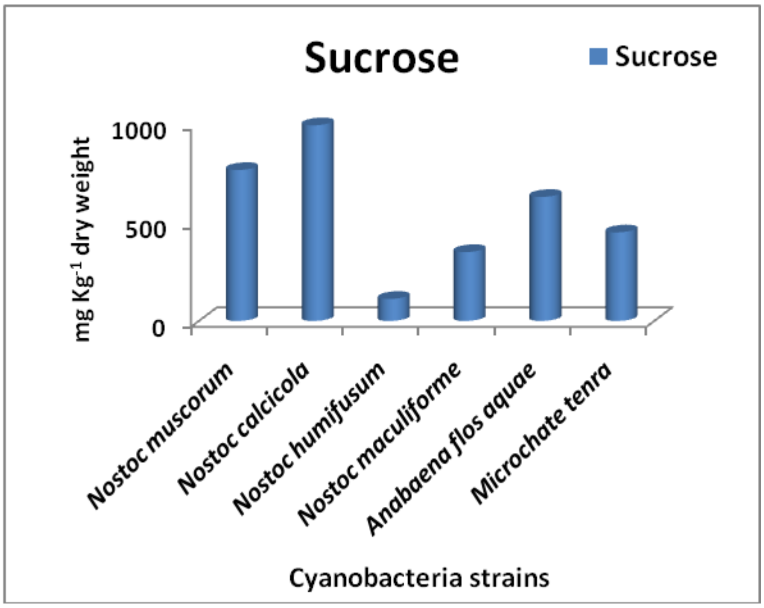

**(4) Xylose**



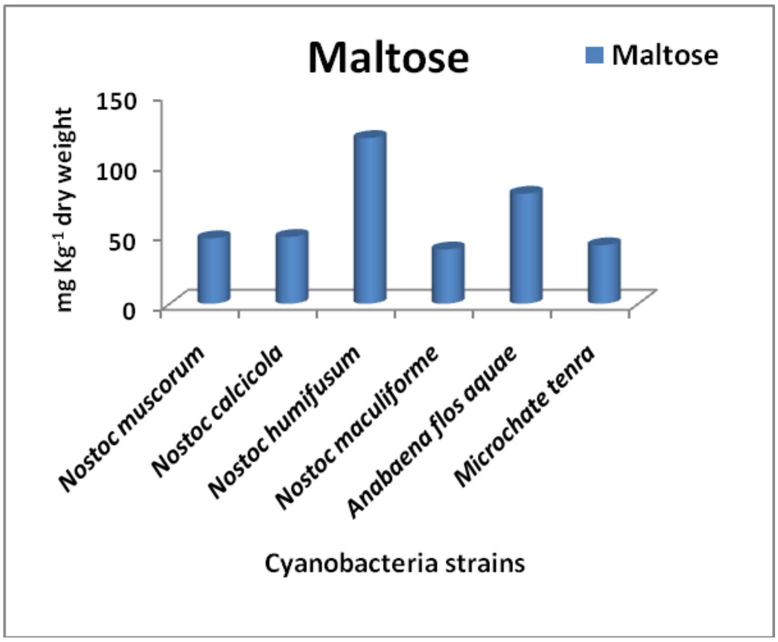

**(5) Galactoronic**



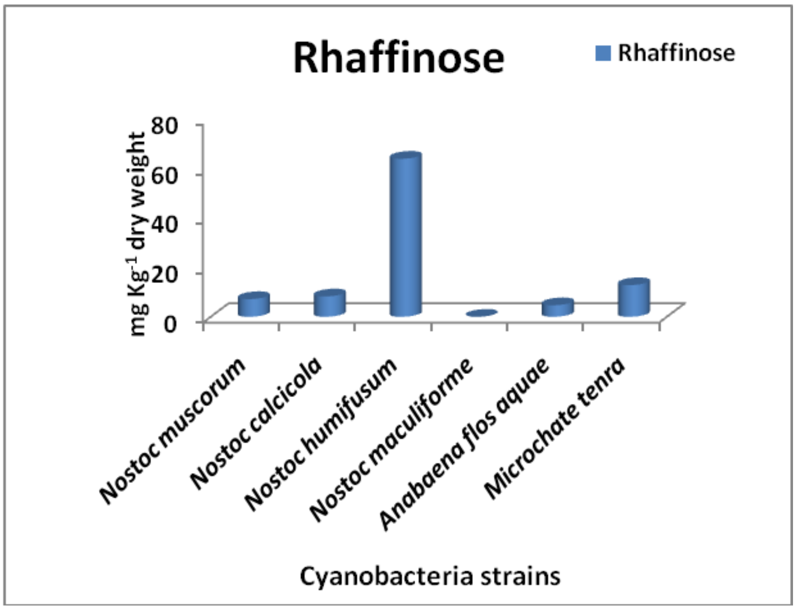

**(6) Sorbitol**




**(7) Ssucrose**




**(8) Maltose**




**(9) Rhaffinose**

### 4.4.3. Total lipids content:

Data in Table (7) and Fig. (5) revealed that culturing all the tested isolated cyanobacteria strains on BG11 medium at incubation periods of 7 to 28 days under continuous illumination led to increase the total lipids contents (TLC) along with increasing the time of incubation. However, the total lipids contents ranged from 0.34% for *A. flos aquae* after 7 days and 3.75 % for *Nostoc calcicola* after 28 days. Relatively, all the tested cyanobacteria strains had recorded their maximum lipids content at the 28$^{th}$ day of incubation. On the other respect, *Anabaena flos aquae* gave the least TLC at all examined incubation periods compared to the other tested cyanobacteria strains. In the contrary, *Nostoc calcicola* gave the highest TLC contents compared to the other tested cyanobacteria strains.

**Table (7): Total lipids content for the selected isolated cyanobacteria strains measured at different growth stages**

| **Cyanobacterial strain** | **Total lipids content (%)** | | | |
|---|---|---|---|---|
| | **Growth stages (days)** | | | |
| | **7** | **14** | **21** | **28** |
| ***Nostoc calcicola*** | **2.09** | **2.70** | **3.04** | **3.75** |
| ***Nostoc muscorum*** | **0.42** | **0.56** | **1.20** | **1.90** |
| ***Nostoc maculiforme*** | **0.90** | **1.20** | **1.45** | **2.10** |
| ***Anabaena flos aquae*** | **0.34** | **0.57** | **1.10** | **1.80** |
| ***Anabaena laxa*** | **1.50** | **2.10** | **2.80** | **3.20** |
| ***Microchate tenra*** | **1.90** | **2.30** | **2.90** | **2.98** |

