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Effect of Organic and Bio-Fertilization on Fruit Chemical and Oil Properties of Manzanillo Olives.

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ABSTRACT

This study was conducted during 2012, 2013 and 2014 seasons to investigate the effect of different sources of organic fertilization alone or in conjunction with bio-fertilizer on fruit chemical and oil properties of Manzanillo olive trees grown in Cairo-Ismailia desert road, Egypt. The experiment was conducted on 15-year-old olive trees cultivar of Manzanillo, planted at 5 X 5 m away grown in sandy soil, beneath drip irrigation system, and uniform in form and received the common and recommended horticultural practices. Seven treatments were used in this experiment. The obtained results showed that SMB2 and PMB2 were the best treatments with regards to The highest rate of fruits from oil content ,lower fruits content from nitrates, the lowest rate of oil from the acidity and peroxide number and the highest rate of oil from iodine value. In addition, the best treatments, which gave the highest total microbial count in the rhizosphere of soil.

Keywords: olive, Manzanillo, Organic fertilization, Bio fertilization, fruit chemical properties, oil quality

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INTRODUCTION

Olive *(Olea europaea L.)* is one of the oldest planted tree crops in the history of the world about 8000 years age. It was originated in antiquity in the eastern side of the Mediterranean Sea. Olive has speared to all the countries throughout the Mediterranean basin, which is still the main region of olive production until today. According to statistics of Food and Agriculture Organization [1] the world area olive groves in 2013 is about 10,244,194 hectares and global production of olive is 20,344,343 tons, most of which is extracted in olive oil while the remaining is used to the table olive.

The organic material necessary for plant nutrition, not only as slow release fertilizers but also essential for the efficient plant production system [2]. Compost was more efficient in improving the physical and chemical properties of the soil and promote the growth of the olive trees. Therefore, these media are recommended for olive cultivation under the arid and semi-arid areas, which are limited in water resources, especially sandy soil [3]. the leaf mineral content during the growth cycles of the olive trees about having an economical yield, and also increase the percentage of fruit set, reduces the fruit dropping weaves and improves the oil characteristics [4]. Organic virgin olive oil was superior qualities compared with the conventional virgin olive oil, i.e. lower acidity value [5,6]. In additions, the use of biofertilizers is one of the important ingredients of integrated nutrient management, as they are cost effective and renewable source of plant nutrients for the completion of chemical fertilizers for sustainable agriculture. Take advantage of many of the micro-organisms and their association with crop plants in the production of bio-fertilizers. Furthermore, Biofertilizers are live Forge of microorganisms (useful bacteria and fungi) that are willing to be used and plant varieties by increased the availability of nutrients in the soil and Flora. Biofertilizers naturally activate the microorganisms found in the soil, recover the soil's natural fertility and protect against drought and soil diseases and thus stimulate plant growth [7]. In addition, Biofertilizer is a natural product carrying living microorganisms derived from the root or planted soil. So they don't have any bad effect on soil health and the environment. Besides their role the atmospheric nitrogen fixing and phosphorous solubilisation, also these assists in stimulating the plant growth hormones, providing better absorption of nutrients and increased tolerance to drought and moisture stress. Therefore, the present study was to evaluate the effect of fertilizing Manzanillo olive trees with organic and bio-fertilization on fruit chemical and oil characteristics.

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 analysis are given in (table 1) and water irrigation analysis are given in (table 2) according to procedures which are outlined by [8].

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

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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 seven treatments, 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, beside the organic manures sheep manure and poultry manure as a source of nitrogen.

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. Organic fertilizer was added by rate (500 gm N /tree / year) Obtained from 25 Kg sheep manure (2.00 % N) per tree or 20 Kg poultry manure (2.5 % N) per tree, organic fertilizer superficially and dug in the soil at the second week of December. The Chemical and physical analysis of organic manure sources for three seasons are given in (Table 3). Mineral phosphate and potassium fertilizer were added by rate 1.75 Kg of superphosphate (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 into two equal doses, firstly at the second week of December combined with phosphate and organic fertilizers and secondly at the first week of June. 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 [9] to give a cell suspension of $5x10^5$; $6x10^7$ and $4x10^7$ cells /ml for *Azotobacter chroococcum*, *B.megaterium* and *B. circulans*, respectively. Cultures were mixed on site, then each tree received either 1 or 2 liters of the mix, and this treatment was repeated every two months for three times during the season.

Table 3: Chemical and physical analysis of organic manure sources.

