Soil Algae of El-Farafra Oasis (The Western Desert, Egypt), and N₂-fixation Efficiency of Five Heterocytous Cyanophytes

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IN TOTAL 47 soil algal morphospecies were cultured and identified from the desert soils of El-Farafra Oasis, The Western Desert (Egypt). The most of them were related to Cyanophyta (34 algal taxa), followed by Chlorophyta and Xanthophyta (5 algal taxa belonging to each). Bacillariophyta represented only by 3 species. Moreover, this work enriched the Egyptian soil algal flora with 5 different algal taxa: *Westiellopsis prolifica* Janet, *Cylindrospermum gregarium* (Zakrz.), *Cylindrospermum. licheniforme* (Bory) Kütz., *Chlorocloster caudatus* Pasch. and *Tetraktis aktinastroides* Pasch. Furthermore, the potentialities of atmospheric N₂-fixation efficiency of *Calothrix elenkinii* Kossinsk., *Nodularia harveyana* f. *sphaerocarpa* (Born. et Flah.) Elenk., *Scytonema ocellatum* Lyngb., *Stratonostoc linckia* (Roth) Elenk. and *Westiellopsis prolifica* Janet were investigated. However, *C. elenkinii, W. prolifica* and *S. ocellatum* exhibited to a large extent the highest fixation rates with mean values of 18.89, 30.52 and 33.01 μmole ml⁻¹ h⁻¹, respectively, followed by *S. linckia* (16.71 μmole ml⁻¹ h⁻¹) and *Nodularia harveyana* f. sphaerocarpa (12.01 μmole ml⁻¹ h⁻¹). In conclusion, *Scytonema ocellatum* and *Westiellopsis prolifica* can used as promising eco-friendly natural bio-fertilizers for the sustainable development in the desert habitats.

Keywords: Soil algal flora, N₂-fixation, Heterocytous cyanobacteria, Desert habitats, Soil amelioration, El-Farafra Oasis, Egypt.

Introduction

Desert and arid lands not only represent biologically important challenging ecosystems that extent over one third of the earth's land surface, but also cryptic algal biodiversity-rich habitats (Bohunická et al., 2015 and Mareš et al., 2015). Edaphic algae are greatly prevalent in soils of all continents in particular, cyanophytes (Řeháková et al., 2007; Zhang et al., 2011 and Venter et al., 2015) followed by eukaryotic algae specifically members of green algae and diatoms (Flechtner et al., 2013 and Fučíková et al. 2014a, b) are the widely-distributed.

Arid and hyper-arid desert habitats are inherently low in nutrients specifically C, N and P (Housman et al., 2006 and Mager & Thomas 2011). To overcome this economicallyimportant problem, numerous studies on Cyanophytes particularly the heterocytous forms (Nostocales), have largely been carried out in order to characterize their highest potentialities on atmospheric N₂-fixtion and to be widely used as natural bio-fertilizers instead of agrochemicals (Osman et al., 2010 and Gheda & Ahmed, 2015). During the last decades, the algal bio-fertilizers technology has been proven to be a highly applicable and a key player instead of inorganic chemical fertilizers (Maqubela et al., 2009). Furthermore, this technique is commonly avoiding soil pollution by adding chemical fertilizers affecting human health (Abdel-Raouf et al., 2012), improves the nutrients-poor soils specifically those important for seedlings germination and crops productivity (Gheda & Ahmed, 2015) and they continuously fix atmospheric nitrogen into the soil even after crop harvest (Sahu et al., 2012).

The main scope of this work is to unearth and identify species composition of soil algal assemblages in El-Farafra Oasis, as well as evaluation of N_2 -fixation efficiency of certain isolated heterocytous cyanophytes.

Materials and Methods

Materials collection

Soil samples were collected through two meteorologically different seasons: summer 2011 and winter 2012. The soil collection was performed according to John (1942). The subsurface soil layers, normally to a depth of about 17 cm, were removed and freed from gravels and debris and finally had been collected in sterile clean air-tight plastic bags. In general, 30 soil samples were collected in this study. The soil materials were transported to the laboratory for culturing, isolation, identification and finally purification from other microorganisms. To evaluate the N₂-fixation efficiency of axenic mass cultures the most widely-distributed heterocytous blue-green algae were used.