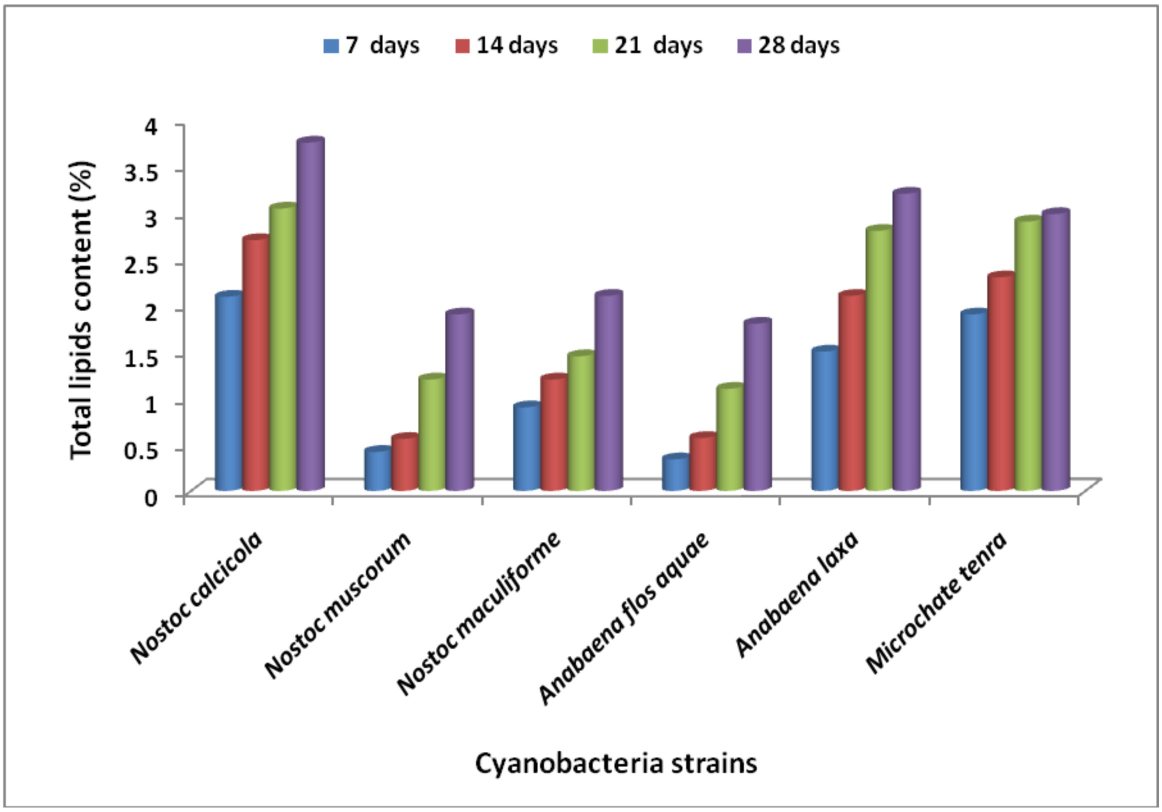



**Figure (5): Total lipids contents of the selected cyanobacteria strains measured at different growth periods.**

### 4.4.3.1. Fatty acids differentiation for cyanobacteria lipids:

Fatty acids are primary metabolites of acetyl Co A pathway, which is genetically determined, evolutionary very old, and therefore conservative. Data in Table (8) and Fig. (6) indicate the presence and variation of fatty acids content of the tested cyanobacteria strains. However, lauric, myristic, palmetic, palmetoleic and stearic represent the highest contents of fatty acids in all examined strains compared to those of oleic, linoleic, linolenic, arachidic and arachidonic that found in less contents. On the other hand, *N. muscorum* gave the highest lauric content (20.35%), *A. flos aquae* gave the highest of 37.5% (myristic), *N. maculiforme* gave the highest of 17.67, 13.35 and 25 29 % for palmetic, palmetoleic and stearic, respectively.

Lipids are the most effective storage energy, function as insulators of delicate internal organs and hormones and play an important role as structural constituents of most of the cellular membranes. They also have a vital role in tolerance to several physiological stressors in a variety of organisms including cyanobacteria. In the present study, the isolated cyanobacteria strains contained lipids as natural product, which is ranged between the least of 0.34% for *A. flos aquae* and the highest of 3.75% for *N. calcicola*. Lipids of cyanobacteria are generally esters of glycerol and fatty acids. They may be either saturated or unsaturated **(Borowitzka, 1988 a)**. The lipids of some cyanobacterial species are also rich in essential fatty acids such as $C_{18}$ linoleic and linolenic acids and their derivatives **(Singh *et al*., 2002)**. They also added that these fatty acids are essential components of the diet of humans and animals and are becoming important feed additives in aquaculture. The lipids and their fatty acids contents have a role in the environmental stress tolerance in cyanobacteria such as acute water deficiency and salinity **(Potts, 2001). Caudales and Wells (1992)** investigated the cellular fatty acids of cyanobacteria belonging to the genera *Anabaena* and *Nostoc* and found significant differences between these genera that confirm the results of the present study that showed different composition of the fatty acids differentiated from the isolated cyanobacteria strains. lauric, myristic, palmetic, palmetoleic, stearic, oleic, linoleic, linolenic, arachidic and arachidonic are the fatty acids dominated in all examined cyanobacteria strains. In this concern, **Rezanka and Dembitsky (2006)** confirmed these results when analyzed the fatty acids composition of several cyanobacteria species of the family *Nostocaceae* (*Anabaenopsis* sp.) and proved the predominantly of palmetic (45.5%), oleic (8.2%), linoleic (16.2%), α linoleic (16.8), γ linoleic(3.6%) and stearidonic (4.6%). In addition, *Anabaenopsis* sp. cells biomass contains 11.4 % lipids. However, the cyanobacteria with high lipids contents can be a source for economic biofuel production **(Widjaja, 2009)**.

