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Cyanobacteria cultivation using olive milling wastewater for bio-fertilization of celery plant

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ABSTRACT

Olive milling wastewater is a major problem facing the Mediterranean countries producing olive oil like Egypt. In the present study, olive milling wastewater rich with organic phenolic compounds, macro and micro nutrients was used as growing media for cyanobacteria. The cyanobacteria were grown on wastewater to obtain two biofertilizers, one bioformulated from single culture of Spirulina platensis and the second from mixed culture of S. platensis, N. muscorum and A.oryzae. The produced biofertilizers, were applied on a sandy soil to grow celery plant under different levels (25, 50 and 75%) of the recommended chemical fertilizers, while the control did not receive any fertilizers in a greenhouse experiment at Giza Research station, Agricultural Research Center, Egypt during the summer season of 2018. Results indicated that application of biofertilizers led to a significant (p<0.05) increase in the height of plant, root and stem lengths over the control group. The number of leaves per plant as well as chlorophyll content were highest in the treatments of Bio-Mix 25 and 50%. Also, these treatments increased the total macro- and micronutrients of celery. There was very remarkable enhancement in some recorded sandy soil properties after harvest i.e., pH, total organic matter, total nitrogen, phosphorus and potassium by the treatments of Bio-Mix with 25 and 50%. The present study concluded that 1/4 or 1/2 of the recommended dose of NPK fertilizers could be saved for celery growth by using Bio-Mix product from cyanobacteria and olive milling wastewater as a promising eco-friendly bio-organic fertilizer.

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INTRODUCTION

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Nowadays, wastewater management and reuse is an urgent ecological task. Wastewater represents a major source of pollution to the environment and

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hazard to human health when released without treatment (Zhang et al., 2015). The limited fresh water resources along with the increasing need of fresh water for human consumption create a challenge for reusing wastewater in different activities. Celery (Apiumgraveolens L.) is a widely cultivated herb of the Apiaceous family used extensively for garnishing and seasoning foods, and for production of essential oil (Li et al., 2018). Celery is cultivated in Egypt as

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leafy vegetable crop or medicinal herb crop in both seasons (Ahmed, 2017). Olive milling wastewater is considered as a major problem that faces the Mediterranean countries producing olive oil like Egypt (Khoufi et al., 2006). The olive milling process generates about 1.2-1.8 m³ of wastewater for each tone of olive which accounts to more than 30 million m³ olive milling wastewater(OMWW) per year in the Mediterranean region (Khatib et al., 2009). OMWW composition differs following the production method but its main components are: water (up to 92%), organic matter (up to 16%) and minerals (up to 2%) (Tunalioğlu and Bektaş, 2012). Other characteristics of OMWW include, high salinity, low acidity and richness in nitrogen, phosphorus, potassium and magnesium (Tunalioğlu et al., 2016). The direct discharge of olive milling wastewater into agricultural soil for irrigation purpose is not recommended especially due to its high phenolic contents (Mohawesh et al., 2014). Reusing wastewater for microalgae cultivation is regarded as an environment friendly and cost effective technique. Microalgae can grow in many types of water with varying chemical composition and characteristics such as fresh or saline waters as well as wastewater from agriculture, industry or domestic wastewater (Cheah et al., 2016; Shitu et al., 2015). Cyanobacteria are prokaryotic oxygenic phototrophs microorganisms that are prosperous source of bioactive compounds such as antiviral, antibacterial, antifungal and anticancer compounds (Priyadarshani and Rath, 2012). Some cyanobacteria form heterocysts and have the ability to fix atmospheric nitrogen (Kumar et al., 2010). In addition, cyanobacteria can be used in treating wastewater, fish feeding, some food industries, production of fertilizers as well as producing secondary metabolites like exopolysaccharides, vitamins, toxins, enzymes and pharmaceuticals (de Morais et al., 2015; Kumar et al., 2010). Phycoremediation of OMW is a technology that uses algae or cyanobacteria for pollutants removal from wastewaters and biomass propagation for sustainable biofertilizers production (El Shimi and Mostafa. 2016). In agriculture practice, fertilizers are usually applied to enhance soil properties, crops productivity and nutrient quality by compensating the inadequate levels of nitrogen, phosphorus and potassium. Presently, it is a strategy for sustainable green agriculture to use environmental friendly and low-cost farming methods involving microorganisms (Singh et al., 2016). Biofertilizers which are organic compounds from living microorganisms (Debska et al., 2016; Win et al., 2018) can be used as a safe alternative to chemical fertilizers with the advantages that they minimize the ecological disturbance and are cost effective (Mohapatra et al., 2013). In this respect, the aim of the present work is to use olive mill wastewater as a cultivation media for cyanobacteria and to investigate the effect of the resulting biofertilizers on the growth of celery plant in a newly reclaimed soil. This study has been carried out in Agricultural Research Center, EGYPT during 2017-2018.

