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Effect of mineral and bio-fertilization on yield and fruit quality of Manzanillo Olive trees

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Abstract: This study was carried out during 2012, 2013 and 2014 seasons to investigate the effect of different levels of mineral nitrogen fertilization with bio-fertilizer on fruit set, yield, fruit quality, oil properties and total microbial count in soil of Manzanillo olive trees grown in Ismailia governorate, Egypt. The study was conducted on 15 years old olive trees of Manzanillo cv., planted at 5 X 5 m apart grown in sandy soil, under drip irrigation, system and uniform in shape and received the common horticultural practices.Four treatments were used in this experiment: 100% mineral nitrogen fertilization (control), 75% mineral nitrogen fertilization + bio-fertilizer (B+MNF75%), 50% mineral nitrogen fertilization + bio-fertilizer (B+MNF50%), 25% mineral nitrogen fertilization + bio-fertilizer (B+MNF25%). The results indicate that B+MNF75% gave the highest values of initial and final fruit set, yield, fruit qualityas well as ; weight, volume, length diameter of fruit, pulp weight, flesh oil content and ioden value. Bio-fertilizer with lower levels of mineral nitrogen fertilization resulted in decreasing nitrate content of fruit and peroxide value of oil. Trees treated by B+MNF75% gave the lowest oil acidity value.Total microbial count in the rhizosphere also was the best from trees treated bythe bio-fertilizer treatments.
Key words: Olive, Manzanillo, Biofertilization, fruit set, yield, fruit quality and oil properties.

Introduction

Olive tree (*Olea europaea* L.) belongs to the family Oleaceae. It can thrive and produce in new reclaimed areas where other crops can't grow. Beside, the nutritional importance of olive fruits, either as a table or for oil production. Hence, olive areas increased rapidly in Egypt and reached about 202,743 feddans, which in turn produced about 563,070 tonsof fruits in the 2012 year according to the statistics[1]. Although olive trees can survive and grow under low soil fertility and water availability conditions, many research studies have been indicating that improving soil fertility and satisfying water requirement are essential factors to obtain a high production. However, increasing olive tree productivity under desert conditions must be based on appropriate technical and economic management to the natural resources scarcity.

The trees are also tenacious, easily sprouting back even when chopped to the ground.According to statistical of Food and Agriculture Organization[2] the world area cultivated with olive trees in 2013 is about 10,244,194 hectares and world production of olive is 20,344,343 tons, most of which is extracted to olive oil and the rest processed mainly to table olive.

Biofertilizers have been developed to enhance nutrient uptake and satisfy requirements of several composts for fruit trees. Hence, several beneficial micro-organisms can be effectively used as alternative to chemical fertilizers to minimize the environmental pollution. N-fixing bacteria like Azotobacter sp. have been developed in several laboratories in Egypt[3]**.** Now a days, clean agriculture has received more attentions by application of different compost sorts and biofertilizers to minimize environmental problems as well as improving structure and fertility in calcareous soil where fruit orchards[4]**.** The aim of this study is to evaluate the effect effect of different levels of mineral nitrogen fertilization with bio-fertilizer on fruit set, yield, fruit quality, oil properties and total microbial count in soil of Manzanillo olive trees.

Materials and Methods

This study was carried out during three successive seasons, (2012, 2013 and 2014) in a private orchard located at Ismailia governorate, Egypt. The study was conducted on 15 years old olive trees of Manzanillo cv., planted at 5 X 5 m apart grown in sandy soil, under drip irrigation system and uniform in shape and received the common horticultural practices. The orchard soil and water irrigation analysis are given in (Table 1) and (Table 2) according to procedures[5].