Character	Sheep manure			Poultry manure		
	2012	2013	2014	2012	2013	2014
Weight of m^3(kg)	550	550	540	530	535	530
Humidity (%)	15	15	13	15	20	15
pH	8.01	8.04	8.09	7.22	7.13	7.20
EC (mm /cm)	13.7	11.59	11.77	14.17	14.4	16.17
Organic matter (%)	31.25	30.45	30.12	33.65	35.16	32.55
Organic carbon	30.22	31.26	30.15	28.00	27.90	27.88
C / N ratio	15.19	15.63	15.08	10.89	11.25	10.89

Total nitrogen (%)	1.99	2.00	2.00	2.55	2.48	2.56
Total phosphorus(%)	0.44	0.51	0.50	1.24	1.33	1.22
Total potassium (%)	5.00	5.16	5.15	5.55	5.58	5.66
Fe (ppm)	817.6	815.4	785.4	577.2	559.6	584.3
Mn (ppm)	346.6	333.2	325.8	239.6	221.8	220.5
Zn (ppm)	56.4	58.9	54.6	105.1	100.3	100.8

Treatments: this experiment included seven treatments as follows:

T1 - 100 % mineral nitrogen fertilizer (1000 g N/tree) (control).
T2 - Sheep manure + bio-fertilizer (2 liter/ tree)(SMB2).
T3 - Sheep manure + bio-fertilizer (1 liter/ tree)(SMB1).
T4 - Sheep manure without bio-fertilizer(SM).
T5 - Poultry manure + bio-fertilizer (2 liter/ tree)(PMB2).
T6 - Poultry manure + bio-fertilizer (1 liter/ tree)(PMB1).
T7- Poultry manure without bio-fertilizer(PM).

Measurements

Chemical properties of fruit:

1. Fruit moisture %: moisture percentage of fruit in the previous fruit samples was estimated, samples were dried at 60-80 º C in an electrical air oven until constant weight, the fruit moisture percentage was calculated according to [10].
2. Fruit oil content %: Fruit oil content as a dry weight was determined according to [10] method of extraction the oil from the dried flesh fruit with soxhelt for extraction apparatus using petroleum ether 60-80 º C of boiling point.
3. Nitrate content NO_3- N ($mg.g^{-1}$): NO_3- N was determined in distilled water extracts of dried tissue, according to the procedure of [11] as modified by [12] by nitration of salicylic acid.

Chemical properties of oil:

1. Acid value:

It was determined according to [13]. Five grams of oil were accurately weighed in 250 ml dry conical flask with about 100 ml of neutralized 50% ethanol + 50% petroleum 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.

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

Where

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

2. Peroxide number:

The peroxide volue was determined according to [14] 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.

Peroxide value =	0.5 X N X V × 100
	Weight of sample

Where

N = Normality of sodium thiosulpahte solution
V = Volume in ml. of sod. thiosulphate needed for titration
3. Iodine value

The degree of un saturation of oil was determined by measuring the amount of halogen absorbed by the oil as stated in [10]. (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 a starch indicator.

4. 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 by [15].

Microbiological measurements:

To determine the effect of different fertilization treatment on the 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 the plate count technique on Nutrient agar according to [16]. Plates were incubated at 30C 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) according to [17] using MSTAT program. Least significant ranges (LSR) were used to compare between the means of treatments according to [18] at probability of 5 %.

RESULTS AND DISCUSSION

Fruit chemical properties:

Fruit moisture (%):

Data in Table (4) showed that the fruit moisture content was significantly influenced by different treatments in the second and third seasons, respectively, except in the first season there was no significant differences between treatments were detected . In the second season PMB1 treatment recorded the highest fruit moisture content (56.63). Meanwhile PM recorded the lowest one in this respect since it was (51.49). As for the third season MNF100 % and PMB1 gave the highest fruit moisture content since it was (57.34 and 57.71) respectively. On the other contrary SMB2, PMB2 and PM gave the lowest value in this respect (55.57, 55.57 and 55.94) respectively. On the other hand other treatments in the second and third seasons were in between ranges.

Flesh oil content(%):

Data presented in Table (4) indicated that flesh oil content was significant affected by various treatments in the second and third seasons, respectively. Where, in the first one there was no significant difference between treatments. In general, flesh oil content was higher in 2014 than in 2012 and 2013

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seasons. The highest flesh oil content was observed in 2013 season under treatment MNF100% since it was (33.33). On the other contrary SM treatment recorded the lowest one in this respect (30.50). Concerning the third season MNF100% and PMB1 treatments gave the highest flesh oil content (40.27 and 41.30) respectively. On the other hand SMB1 and SM treatments recorded the lowest one in this respect (35.33 and 35.07) respectively. Other treatments were intermediate in 2013 and 2014 seasons respectively.