Applied media, culture conditions, isolation and purification of algal taxa

Three different algal growth media: Chu #10 (Chu, 1942), BBM (Bold, 1949 and Bischoff & Bold, 1963) and COMBO medium (Kilham et al., 1998) were virtually used for cultivation of the soil algae based on their nutrients divergences and their highly applicable ranges for flourishing a wide variety of different algal divisions. Traditional technique for culturing and isolation of soil algae were recommended by Andersen & Kawachi (2005). The classification systems of Gollerbach et al. (1953), Desikachary (1959), De Desenko-Schegolova et al. (1959), De Desenko-Schegolova & Gollerbach (1962) and Philipose (1967) had been used for algal identification.

Evaluation of atmospheric N_2 *-fixation capacity*

Five heterocytous cyanophytes that easily give axenic and highest growth masses were recommended for the evaluation of atmospheric N_2 -fixation capacity using N-free Allen's medium (Allen, 1968). Then 1-ml of each cultured strain had been used as a standard inoculum to estimate the total number of individuals using Sedgewick Rafter Counting Cell Slide. Moreover, Acetylene Reduction Assay "ARA" of Hardy et al. (1973) [using digital gas chromatography DANI GC1000] was followed for determining N_2 fixation.

i.e., R is the amount of ethylene gas formed, air space of vial was 400 ml, algal culture volume was 100 ml, gas mixture injected into the column was 1 ml, incubation time was 24 h and 1000 = to convert ethylene concentration from nmole into μ mole.

Statistical analysis

Data for the N_2 -fixation evaluation were expressed as mean of three replicates \pm SD using the T test.

Results and Discussion

In total 47 soils algal morpho-species had been identified. These belonging to four algal divisions: Cyanophyta contributed the dominant division with 33 different species belonging to 18 genera, followed by 5 taxa belonging to each of Chlorophyta and Xanthophyta. Diatoms represented only by 3 species. Most of the identified Cyanophytes were related to order Nostocales and order Oscillatoriales. In agreement with these results, contributions of Flechtner et al. (1998), Johansen et al. (2001) and Alwathnani & Johansen (2011) on the hottest and driest Deserts of California, and Patzelt et al. (2014) on the hyper-arid Atacama Desert in Chile pointed out that the filamentous and heterocystous bluegreen algae like Schizothrix, Nostoc, Scytonema and Calothrix compose the main soil algal taxa. Westiellopsis prolifica is also considered as one of the main subtropical arid soil habitats (Tiwari et al., 2005). Concerning green algae, there are some recent studies pointed out their remarkable distribution in dry desert habitats (Cardon et al., 2008; Flechtner et al., 2013 and Fučíková et al. 2014a, b). Scarcity of diatoms and xanthophytes in soil algae was also confirmed by contributions of Zancan et al. (2006).

The most frequent taxa on all applied growth media were represented by: Anabaena variabilis Kütz., Lyngbya limnetica Lemm., Stratonostoc. linckia f. calcicola (Bréb.) Elenk., Westiellopsis prolifica Janet, Chloridella simplex Pasch, Pleurochloris pyrenoidosa Pasch, Chlamydomonas dactylococcoides Scherff. et Pasch and Chlamydomonas globosa Snow.

Where ARA (μ mole C₂H₄/ml/h) =

Reading (R) x air space in the vial (ml) x 1000

culture volume (ml) x gas voulume injected for ethylene measurement (ml) x incubation time (h)

Based on available literature (El-Gamal, 1990; Shaaban, 1994; Mansour, 2004; Hamed, 2008 and Mansour & Shaaban, 2010), this work enriched for the first time the Egyptian soil algal flora with 5 different algal taxa: *Cylindrospermum gregarium*, *C. licheniforme, Chlorocloster caudatus* and *Tetraktis aktinastroides* (Fig. 1; A-D), in addition to Westiellopsis prolifica (Fig. 2; E1-E2).