Table (1): Some physical and chemical analysis of the orchard soil:

parameters	Depth of simple (cm)		
	Surface sample	30 cm depth	60 cm depth
pH	8.02	8.70	8.11
EC(dSm-1)	3.80	0.80	1.70
	Soluble cations (meq\l)		
Ca^{++}	6.00	2.50	3.00
Mg^{++}	4.00	1.50	1.50
Na^{+}	28.60	4.40	12.90
K^{+}	0.12	0.14	0.78
	Soluble anions (meq\l)		
$CO3^{-}$	-	-	-
$HCO3^{-}$	4.40	2.40	2.00
Cl^{-}	27.20	5.00	13.00
$SO4^{=}$	7.12	1.14	3.18

Table (2): **Chemical characteristics of water weal used for the present study**:

parameters	Values
pH	7.49
$EC(dSm^{-1})$	4.40
Soluble cations (meq\l)	
Ca^{++}	7.50
Mg^{++}	5.00
Na^{+}	33.1
K^{+}	0.16
Soluble anions (meq\l)	
$CO3^{=}$	-
$HCO3^{-}$	1.60
Cl^{-}	40.00
$SO4^{=}$	4.16

Experimental design

The treatments will be arranged in a randomized complete block design (RCBD), the experiment contains four treatments, and each contains three replicates and the replicate represented by one tree.The normal horticulture practices that used in the farm were applied to all Manzanillo olive trees except those dealing with bio-fertilization.

Experimental material

According to the recommendation of Ministry of Agriculture, Egypt, the olive trees required actual nitrogen yearly (1000 gm / tree / year) equal 5 Kg ammonium sulfate (20.6 % N) or 3 kg ammonium nitrate (33.3 % N)(control). Under the experiment condition ammonium sulfate (20.6 % N) was used.

Mineral phosphate and potassium fertilizer was added by rate 1.75 Kg of super phosphate (15.5 % P_2O_5) per tree. In addition, 1.50 Kg of potassium sulfate (48 % K_2O) per tree was added as a soil application divided to two equal doses, firstly at the second week of December combined with phosphate.

Microbial cultures and biofertilizers inoculation. Biofertilizer consisted of liquid cultures of three bacteria; *Azotobacter chrococcum; Bacillus megaterium and Bacillus circulans*, kindly provided by the Unit of Biofertilizers, Faculty of Agriculture, Ain Shams University. Each organism was grown separately in batch culture to the late exponential phase of each microorganism[6] to give a cell suspension of $5x10^5$; $6x10^7$ and $4x10^7$ cell /ml for *Azotobacter chroococcum*, *B.megaterium* and *B. circulans*, respectively. Cultures were mixed on site then each tree received 2 liters of the mix, and this treatment was repeated every two months for three times during the season.

Treatments: this experiment included four treatments as follows:

T1-100% mineral nitrogen fertilization (1000 g N/tree) (control).
T2-75% mineral nitrogen fertilization (750 g N/tree) + bio-fertilizer (2liter / tree).
T3-50% mineral nitrogen fertilization (500 g N/tree) +bio-fertilizer (2liter / tree).
T4-25% mineral nitrogen fertilization (250 g N/tree) +bio-fertilizer(2liter / tree).

Measurements

a. Fruit set and fruit drop: fruit set percentage as number of fruits / meter at two times first after 20 days from full bloom as initial fruit set and the second 60 days after full bloom as final fruit set[7].

Initial fruit set, final fruit set & fruit drop percentages were estimated as follows:

Initial fruit set (%) = [Number of fruit set (20 days after pollination) / shoot length (cm)] × 100

Final fruit set (%) = [Number of fruit set (60 days after pollination) / shoot length (cm)] × 100

Fruit drop (%) = [(Initial fruit set - Final fruit set) / Initial fruit set] × 100

b. Yield: at maturity stage (mid October), fruits of each tree were separately harvested, then weighed and yield as Kg / tree was estimated.

c. Fruit quality

1. Thirty fruit per each tree were randomly selected for carrying out the fruit quality measurements:
2. fruit length L (cm), fruit diameter D (cm) and fruit shape (L/D ratio).
3. Average fresh weight of (fruit, flesh and seeds in g).
4. Fruit volume (cm^3) and specific gravity (weight/volume ratio).
5. The weight ratio of flesh / seed of fruit were calculated.
6. Fruit moisture %: moisture percentage of fruit in the previous fruit samples was estimated, samples were dried at 60-80 º C in electrical air oven until constant weight, the fruit moisture percentage was calculated[8]
7. Fruit oil content %: Fruit oil content as a dry weight was determined[8] method by extraction the oil from the dried flesh fruit with soxhelt for extraction apparatus using petroleum ether 60-80 º C of boiling point.
8. Nitrate content NO_3- N (mg.g $^{-1}$): NO_3- N was determined in distilled water extracts of dried tissue according to the procedure[9] as modified by nitration of salicylic acid[10].

