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Chlorella vulgaris and *Chlorella pyrenoidosa* live cells appear to be promising sustainable biofertilizer to grow rice, lettuce, cucumber and eggplant in the UAE soils

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ABSTRACT

The culture conditions of *Chlorella vulgaris* and *Chlorella pyrenoidosa* under laboratory and field conditions have been standardized. *C. pyrenoidosa* could survive under field conditions at 40°C in 1 L cultures bottles, whereas *C. vulgaris* could not survive at this temperature. When the salt tolerance of the two species was tested against three levels of salt, *C. vulgaris* could survive in 1.0% NaCl with a density of 87 $\times$ 10^4/ml cells while *C. pyrenoidosa* did not survive at this level of salt. The total chlorophyll content (chlorophyll a and chlorophyll b) of *C. pyrenoidosa* is higher (1780 mg/g fresh weight) than that of *C. vulgaris* (12360 mg/g fresh weight) for the same mass of cells. When chlorophyll rich, salt tolerant *C. pyrenoidosa* live cells (11.8 $\times$ 10^4/ml) were used as biofertilizer, rice seedlings had enhanced root system, cucumber seeds had quicker germination, and 1 month old seedlings had green leaves with 2 times higher chlorophyll content than the respective control seedlings. Enrichment of salt affected soils with these green algae *C. vulgaris* and *C. pyrenoidosa* may be a possible option to grow these crops in the UAE.

KEY WORDS: Biofertilizer, *Chlorella pyrenoidosa*, *Chlorella vulgaris*, hemocytometer, molecular markers, salt tolerance

INTRODUCTION

The UAE soils are infertile without humus and the high temperatures during summer seasons do not promote microorganisms that can enrich the soils. In addition to this condition, soils also contain a high amount of salt, more than 8000 parts a million (8-16 dS/m), which is a threat to agriculture (Henzell, 2009). Efforts to grow vegetable crops in these soils are being explored through different ways, of which enriching the soils with biofertilizers is one method. Supplementing the soils may meet the nutrient need of the crop and allow the crop with a better yield. Nitrogen or phosphorous fertilizers also can be added to soil, however, biofertilizers are preferred to chemical fertilizers because they do not cause adverse effects to soil or environment or the crops. Usually, nitrogen fixing bacteria and growth promoting bacteria, mycorrhizae, and free floating ferns are used as biofertilizers for different crops. During the last decade, chlorophycean members were also being explored as biofertilizer as they are rich in carbohydrates, proteins, lipids, and growth hormones.

Among the members of Chlorophyta, *Chlorella* strains are considered as competent candidates, which promoted plant growth when used individually as in lettuce (Faheed and Abd-El Fattah, 2008) or along with rhizobacteria as in clovers (Raposo and De Morias, 2011). Species of *Chlorella* have varied tolerance to salt and also to a range of temperature from 25°C to 40°C (Sankar and Ramasubramanian, 2012; Sawant *et al.*, 2014).

In this study, an attempt is made to culture *Chlorella vulgaris* and *C. pyrenoidosa* under laboratory and field conditions with the use of *C. pyrenoidosa* as biofertilizer to grow vegetable crops viz., lettuce, rice, eggplant and cucumber in salt affected soils. *C. vulgaris* was chosen for

the present study because it contains high amounts of minerals, protein, vitamins and carotenoids, and is also reported to survive in cultures with 5% petrol/kerosene and up to 15 days in 20% petrol/kerosene (Agrawal *et al*., 2007). *C. pyrenoidosa* was better than fytozyme to enhance the yield of soybean (Dubey and Dubey, 2010).

As it is extremely difficult to identify the phytoplankton through light microscopic study, we used specific 18S DNA primer to authenticate the genus (Sawant *et al*., 2014) and attempted to identify the strain with microsatellite markers (Jo *et al*., 2014). The present work includes:

- Authentication of *Chlorella*
- Standardization of culture conditions for the two species and
- Use of live cells of *C. pyrenoidosa* as biofertilizer, for-lettuce, rice, eggplant, and cucumber.

To the best of our knowledge, this is the first report on the use of *Chlorella* live cells as biofertilizer to grow these crops in salt affected, infertile UAE soils.

MATERIALS AND METHODS

Source of *Chlorella* Cells

C. vulgaris culture was obtained from Shalimar Biotechnology LLC, located in Dubai, UAE. *C. pyrenoidosa* cells were retrieved from the tablets of Sun *Chlorella* A, USA. One 200 mg tablet was dissolved in 1 L of water; the bottles were maintained in the laboratory at 22 ± 1°C or in the field with 14/10 h light/dark conditions. The temperature of the field varied between 32°C and 40°C during February to May 2015. The culture was checked periodically every 3 days once for the retrieval of cells.