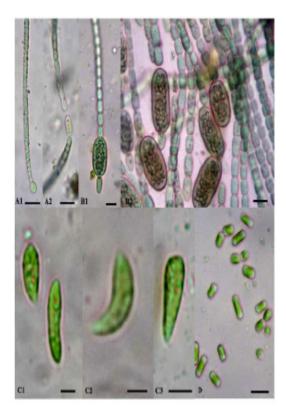


Fig. 1 (A–D): A1-2, Cylindrospermum gregarium (Zakrz.) Elenk; B1-2,Cylindrospermum licheniforme (Bory) Kütz.; C 1-3, Chlorocloster caudatus Pasch. and D, Tetraktis aktinastroides Pasch. Scale bar = 10 μm.

In the late exponential phase, Calothrix elenkinii Kossinsk, Nodularia harveyana f. sphaerocarpa (Born. et Flah.) Elenk, Scytonema ocellatum Lyngb, Stratonostoc linckia (Roth) Elenk. and Westiellopsis prolifica Janet (Fig. 2. A-E2) were easily give mass growth and axenic cultures. So, these five heterocystous cyanophytes were selected and recommended for the evaluation of atmospheric N_2 -fixation efficiency.

 N_2 fixation rates presented in Table 1 and Fig. 3 showed that, there are remarkable differences

among the studied heterocytous cyanophytes. In particular, *Calothrix elenkinii, Westiellopsis prolifica* and *Scytonema ocellatum* exhibited to a large extent the highest fixation rates with mean values of 18.89, 30.52 and 33.01 µmole ml⁻¹ h⁻¹, respectively. *Stratonostoc linckia* and *Nodularia harveyana* f. *sphaerocarpa* had the 4th and 5th ranks with mean values of 16.71 and 12.01 µmole ml⁻¹ h⁻¹, respectively.

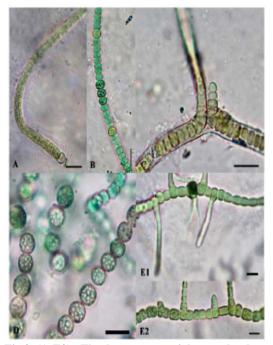


Fig.2 (A-E2). The late exponential growth phase of: A, Calothrix elenkinii Kossinsk; B, Nodularia harveyana f. sphaerocarpa (Born. et Flah.) Elenk; C, Scytonema ocellatum Lyngb; D, Stratonostoc linckia (Roth) Elenk and E1 & E2, Westiellopsis prolifica Janet. Scale bar = 10 μm.

Variations in amounts of N₂ fixation rates in different blue-green algae taxa were discussed in some previous studies (Moisander et al., 1996; Osman et al., 2010; Gheda & Ahmed, 2015 and Khanna et al., 2016). This study pointed out that Calothrix elenkinii, Scytonema ocellatum and Westiellopsis prolifica have the highest potentials of N₂ fixation on the basis of individual unit. Therefore, they could be widely-used as excellent natural bio-fertilizers and in reclamation of the nutrients-poor desert soils of El-Farafra Oasis. In agreement with these results, recent contribution of Priya et al. (2015) showed that there is a significant increase in rice plant growth inoculated with Calothrix elenkinii. Goyal (1993) confirmed that Scytonema grows well on the soil surface and fixes more nitrogen than the most

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widely-distributed genera such as *Nostoc* and *Anabaena*. In addition, González (2003) stated that, *Scytonema. ocellatum* isolated from the tropical soil in Mexico not only could be used as an excellent soil bio-fertilizer but it also improves

the soil water-holding capacity. As regard to *Westiellopsis prolifica*, there are some studies supported well its utilization as soil bio-fertilizers (Jha et al., 1987; Jha & Kaushik, 1988; Prabu & Udayasoorian, 2007; Singh & Dhar, 2010 and Paudel et al., 2012).

TABLE 1. Mean values (mean \pm SD) of the produced C_2H_4 and the fixed atmospheric N_2 by the heterocytous cyanophytes.