d- Chemical properties of oil

Acid value

It was determined according to[11]**.** Five grams of oil were accurately weighed in 250 ml dry conical flask with about 100 ml of neutralized 50% ethanol + 50% petrolium ether to dissolve the oily sample. Acidity of the sample was determined by titration with 0.1 N potassium hydroxide solution in the presence of phenol phthalein as an indicator. The acid value was calculated according to the following equation.

$$\text{Acid percentage} = \frac{\text{V X N X 5.61}}{\text{Weight of sample}} \text{ X100}$$

Wher,
V = Volume of KOH solution
N = Normality of potassium hydroxide solution

e. Peroxide number

The peroxide volue was determined[12] by dissolving 5 gm of the oil in a mixture consisting of 60% glacial acetic acid + 40% chloroform. The solution was treated with approximately 0.5 ml of saturated solution of potassium iodide in glass stoppered flask. The flask was shacked in rotary for exactly two minutes, after which 30 ml of distilled water added, and the liberated iodine was titrated with 0.01 N sodium thiosulphate using 1% starch solution as external indicator.

The results were calculated in milli moles per kilogram oil according to the following equation.

$$\text{Peroxide value} = \frac{\text{0.5 X N X V} \times 100}{\text{Weight of sample}}$$

Where,
N = Normality of sodium thiosulpahte solution
V = Volume in ml. of sod. thiossulphate needed for titration

Iodine value

The degree of unsaturation of oil was determined by measuring the amount of halogen absorbed by the oil as stated[13]**.** (Ca 0.1 – 0.5) of oil was dissolved in 10 ml of chloroform and 25 ml of Hanus iodine solution were added. After 30 min. 10 ml of 15 % potassium iodine solution and 100 ml of freshly boiled cooled distilled water were added. The liberated iodine was titrated with 0.1 N sodium thiosulphate using starch indicator.

Refractive index

The oil samples were determined using a refractometer (Rudolph model J157 at 20˚C, for each test performed five repetitions. Refractive index was determined according to the method described[8]**.**

f. Microbiological measurements

To determine the effect of different fertilization treatment on total bacterial count, soil samples were taken 15 days after each addition by withdrawing about 500g soil at a depth of 15cm around the root of the olive tree. Total bacterial counts were determined in these samples using plate count technique on Nutrient agar according to the method[14] . Plates were incubated at 30˚C for 3days and cell concentration was calculated by counting the grown colonies.

Statistical analysis

All obtained data during 2012, 2013 and 2014 experimental seasons were subjected to analysis of variances (ANOVA) [15]using MSTAT program. Least significant ranges (LSR) was used to compare between means of treatments[16] at probability of 5 %.

Results and Discussion

1. Fruit set and yield

Initial fruit set %

Data presented in Table (3) reveled that percentage of initial fruit set were significantly affected by the different mineral and bio-fertilization treatments in three seasons of studied. The highest initial fruit set was found under treatment B+MNF75% (44.92, 33.46 and 43.79 %) during the first, second and third seasons respectively. On the other hand in this respect the lowest initial fruit set was found in B+MNF25% (31.33, 18.85 and 35.54 %) during three studied seasons respectively.

Final fruit set %

It is clearly noticed that final fruit set were significantly affected by different fertilizers treatments Table (3) in three seasons of study. The highest final fruit set was found under treatment B+MNF75% (29.48, 23.88 and 31.94 %) during the first, second and third seasons respectively. On the other contrary, in this respect, the lowest final fruit set was found in B+MNF25% (20.26, 14.03 and 23.54 %) during the first, second and third seasons respectively.

Fruit drop %

Data in Table (3) showed that, there was no clear trend concerning different fertilizer treatments on fruit drop. The highest fruit drop (%) was found under B+MNF50% treatment in the first season (40.05 %). Meanwhile, in the second one MNF100% recorded the highest one in this respect since it was 33.53, additionally in the third season the highest fruit drop founded with MNF25% treatment since it was (33.76 %). On the contrary, the lowest values were recorded with B+MNF75%(bio-fertilizer + 75% Mineral Nitrogen Fertilization)34.35 and 27.06 % in the first and third seasons respectively. Also, in second season the lowest significant fruit drop (%) was observed with B+MNF50% (17.53 %).