Pre Inoculum and Maintenance of Mother Culture

Two species of *Chlorella* cells were grown in water in the laboratory and also in the field conditions. The cells were suspended in fresh water once a week. The cells were also screened against three concentrations of salt viz., 0.01%, 0.1%, and 1.0%. A small volume of 20 µl of pre-inoculum was checked under a light microscope for the presence of single cells, auto-sporangia, and akinetes before it was used as pre-inoculum to inoculate the different culture media.

Media Preparation and Culture of *Chlorella* Cells in Bold's Basal and Chu 10 Media

Bold's basal medium (Bischoff and Bold, 1963) and Chu 10 medium (Chu *et al*. 1942), were the most common used nutrient media for culture of green algal cells. Bold's and Chu 10 media, each supplemented with 0.0, and/or 0.01%, and/or 0.1%, and/or 1.0% NaCl was used to culture the two species of *Chlorella* cells in the present study. The pH of the medium was adjusted to 7.0 with 1 N NaOH before the medium was autoclaved. About 10% of the 1-week old *Chlorella* culture was used as a pre-inoculum. For instance, 10 ml of the culture was inoculated with 1 ml of pre-inoculum. The cells were then harvested by spinning the culture at 13000 rpm for 30 s, the supernatant is discarded, and the pellet is washed twice using the medium, the loose pellet after the 2nd wash was used as pre-inoculum, to culture the media. The culture vials and bottles were maintained with one set in the growth chamber at 22 ± 1°C with light intensity 150 mol/m^2/S, photoperiod 14 h/10 h light/dark; while the other set of cultures were maintained in the field conditions at 32-40°C, with 14 h/10 h light/dark cycle. The duration of light/dark cycle in the growth chamber was so adjusted to match with the photoperiod of the field conditions. The experiment is repeated 3 times, cell counts – mean and standard deviations were made for each replicate.

Light Microscopy and Determination of Cell Density on Hemocytometer

For light microscopy, the cells were mounted on flat glass slides and examined under light microscope (XSZ-106T, NO 000058). Photographs were taken under ×40 and oil immersion ×100 objective lens.

The cell density for each of the media was estimated using hemocytometer (Neubauer-improved). The formula used for counting the cell determinations was:

$$\text{Number of cells in the original sample} = \frac{\text{Number of cells counted}}{\left(\text{Proportion of chamber counted}\right)\left(\text{Volume of squares counted}\right)} \times 10^4$$

Culture of *Chlorella* Cells in Solid Media

C. vulgaris and *C. pyrenoidosa* cells were harvested from 1 ml cultures by spinning the cultures at 13000 rpm for 30 s; 100 µl of the loose pellet was streaked on Bold's basal or Chu 10 medium each supplemented with 0.1% NaCl. The culture plates were then maintained in the growth chamber at 14 h/10 h light/dark at 22 ± 1°C temperature.

After the development of colonies on the solid media, to obtain pure cultures, the single colonies from each plate were cultured in 10 ml liquid cultures for a week.

Verification of *Chlorella* Cells with 18S rDNA Primer and Strain Identification with Specific Molecular Markers

DNA extraction

DNA was isolated from *C. vulgaris* and *C. pyrenoidosa* cells using Accuvis bio DNA isolation kit (Norgen, Canada) following the manufacturer's instructions. The isolated DNA samples were electrophoresed on 2% agarose gel at 100 V, the gels were then viewed and photographed under UV illumination.

Polymerase Chain Reaction (PCR) Amplification

PCR reaction was performed in a 20 µl reaction mixture containing 1 µl (15-25 ng) of extracted genomic DNA, 10 µl ×2 PCR master mix from Accuvis Bio, 1 µl (10 µM) of each primer set and 7 µl of sterile distilled water)using a thermal cycler programmed for 35 cycles (Veriti PCR Machine, Applied Bio systems, USA). A sample without a template DNA was included in the reaction as negative control. The PCR amplification conditions were 95°C for 5 min, followed by 35 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s with a final extension at 72°C for 7 min. The PCR products were electrophoresed on 1% agarose gel at 100 V, the gels were viewed and photographed under UV illumination (Table 1).