Table 4: Effect of organic and bio-fertilization on chemical fruit properties of "Manzanillo" olive trees during in 2012, 2013 & 2014 seasons

Treatments	Fruit moisture (%)			Flesh oil content(%)			Fruit content of NO3(mg.kg $^{-1}$)		
	2012	2013	2014	2012	2013	2014	2012	2013	2014
MNF100%*	60.26 a	52.34 d	57.34 a	28.44 a	33.33 a	40.27 a	9.07 a	7.14 a	7.22 a
SMB2	61.06 a	55.02 b	55.57 b	27.89 a	32.70 ab	37.70 b	5.09 e	3.06 g	1.39 e
SMB1	63.04 a	52.85 cd	56.48 ab	27.09 a	32.00 ab	35.33 c	5.28 d	5.19 b	2.07 c
SM	59.54 a	53.51 c	56.58 ab	27.04 a	30.50 c	35.07 c	6.02 b	3.61 f	1.76 d
PMB2	59.04 a	54.65 b	55.57 b	28.52 a	32.96 ab	41.30 a	5.37 d	3.86 e	1.67 d
PMB1	60.16 a	56.63 a	57.71 a	27.81 a	31.89 abc	38.00 b	5.68 c	4.11 d	2.41 b
PM	61.59 a	51.49 e	55.94 b	27.24 a	31.57 bc	38.13 b	5.77 c	4.41 c	1.85 cd

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

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

As shown in Table (4) data showed that, the fruit content of NO_3 as (mg.kg) was significant affected by different treatments in the three studied seasons. In general, the fruit content of NO_3 was lower in 2014 season than in 2012 and 2013 seasons. MNF100% mineral nitrogen fertilization gave the highest fruit content of NO_3 in three seasons of the study since it was (9.07, 7.14 and 7.22 mg.kg $^{-1}$ respectively). Meanwhile, treatment SMP2 (5.09, 3.06 and 1.39) recorded the lowest fruit content of NO_3 in the three seasons consecutive.

These results are approving with those obtained by [19] who found that, Koroneiki olive cv. When provided with farmyard manure + compost + bio-fertilizers (as combination between phosphorene and Nitrobein) produced the highest fruit oil content. in addition, [20] reported that soil application of manure tea with yeast + humic acid gave a better effect on fruit moisture content. Whereas, using compost tea to the soil with yeast + humic acid extracts gave a better effect on fruit flesh oil contents. Furthermore [21] used aqueous extract of three compost sources *i.e*. farmyard manure, town refuse and sewage sludge at two rates (20% and 30%), either alone or with bacterial suspension of *Azotobacter chroococcum* and showed obvious beneficial effects of different measurements composts at rate 30% that reflected on flesh oil content. The best results were received with sewage sludge treatment, as compared with control. Also, [22] reported that Valencia orange trees fertilized with N as 100% inorganic N, as well as 75% inorganic N + 12.5 % organic + 50 to 400 ml EM and/or foliar with amino acids enriched with N, P, K, Mg, Fe, Zn, Fe and B. and found that a progressive reduction on both nitrate and nitrite in the juice was observed with reducing the percentages of inorganic N and increasing levels of effective microorganisms.

Oil properties:

Oil acidity (%):

Regarding data in Table (5) it is obvious that oil acidity percentage was significant affected by different treatments in the three seasons. In general, oil acidity was lower in the third season than in the first and second seasons. SMB1 and SM treatments gave the highest oil acidity percentage since it was (0.47 and

0.45 %) in the first season and second season respectively. In addition, in the third season the highest oil acidity was found under SM and PM treatments being (0.34 and 0.37 %). On the other side, the lowest oil acidity percentage was obtained by PMB2 and PM treatment (0.32 and 0.32 %) in the first season. Meanwhile SMB2 treatment recorded the lowest oil acidity (0.32 and 0.20 %) during the second and third seasons consecutive.

Peroxide, Iodine value and Refractive index:

Table 5 indicated that the peroxide value was significant affected by different treatments during three seasons of study. MNF100%, SM and MNF100% treatments recorded the highest peroxide value was (6.98, 6.72 and 5.92) in the first, second and third seasons consecutive. In return, in the first season the lowest peroxide value was observed with SMB2 (4.91), while SMP2 and PMP2 treatments gave the lowest peroxide value (5.36 and 5.39) in the second one respectively followed by PMP2 treatment which showed the lowest peroxide value 4.56 in the third one. Other treatments were in between range in this respect. As for iodine value data shown in Table (5), cleared that iodine value was significant affected by different treatments in all seasons under study. PMB2 and SMB2 treatments recorded the highest iodine value (84.26 and 79.46) in the first and second seasons respectively. Meanwhile the lowest iodine value was found under treatment SM since it was (73.77 and 71.42) during both seasons respectively. In addition in the third one the highest iodine value was found under treatments MNF100% and PMB2 (79.79 and 79.81) respectively. On the other contrary, the lowest iodine value in this respect was observed by SM and PM (77.70 and 77.56) respectively. Concerning refractive index data in the same Table revealed that there was no significant difference in refractive index in all seasons between all different where all treatments gave there refractive index in the normal range of olive oil it was between (1.4680 – 1.4707) at 20°C.

