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EFFECT OF DIFFERENT IRRIGATION REGIME AND OLIVE VEGETATION WATER (OVW) TREATED WITH CYANOBACTERIA ON SOIL CHEMICAL PROPERTIES, GROWTH, YIELD AND FRUIT QUALITIES OF OLIVE TREES.

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ABSTRACT

The experiment was conducted during the two growing seasons of 2013 and 2014 on 13-year old *Manzanillo* olive trees in sandy loam soil, under drip irrigation system in a private farm located at km 50 of Cairo –Alexandria road in the north west of Egypt. The trees are cultivated at 5×8 m. apart and grown under standard cultural practices. The objective of this study was to evaluate the effect of olive vegetation water (OVW) treated with mixed culture of cyanobacteria strains (*Nostocmuscorum*, *Anabaena oryzae* and *Spirulina platensis*) on Manzanillo olive trees under different irrigation levels and to highlight the economic advantages of these practices in improving fruit quality and crop yields. Processes to reduce the toxicity of OVW and make it acceptable for disposal onto soil or into receiving waters were reviewed. The effect of four treatments of different irrigation regime and olive vegetation water(OVW) with Cyanobacteria. Treaments were:a- control: 3400m3/feddan/year(100%) b.3400m3/feddan/year(100%)+2 liter OVW plus cyanobacteria was diluted in 22 liter of farmwater/tree, C- 2550m3/feddan/year(75%) of the control+2 liter OVW plus cyanobacteria was diluted in 34 liter of farm water/tree, d- 1700m3/feddan/year(50%) of the control +2 liter OVW plus cyanobacteria was diluted in 46 liter of farm water/tree. Data showed that application of irrigation at 100% (irrigated with 2 liter/ tree of OVW plus Cyanobacteria) and diluted in 22 liter of farm water/tree and added in six months (January up to June) each two weeks gave the highest vegetative growth, flowering characteristics, yield and fruit oil content. Moreover, the same treatment significantly increased N, P, and Mn. This treatment would be recommended under this study and also under similar conditions and which confirmed by the economic study.

Keywords: Cyanobacteria, Olive Vegetation Water (OVW), irrigation

INTRODUCTION

Olive vegetation water (OVW) is the liquid of by-product obtained during olive processing of extracting the virgin olive oil (Di-giovacchino *et al.*, 2002). The disposal of waste water from olive oil extraction processes is a major and unresolved economic and environmental problem for olive oil producing countries such as those in Mediterranean areas. The mechanical extraction process of oil does not involve the addition of any inorganic or organic products, which could pollute the oil and the waste water derived from this process (Gamba *et al.*, 2005). It can be assumed that the benefit of organic wastes recycling occurred only when they are applied according to best agronomical practices (Van-Camp *et al.*, 2004). Many researchers have established that these wastes have a high value when applied to the soil (Garcia Barrionuevo *et al.*, 1992 and Parades *et al.*, 1999). However, the direct application to agricultural soils as organic fertilizers is the most frequently used method nowadays (Mekki *et al.*, 2006) and also using OVW for irrigation offsets the water scarcity and low soil fertility in the Mediterranean region. Moreover, agricultural irrigation with waste water effluents became a common practice in arid and semiarid regions, where it was used as a readily available and inexpensive option to fresh water. Moreover, authors confirmed that the recycling of the OVW and its use as water for irrigation in agriculture provided that its impact on soil and plant was established as an attractive possibility for the Mediterranean countries (Mechri *et al.*, 2007). In order to find convenient solutions for spreading this by-product into agricultural lands without harmful environmental effects, many studies were conducted in several olive producing oil countries (Casa *et al.*, 2003 and El-Hadrami *et al.*, 2004). Most of these studies were focused on the pretreatment of OVW using different microorganisms (Piperidou *et al.*, 2000). Cyanobacteria or blue green algae (BGA) are photosynthetic N- fixers free microorganisms play a key role in improving growth of many plants when applied as bio-fertilizers (Sahu *et al.*, 2012). Cyanobacteria considered the ideal solution for treating olive vegetative waste water (Amores-Sanchez *et al.*, 2015). In the area of agriculture and horticulture, microalgae have been shown to stimulate the growth of plants, due to the presence of auxine, cytokinins, gibberellins and related growth regulator substances (Aly *et al.*, 2008). In addition, cyanobacteria helped to save about 25% of irrigating water in calcareous soil and did not affect the productivity of crop yield (Mostafa *et al.*, 2011). On the basis of these findings, this work built on to assess the benefits of reusing treated OVW via cyanobacteria as a bio fertirrigation, evaluate its influences on soil and olive trees and to highlight the economic advantages of these practices in improving fruit quality and crop yields under different water levels.

MATERIALS AND METHODS

1. Field practices:

The present study was carried out during 2013 and 2014 on Manzanillo olive trees grown in sandy soil. The trees were grown in a private orchard located at the Cairo-Alexandria desert road(about 50- Kilometer, Cairo). Considered trees were irrigated with deep well water using a bi-lateral drip irrigation system with 4 adjustable discharge emitters/tree. Water requirements were as following: November, December, January, February: two times/week(550 m^3/ feddan); March, April, May, June: three times/week (1100$m^{3/}$feddan); July, August, September, October: six times/ week (1750^3/ feddan). Total = 3400m^3/fed. Variations in management were only in irrigation regimes which varied according to the experiment and conducted treatments. Variations were adjusted by emitter discharge without altering emitter's locations.

The olive waste water originates from a traditional process Cyanobacteria extract in tap water was applied in three rates (24, 36, 48 liters), each rate contains two liter of cyanobacteria.

Conventional management practices recommended by the Ministry of Agriculture were applied to all trees on equal bases. Farm fertilization (compost and mineral fertilization) were added at the second week of November and was applied in two parallel ditches of 100 x 40 x 30 cm, for length, width and depth respectively. The ditches surrounded th tree from two direction in the end of canopy shade. The amount of compost was 50 Kg/tree. the rate of mineral fertilization was 100 gm nitrogen + 270 gm potassium + 0.5 Kg of $MgSO_4$ (9.6 % Mg) + 0.25 Kg as sulphur + 0.25 Kg Boron per tree.