MATERIALS AND METHODS

Chemical fertilizer

NPK (20-10-10) chemical fertilizer with the following composition: Total N (20%), P_2O_5 (10%) and K_2O (10%) was obtained from a local company in Egypt. The fertilizer was applied at the rates recommended by the Egyptian ministry of Agriculture.

Preparation of cyanobacteria based fertilizers

Olive mill wastewater used for cyanobacteria cultivation was obtained from local olive oil production factory in Egypt. The characteristics of olive mill wastewater are given in Table 1. The analysis of wastewater was done according to the standard methods for the examination of water and wastewater (APHA, 2005). Total phenols in wastewater were extracted according to the method of De Marco et al. (2006) and were determined spectrophotometrically as described by Swain et al. (1959). Cyanobacteria strains (Spirulina platensis, Nostoc muscorum and Anabaena oryzae) were kindly provided by the Microbiology Department, Soils, Water and Environment Research Institute (SWERI), Agricultural Research Center (ARC). Cyanobacteria culture suspensions at log phase, either spirulina or mixture

Table 1.Olive mill wastewater characteristics

ppm	
13 5.80 3	314.75
.06 ±0.63 ±	±53.18

Table 2. Mean values of physical and chemical soil characteristics

Mechanical and chemical analysis								
Fine	Coarse	C:I+	Cl	Soil	Total organic	mll	EC	CEC
Sand	Sand	Silt	Clay	ay texture	matter (TOM)	рН	dsm ⁻¹	meq/100g soil
43.50	40.30	11.70	4.5	Sandy	0.21	7.97	3.25	7.60
Available macronutrients (%)								
N P K			K					
0.002		0.0004			0.006			

of Spirulina + Nostoc + Anabenae in the ratio (1:1:1), were inoculated at the rate of 20% and the media was OMW diluted to 50%. The media for *Spirulina platensis* was supplemented with 5 g/L NaHCO₃. The cultures were incubated at 27±2°C under continuous shaking (150 rpm) and illumination (2000 Lux) for one month. The cultivation procedure was done according to the method of El Shimi and Mostafa (2016). The two bioformulated fertilizers produced by cultivating a) *Spirulina platensis* and b) mixed culture (*Spirulina platensis*, *Nostoc muscorum* and *Anabaena oryzae*) were named: BIO-Spi (Biofertilizer from Spirulina platensis) and BIO-Mix (Biofertilizer from three cyanobacteria), respectively.

Soil samples and celery transplanting

Soil samples were obtained from Ismailia governorate. Soil texture was determined by using hydrometer method. EC and pH were measured in a 1:2.5 (soil: water) suspension after stirring for 30 min. Cation exchange capacity (CEC) of the soil was determined following the 1 N ammonium acetate (pH 7) method. Soil organic matter was determined by the Walkley-Black method. Total N was measured by the semi-micro Kjeldhal method, total P was measured by the molybdenum antimony blue colorimetry method and total K concentration was measured spectrometrically using atomic absorption (Estefan et al., 2013; Shaaban, 2001; Xu et al., 2016). The physiochemical characteristics of the soil are shown in Table 2. Celery seedlings 20 days old from local Egyptian cultivars "Balady" were purchased from local Egyptian farm. Each health seedling was transplanted in 15 cm diameter polyethylene pots using 2 kg of soil per pot. The pots were irrigated regularly each two days. The plants were harvested after 50 days. Celery growth parameters including; Number of leaves, plant height (cm), fresh and dry weight (g) and chlorophyll contents (%) were determined. The macro and micronutrients in celery leaves were determined using the official methods of analysis (AOAC, 1990). Total chlorophyll content was measured by spectrophotometer using the modified method of Arnon (Jia et al., 2017).