Estimation of Chlorophyll Content in *C. vulgaris* and *C. pyrenoidosa*

About 500 mg of *C. vulgaris* and of *C. pyrenoidosa* cells were harvested from the respective cultures, incubated in 80% acetone for 3 h and/or 24 h in the dark. Total chlorophyll content of the cells was estimated by measuring the absorption at 652 nm (Gillmartin and Bowler, 2002) on a spectrophotometer (Digital UV Plus RS, UV 2502 model from Labomed) implementing the following the formula (Jiwan, 1990).

C = Chlorophyll content µg/ml C = A 652/36 acetone extracts

C = (1000 × A 652 nm/36) × 500 mg/g fresh weight of leaf tissue

C. pyrenoidosa as a Bio-fertilizer

Seed germination in Petri dishes

Seeds of cucumber, eggplant, rice and lettuce were incubated in each Petri plate on Whatman filter paper, and watered with 2 ml of water or 2 ml of *C. vulgaris* solution containing live cells 289 × 10^4/ml and/or *C. pyrenoidosa* of cell density 11.8 × 10^4/ml twice a day. The experiments were done in three replicates of about 20 seeds per each replicate. The percentof seed germination was calculated for each plant.

Seed germination in potted soils

As the chlorophyll content of *C. pyrenoidosa* was higher, only *C. pyrenoidosa* was used as biofertilizer for potted plants. The seeds were sown in potted soil and were watered with water or *C. pyrenoidosa* solution (≈11 × 10^5 cells). The seedlings were grown up to 4 weeks; the chlorophyll content was estimated from the leaves of the control and the treated plants. The protocol for chlorophyll estimation was similar to that carried for *Chlorella* cells. For each plant 500 mg of leaves was weighed and incubated in 10 ml of 80% acetone in dark conditions for a period of 3 h and 24 h, from which the total chlorophyll content was estimated.

RESULTS

Pre Inoculum and Maintenance of Mother Culture

C. vulgaris cells could grow in water and also in water containing NaCl under the laboratory 22 ± 1° conditions (Table 2). Light microscopic observation revealed the cells which are green, spherical each with a chloroplast, nucleus and colorless cell wall. 1-week old cultures had single cells and akinetes without anyautosporangia. 1-month old mother culture had 63.6% of single cells and 36% of akinetes (Table 2). Presence of akinetes indicates unfavorable conditions; probably the water was depleted of nutrients resulting in high per cent (36%) of akinetes.

Table 1: List of PCR primers and their sequences used in this study

Primer	Sequence from 5′ to 3′
18S rDNA	F: CTG CGA ATG GCT CAT TAA ATC
	R: AAG GCC AGG GAC GTA ATC AA
mChl-001	F: CCT ATT GCT CTA TGT TAA CAT ATG
	R: GTT TTG AAT TTT TCC CCA TTG CTG
mChl-002	F: ACA GGC CAG TCA ATT TAT TT
	R: CAC TAC ATC GTC TAT TTG ACA TTG AG
mChl-005	F: CAA GCC AAT TTT ATT TAA AAT C
	R: AGG TTC ACC TCT TCG CCT AA
mChl-011	F: CAG TAT AGA GTA CAC GAT TTT CC
	R: GAG CGT GTA ATT GTT ATA ACT TC
mChL-0012	F: CGC TAT AGT CAT AGC GTC GTG ACG
	R: CTT GAA AGC TTC ATG AGG AGT GCC

PCR: Polymerase chain reaction

Table 2: Salinity tolerance of *Chlorella vulgaris* cells in water after 1 month of culture

Cell cultured in	% of single cells	% of akinetes	Total number of cells/ml
Water	63.6	36	845
Water+0.01% NaCl	93	7	876
Water+0.1% NaCl	90.1	9.8	950
Water+1.0% NaCl	66.3	33.6	666

The percent of akinetes was reduced in the 0.01% and also in 0.1% of NaCl concentrations indicating that akinetes could disperse the spores resulting in increased single cells and a decrease in per cent of akinetes. However, in 1% NaCl, high per cent of akinetes and decrease in total cell numbers reveal unsuitable culture conditions.

The cells in the field conditions did not survive in water with or without NaCl, inferring their sensitivity to high temperature 35°C.

Retrieval of *C. pyrenoidosa* Cells

C. pyrenoidosa cells could be retrieved from the tablet in water under the field conditions in 4 weeks (Figure 1c). The retrieved cells were cultured in Bold's basal medium and Chu 10 medium each containing different concentrations of salt.