The experiment consisted of 4 treatments; replicated 3 times in a randomized block design.

The selected experiment trees were nearly uniform and vigor growth and free from pathological and physiological disorders received the same culture management (irrigation, weed, pests, and diseases control usually applied in the orchard except for the irrigation treatments).

The treatments involved:

a. Control of irrigated at 100 % only (four drippers under the canopy of the trees and each one received 8 liter/hour per tree (total 32 liter/ hour/tree), that mean 3400m3/feddan/year.
b. Application of irrigated at 100% with 24 liter/ tree of OVW plus Cyanobacteria (four drippers without blocked irrigated), that mean 3400m3/feddan/year(diluted in 22 liter of farm water/tree).
c. Application of irrigated 75% with 36 liter/tree of OVW plus Cyanobacteria (one drippers were blocked under the trees irrigated), that mean (2550 m3/feddan/year(diluted in 34 liter of farm water/tree).
d. Application of irrigated 50% with 48 liter/tree of OVW plus Cyano bacteria (two drippers was blocked under the trees irrigated), that mean (1700m3/feddan/year(diluted in 46 liter of farm water/tree).
e. Three application of the olive oil vegetable water were used, were add to each tree at the end of canopy shade each two weeks during the period from the first of January till the end of fruit set.

2-Soil, olive vegetable water (OVW),water samples and compost:

Soil samples were randomly collected from the zone of the end of root ramification of the canopy in November. Depth of the soil sampling was 0-60cm. (Fernandez Je *et al.*, 1991). Soil texture characterization and chemical analyses were determined according to the methods described by (Page *et al.* 1982) as shown in Table (1). Analysis of the tested olive vegetable water is shown in Table (2). Samples of irrigation water and compost were collected during the experiment and analyzed as shown in Table (3) and Table (4).

3- Soil measurements

Soil enzymes activity

Fresh soil samples of the root zone were tested for dehydrogenase (Casida *et al*., 1964) and nitrogenase (Hardy *et al*., 1973) enzymes activity two weeks after the last biological treatments dose application.

Soil chemical analyses

Air dried soil samples were ground to pass through a 2 mm sieve using a wooden grinding and stored in plastic bottles prior to the physical and chemical analysis.

Available nitrogen in soil was determined according to the method described by (Black *et al.* 1982). Available phosphorus was determined spectrophotometrically as mentioned by Watanabe and Olsen (1965). Available potassium was determined using flame-photometric method (Black *et al*., 1982). Microelements (Fe, Mn and Zn) were extracted using DPTA (Lindsay *et al.*, 1954). Soil reaction (pH) was measured in 1:2.5 soil water extract using glass electrode pH meter Model (955), and electric conductivity (EC) was measured in 1:5 soil water extract using glass electrode conductivity meter Model Jenway 4310. Soil organic matter (O.M %) was determined using potassium dichromate (Chapman and Pratt, 1961). Phenolic compounds from the soil samples were extracted with ethyl-acetate. The soil phenols were extracted with ethyl-acetate using a ratio of 5:1 (v/w) and total phenol was determined by Folin-Ciocalteau colorimetric method using Gallic acid as a standard (Box, 1983).

Olive vegetable water (OVW)

Olive vegetable water (OVW) was obtained from a private farm located at km50 of Cairo-Alexandria, Egypt. The raw OVW was generated by the three-phase olive-oil extraction process and was left to settle for 24 hr. then; the light supernatant after the sedimentation was filtered by passing through a sieve (mesh size about 200 μ) and was kept to be used as substrate for cyanobacteria cultivation. Physical and chemical properties of OVW obtained from olive oil three phase extraction mill collected during the late fall and winter season was determined at Soil and Water Research Institute (SWERI), Agricultural Research Centre (ARC). Physical and chemical properties of OVW obtained from olive oil three phase extraction mill collected during the late fall and winter season was determined at Soils, Water and Environment Research Institute (SWERI), Agricultural Research Centre (ARC), Egypt.

Table1. Physical and chemical characteristics of the testing soil

Character	2013	2014
Particles size distribution		
Coarse sand (%)	4.45	5.41
Fine sand (%)	76.35	78.00
Silt (%)	12.9	11.08
Clay (%)	6.30	5.51
Texture	Loamy sand	Loamy sand
Chemical analysis		
EC dS/m (1:2.5)	4.40	4.30
pH (1:2:5)	8.46	8.10
Organic matter (%)	0.18	0.26
Organic carbon (%)	0.11	0.15
Total nutrients mg/100g		
N	0.13	0.22
P	0.40	0.50
K	7.40	16.82
Total nutrients mg/kg		
Mn	1.3`5	1.90
Zn	0.46	0.61
Fe	1.60	1.82

Before the application of the treatments, a compost sample was taken and analyzed at Soils, Water and Environment Research Institute (SWERI), Agricultural Research Centre (ARC), Egypt and illustrated in Table(4).

Table 2. Chemical analysis of OVW samples (Values are average of two seasons)

Parameters	Olive Vegetative water	Olive vegetative water treated with cyanobacteria
pH (1:2.5)	5.01	8.00
EC ds/cm	5.52	3.10
Total nitrogen (%)	1.10	3.10
Organic Matter (%)	20.81	2.80
Organic carbon (%)	12.10	1.04
Total phenols (%)	0.80	0.28
C/N ratio	8.64	0.45
Total P (%)	0.60	1.90
Total K (%)	1.30	2.50
N-NH_4 (ppm)	25.50	T.A.N. (ppm)*
N-NO_3 (ppm)	8.90	2002.00

*** T.A.N. = total available nitrogen.**

Table 3. Chemical analysis of well`s water

Parameter	Value	Parameter	Value
EC (dS/m)	1.53	Hard soluble Mg	50
SAR	4.10	Mg	12.15
pH	7.26	Na	313
ppm		K	7.52
NO_3	Non	Fe	1.02
Po_4	0.80	Mn	0.24
Cl	147	Zn	0.14
Phenols	Non	Cu	0.07
Ca	123	B	0.05