Table (3): Effect of mineral and bio-fertilization on Initial fruit set, Final fruit set, final drop content and yield of "Manzanillo" olives in 2012, 2013 & 2014 seasons.

Yield (kg/tree)			Fruit drop (%)			Final fruit set (%)			Initial fruit set (%)			Treatments
2014	2013	2012	2014	2013	2012	2014	2013	2012	2014	2013	2012	
68.07 b	26.38 b	57.37 b	28.92bc	33.53 a	34.92 b	30.30 b	22.18 b	26.68 b	42.62 b	33.42 a	40.99 b	**MNF100%***
71.67 a	28.13 a	59.97 a	27.06 c	28.57ab	34.35 b	31.94 a	23.88 a	29.48 a	43.79 a	33.46 a	44.92 a	**B+MNF75%**
64.34 c	26.37 b	59.10 a	29.38 b	17.53 c	40.05 a	28.83 c	20.24 c	25.35 b	40.83 c	24.60 b	42.27 b	**B+MNF50%**
51.47 d	22.67 c	52.33 c	33.76 a	25.60 b	35.08 b	23.54 d	14.03 d	20.26 c	35.54 d	18.85 c	31.33 c	**B+MNF25%**

Mean in each column with similar letter(s) are not significantly different at 5 % level.

(*)MNF100% =100% Mineral Nitrogen Fertilization (control**), B+MNF75%**= bio-fertilizer 2liter + 75%Mineral Nitrogen Fertilization**, B+MNF50% =** bio-fertilizer 2liter + 50%Mineral Nitrogen Fertilization, **B+MNF25%** = bio-fertilizer 2liter + 25%Mineral Nitrogen Fertilization.

Yield (kg/tree)

It is clear from the obtained data in Table (3) that mineral and bio-fertilizer significantly increased total yield compared with the control. In three seasons, the highest values of yield were recorded by trees treated with B+MNF75%scince it was 59.97, 28.13 and 71.67 kg. On the other hand, the result showed that application of B+MNF25% gave the lowest significant value of yield per tree compared with other treatments in the three seasons (52.33, 22.67 and 51.47 kg) respectively. Other treatments were in between.

From the previously results it could be concluded that application of bio-fertilizer with 75% mineral nitrogen fertilization increased fruit set and yield, this may be due to improvement of soil chemical and physical properties after bio-fertilizer application. Furthermore, the stimulus effect of biofertilizers application may be attributed to the promotion effect on the parameters of plant growth which are enable to absorb minerals by its root system and thus reflected on the fruit yield.

The present's results are in an agreement with those found (Abd El- Hameed, S.A.) [17]who mentioned that the interaction between 100% N and BF gave the highest significant fruit set%. Moreover, the highest significant fruit set % was observed with BF.In this respect, On Olive[18], mentioned that inoculation with Bacillus + $(NH4)_2SO_4$ at 500 g N / tree was the most effective treatment on yield as kg / tree. Meanwhile, on olive trees, El-Khawaga, A.S.[19] reported that, it is recommended use N at 400 g/tree through 972g ammonium suphate + 5.0 kg filter cake mud + 12.5g Bio azotene/tree to obtain an economical yield.

Such observations were also recorded by Abd El-Moneim[20] who showed that, using of 50% mineral N + 50% organic N + bio-fertilizer is the promising treatment to improve both fruit yield and quality of Washington Navel orange trees. Moreover, On mango Amrapali cv. Kundu, S. et al[21] indicated that higher yield and fruit wieght was obtained when the plants were treated with 100% NPK + *Azotobacter* + VAM (98.l kg/plant) or 75%NPK + *Azotobacter* + VAM (93.5 kg/plant) as compared to much lesser yield (60 Kg/plant) with 100% NPK. Also, On Valencia Orange Trees[22] indicated that, the application of biofertilizer plus 750 gm Magnetite treatment was the best combination for achieving the highest total yield (51.44, 38.22 % over control) during two seasons.