Pot experiment

The experiment was laid out at Giza Research station, Agricultural Research Center (ARC), Egypt during the summer season of 2018 under greenhouse conditions in a randomized block design with three replications. The experiment consisted of one control group without any fertilizers and 9 treatments as follows: T1= 100% NPK, T2= 50% NPK, T3= 25% NPK, T4=75 % NPK + 25 % BIO-Spi, T5= 50 % NPK + 50 % BIO-Spi, T6= 25 % NPK + 75 % BIO-Spi, T7=75 % NPK + 25 % BIO-MIX, T8= 50 % NPK + 50 % BIO-MIX, T9= 25 % NPK + 75 % BIO-MIX and T10= Control without any fertilizers.

Statistical analysis

The data were statistically analyzed by SPSS software with one way ANOVA and the means were separated using the least significant difference (LSD) test at 5%.

RESULTS AND DISCUSSION

The selected growth media for cyanobacteria strains in the present study was olive mill wastewater rich with several organic compounds as well as nutrients. The chemical composition of the cyanobacteria biofertilizers formulated by algae cultivation using olive mill wastewater as growth media is given in Table 3.

Table 3. Composition of cyanobacteria – OMW biofertilizers

Parameters	Bio-Spi	Bio-MIX
рН	8.70	8.92
EC; μs/cm	7852	7260
N%	4.80	5.50
P%	2.29	2.84
K%	2.67	3.09
Zn (μg/g)	3.08	5.38
Fe(μg/g)	105.90	126.67
Cu(µg/g)	0.08	0.09
Mn (μg/g)	21.63	23.17

Data show that the two biofertilizers BIO-Spi and BIO-MIX are characterized by their alkaline pH which upon application to the soil may enhance the availability and uptake of nitrogen, phosphorus, and potassium (Pimratch *et al.*, 2015). BIO-Spi and BIO-MIX were found to contain good amount of macronutrients (N, P and K) and some micronutrients (Fe, Zn, Cu and Mn) which could be beneficial to soil and crops.

Effect of cyanobacteria-OMW biofertilizers on celery growth and chlorophyll content

Besides N₂ fixation, the cyanobacteria contain several extracellular products like growth promoters, vitamins, useful enzymes and nutrients (Jaiswal et al., 2018) which are recognized as a key factors in plant growth promotion. The effect of cyanobacteria-OMW biofertilizers on celery growth parameters is presented in Table 4. The results show that the application of BIO-Spi and BIO-MIX significantly (p<0.05) increased plant heights and the lengths of roots and stems over the control group. It was also observed that the treatments T7 and T8 showed significantly (p<0.05) higher plant, root and stem heights compared to other treatments. This indicates that the mixed cyanobacteria biofertilizers, at either 25 or 50% levels were more efficient compared to other fertilizers. The application of cyanobacteria fertilizers caused a significant increase (p<0.05) in the leaves number per plant in all treatments in comparison with control. At the same time the results revealed that the application of 50% and 25% of either BIO-Spi or BIO-MIX did not show any significant difference in the leaves' number per plant as compared to the chemical fertilizer treatment (100% NPK). This may be attributed to the efficacy of cyanobacteria-OMW biofertilizers in supplying plant nutrients and thus improving soil fertility, soil biological processes and release of growth promoting substances and vitamins (Jaiswal et al., 2018). The cyanobacteria-OMW fertilizers have shown positive effect on the fresh and dry weights of celery. The treatments T7 and T8 had significantly higher (p<0.05) fresh weight and dry weight compared to control and compared to the chemical fertilizer treatments. Cyanobacteria-OMW as biofertilizer improved the weight of celery by providing essential nutrients resulting in maximum cell growth and thus plant growth. These observations agree with the results of Sanaa et al., (2014) who reported that application of cyanobacteria biofertilizer increased biomass weight compared to unfertilized ones and attributed this to the algal enzymes activities such as nitrogenase and nitrate reductase and also it could be due to production of amino acids and peptides produced by algal suspension and other plant growth stimulants (Abdel-Raouf, 2012; Osman et al., 2010). Chlorophyll content in celery plants significantly varied among different treatments. The application of T7 and T8 significantly (p<0.05) increased the chlorophyll content compared with other treatments with no significant difference with the full dose chemical fertilization treatment (T1). Previous studies have shown that cyanobacteria suspension contains some unique bioactive compounds such as plant growth regulators, which can reduce the transpiration and senescence and increase the chlorophyll content of the leaves (Ibrahim et al., 2008). In agreement with the results of the present study, the use of cyanobacteria biofertilizers was effective for different crops such as pea (Osman et al., 2010), rice (Pimratch et al., 2015), tomato (Abuye and Achamo, 2016), common bean (Hegazi et al., 2010).