Table 4. Physical and chemical analysis of compost

Parameter	Value	Parameter	Value
pH (1: 2.5)	9.01	Total P (%)	0.54
EC (%)	7.89	Total K (%)	2.64
TN (%)	1.95	N NH_4 (ppm)	657.9
Organic carbon (%)	28.50	N No_3 (ppm)	152.9
C/N ratio	14.62		
Organic matter(%)	49.02		

4- Cyanobacteria source and growth conditions:

Three cyanobacteria strains (*Nostocmuscorum*, *Anabaena oryzae* and *Spirulinaplatensis*) were obtained from the Microbiology Department; Soils, Water and Environment Research Institute (SWERI), Agricultural Research Center (ARC), Giza, Egypt. N_2-fixing strains (*Nostocmuscorum* and *Anabaena oryzae*) were maintained separately on BG11 medium (Rippka *et al.*, 1979). While, the non N_2-fixing alga *Spirulinaplatensis* was grown on Zarrouk medium (Zarrouk, 1966). Cultures were incubated in growth chamber under continuous shaking (150 rpm) and illumination (2000 lux) at 27 °C ± 2 °C for 30 days to be used as inoculums for lab experiment.

5- Biofertilizer production for field experiment:

Fifty-liter plastic tanks were used to prepare Cyano-OVW biofertilizer by diluting non sterilized OVW with tap water (1:1 v/v). Tanks were under continuous aeration for two days by air pumps before cyanobacterial inoculation. Ten L of cyanobacterial mixed culture at log phase (1:1:1 v/v) were added to 40 L of 1:1 OVW tap water diluted and incubated for two weeks under lab. conditions and continuous aeration to be used for field experiments.

6- Measurements:

Vegetative growth parameters

At the end of each growing season during first week of August the following characteristics were measured:

a. Number of leaves per shoot.
b. Average shoots length (cm).
c. Leaf area (cm^2): using a planimeter according to Aly (2005).

Leaf minerals and phenols contents:

At the first week of August of each season, leaf samples were taken from mid of the current of growing shoot (Piper, 1950) and then water washed; air dried at 70^0C till constant weight and finally grounded. Thereafter the following determinations were carried out in the acid digested solutions as reported by Van Shouwenburg (1968).

- Nitrogen was determined by the Micro kjeldahl method (Pregl, 1945).
- Phosphorous was estimated by the method described by Murphy and Riely (1962)
- Potassium was flame-photometerically determined according to the method described by (Brown and Lilleland, 1946).
- Calcium was flame-spectrometerically determined according to the method described by Chapman and Partt, (1961)
- Microelements (Fe, Mn, Zn, Cu) as ppm was spectrophotometerically determined using atomic absorption (Model, spectonic 21D) as the method described by Jackson (1973).
- Total phenolic compounds were determined using the Folin–Ciocalteu reagent method (Skerget *et al.,* 2005)

Flowering parameters:

a. Inflorescence length (cm):

Thirty inflorescences were randomly taken from each replicate and the length of their axis was measured.

b- Flowering density: number of inflorescence per meter on the labeled twenty shoots were calculated. It was calculated by the following formula.

Flowering density (FD) = (av. No. of inflorescences/ Av. Shoot length (cm)* 100

c. Average number of total flowers per Inflorescence

Samples of 30 inflorescences (located in inner and outer portions of the tree) were taken from the middle portions of shoots and the total number of flowers per inflorescence were counted and tabulated.

d. Percentage of perfect flowers

Twenty inflorescences at balloon stage were collected from the middle portions of shoots from each tree. The percentage of perfect flowers to the total number of flowers was calculated.

The perfect flowers % = No. of perfect flowers/No. of total flowers x 100

Fruiting (fruit set and yield)

Number of fruitless tagged on each replicate was recorded after 60 days from full bloom according to (Mofeed, 2002).

Percentage of Initial fruit set was calculated according to the following equation.

Initial fruit set (%) = (Total number of fruitless/total number of perfect flowers)
* 100.

a. Yield:

At the stage of green maturity fruits from each considered tree were picked, then weighed in Kg and counted and recorded.

Fruits and Stones characterizes:

b- Fruits:

Fifty fruits per each tree at maturity were randomly selected in both seasons, from each replicate to study different physical and chemical characteristics of fruit in different treatments according to the following basis:

- Fruit characters:
- Fruit length (cm).
- Fruit diameter (cm).
- Fruit weight(gm).

c- Stones:

- Stones were extracted from the selected fruits to determine the following physical characteristics:- stone length (cm), Stone diameter (cm) and stone weight (g).

Flesh/fruit weight percentage was calculated according to the following equation.

Flesh weight = average fruit weight- average stone weight (g)

The flesh/fruit (%) = Flesh weight/ average fruit weight x100 according to Fouad *et al.* (1992).

Fruit oil content (%)

Fruit oil content as a dry weight was determined according to (**AOAC, 1995)** method by extraction the oil from the dried fruits with soxelt fat extraction apparatus using petroleum ether 60-80^0 C of boiling point.

7- Economic evaluation:

Economic evaluation was calculated according to **Heady and Dillon (1961)** as follows:

- Total gross income = total yield (kg) × 3 L.E.
- Gross margin= total gross income-total cost.

Annual applied water quantities/fed of control was 3400 m^3/feddan.

8- Statistical analysis:

The obtained data was subjected to the method described by analysis of variance (ANOVA) according to (Snedecor and Cochran, 1980). Differences between treatments were compared by Duncan's multiple range tests SAS (SAS, 1994).