Table 5: Effect of organic and bio-fertilizationon oil properties of "Manzanillo" olives in 2012, 2013 & 2014 seasons

Treatments	Oil acidity(%)			Peroxide value (meq / kg oil)			Iodine value			Refractive index (RI)		
	2012	2013	2014	2012	2013	2014	2012	2013	2014	2012	2013	2014
MNF100%*	0.34 cd	0.36 bc	0.23 bc	6.98 a	6.31 b	5.92 a	82.58 b	78.89a b	79.79 a	1.4687 a	1.4684 a	1.4681 a
SMB2	0.43 ab	0.32 c	0.20 c	4.91 e	5.36 e	4.83 d	77.69 d	79.46 a	78.87a b	1.4689 a	1.4685 a	1.4680 a
SMB1	0.47 a	0.41 ab	0.24 bc	5.73 c	5.63 c	5.52 c	77.40 d	77.98 b	71.76 d	1.4686 a	1.4680 a	1.4689 a
SM	0.38 bc	0.45 a	0.34 a	5.40 d	6.72 a	5.68 b	73.77 f	71.42 d	77.70 c	1.4680 a	1.4681 a	1.4689 a
PMB2	0.32 d	0.34 c	0.27 b	5.47 d	5.39 e	4.56 f	84.26 a	76.45 c	79.81 a	1.4686 a	1.4680 a	1.4685 a
PMB1	0.33 cd	0.42 ab	0.28 b	5.64 c	5.51 d	4.62 e	76.71 e	76.20 c	78.51b c	1.4681 a	1.4685 a	1.4684 a
PM	0.32 cd	0.38 bc	0.37 a	5.85 b	5.66 c	5.63 b	79.03 c	75.50 c	77.56 c	1.4683 a	1.4680 a	1.4680 a

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

These results are in agreement with those obtained by [23] investigated that the oils were extracted from Leccine and Frantoio varieties, grown in the same geographic ant area under either organic or conventional methods. Found significant differences among organic and traditional oils differences in some years, but inconsistent trends across the 3 years were found. The acidity oils of organic Leccino was higher than traditional oils in first and second but not in third season; Frantoio oils were never different. Organic Leccino oils had a lower peroxide index in 2001, but the results were not statistically different in the other years. The concentrations of phenols, tocopherols, the antioxidant amplitude and the volatile compounds made clear differences on olive trees,. In this concern, On olive (Roghiani cv.), [20] reported that manure tea gave the highest oil peroxide and iodine values compared to the compost tea compared with control.

Total microbial count (x10^6 / g dry soil) in the rhizosphere :

Data presented in the Table (6) investigated that total microbial count increased at a great rate after each addition and total microbial count was higher after the third addition than after the first and second additions compared with zero time (before addition in beginning growing season). Secondly, the best treatments, which gave the highest total microbial count in the rhizosphere of soil was obtained by SMB2 (Sheep Manure + Bio-fertilizer 2liter) and PMB2 (Poultry Manure + Bio-fertilizer 2liter)since there were (15.9, 26.2 and 40.4) for SMB2 and (20.6, 22.5 and 44.2) for PMB2 after the three additions respectively. On the other hand, the lowest total microbial count was recorded with the treatments (SM, PM and MNF100% without bio-fertilizer) since there were (9.7, 7.9 and 4.7) after the first one and (10.7, 11 and 8.5) after the second addition and (11.9, 13.8 and 10.7) after third addition respectively. The beneficial effects of effective microorganism on orange trees were mainly ascribes to its positive action on enhancing soil fertility and uptake of majority total counts of bacteria, nutrients and nitrogenase activity [24]. These results were supported by the findings of [25,26] .

Table 6: Effect of organic and bio-fertilizationon total microbial count (x10^6 / g dry soil) in the rhizosphere of "Manzanillo" olive.

Treatments	Zero time®	first addition®	second addition®	Third addition®
MNF100%*	6.5	7.4	8.5	10.7
SMB2	6.2	15.9	26.2	40.4
SMB1	6.1	13.8	18.8	26.1
SM	5.8	9.7	10.7	11.9
PMB2	6.3	20.6	22.5	44.2
PMB1	5.9	14.2	19.5	28.7
PM	6.1	7.9	11	13.8

® Counting is done 15 days after each addition according to the method described in the materials and methods section.

CONCLUSION

From the previous results it could be concluded that, SMB2 and PMB2 were the best treatments with regards to The highest rate of fruits from oil content ,lower fruits content from nitrates, the lowest rate of oil from the acidity and peroxide number and the highest rate of oil from iodine value . In addition, the best treatments, which gave the highest total microbial count in the rhizosphere of soil .

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