Table 4. Effect of different fertilizers on celery growth

Treatment	Plant height (cm)	Root height (cm)	Stem length (cm)	Fresh weight (g/plant)	Dry weight (g/plant)	Number of leaves /plant	Chlorophyll (%)
T1	47.67 ^b	17.67 ^{ab}	30.00 ^b	130.00 ^b	5.18 ^b	57.67ª	2.35ª
T2	45.00°	17.00 ^b	28.00 ^b	116.67°	3.28 ^d	54.00 ^b	2.18 ^b
T3	35.00 ^e	13.00 ^{cd}	22.00°	107.67 ^d	2.41 ^e	42.33c	1.43 ^e
T4	42.33 ^d	16.67 ^b	25.67 ^c	135.00 ^{ab}	3.63 ^d	58.33°	1.92°
T5	43.67 ^{cd}	14.67 ^c	29.00 ^b	132.00 ^{ab}	4.28 ^c	55.67 ^{ab}	1.76 ^d
T6	29.33 ^f	12.00 ^d	20.00 ^d	113.33 ^{cd}	3.48 ^d	34.00 ^e	1.33 ^f
T7	51.00 a	19.33 ^a	31.67 ^a	134.67 ^a	6.15 ^a	57.33°	2.37 ^a
T8	50.67 a	19.33 ^a	32.67 ^a	136.67 ^a	6.16 ^a	57.33°	2.34 ^a
Т9	41.67 ^d	13.33 ^{cd}	28.33 ^b	111.00 ^{cd}	3.33 ^d	38.33 ^d	1.22 ^g
T10	28.00 ^f	11.33 ^d	16.00 ^e	107.00 ^e	2.32 ^e	31.67 ^e	0.90 ^h
LSD 0.05	2.35	1.97	2.18	5.62	0.58	3.88	0.041

a,b,c,....,h: different superscripts within the same column are significantly different (p<0.05)

Table 5. Effect of biofertilizers on macronutrients content in celery

Treatment -	N	Р	K
rreatment -		(%)	
T1	3.22 ^a	0.54 ^a	6.61 ^a
T2	2.89 ^b	0.50 ^{abc}	6.34 ^b
T3	1.83 ^e	0.39 ^d	5.80°
T4	2.84 ^b	0.51 ^{ab}	5.63 ^d
T5	2.32 ^d	0.49 ^{bc}	5.10 ^e
T6	1.91 ^e	0.45 ^c	3.80 ^g
T7	3.16 ^a	0.56 _a	6.50 ^a
T8	3.14 ^a	0.53 ^{ab}	6.63 ^a
Т9	2.48 ^c	0.46 ^c	4.80 ^f
T10	1.66 ^f	0.26 ^e	2.97 ^h
LSD 0.05	0.0903	0.0486	0.1356

a,b,c,....h different superscripts within the same column are significantly different (p<0.05)

Table 6. Effect of biofertilizers on micronutrients in celery

	Mn	Fe	Zn	Cu		
Treatment	(mg/kg)					
T1	144.67°	254.33°	130.00°	25.00 ^a		
T2	140.67 ^b	253.67 ^a	127.33 ^b	22.00 ^b		
T3	124.00°	250.33 ^a	99.67°	15.33 ^e		
T4	141.00 ^b	244.00 ^b	117.33 ^c	20.67 ^c		
T5	127.00 ^c	240.33 ^b	109.67 ^d	19.33 ^d		
T6	94.67 ^e	225.00°	91.00 ^f	11.67 ^f		
T7	144.00°	254.67 ^a	128.00 ^{ab}	23.33 ^b		
T8	144.00°	254.33 ^a	126.00 ^b	23.33 ^b		
Т9	107.00 ^d	241.33 ^b	97.67 ^e	16.33 ^e		
T10	86.33 ^f	149.67 ^d	47.67 ^g	11.67 ^f		
LSD 0.05	2.176	4.986	2.488	1.163		