Algal taxa	C ₂ H ₄ concentrations (nmole ml ⁻¹ h ⁻¹)	Nitrogen fixation rate (μmole ml ⁻¹ h ⁻¹)
<i>Calothrix elenkinii</i> (45 x 10 ³ individuals/ml)	4721.89 ± 557.67	18.89 ± 2.23
Nodularia harveyana f. sphaerocarpa (91 x 10 ³ individuals/ml)	3003.67 ±1541.70	12.01 ± 6.17
Scytonema ocellatum (198 x 10 ³ individuals/ml)	8252.20 ±5870.01	33.01 ± 23.48
Stratonostoc linckia (485 x 10 ³ individuals/ml)	4177.89 ±3462.10	16.71 ± 13.85
Westiellopsis prolifica (223 x 10 ³ individuals/ml)	7630.50 ±1775.20	30.52 ± 7.10

Values are expressed as mean \pm SD, n=3.

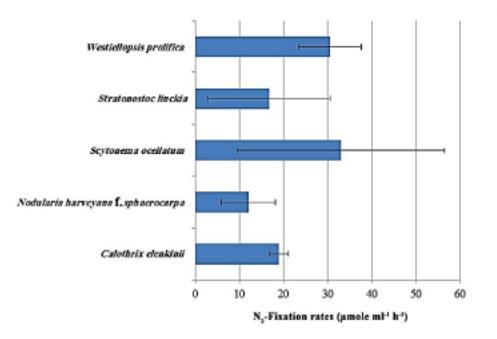


Fig.3. Atmospheric N_2 -Fixation rates (mean \pm SD) in the selected five axenic heterocystous cyanophytes.

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Conclusion

In conclusion, the isolated blue-green algal taxa of El-Farafra Oasis, specifically *Scytonema ocellatum* and *Westiellopsis prolifica* can be considered as promising eco-friendly natural biofertilizers for the sustainable development in the desert habitats.

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طحالب تربة واحة الفرافرة (الصحراء الغربية، مصر) و كفاءة تثبيت النيتروجين الجوى لخمسة من الطحالب الخضراء المزرقة ذات الحويصلات المغايرة

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تم زراعة و تعريف 47 نوع مختلف من طحالب تربة واحة الفرافرة (الصحراء الغربية، مصر). أغلب الأنواع التي تم تسجيلها تتبع الطحالب الخضراء المزرقة (34 نوع)، متبوعة بـ 5 أنواع لكل من الطحالب الخضراء و الخضراء المصفرة. و تم أيضا تمثيل الدياتومات من خلال 3 أنواع فقط. هذا وقد أضافت هذه الدراسة 5 أنواع طحلبية جديدة على ما تم تعريفه من طحالب التربة المصرية، وهي:

Westiellopsis prolifica Janet, Cylindrospermum gregarium (Zakrz.), Cylindrospermum. licheniforme (Bory) Kütz., Chlorocloster caudatus Pasch. and Tetraktis aktinastroides Pasch.

علاوة على ذلك، تم تقدير كفاءة تثبيت النيتروجين الجوى لخمسة من الطحالب الخضراء المزرقة ذات الحويصلات المغايرة مثل:

Calothrix elenkinii Kossinsk., *Nodularia harveyana* f. *sphaerocarpa* (Born. et Flah.) Elenk., *Scytonema ocellatum* Lyngb., *Stratonostoc linckia* (Roth) Elenk. and *W. prolifica* Janet.

ولقد أسفرت النتائج أن كل من C. elenkinii. S. ocellatum, W. prolifica لديهم القدرة الأعلى على تثبيت النيتروجين الجوى بمتوسط قيم 33.01 , 30.52 g mole ml⁻¹ h⁻¹18.89 بالتتابع، و يليهم فى ذلك كل من الطحالب:

S. linckia (16.71 µmole ml-1 h-1) ٹ
مN. harveyana f. sphaerocarpa (12.01 µmole ml-1 h-1)

و نستنتج من هذه الدراسة على إمكانية إستخدام الخمس طحالب سالفة الذكر خاصا (Scytonema ocellatum , Westiellopsis prolifica) كمخصبات بيولوجية, واعدة وصديقة للبيئة للتنمية المستدامة في البيئات الصحر اوية.