**Table (8)**

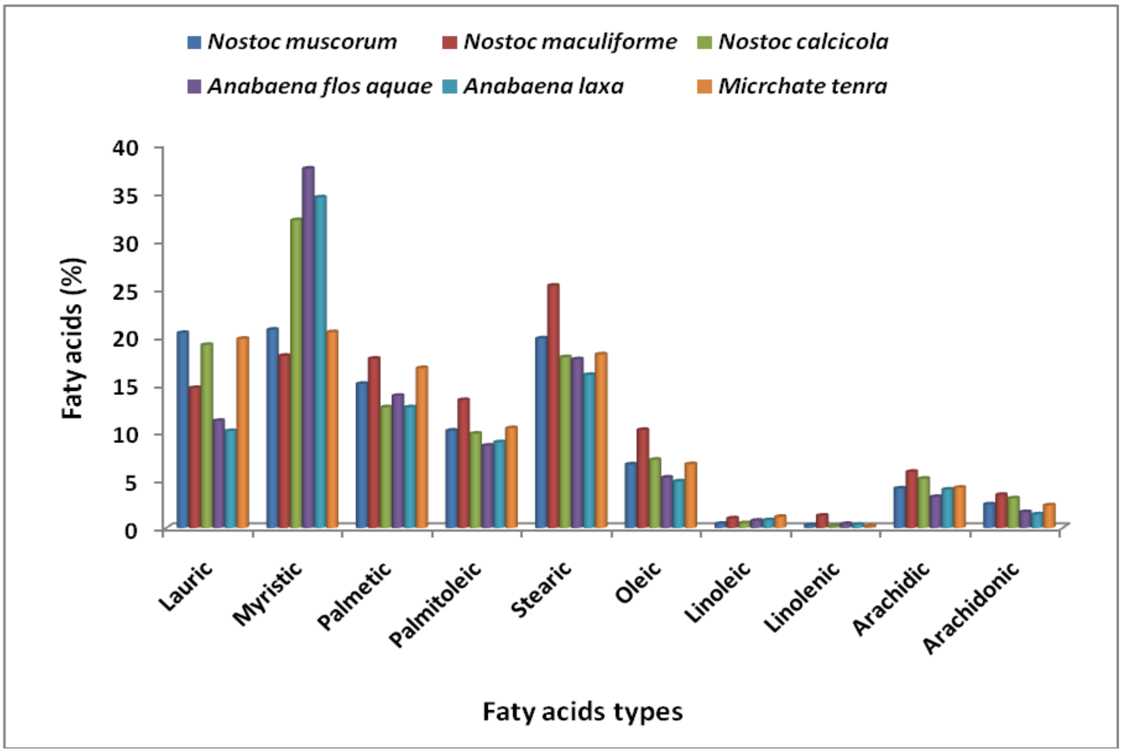



**Figure (6): Fatty acids differentiation in lipids contents of the selected cyanobacteria strains.**

#### 4.4.5. Pigments contents:

Data in Table (9) and Fig. (7) indicate the Chlorophyll a, carotene and phycopillin pigments concentrations in the selected cyanobacteria strains measured at the end period of incubation (28 days). Results revealed that chlorophyll a values were higher than those recorded by both carotene and phycopillin pigments. However, the highest chlorophyll a concentration of 63.00 mg 100 $ml^{-1}$ medium was due to *M. tenra* followed by 54.00 and 51.00 mg 100 $ml^{-1}$ medium for both *A. laxa* and *N. maculiforme*, respectively, While the least chlorophyll a concentration of 21. 90 mg 100 $ml^{-1}$ medium was due to *N. cacicola*.

Due to carotene concentrations, *A. laxa* recorded the highest carotene concentration of 25.14 mg 100 $ml^{-1}$ medium against 18.52 mg 100 $ml^{-1}$ medium the least carotene concentration due to *N. muscorum*. However, the carotene concentration due the other tested cyanobacteria strains were 19.87, 20.32, 21.26 and 22.46 mg 100 $ml^{-1}$ medium for *A. flos aqua*, *N. maculiforme*, *N. cacicola* and *M. tenra*, respectively.