RESULTS AND DISCUSSION

1- Soil enzymes:

Effect of different irrigation regime with Cyanobacteria and (OVW) on soil enzymes:

Results in Table (5) showed that all irrigation levels that received Cyanobacteria + OVW biofertilizers increased soil enzymes activity in terms of dehydrogenase and nitrogenase even with 50 % reduction of irrigation water over than control in both studded seasons. The highest significant values (3.39 & 3.50 and 491.45 & 498.68) in both seasons, respectively recorded with received 100 % irrigated plus Cyanobacteria with 24 liters/tree of OVW. These results indicated that the produced biofertilizer stimulate total soil microorganisms as well as the N_2-fixers. Cyanobacteria have emerged as promising detoxification microorganism Degradation of diversehazardous contaminants, synthesis of high-value secondary metabolites, use of sunlight as energy source ,production of photosynthetic oxygen that reduces the need for external aeration, are advantages that render these microorganisms as attractive "pollutant removers "(Amores-Sanchez *et al.*, 2015). These results are compatible with that reported by Piotrowska *et al.* (2006) and are in accordance with many previous works like by Gamba *et al.* (2005) and Ibrahim *et al.* (2007) and Magdich *et al.* (2012).

Table 5. Effect of different irrigation regime and OVW treated with Cyanobacteria on soil enzymes activity during seasons of 2013 and 2014.

Treatments	Dehydrogenase (µgTPF.g^{-1} dry soil)		Nitrogenase (µ mol $C_2H_4.g^{-1}.h^{-1}$)	
	2013	2014	2013	2014
Control (100 %) Irrig.	0.67 C	0.70 D	13.70 D	14.60 D
I_1 100 % Irrig. + OVW +Cy. Ba.	3.39 A	3.50 A	491.45A	498.68A
I_2 75 % Irrig. + OVW +Cy. Ba	1.14 B	1.26 B	79.86 B	91.89 B
I_3 50 % Irrig. + OVW +Cy. Ba	0.85 C	0.92 C	43.08 C	51.97C

I = irrigation Cy.Ba = Cyanobacteria OVW = **olive vegetation water**

Means having the same letter(s) within the same column are not significantly differ at the probability of 5 % level according to Duncan's Multiple Range Test.

2- Chemical properties

Effect of different irrigation regime and OVW with Cyanobacteria on soil chemical properties

Data in Table (6) showed that all irrigation levels that received Cyanobacteria + OVW biofertilizer increased the availability and total soil contents of N, P, K, Fe and Zn as compared with control in both seasons. Applications of irrigation levels that received Cyanobacteria + OVW biofertilizer had insignificant effect on pH values in the first season. Regarding the second season, the pH values significantly decreased by treatments received 100 % irrigated plus Cyanobacteria with 24 liter/tree of OVW comparing with other irrigation levels. On the other hand, the decrease in pH values in the second season was due to the reduction of water irrigation at the rates of 25-50 % in treatments that received 36 and 48 L/tree (Table 6).

The decrease of pH could be due to the degradation of some phenolic acids present in the waste. The level of pH reached at the end of the process (7.84 – 7.91) was suitable for the microbial growth. The increase in the soil salinity could result from the main ionic species, sodium chloride and sulphate, which came from the treated or untreated OVW. The OVW also has a high potassium concentration and not able levels of phosphorus, calcium, magnesium and iron (Parades *et al*, 1999).

The enhancement of organic and mineral nutrient contents might have a beneficial effect on soil fertility. The increase in C/N ratio (Table 6) in both seasons reflected the increase in nitrogen fixation by cyanobacteria (Chartzoulakis *et al.* 2010 and Sahu *et al.*, 2012) showed that after 3 years of raw OVW application, there were no significant differences in pH, electrical conductivity (EC), P, Na and organic rates between the control and OVW treated soils. Further, advantage of OVW is the increase of soil aggregate stability (Mahmoud *et al.*, 2012).

The phenolic compound concentration in plant and soil amended with 24, 36 and 48 L/tree of cyanobacteria + OVW biofertilizer under different irrigation levels are shown in Table (7). Regarding, total phenols in leaves, all treatments were lower than control in both seasons. This reduction of total phenols in soil treated with cyanobacteria + OVW could be due to the biodegradation ability of cyanobacteria to phenolic compounds (Amores-Sanchez *et al.*, 2015).

OVW organic matter and contained residues of oil and grease form a coating on soil aggregates and pore walls, which may reduce anion diffusion into soil aggregates. OVW has a beneficial influence on soil aggregation, soil structure stability and hydrodynamic properties of a sandy soil. Thus, land application of OVW can be sustainable and cost-effective recycling of nutrients and organic matter of this effluent to soil (Mohawesh *et al.*, 2014)

Table (6): Effect of different irrigation regime and OVW treated with Cyanobacteria on soil chemical properties during seasons of 2013 and 2014.

Treatments	Total (%)			Available (mg/kg)			Available (mg/kg)			pH	EC	OC%	OM%	C/N ratio
	N	P	K	N	P	K	Mn	Zn	Fe					
Season of 2013														
Control (100 %)Irrig.	0.16c	0.04a	0.10b	33.40c	8.65b	237.44d	1.40c	0.78c	2.22c	8.46a	4.46ab	0.29c	0.502a	1.79a
I_1 100 % Irrig. +OVW+Cy.Ba	0.25a	0.049a	0.23a	52.17a	9.11Ab	520.33a	1.85a	0.98a	2.27c	8.00a	3.14c	0.36a	0.610a	1.40b
I_2 75 % Irrig. + OVW.+ Cy.Ba	0.22b	0.04a	0.21a	44.78b	9.75a	501.78c	1.77b	0.90b	2.75b	8.22a	3.87b	0.33b	0.571a	1.52Ab
I_3 50 % Irrig. + OVW +Cy. Ba	0.22b	0.04a	0.22a	45.61b	9.81a	515.16b	1.85a	0.86bc	3.39a	8.30a	4.99a	0.35ab	0.600a	1.59ab
Season of 2014														
Control(100%)Irrig.	0.13d	0.04a	0.04b	36.54c	7.96a	91.35c	1.90b	0.99a	2.54Ab	7.96ab	4.39a	0.34b	0.58b	2.66a
I_1 100 % Irrig. + OVW +Cy. Ba	0.28a	0.04a	0.07a	51.16a	8.01a	148.15a	2.00Ab	1.05a	2.86a	8.01a	2.53b	0.36a	0.62a	1.25c
I_2 75 % Irrig. + OVW+ Cy. Ba	0.21b	0.03a	0.04b	42.36b	7.84b	93.86c	2.50a	1.00a	2.34b	7.84c	3.09b	0.34b	0.58b	1.61b
I_3 50 % Irrig. + OVW+ Cy. Ba.	0.19c	0.03a	0.04b	37.15c	7.91ab	102.11b	2.05ab	0.97a	2.55ab	7.91bc	5.00a	0.31c	0.53c	1.66b

I = irrigation Cy.Ba = Cyanobacteria OVW = **olive vegetation water**

Means having the same letter(s) within the same column are not significantly differ at the probability of 5 % level according to Duncan's Multiple Range Test.