Culture of *C. vulgaris* and *C. pyrenoidosa* cells in Bold's Basal and Chu 10 Media

1-week old *C. vulgaris* cells grew better in Chu 10 medium with the highest cell number (Figure 2a and b). A few tetra-sporangia and a few auto-sporangia and 4-celledsporangia were also observed in the media (Figure 1a and b). Sporangia with 4 spores (cells) were more frequent than the multispore auto-sporangia. Although, *C. vulgaris* cell numbers were higher in Bold + 1.0% NaCl medium (Figure 2a), auto-sporangia were not detected in any of the three replicates after 1-week of culturing. This indicates that the initial sporangia must have released the spores and new sporangia are not formed at this salt concentration. The cells did not survive when the cultures were in the field at 32-40°C.

C. pyrenoidosa cells grew better in Bold's basal medium, tolerating salinity up to 0.01% only in the laboratory conditions (Figure 2c and d). Under field conditions, the cells survived in both the media and also in water, only in 1 L culture bottles without salt. The cells did not survive in small-scale cultures (10 ml and 100 ml), probably because the small volume of the cultures get heated up to 40°C, with the outside environment which must be detrimental to cell survival (Figure 3a, b and f).

After the *C. pyrenoidosa* cells were retrieved from the tablet, they did not retain their colonial form, but only single cells, dyads and tetrads and auto sporangia were observed in the water and media cultures (Figure 1d and e).

Culture of *Chlorella* Cells in Solid Media

C. vulgaris colonies developed in 2 days in the solid media under laboratory conditions (Figure 3d) while *C. pyrenoidosa* colonies developed in 2-3 weeks (Figure 3e). *C. pyrenoidosa* cultures when observed under the light microscope, the cultures of seemed to be maintained ascolonies on solid culture media (Figure 3c). The growth of *C. vulgaris* in solid media is faster than the growth of *C. pyrenoidosa* under similar culture conditions.

DNA Isolation and PCR Verification of *Chlorella*

Genomic DNA of the two species of *Chlorella* were isolated and amplified with the 18S rDNA and specific microsatellite primer (4), of the product size of 175 bps (Figure 4a-c).

Estimation of Chlorophyll Content in *C. vulgaris* and *C. pyrenoidosa*

The estimated Chlorophyll content of 500 mg of *Chlorella* cells incubated in 80% acetone for 3 h samples was lesser compared to 24 h incubation samples in both the species. The chlorophyll estimates were made from 24 h incubated samples. The pigment concentration (Chlorophyll a and b)

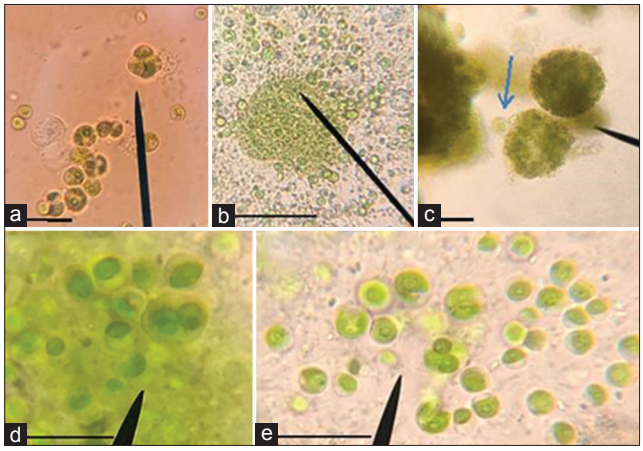

Figure 1: *Chlorella* cells in culture media. (a and b) *Chlorella vulgaris* cells in Chu 10 culture medium, magnification 100 µ (b) single multi-celled auto-sporangium, magnification 10 µ, (c-e) *Chlorella pyrenoidosa* colonial cells retrieved from the tablet dissolved in Bold's basal medium after 20 days of culture in 1 L culture under field conditions. (c) 2 Colonies, individual cells can be seen in colony 1 (arrow) magnification 10 µ. (d and e) Dyads (d) and tetrads (e) magnification 10 µ

Table 3: Seed germination percent in control and treated plants of eggplant, coriander, lettuce and cucumber (Petri plates are at 22°C, 14 h/10 light/dark)

Plant	Number of seeds incubated		% of seed germination			
	Water	*C. vulgaris*/ *C. pyrenoidosa*	Water	*C. pyrenoidosa*	water	*C. vulgaris*
Lettuce	60	60	83	75	98	90
Cucumber	60	60	80	97	60	70
Egg plant	60	60	100	100	74	76.5
Rice	60	60	60	61.5	60	62