2. Physical fruit characteristics

Fruit weight (gm) and volume (cm^3)

It is clearly noticed that fruit weight and volume were significantly affected by different fertilizers treatments Table (4) in three seasons of study. From the obtained results, trees treated by B+MNF75% treatment gave the highest fruit weight (6.87, 8.96 and 5.73 gm) and volume (6.26, 8.00 and 5.53cm^3) during the first, second and third seasons respectively. On the other contrary B+MNF25% recorded the lowest fruit weight and volume in the first, second and third seasons since it was (5.38, 7.93 and 4.44 gm) and (5.03, 6.95 and 4.23cm^3) for fruit weight and volume respectively.

Table (4): Effect of mineral and bio-fertilization on fruit properties (fruit weight, fruit volume, fruit length and fruit diameter)"Manzanillo" olives in 2012, 2013 & 2014 seasons.

Fruit diameter (D cm)			Fruit length (L cm)			Fruit volume (cm^3)			Fruit weight (gm)			Treatment
2014	2013	2012	2014	2013	2012	2014	2013	2012	2014	2013	2012	
1.99 a	2.21 b	2.00 b	2.45 ab	2.74 b	2.54 ab	5.01 b	7.64 b	5.60 b	5.22 b	8.60 b	6.09 b	**MNF100%***
2.01a	2.28 a	2.09 a	2.50 a	2.84 a	2.60 a	5.53 a	8.00 a	6.26 a	5.73 a	8.96 a	6.87 a	**B+MNF75%**
1.96a	2.23 ab	1.98 b	2.40 b	2.72 b	2.50 bc	4.97 b	7.80 ab	5.20 c	5.19 b	8.57 b	5.65 c	**B+MNF50%**
1.85 b	2.17 b	1.97 b	2.33 c	2.65 c	2.44 c	4.23 c	6.95 c	5.03 c	4.44 c	7.93 c	5.38 d	**B+MNF25%**

Mean in each column with similar letter(s) are not significantly different at 5 % level.

(*)MNF100% =100% Mineral Nitrogen Fertilization (control**), B+MNF75%** = bio-fertilizer 2liter + 75%Mineral Nitrogen Fertilization**, B+MNF50% =** bio-fertilizer 2liter + 50%Mineral Nitrogen Fertilization, **B+MNF25%** = bio-fertilizer 2liter + 25%Mineral Nitrogen Fertilization

Fruit length, diameter (cm) and shape index

Data in Table (4) showed that fruit length and diameter cm was significantly affected with different treatments. The highest fruit length and diameter was recorded from B+MNF75% (2.60, 2.84 and 2.50 cm) for fruit length and (2.09, 2.28 and 2.01 cm) for fruit diameter during three studied seasons respectively. Meanwhile, the lowest fruit length was recorded due to treatments B+MNF25% since it was (2.44, 2.65 and 2.33 cm) for fruit length and (1.97, 2.17 and 1.85cm) for fruit diameter in the three studied seasons respectively. Concerning fruit shape index data given in Table (5) revealed that all fertilizer treatments had no significant effect on shape index in three studied seasons.

Pulp weight (gm)

It is clearly noticed thatpulp weight were significantly affected by different fertilizers treatments in three seasons of study. Data presented in Table (5) revealed that B+MNF75% (bio-fertilizer 2liter + 75%Mineral Nitrogen Fertilization) treatment gave the highest pulp weight (5.69, 5.53 and 5.01 gm) through studied seasons respectively. On the other hand, under B+MNF25% treatment pulp weight recorded the lowest values (4.28, 5.14 and 4.77) in the first, second and third seasons respectively.

Table (5): Effect of mineral and bio-fertilization on fruit properties (fruit shape index, Pulp weight, seed weight and Pulp/Seed ratio) "Manzanillo" olives in 2012, 2013 & 2014 seasons.

Pulp/Pit ratio			Seed weight (gm)			Pulp weight (gm)			Fruit shape index (L/D)			Treatments
2014	2013	2012	2014	2013	2012	2014	2013	2012	2014	2013	2012	
7.72 a	2.30 ab	5.83 a	0.600 b	2.617ab	0.900 b	4.62 b	5.98 b	5.19 b	1.23 a	1.24 a	1.13 a	**MNF100%***
6.93 a	2.70 a	4.87 ab	0.723 a	2.427ab	1.173 a	5.01 a	6.53 a	5.69 a	1.25 a	1.25 a	1.25 a	**B+MNF75%**
5.70 b	2.75 a	4.33 b	0.776 a	2.307 b	1.067ab	4.41 c	6.26 ab	4.58 c	1.22 a	1.22 a	1.26 a	**B+MNF50%**
5.71 b	1.85 b	3.91 b	0.666ab	2.790 a	1.100 a	4.77 d	5.14 c	4.28 d	1.26 a	1.22 a	1.23 a	**B+MNF25%**

Mean in each column with similar letter(s) are not significantly different at 5 % level.