Table 7. Effect of different irrigation regime and OVW treated with Cyanobacteria on total phenolic contents of soil and olive leaves during seasons of 2013 and 2014

Treatments	Total phenols in leaves (mg *GAE g^{-1} DM)		Total phenols in soil (mg *GAE g^{-1} DM)	
	2013	2014	2013	2014
Control (100 %) Irrig.	25.50a	28.00a	82.69a	76.02a
I_1 100 % Irrig. + OVW +Cy. Ba.	20.66c	16.40c	59.66b	46.43b
I_2 75 % Irrig. + OVW +Cy. Ba.	13.89d	21.74b	57.04c	55.31c
I_3 50 % Irrig. + OVW +Cy. Ba.	22.99b	22.58b	54.63d	53.93d

I = irrigation Cy.Ba = Cyanobacteria OVW = olive vegetation water

* Gallic acid (GAE)

Means having the same letter(s) within the same column are not significantly differ at the probability of 5 % level according to Duncan's Multiple Range Test.

3- Vegetative growth parameters

Effect of different irrigation regime and OVW with treated Cyanobacteria on vegetative growth parameters

Data in Table (8) depict the effect of applications of OVW plus cyanobacteria with three concentrations (24 L/tree, 36 L/tree and 48 L/tree) on some growth parameters. In this concern application of 100 % irrigated plus cyanobacteria with 24 liter/tree of OVW presented the highest significant No. of leaves (24.21 & 25.00), shoot length (24.10 & 25.50) and leaf area (3.84 & 3.93) in both seasons, respectively.

This may be ascribed to the synergistic effect of organic matter as expected; the organic treatments produced better quality plants with respect to control (Shereen *et al.*, 2011). The previous results are in agreement with those obtained by Mechri *et al.* (2007), who reported that recycling of the OVW and its use as water for irrigation in agriculture provided its impact on soil and plant. Also, several studies have shown that bio-fertilizers affected soil properties. Through its contents of different strains of a symbiotic associative diazotrophes, solubilizing microorganisms of phosphate, silicate dissolving microorganisms (Saber, 1993; Baradi and Malusa, 2012).

Table 8. Effect of different irrigation regime and OVW treated with Cyanobacteria on vegetative growth of Manzanillo olive leaves during 2013 and2014 seasons

Treatments	No. of leaves		Shoot length		Leaf area	
	2013	2014	2013	2014	2013	2014
Control (100 %) Irrig.	15.65d	16.00c	17.20c	19.73c	2.34c	2.58c
I_1 100 % Irrig. + OVW +Cy. Ba.	24.21a	25.00a	24.10a	25.50a	3.84a	3.93a
I_2 75 % Irrig. + OVW +Cy. Ba.	20.76b	20.90b	18.90b	22.13b	3.06b	3.15b
I_3 50 % Irrig. + OVW +Cy. Ba.	18.69c	15.57d	14.70d	16.47d	2.04d	2.23d

I = irrigation Cy.Ba = Cyanobacteria OVW = olive vegetation water

Means having the same letter(s) within the same column are not significantly differ at the probability of 5 % level according to Duncan's Multiple Range Test.

4- Leaf mineral contents:

Effect of different irrigation regime and OVW with Cyanobacteria on leaf mineral contents:

Data in Table (9) showed that application of 100 % irrigated plus cyanobacteria with liter/tree of OVW raised up the macro nutrients in leaves of Manzanillo olive trees compared to untreated control in both seasons. The highest nitrogen and phosphorus percent in leaves (1.41 & 1.43% and 0.82 & 0.86 %, respectively) obtained for treatment of 100 % irrigated plus cyanobacteria with 24 liter/tree of OVW, while the highest values of potassium content (1.52 and 1.56%) recorded for 75% .

liter/tree of OVW in the first and second seasons, respectively. Concerning micronutrients in leaves, results cleared that the application of 100 % irrigated plus cyanobacteria with 24 liter/tree of OVW was the superior treatment increase of Mn (24.23 and 25.07 ppm) in 2013 and 2014 seasons, respectively. Regarding the Zn and Fe the untreated control gave the highest values (48.57 & 48.80 ppm and 120.30 & 128.20 ppm) in both seasons, respectively.

These increases are related to improving soil quality, as a result of a rising organic matter content of OVW and an enrichment in N2- fixing bacteria which is a very important factor for soil fertility (Lopez *et al*., 1996). Recently, Bardi and Malusa (2012) mentioned that the positive influence of rhizosphere microorganisms on nutrient uptake is well established. The previous results agree with those obtained by Montemurro *et al.*(2011) and Lozano *et al*. (2013).