C. vulgaris: Chlorella vulgaris, C. pyrenoidosa: Chlorella pyrenoidosa

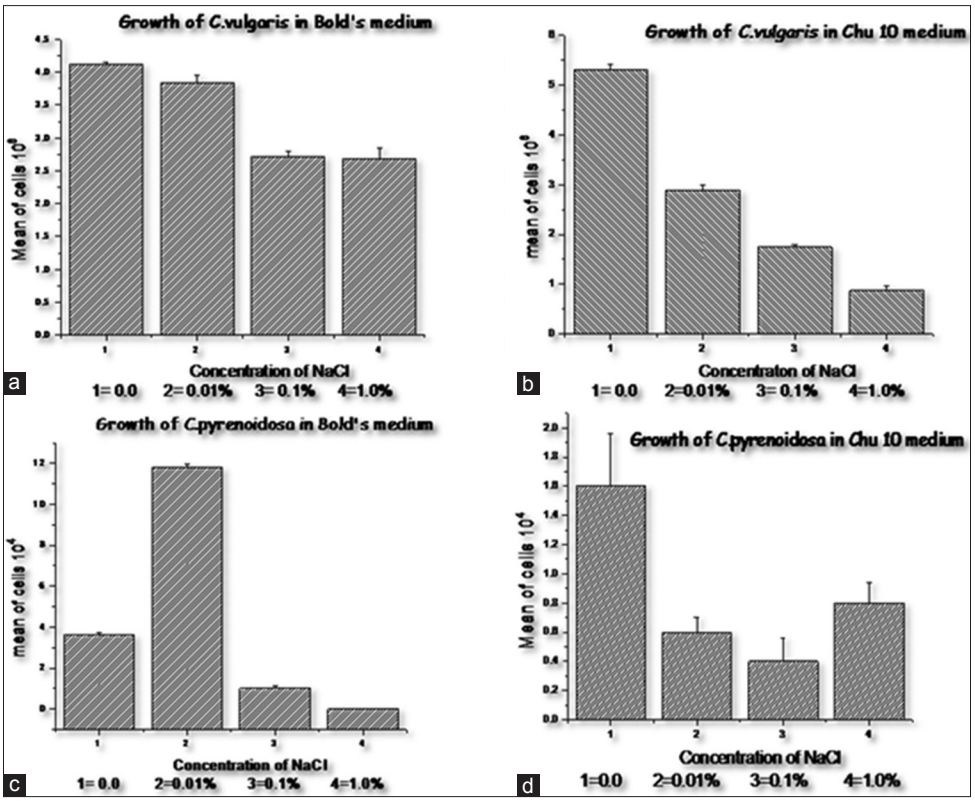

Figure 2: (a-d) Cell densities (mean and standard deviation) of 1 week old cultures of *Chlorella vulgaris* and *Chlorella pyrenoidosa* in Bold's basal and Chu 10 media ± NaCl. (Cultures were at 22°C, 14 h/10 h light/dark)

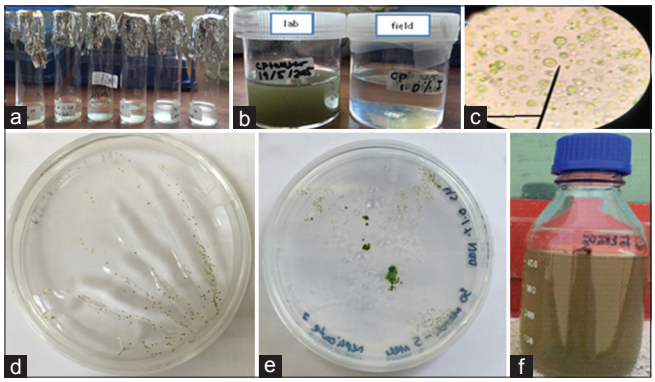

Figure 3: Liquid and solid cultures of *Chlorella vulgaris* and *Chlorella pyrenoidosa*. (a, b and f) 10 ml and 1 L (f) cultures under field conditions. (c) Colonies of *C. pyrenoidosa* from culture plates, magnification 100 µ. (d) *C. vulgaris* colonies in Chu 10 plates 2 days after culture, at 22 ± 1°C with 14/10 h light/dark. (e) *C. pyrenoidosa* colonies in Bold's basal medium 3 weeks after culture, at 22 ± 1°C with 14/10 h light/dark

of *C. vulgaris* is lesser than that of *C. pyrenoidosa* for same mass of cells (500 mg) in all three replicates (Figure 5a).