a,b,c,....g: different superscripts within the same column are significantly

Effect of biofertilizers on NPK concentration of celery

Nitrogen, phosphorus and potassium concentrations in celery leaves were significantly impacted by fertilizer types as shown in Table 5. Compared to chemical fertilizer 100%, the two treatments T7 and T8 showed no significant differences (p>0.05) in nitrogen, phosphorous or potassium concentrations. Also, these treatments recorded significantly (p<0.05) higher N, P and K concentrations compared to the other treatments. The increase in the total nitrogen could be attributed to the fixation of nitrogen by cyanobacteria, the activity of nitrate reductase enzyme and to uptake of ammonium, amino acids and peptides formed by cyanobacteria (Al-Sherif el al., 2015). The application of biofertilizers increased the phosphorus concentration in celery as cyanobacteria have phosphorus solubilization activity beside their ability to produce several organic acids that decrease soil pH resulting in the transformation of phosphorus from unavailable to available form (Osman et al., 2010; Sharma et al., 2013). The phosphorus in soil is made available for plants by the chelating compounds produced by different microorganisms which solubilize phosphates (Nisha et al., 2014). Cyanobacteria are capable of mobilizing inorganic phosphate insoluble forms existing in soil and mineralizing inorganic phosphorus into soluble forms (Hegazi et al., 2010). Also, microalga in soil may assist the solubilization of potassium by releasing exopolysaccharides and maintaining the growth of potassium-solubilizing bacteria (Han et al., 2006; Renuka et al., 2017).

Effect of treatments on some micronutrients concentration of celery

The effect of cyanobacteria-OMW biofertilizers on micronutrients content of celery is shown in Table (6). Significant increase in zinc, iron, Copper and Manganese content, was observed with cyanobacteria-OMW biofertilizers in comparison with control. The availability of micronutrients to the

plant is enhanced by the application of cyanobacteria fertilizers in conjunction with the production of organic acids with chelating characteristics. The micronutrients availability to the growing plants can be increased by these chelating molecules (Renuka et al., 2017). Based on the results of celery growth and nutrients contents, it could be concluded that the treatments T7 and T8 had the highest values for all measured parameters. Thus in order to get more insights into the beneficial application of these two treatments on soil parameters, the soils amended by (T8) and (T7) were analyzed after celery harvesting. It was noticed that in case of (T8), the soil parameters were: pH (7.1),TOM (0.51%), N (0.024%), P (0.007%), K (0.0088%) and these parameters were pH (7.3), TOM (0.45 %), N (0.022%), P (0.0067%), K (0.0085%) for (T7). This indicates that application of both biofertilizers enhanced the total organic matter as well as available macro-nutritive elements which in turn was reflected on celery plant growth.

CONCLUSION

In the present work, a prominent growth of cyanobacteria on wastewater generated from olive milling was achieved. These cyanobacteria grown on olive mill wastewater were successfully applied as biofertilizers for celery planting in sandy soil. The inputs of cyanobacteria biomass obtained from olive milling wastewater significantly improved soil properties and enhanced celery plant growth. Application of cyanobacteria biofertilizer enhanced plant growth in terms of height, number of leaves, chlorophyll, and further improved celery macro and micronutrients content. The results of the present study suggest the beneficial use of an environmental waste such as olive mill wastewater in the production of economic green biofertilizers.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/

or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

ABBREVIATIONS

ADDICEVIALI	
ANOVA	Analysis of Variance
Bio-mix	Biofertilizer from cyanobacteria (Spirulin platensis, Nostoc muscorum and Anabaen oryzae).
Bio-Spi	Biofertilizer from Spirulina platensis
Са	Calcium
CEC	Cation exchange capacity
Ст	Centimeter
CO ₂	Carbon dioxide
Cu	Copper
EC	electrical conductivity
Fe	Iron
g	Gram
K	Potassium
K_2O	Potassium oxide
Кд	Kilogram
LSD	least significant difference
m³/ton	Cubic meter per ton
Mg	Magnesium
mg/L	milligrams per liter
Mn	Manganese
N	Nitrogen
NPK	nitrogen, phosphorus and potassium
ОМ	Organic matter
OMW	Olive milling wastewater
OMWW	Olive milling wastewater
P	Phosphorus
$P_{2}O_{5}$	Phosphorus pentoxide
ppm	Part per million

Sulfur

SWERI Soils, Water and Environment Research

Institute

Zn Zinc

% Percentage

 $\mu g/g$ Microgram per gram

μs/cm Micro Siemens per centimeter

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