Table 9. Effect of different irrigation regime and OVW treated with Cyanobacteria on some macro and micro elements of Manzanillo olive leaves during 2013 and2014 seasons

Treatments	N (%)	P (%)	K (%)	Mn (ppm)	Zn (ppm)	Fe (ppm)
Season of 2013						
Control (100 %)	1.10c	0.70bc	1.22c	13.50d	48.57a	120.30a
I_1 100 % Irrig. + OVW +Cy. Ba.	1.41a	0.82a	1.39b	24.23a	39.80c	105.30d
I_2 75 % Irrig. + OVW +Cy. Ba.	1.27b	0.64c	1.52a	21.30b	43.10b	109.80c
I_3 50 % Irrig. + OVW +Cy. Ba.	1.00c	0.72b	1.27c	17.10c	39.07d	115.30b
Season of 2014						
Control (100 %)	1.15c	0.71c	1.27d	16.63d	48.80a	128.20a
I_1 100 % + OVW +Cy. Ba.	1.43a	0.86a	1.44b	25.07a	45.07c	108.40d
I_2 75 % + OVW +Cy. Ba.	1.30b	0.68d	1.56a	22.70b	48.50b	115.40c
I_3 50 % + OVW +Cy. Ba.	1.09c	0.74b	1.34c	19.00c	41.47d	118.65b

I = irrigation Cy.Ba = Cyanobacteria OVW = olive vegetation water

Means having the same letter(s) within the same column are not significantly differ at the probability of 5 % level according to Duncan's Multiple Range Test.

5- Flowering parameters

Effect of different irrigation regime and OVW with Cyanobacteria on flowering parameters:

Table (10) shows the effect of different irrigation regime and OVW with cyanobacteria on flowering parameters (Length. of inflorescences (cm), flowering density, No. of total flowers/ infl. and perfect flower percentage). These parameters affected the aforementioned treatments compared to the control in both seasons. The application of 100% irrigation plus Cyanobacteria + OVW (24 liter/tree) gave the highest significant values comparing to other treatments in both seasons. (Kulk 1995 and Adam 1999) reported that the growth promotion in response to application of nitrogen fixer cyanobacterium (*Nostocmuscom*) could be attributed to the nitrogenase as well as nitrate reductase activities of algae associated with the surface of plants, or the amino acids and peptides produced in algal filtrate and / or other compounds that stimulated growth of crop plants.

Table 10.Effect of different irrigation regime and OVW treated with Cyanobacteria on flowering parameters of Manzanillo olive leaves during 2013 and2014 seasons

Treatments	Length of inflor.(cm)		Flowering density		No. of total flowers/infl.		Perfect flowers %	
	2013	2014	2013	2014	2013	2014	2013	2014
Control (100 %)	1.80c	2.08c	15.43c	15.70c	9.30bc	10.74c	43.80c	45.80c
I_1 100 % Irrig. + OVW +Cy. Ba.	2.80a	2.91a	30.50a	30.93a	13.83a	14.43a	62.47a	73.70a
I_2 75 % Irrig. + OVW +Cy. Ba.	2.10b	2.35b	20.10b	20.43b	9.83b	11.70b	55.73b	57.80b
I_3 50 % Irrig. + OVW +Cy. Ba.	1.73c	1.86d	13.80d	14.67d	8.86c	10.53d	42.30d	41.32d

I = irrigation Cy.Ba = Cyanobacteria OVW = **olive vegetation water**

Means having the same letter(s) within the same column are not significantly differ at the probability of 5 % level according to Duncan's Multiple Range Test.

6- Fruiting (fruit set and yield)

Effect of different irrigation regime with Cyanobacteria and OVW on fruiting.

Data presented in Table (11) indicated that all the tested treatments except 50% irrigation increased significantly initial and final fruit set of Manzanillo olive trees as compared with control during both seasons. In this concern application of 100 % irrigated plus Cyanobacteria with 24 liter/ tree of OVW presented the highest significant percentage of initial fruit set (6,81 and 6,78) and final fruit set(4.78 and 5,54%) in both seasons, respectively. Concerning yield (Kg/tree) of Manzanillo olive trees, the results also revealed that using 100 % irrigated plus Cyanobacteria with 24 liter/tree of OVW significantly improved the yield (23,70 and 34.20 kg/tree) as compared to control in both seasons. Respectively. The present results are in harmony with those of Di-Giovacchino *et al.*, (2002) on olive and Altieri *et al.*, (2008) on olive who reported that OVW land application promoted crop yield of olive trees. Also, Cyanobacteria + OVW liquid bio-fertilizer resulted from cultivating three cyanobacteria strains on (1:1) water diluted OVW has the average values of the pH (7.5), EC ($1.45 dS.m^{-1}$), dry matter ($28.32\ g.l^{-1}$). COD ($2.2 g.l^{-1}$) organic matter ($1.4\ g.l^{-1}$) and total phenolic compounds ($0.27\ g.l^{-1}$) after 15 days of incubation process. These results reflected that Cyanobacteria + OVW could be recommended as liquid bio-fertilizer for fertigation. Fertigation is a highly recommended technique to apply both water and nutrient into crops at the same time and reduce water and nutrient losses; producing soluble fertilizers benefits plants, soil, humans, and the environment by improving soil fertility and crop growth, while reducing nutrient leaching into the ground water (Bres, 2009).

Table 11. Effect of different irrigation regime and OVW treated with Cyanobacteria on fruit set and yield of Manzanillo olive leaves during 2013 and2014 seasons

Treatments	Initial Fruit set(%)		Final Fruit set(%)		Yield (Kg/tree)	
	2013	2014	2013	2014	2013	2014
Control (100 %)	4.28b	5.06c	1.87c	2.18c	8.74c	17.47c
I_1 100 % Irrig. + OVW +Cy. Ba.	6.81a	6.78a	4.78a	5.54a	23.70a	34.20a
I_2 75 % Irrig. + OVW +Cy. Ba.	4.64b	6.43b	3.08b	4.03b	10.60b	27.90b
I_3 50 % Irrig. + OVW +Cy. Ba.	3.80c	4.90d	1.54d	1.88d	6.43	11.23d

I = irrigation Cy.Ba = Cyanobacteria OVW = olive vegetation water

Means having the same letter(s) within the same column are not significantly differ at the probability of 5 % level according to Duncan's Multiple Range Test

7- Fruit quality.