Seed Germination in Petri Dishes and Pots

Seed germination in the treated samples is either the same or higher than in the controlsin all crops except in lettuce (Table 3). Treated 2 weeks old seedlings of rice in the petri dishes were healthier with enhanced root system (Figure 6b and d). The germinated seedlings of lettuce survived for a week when they were not watered with *Chlorella* solution while the control seedlings withered in 3-4 days (Figures 6a and c). Three to 4 weeks old seedlings of cucumber and egg plants in pots had greener and bigger leaves (Figure 7a-c and e); cucumber seedlings were disease resistant (Figure 7d) when watered with chlorella solutions.

Estimation of Chlorophyll Content in 1-month Old Seedlings

The estimated chlorophyll content from 3 h incubation samples were very low (875 mg/g vs. 1780 mg/g fresh weight) henceforth, the experiments were continued with 24 h incubation samples. The Chlorophyll a and b content was higher in the treated leaf samples than in the controls in all the crops except in rice (5b), the difference is more evident in cucumber seedlings (Figures 5b and 7c). Although the total chlorophyll content of rice seedlings was lesser in the treated rice seedlings than in their controls, the enhanced root growth reflects the effect of *C. pyrenoidosa* as the biofertilizer.

Each sample had 20 seeds, the experiment is done in 3 replicates and the data for the 3 replicates is pooled.

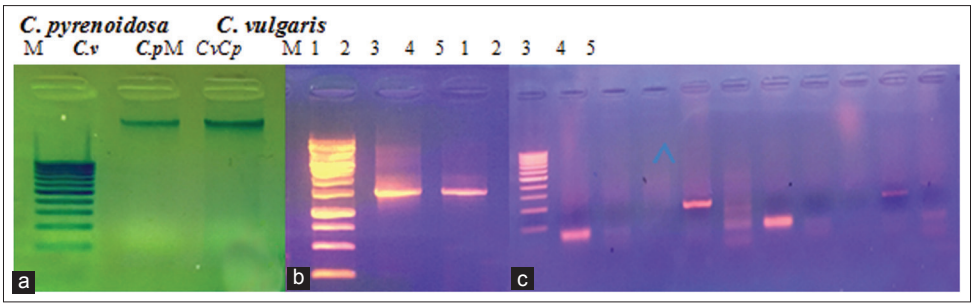

Figure 4: Agarose gels (1%) showing genomic DNA and polymerase chain reaction (PCR) products. (a) Genomic DNA of *C. vulgaris* and *Chlorella pyrenoidosa*. (b) PCR amplification products with 18S rDNA primer. (c) Lane 4 and 9 showing PCR products with primer set 4

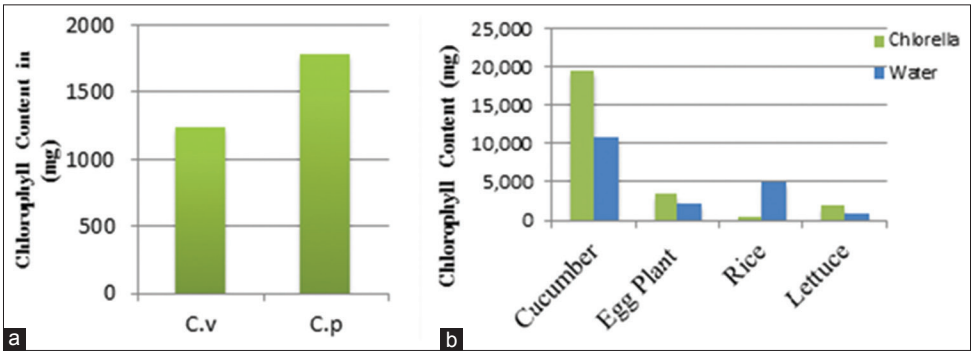

Figure 5: Estimation of Chlorophyll a and b (a) Chlorophyll content in *Chlorella vulgaris* and *Chlorella pyrenoidosa*. (b) Chlorophyll content in control and treated cucumber, eggplant, coriander and lettucex