(*)MNF100% =100% Mineral Nitrogen Fertilization (control**), B+MNF75%**= bio-fertilizer 2liter + 75%Mineral Nitrogen Fertilization**, B+MNF50% =** bio-fertilizer 2liter + 50%Mineral Nitrogen Fertilization, **B+MNF25%** = bio-fertilizer 2liter + 25%Mineral Nitrogen Fertilization

Seed weight (gm)

Data given in Table (5) revealed that seed weight was significantly affected with different mineral and bio-fertilizer treatments. Results indicated that seed weight had no aclear trend in three studied seasons.The highest seed weight was found under treatment B+MNF75% (1.17 gm),B+MNF25% (2.79 gm) and B+MNF50% (0.78 gm) in three studied seasons respectively. On the other hand the lowest seed weight was recorded from MNF100% (1.07 gm), B+MNF50% (2.31 gm) and MNF100%(0.60 gm) in the first, second and third seasons respectively.

Pulp/seed ratio

Data given in Table (5) revealed that pulp/seed ratio was significantly affected with different mineral and bio-fertilizer treatments. The highest pulp/seed ratio was found under treatments MNF100%(5.83), B+MNF50% (2.75) and MNF100% (7.72) in the first, second and third seasons respectively. Meanwhile the lowest pulp/seed ratio was found on B+MNF25% (3.91 and 1.85) in the first and second season and B+MNF50% (5.70) during the third one.

These results are in agreement with[18] who reported that, on olive trees inoculation with Bacillus + $(NH4)_2SO_4$ at 500 g N / tree was the most effective treatment on yield as kg/ tree, fruit length, width and weight, flesh % and seed weight, while values of fruit shape index were decreased. In this manner [23] reported that fertilizing Washington Navel orange trees with N at 1000 g / tree in the form of ammonium sulphate at 2.43 kg/ tree, Compost El- Neel at 11.63 kg/ tree and Biogen at 250 g / tree gave a striking effect on fruit quality (fruit weight). Also, on mango Amrapali cv.[21] concluded that the treatments 100%NPK + *Azotobacter* + VAM and 75% NPK + Azotobacter + VAM were effective and may be adopted to improve the productivity with quality fruits. Furthermore on Flame seedless grapevines, [24] proved that yield and clusters weights were significantly increased as microbial biofertilization were applied. 75% and 50% mineral fertilization plus Biofertilizers treatments recorded the highest clusters weight. Additionally**,** [25] found that the best results with regard to fruit quality of Anna apples were obtained with using N via 50 % mineral + 50 % compost enriched with effective microorganisms EM at 100 ml and molybdenum at 5 g/ tree.

3. Chemical fruit characteristics

Fruit moisture (%)

Data in Table (6) indicated that fruit moisture was not significantly affected by different fertilization treatments in the first season of the study exception for the second and third one there was significantly affected by different fertilization treatments on fruit moisture content B+MNF50% and MNF100% gave the highest fruit moisture (54.20 and 57.34 % respectively). WhileMNF100%and B+MNF50% gave lowest fruit moisture (52.34 % and 53.05 %) in the second season and third seasons respectively.

Flesh oil content (%)

Data presented in Table (6) showed that flesh oil content was significantly affected by different fertilization treatments in all seasons under study**.** In addition, flesh oil content was higher in the third season than in the first and second seasons. The highest flesh oil content was observed in the three seasons with B+MNF75% (29.80 and 41.13 %) in the first and third seasons, also with MNF100% (33.33 %) in the second one. While B+MNF25% gave the lowest flesh oil content (27.44, 30.89 and 35.07 %) in the three studied seasons respectively. Other treatments are in between.