Effect of different irrigation regime and OVW with treated Cyanobacteria on fruit length, diameter and weight

The effect of tested treatments on fruit length, diameter, and fruit weight of Manzanillo olive trees is shown in Table (12). As for fruit length, control was the superior(2.65 cm) in the first season, while in the second one the superior values (2.59cm) recorded with application of 50 % irrigation plus Cyanobacteria with 48 liter/ tree of OVW.

Concerning, fruit diameter, control had treatment the highest value in the first season(2.30 cm) while, in the second season the highest value with application 50 % irrigation plus Cyanobacteria 48 liter/ tree of OVW (1.95 cm). As for fruit weight, control treatment gave the heaviest weight (6.02 a) in the first season, while in the second one the superior values (5.33) were recorded with application of 75 % irrigation plus cyanobacteria 36 liter/ tree of OVW.

Table 12. Effect of different irrigation regime and OVW treated with Cyanobacteria on fruit length, fruit diameter and fruit weight of Manzanillo olive during 2013 and2014 seasons

Treatments	Fruit length (cm)		Fruit diameter (cm)		F. weight (g)	
	2013	2014	2013	2014	2013	2014
Control (100 %) Irrig.	2.65a	2.20d	2.30a	1.90b	6.02a	4.35bc
I_1 100 % Irrig. + OVW +Cy. Ba.	2.45b	2.26c	2.00c	1.90b	5.26c	4.47b
I_2 75 % Irrig. + OVW +Cy. Ba.	2.36c	2.30b	2.00c	1.80c	5.51b	5.33a
I_3 50 % Irrig. + OVW +Cy. Ba.	2.24d	2.59a	2.09b	1.95a	5.23c	4.20c

I = irrigation Cy.Ba = Cyanobacteria OVW = **olive vegetation water**

Means having the same letter(s) within the same column are not significantly differ at the probability of 5 % level according to Duncan's Multiple Range Test.

Stone length, diameter and weight:

Data in Table (13) indicated that shows the effect of different irrigation regime and OVW with cyanobacteria on stone length, diameter and weight. As for stone length, 50 % irrigation regime and OVW with Cyanobacteria has the highest values (1.64 and 1.80) in the first and second seasons, respectively . As for stone diameter, 50 % irrigation regime and OVW with Cyanobacteria was the superior in the first season(0.99) while in the second one the superior values were recorded with the control. Regarding the weight, control gave the highest values (0stone.96,1.02) during 2013 and 2014 growing seasons, respectively. These results are nearly in the same line with these obtained by Haynes and Swift (1990) and Noval and Rezk (2009), Who reported that the length of organic wastes recycling when they are applied for soil in improving the soil physical properties such as densities, porosities, structure, aggregation, water retention and transmission, due to direct effect on retention water (hydrophilic mature) and indirect effect of the modification of the soil structure. Also, Laor *et al.* (2011) reported that soil microbial activity was generally by OVW application.

Table 13. Effect of different irrigation regime and OVW treated with Cyanobacteria on stone length, stone diameter and stone weight of *Manzanillo* olive during 2013 and 2014 seasons

Treatments	Stone length (cm)		Stone diameter(cm)		Stone weight (g)	
	2013	2014	2013	2014	2013	2014
Control (100 %) Irrig.	1.60a	1.57b	0.98a	0.99a	0.96a	1.02a
I_1 100 % Irrig. + OVW +Cy. Ba.	1.43b	1.50c	0.87c	0.92b	0.75d	0.78c
I_2 75 % Irrig. + OVW +Cy. Ba.	1.62a	1.60b	0.94b	0.86c	0.81c	0.84b
I_3 50 % Irrig. + OVW +Cy. Ba.	1.64a	1.80a	0.99a	0.90b	0.92b	0.78c

I = irrigation Cy.Ba = Cyanobacteria OVW = olive vegetation water

Means having the same letter(s) within the same column are not significantly differ at the probability of 5 % level according to Duncan's Multiple Range Test.

Flesh weight, flesh/fruit weight % and oil content:

Data in Table (14) revealed that control gave the heaviest flesh weight (5.06 g) in the first season, while in the second season the heaviest weight (4.49 g) with application of 75% irrigation with cyanobacteria 36 liter/ tree of OVW. As for flesh /fruit weight percentage, application of 100% irrigation with cyanobacteria 24 liter/ tree of OVW in the first season gave the highest percentage (84.78) while in the second one the highest percentage(86.37)recorded with application of 50% irrigated cyanobacteria with 48 liter/ tree of OVW. Oil content was significantly affected by application of 100 % irrigated cyanobacteria with 24 liter/ tree of OVW in both seasons. El-Hassani *et al.* (2010) pointed to an increase in oil content by 17% in comparison with control in response to application of olive mill waste water as an amendment for *Menthaspicata* L., but other worker report that, the oil content expressed as a percentage of dry weight, decreased significantly after agronomic application of OVW (Mechri *et al.,* 2009).

Table 14. Effect of different irrigation regime and OVW treated with Cyanobacteria on flesh weight, flesh/fruits % and fruit oil content of Manzanillo olive during 2013 and 2014 seasons

Treatments	Flesh weight (g)		Flesh/fruits (%)		Fruit oil content (%)	
	2013	2014	2013	2014	2013	2014
Control (100 %) Irrig.	5.06a	3.33d	84.29b	76.62d	30.14b	32.08b
I_1 100 % Irrig. + OVW +Cy. Ba.	4.51c	3.69b	84.78a	82.56b	35.91a	37.85a
I_2 75 % Irrig. + OVW +Cy. Ba.	4.70b	4.49a	84.34b	79.34c	30.91b	31.43b
I_3 50 % Irrig. + OVW +Cy. Ba.	4.31d	3.42c	83.57c	86.37a	30.72b	31.19b

I = irrigation Cy.Ba = Cyanobacteria OVW = olive vegetation water

Means having the same letter(s) within the same column are not significantly differ at the probability of 5 % level according to Duncan's Multiple Range Test.