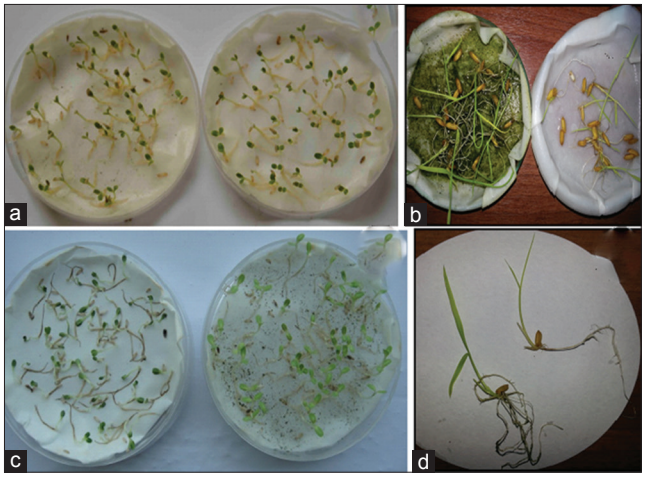

Figure 6: Seed germination in Petri plates (at 22 ± 1°C) and in pots (35-40°C), both at 14 h/10 h light/dark (a) 4 days old seedlings of lettuce at the beginning of water stress (b and d) 2 weeks old rice seedlings (b), treated seedlings with enhanced root system (d). (c) Wilted (control) and healthy (treated) seedlings after 7 days of water stress at 22 ± 1°C

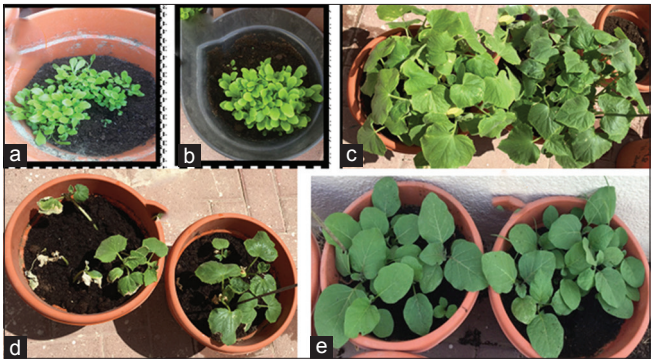

Figure 7: 3 to 4 weeks old seedlings in pots irrigated with biofertilizer and/or water under field conditions: (at 35-40°C, and 14 h/10 h light/dark). (a and b) 3 weeks old seedlings of lettuce control (a) and treated (b). (c) 3 weeks old cucumber seedlings, with greener leaves in treated (right side). (d) 3 week old cucumber seedlings, treated are disease resistant. (e) 4 weeks old eggplant seedlings with bigger leaves in treated

DISCUSSION

C. vulgaris cells grew in both the media - Bold's basal medium and Chu 10 medium under the laboratory conditions. Chu 10 medium is reported to be the most suitable medium for some strains of *C. vulgaris* (Sharma *et al*., 2011) and the present strain is in accordance with the report.

Although, the tolerance of *C. vulgaris* to 1.0% salt was better in Bold's basal medium, no auto-sporangia could be detected in any of the three replicates, in 1-week old cultures, indicating that they require longer duration to reproduce or fail to do so under the salt stress. The microalgal growth is also controlled by the light and temperature besides the nutrient media, *C. vulgaris* cells grew better under natural light at 25-30°C than at 30-35°C (Sharma *et al*., 2012). The present strain of *C. vulgaris* also is temperature sensitive, as they did not

survive at 35°C. Nutrient media, temperature and light intensity are reported to have an effect on the phenotype of the microalgae (Soylu and Gonulol, 2012), which could be a possible reason for the loss of colonial state of *C. pyrenoidosa*, in the present study.

DNA Isolation and PCR Verification of *Chlorella*

PCR amplification of the genomic DNA of the two species with 18SrDNA primers conforms that the cells are *Chlorella*. However, it cannot be concluded at this stage that *C. pyrenoidosa*is a strain of *C. vulgaris* and not a different species unless the PCR products are sequenced, which is not the aim of the present work. So also, the amplification product of primer 1 was lesser than the expected 154 bps size, hence it cannot be concluded at this stage whether it had less number of repeats (TTA) or it was a nonrandom amplification product of PCR.

Seed Germination in Petri Dishes and Pots

The treated seedlings of the four crops had a positive effect of the biofertilizer which were with greener leaves and were healthy. Similar response of greater number of leaves with bigger surface area was observed in soybean seedlings irrigated with *C. pyrenoidosa*, and our present study is similar to this earlier report (Dubey and Dubey 2010). Although infection was not induced in the cucumber seedlings, the control seedlings had infections while the treated seedlings were healthy. Resistance to disease among crops irrigated with *Chlorella* cells is not a new phenomenon as was reported in Grape vines (Bileva, 2012). Lettuce seedlings were positively affectedwith increased fresh and dry weight when watered with *C. vulgaris* (Faheed and Abd-El Fattah, 2008). In the present study, lettuce seedlings watered with *C. pyrenoidosa* solution under laboratory conditions could withstand water deficit up to 7 days. Tolerance of water stresses with *Chlorella* solution is being reported for the 1st time.