Fruit content of NO_3(mg.kg^{-1})

As shown in Table (6) data indicated that, fruit content of NO_3 was significantly affected by different fertilization treatments in the three seasons**.** In addition, fruit content of NO_3 was higher in the first season than in the second and third seasons. 100 % mineral nitrogen fertilization (MNF100%) gave the highest fruit content of NO_3 inthe three seasons (9.07, 7.32 and 7.22 mg.kg $^{-1}$ respectively), while treatment B+MNF25% decrease fruit content of NO_3 since it was (4.44, 3.06 and 2.07 mg.kg^{-1}) in the three seasons respectively. Other treatments were intermediate. Such levels of NO3 and NO_2 in berries juice are less than those of maximum contaminant level (MCLs) of nitrate (10 mg/L) in drinking water as recommended [26].

Table (6): Effect of mineral and bio-fertilization on fruit chemical properties of "Manzanillo" olives during in 2012, 2013 & 2014 seasons.

Fruit content of NO3(mg.kg $^{-1}$)			Flesh oil content(%)			Fruit moisture (%)			Treatments
2014	**2013**	**2012**	**2014**	**2013**	**2012**	**2014**	**2013**	**2012**	
7.22 a	7.32 a	9.07 a	40.27 ab	33.33 a	28.44 b	57.34 a	52.34 b	60.26 a	**MNF100%***
4.82 b	5.46 b	7.04 b	41.13 a	32.33 ab	29.80 a	54.16 b	53.62 a	61.70 a	**B+MNF75%**
3.24 c	3.61 c	5.28 c	38.67 b	32.06 b	28.08 b	53.05 c	54.20 a	56.11 a	**B+MNF50%**
2.07 d	3.06 d	4.44 d	35.07 c	30.89 c	27.44 b	54.92 b	53.55 a	61.03 a	**B+MNF25%**

Mean in each column with similar letter(s) are not significantly different at 5 % level.

(*)MNF100% =100% Mineral Nitrogen Fertilization (control**), B+MNF75%** = bio-fertilizer 2liter + 75%Mineral Nitrogen Fertilization**, B+MNF50% =** bio-fertilizer 2liter + 50%Mineral Nitrogen Fertilization, **B+MNF25%** = bio-fertilizer 2liter + 25%Mineral Nitrogen Fertilization.

4. Oil properties

Oil acidity (%)

Concerning data in Table (7) it is obvious that oil acidity was significantly affected by different fertilization treatments in the three seasons. Moreover, oil acidity was lower in the third season than in the first and second seasons. B+MNF25% gave the highest oil acidity in the first, second and third seasons since it was (0.48, 0.46 and 0.29 %). On the other hand, the lowest oil acidity was obtained by B+MNF50% in the first and second seasons it was (0.41 and 0.36 %), while B+MNF75% give the lowest oil acidity (0.22 %) in the third one.

Peroxide value (meq / kg oil)

Table (7) showed that peroxide value was significantly affected by different fertilization treatments in the three seasons. In addition, 100 % mineral nitrogen fertilization (MNF100%) gave the highest peroxide value (6.98, 6.31 and 5.92) in the first, second and third seasons respectively. In contrast, in the first, second and third seasons the lowest peroxide value was observed with B+MNF25% (6.25, 4.09 and 4.20 respectively) compared with other treatment.

Iodine value

As shown in Table (7), iodine value was significantly affected by different fertilization treatments in all seasons under study. In the first season, MNF100% gave the highest iodine value (82.58), in addition, in the second and third season of the study B+MNF75% gave the highest iodine value (82.34 and 84.48) respectively. On the other contrary, the lowest iodine value in the first, second and third seasons was observed by B+MNF25% (76.55, 76.17 and 77.47 respectively).Other treatments were intermediate.

Table (7): Effect of mineral and bio-fertilization on oil properties"Manzanillo" olives in 2012, 2013 & 2014 seasons.