## Table (9)

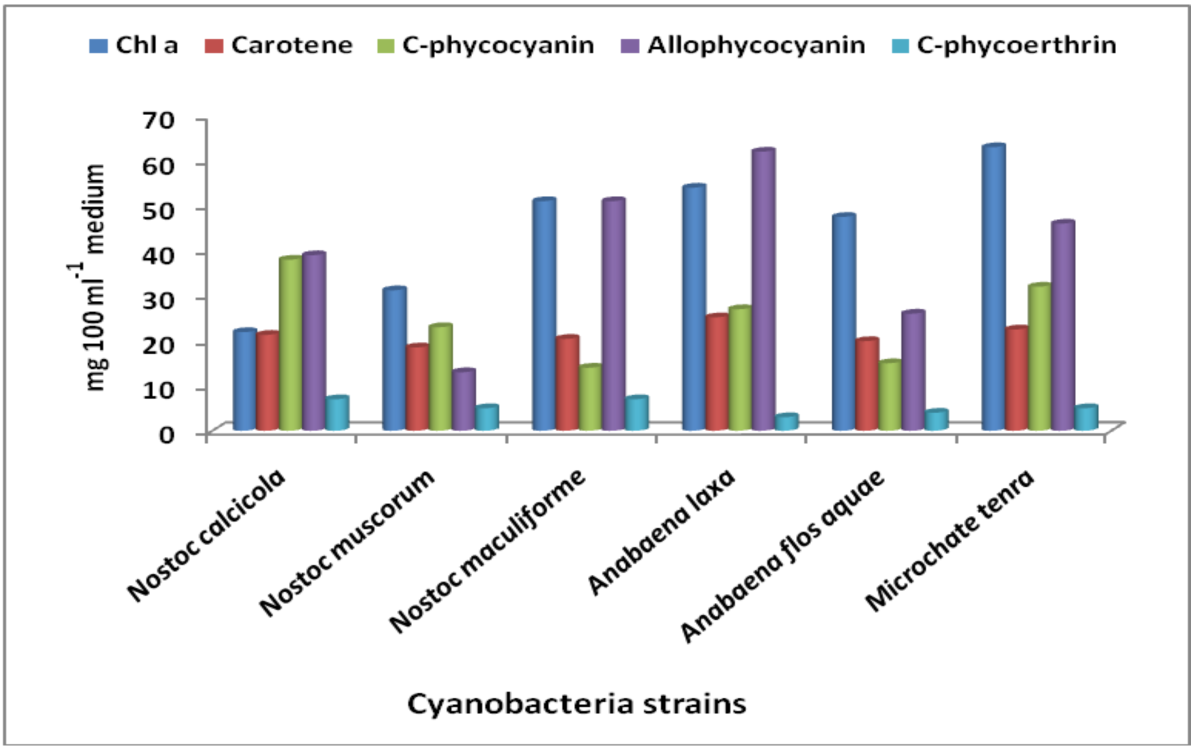



**Figure (7):** **Chlorophyll a, carotene and phycobillin pigments concentrations in the isolated cyanobacteria strains after 28 days incubation.**

In concern to phycobillin pigments (C-phycocyanin, allophycocyanin and C-phycoyanin), results revealed that the concentration of both C-phycocyanin and allophycocyanin were higher than those recorded by C-phycoerthrin. However, the highest C-phycocyanin concentration of 38.00 mg 100 $ml^{-1}$ medium was due to *N. calcicola* followed by 32.00 and 27.00 mg 100 $ml^{-1}$ medium for both *M. tenra* and *A. laxa*, respectively. On the other respect, the least concentration of 14.00 mg 100 $ml^{-1}$ medium (C-phycocyanin) was due to *N. maculiforme*. As for allophycocyanin, the highest concentration of 62.00 mg 100 $ml^{-1}$ medium was belonged to *A. laxa* followed by 51.00 and 46.00 mg 100 $ml^{-1}$ medium in respective to both *N. maculiforme* and *M. tenra*, respectively. Due to C-phycoerthrin, its concentrations were extremely less than those of C-phycoyanin and allophycocyanin and ranged between 3.00 and7.00 mg 100 $ml^{-1}$ medium.