8- Economic study

Table (15) shows total yield of olive Manzanillo cultivars, in Kg/feddan and total return in Egyptian pounds. Price/Kg was 3 Egyptian pounds in 2013 & 2014 and water price/meter was 0.5 Egyptian pounds. Total operation cost included water costs plus costs of fertilizers, labors, pesticides and others. The net income of the irrigation level 3402.5 m^3/feddan applications on yield of Manzanillo olive trees achieved the highest net income during 2013 and 2014 seasons. It's recommended to apply this treatment to get the highest rate of economic

Table (15). Economic evaluation of Cyanobacteria and Olive Vegetation Water (Cyanobacteria+ OVW) on olive trees under different irrigation regime during 2013 and 2014

seasonsTreatments	Mean yield kg/fed		Gross income per treatment ($EGP.fed^{-1}$)		Operation cost (EGP/fed)	Farm irrigation water (m^3/fed)	Water for Cyano/ OVW Dilution (m^3/fed)	Total irrigation water (m^3/fed)	Total water Irrigation cost (EGP/fed)	Cyano/ OVW(m^3/fed)	Cyano/ OVW cost (EGP/fed)	Total cost (EGP/fed)	Net return (EGP/fed)	
	2013	2014	2013	2014	Average for two seasons								2013	2014
Control (100 %)	945	1890	2835	5670	3800	3400	0.00	3400	1700	0.00	0	5500	-2665	170
$I_1$100 % Irrig. + Cy OVW +Cy. Ba.	2520	3570	7560	10710	3800	3400	1.26	3401	1701	1.26	126	5627	1933	5083
I_2 75 % Irrig. OVW +Cy. Ba.	1155	2940	3465	8820	3800	2550	1.68	2552	1276	1.68	189	5265	-1800	3555
I_3 50 % Irrig. + OVW +Cy. Ba.	735	1260	2205	3780	3800	1700	2.52	1703	851	2.52	252	4903	-2698	-1123

I = irrigation Cy.Ba = Cyanobacteria OVW = **olive vegetation water**

***Gross income ($EGP.fed^{-1}$) = Mean yield $kg.fed^{-1}$ x Sell Price of olive fruits (3.0 EGP/Kg)**

***Operation cost ($EGP.fed^{-1}$) = Cost of irrigation, Labors, pesticides and others**

*** Sell Price of Cyano + OVW = 0.1 $EGP.L^{-1}$**

***Total cost ($EGP.fed^{-1}$) = Operation cost + Total water Irrigation cost + Cyano/OMV cost**

***Net return ($EGP.fed^{-1}$) = Gross income - Total cost**

CONCLUSION

The results of the experiment indicated that irrigation level 3402.5 m^3/feddan plus Olive Vegetation Water (OVW)was the optimum water requirement for olive trees at Cairo-Alexandria desert road of Egypt, where this level of irrigation achieved the highest net profit in economic study.

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تأثير مقننات الري المختلفة والماء الخضري المعالج بالسيانو بكتريا على خواص التربة الكيميائية والنمو والمحصول وجودة الثمار لأشجار الزيتون.

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قسم بحوث الزيتون وفاكهة المناطق شبه الجافة-معهد بحوث البساتين-مركز البحوث الزراعية
قسم بحوث الميكروبيولوجى- معهد بحوث الأراضي والمياه و البيئة - مركز البحوث الزراعية

أجريت هذه التجربة خلال موسميين متتاليين ٢٠١٣ و٢٠١٤ على أشجار الزيتون صنف المنزانللو عمرها ١٣ سنه النامية في تربة رملية طفليه تحت نظام الري بالتنقيط في مزرعة خاصة على بعد ٥٠كم من القاهرة- طريق مصر الإسكندرية الصحراوي في شمال غرب مصر. تزرع الأشجار في مسافات ٥×٨ م وتنمو باستخدام ممارسات زراعية موحدة لدراسة تأثير أربعة معاملات رى- (الكنترول ٣٤٠٠م٣ فدان/سنه (١٠٠%) ،ب- ٣٤٠٠م٣ فدان/السنه (١٠٠%)مضافا اليه سيانوبكتريا + ٢ لتر ماء خضرى الزيتون (خففت فى ٢٢ لتر ماء مزرعة وذلك للشجرة)، ج- ٢٥٥٠م٣ /فدان/السنة(٧٥%) من الكنترول+ ٢ لتر ماء خضرى الزيتون مضافا اليه سيانوبكتريا(خففت فى٤ ٣ لتر ماء مزرعة وذلك للشجرة) د- ١٧٠٠م٣ /فدان/السنة (٥٠%) من الكنترول+٢ لتر ماء خضرى الزيتون مضافا اليه سيانو بكتريا (خففت فى ٤٦ لتر ماء وذلك للشجرة) وقد تم اضافة الماء الخضرى للزيتون المضاف اليه السيانوبكتريا مرتين شهريا خلال الفترة من يناير حتى يونيو.

كان الهدف من هذه الدراسة هو تقييم تأثير مياه خضري الزيتون الناتج من المعالجة بخليط من سلالات الطحالب الخضراء المزرقة (نوستوكمسكورم، أنابينا أوريزا، سبيرولينابلاتنسيس) على أشجار الزيتون منزانللو تحت مستويات مختلفة من الري وتسليط الضوء على المزايا الاقتصادية لهذه الممارسات في مجال تحسين جودة الثمار والمحصول على خلفية عمليات المعالجة السابقة لتقليل سمية ماء خضري الزيتون وجعله مقبولا للتربة والمياه.

أظهرت النتائج أن إضافة ٣٤٠٠م٣/الفدان/ السنة(١٠٠%) +_٢ لتر ماء خضري زيتون مضافا إليه سيانو بكتريا والمضافة خلال ستة أشهر من يناير إلى يونيو مرتين كل شهر أعطت أفضل قيم النمو الخضري والإزهار والمحصول علاوة على ذلك نفس المعاملة أدت إلى زيادة معنوية في عناصر النتروجين والفوسفور والمنجنيزوالزنك..

وهذه هي المعاملة التي يمكن أن نوصى بها تحت ظروف هذه الدراسة والظروف المشابهة وهذا ما أكدته دراسة الجدوى الاقتصادية.