Estimation of Chlorophyll Content in 1-month Old Seedlings

The chlorophyll content of the treated seedlings is higher in all crops except in rice seedlings; however, the rice seedlings had enhanced root growth indicating biofertilizer effect. Enhanced root system with rhizobacteria application in rice fields is an established fact, and recently in combination with microalgae is being experimented; a positive effect on plant growth and root system in reported in clover plantlets (Raposo *et al*., 2005). Although the yield of the four crops has not been obtained, based on the seedling growth, chlorophyll content and drought tolerance, irrigation of seedlings with *C. pyrenoidosa* seems to be a promising tool to grow these vegetable crops in the UAE soils. Identification of the strain of *C. vulgaris* is mandatory to recommend it as biofertilizer which is in the process. Further application of *Chlorella* species in hydroponics as a nutrient supplement will be interesting to explore.

CONCLUSIONS

To the best of our knowledge, this is the first report on the use of live *Chlorella* cells as biofertilizer to grow crops in the UAE soils. The study opens the possibility of utilizing *C. pyrenoidosa* live cells as a potential source of biofertilizer without causing environmental pollution. Further studies on evaluation of yield and taste of these vegetable crops are imperative to conclude its use and to recommend to fruit crops.

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REFERENCES

Agrawal SC, Manisha. Growth, survival and reproduction in *Chlorella vulgaris* and *C. variegata* with respect to culture age and under different chemical factors. Folia Microbiol (Praha) 2007;52:399-406.

Bileva T. Influence of green Algae *Chlorella* vulgaris on infested *Xiphinema index* grape seedlings. J Earth Sci Clim Change 2013;4:2.

Bischoff HW, Bold HC. Physiological studies IV. Some Soil Algae from Enchanted Rock and Related Algal Species. Vol. 6318. Texas: University of Texas Publications; 1963. p. 1-95.

Chu SP. The influence of the mineral composition of the medium on the growth of planktonic algae. Part I. Methods and culture media. J. Ecol 1942;30:284-325.

Dubey A, Dubey DK. 2010. Evaluation of cost effective organic fertilizer. 17043. p. 1-7. Available from: http://www.Orgprints.org. [Last accessed August, 2015].

Faheed FA, Abd-El Fattah Z. Effect of *Chlorella* vulgaris as bio-fertilizer on growth parameters and metabolic aspects oflettuce plant. J Agric Soc Sci 2008;4:165-9.

Gillmartin P, Bowler C. Chlorophyll determination as a marker for chloroplasts. Molecular Plant Biology: A Practical Approach. Vol. 2. Oxford University Press; 2002. p. 167.

Henzell J. Soil samples indicate land degradation. In: UAE National; 2009. Available from: http://www.thenational.ae/news/uae-news/environment/soil-samples-indicate-land-degradation.

Jiwan PP. Instrumentation for studying vegetation canopies for remote sending in optical and thermal infrared regions. Remote Sens Rev 1990;5:207-13.

Jo BH, Lee CS, Song HR, Lee HG, Oh HM. Development of novel microsatellite markers for strain-specific identification of *Chlorella* vulgaris. J Microbiol Biotechnol 2014;24:1189-95.

Raposo MF, Oliveira SA, Morais RM. Evaluation of soil fertilization with encapsulates of *Chlorella* vulgaris and a *C. vulgaris*/Pseudomonas putida Consortium for the Growth of Meadow Clover Plantlets; 2005.

Raposo MF, De Morais RM. *Chlorella* vulgaris as Soil amendment: Influence of encapsulation and enrichment with rhizobacteria. Int J Agric Biol 2011;13:719-24.

Sankar M, Ramasubramanian V. Biomass production of commercial algae *Chlorella vulgaris* on different culture media. E J Life Sci 2012;1:56-60.

Sawant SS, Joshi AA, Bhagwat A, Kelkar-Mane V. Tapping the antioxidant potential of a novel isolate- *Chlorella* emersonii. World J Pharm Res 2014;3:726-39.

Sharma R, Singh GP, Sharma VK. Comparison of different media formulations on growth, morphology and chlorophyll content of green alga, *Chlorella vulgaris*. IIJBS 2011;2:B509-16.

Sharma R, Singh GP, Sharma VK. Effects of culture conditions on growth and biochemical profile of *Chlorella vulgaris.* J Plant Pathol Microb 2012;3:1-6.

Soylu EN, Gonulol A. Morphological and 18S rRNA analysis of coccid green algae isolated from lakes of Kizihrmak Delta. Turk J Biol 2012;36:247-54.