One of the most obvious and arresting characteristic of the algae is their color. In general, each phylum has its own particular combination of pigments and an individual color. Aside chlorophylls, as the primary photosynthetic pigment, microalgae also form various accessory or secondary pigments, such as phycobiliproteins and a wide range of carotenoids. These natural pigments are able to improve the efficiency of light energy utilization of the algae and protect them against solar radiation and related effects. Their function as antioxidants in the plant shows interesting parallels with their potential role as antioxidants in foods and humans **(Van den Berg *et al*., 2000)**. Therefore, microalgae are recognized as an excellent source of natural colorants and nutraceuticals and it is expected they will surpass synthetics as well as other natural sources due to their sustainability of production and renewable nature **(Dufossé *et al*., 2005)**. The isolated cyanobacteria strains when examined for its pigment contents, they exhibited the occurrence of chlorophyll a, carotene and phycobillins. As for chlorophylls, all algae contain one or more type of chlorophyll: chlorophyll-a is the primary photosynthetic pigment in all algae and is the only chlorophyll a in cyanobacteria (blue-green algae) and rhodophyta. Apart from their use as food and pharmaceutical colorants, chlorophyll derivatives can exhibit health promoting activities. These compounds have been traditionally used in medicine due to its wound healing and anti-inflammatory properties as well as control of calcium oxalate crystals and internal deodorization **(Ferruzi and Blakeslee, 2007)**. Due to carotenoids, they are naturally occurring pigments that are responsible for the different colors of fruits, vegetables and other plants (**Ben-Amotz and Fishler, 1998)**. Carotenoids are usually yellow to red, isoprenoid polyene pigments derived from lycopene. They are synthesized de novo by photosynthetic organisms and some other microorganisms (**Borowitzka, 1988 b)**. In animals the carotenoids ingested in the diet are accumulated and/or metabolized by the organism, being present in meat, eggs, fish skin (trout, salmon), the egg yolks, the liver and in the feathers of birds (poultry) **(Breithaupt, 2007)**. In the algae the carotenoids seem to function primarily as photoprotective agents and as accessory light harvesting pigment, thereby protecting the photosynthetic apparatus against photo damage. Some microalgae can undergo a carotenogenesis process, in response to various environmental and cultural stresses (e.g. light, temperature, salts, nutrients), where the alga stops growth and changes dramatically its carotenoid metabolism, accumulating secondary carotenoids as an adaptation to severe environments **(Bhosale, 2004)**.

The main carotenoids produced by microalgae are β-carotene which serves as an essential nutrient and has high demand in the market as a natural food coloring agent, as an additive to cosmetics and also as a health food **(Raja *et al*., 2007)**. β-carotene is routinely used in soft-drinks, cheeses and butter or margarines. Is well regarded as being safe and indeed positive health effects are also ascribed to this carotenoids due to a pro-vitamin A activity **(Baker and Gunther, 2004)**.

Besides chlorophyll and carotenoid lipophilic pigments, cyanobacteria contain phycobiliproteins, deep colored water-soluble fluorescent pigments, which are major components of a complex assemblage of photosynthetic light-harvesting antenna pigments - the phycobilisomes **(Glazer, 1994)**. The main natural resources of phycobiliproteins are the cyanobacteria for phycocyanin (blue) and the rhodophyta for phycoerythrin (red). Phycobiliproteins are extensively used for fluorescence applications, as highly sensitive fluorescence markers in clinical diagnosis and for labeling antibodies used in multicolor immunofluorescence or fluorescence-activated cell-sorter analysis **(Becker, 1994).** Phycocyanin is currently used in Japan and China as a natural coloring, in food products like chewing gums, candies, dairy products, jellies, ice creams, soft drinks (e.g. Pepsi® blue) and also in cosmetics such as lipsticks, eyeliners and eye shadows **(Sekar and Chandramohan, 2007)**. Phycocyanin is also used as antioxidant, anti-inflammatory, neuroprotective and hepatoprotective effects **(Benedetti *et al*., 2004).**