Refractive index (RI)			Iodine value			Peroxide value (meq / kg oil)			Oil acidity(%)			Treatments
2014	2013	2012	2014	2013	2012	2014	2013	2012	2014	2013	2012	
1.4681a	1.4684a	1.4687a	79.79 b	78.89 c	82.58 a	5.91 a	6.31 a	6.98 a	0.23 ab	0.36 b	0.34 c	**MNF100%***
1.4684a	1.4680a	1.4685a	84.48 a	82.34 a	79.78 b	4.80 b	5.18 b	6.54 c	0.22 b	0.38 b	0.40 bc	**B+MNF75%**
1.4689a	1.4681a	1.4682a	79.27 b	79.55 b	80.16 b	4.71 c	5.16 b	6.78 b	0.27 ab	0.36 b	0.41 b	**B+MNF50%**
1.4686a	1.4689a	1.4680a	77.47 c	76.17 d	76.55 c	4.20 d	4.09 c	6.25 d	0.29 a	0.46 a	0.48 a	**B+MNF25%**

Mean in each column with similar letter(s) are not significantly different at 5 % level.

(*)MNF100% =100% Mineral Nitrogen Fertilization (control**), B+MNF75%**= bio-fertilizer 2liter + 75%Mineral Nitrogen Fertilization**, B+MNF50% =** bio-fertilizer 2liter + 50%Mineral Nitrogen Fertilization, **B+MNF25%** = bio-fertilizer 2liter + 25%Mineral Nitrogen Fertilization

Refractive index (RI)

There was no significant difference in refractive index in the three seasons Table (7), where all treatments gave the refractive index in the normal range of olive oil it was between (1.4680 – 1.4707) at 20°C.

The present's results are in an agreement with those found [18] who mentioned that inoculation with Bacillus + $(NH4)_2SO_4$ at 500 g N / tree was the most effective treatment on oil % in all seasons of study on olive trees. Also, [27] found that promotive effects were found in flesh oils content as the biofertilizer 'Phosphorine' combined with other treatments.

In this manner, these results are agree with those obtained on Crimson seedless grapevine[28,29,230,31,32].

5.total microbial count (x10^6 / g dry soil) in the rhizosphere

Table (8) illustrates the effect of mineral nitrogen fertilization and biofertilization on total microbial in the rhizosphere of "Manzanillo" olives. Data showed `that total microbial count gradually increased with plant age time and addition of biofertilizers does. This could be related to the change in root exudates, which usually stimulate the growth of rhizosphere bacteria. Data also showed that the highest number of microbial counts were detected with the treatment consisted of biofertilizers with and poultry manure with 75% of mineral nitrogen fertilization, which was significantly higher 100% of mineral nitrogen fertilization. This increase can be explained by that the inoculated biofertilizing bacteria was able to colonize and get adapted to the soil they are injected into, which adds to the total number of rhizosphere bacteria. The increase in total microbial count could be reflected on the yield and quality of the Manzanillo olives.

Table (8): Effect of mineral and bio-fertilization on total microbial count (x10^6 / g dry soil) in the rhizosphere of "Manzanillo" olives.

Third addition©	second addition©	first addition©	Zero time©	Treatments
10.7	**8.5**	**7.4**	**6.5**	**MNF100%***
34.1	**20.9**	**15.9**	**6.3**	**B+MNF75%**
27.3	**17.5**	**15.4**	**7.1**	**B+MNF50%**
22.9	**15.4**	**14.7**	**6.8**	**B+MNF25%**

(©) Counting are done 15 days after each addition according to the method described in the materials and methods section.

(*)MNF100% =100% Mineral Nitrogen Fertilization (control**), B+MNF75%**= bio-fertilizer 2liter + 75%Mineral Nitrogen Fertilization**, B+MNF50% =** bio-fertilizer 2liter + 50%Mineral Nitrogen Fertilization, **B+MNF25%** = bio-fertilizer 2liter + 25%Mineral Nitrogen Fertilization**.**

The beneficial effects of EM on Valencia orange trees was mainly attributed to its positive action on enhancing soil fertility and uptake of most nutrients, nitrogenase activity and total counts of bacteria[33]. These results were supported by the findings of [34,35]**.**

Conclusion

From the abovementioned results, we can conclude that all soil application of different levels of mineral nitrogen fertilization alone or in combination with bio-fertilizer had a positive effect on increased yield as well as fruit physical properties, fruit oil content and improved oil quality and total microbial count. In addition, treatment with (B+MNF75%) was the most effective treatment in enhancing yield and fruit physical parameters as well as; fruit weight, volume, length and diameter. In addition, fruit oil content, oil quality and total microbial count also were the best from trees treated by B+MNF75%.

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