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Effect of Mineral and Bio-fertilization on Vegetative Growth, Leaf Mineral Contents and Flowering 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 alone or in combination with bio-fertilizer on vegetative growth, leaf mineral contents and flowering 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 obtained results showed that(B+MNF75%) gave the highest values of all vegetative growth parameters as well as; shoot length, diameter, number of leaves per shoot, leaf area, pigments content and leaf mineral contents of (N, P, K, Fe, Zn and Mn). In addition flowering characteristics also were the best from trees treated by B+MNF75%.
Key words: Olive, Manzanillo, Biofertilization, Vegetative growth, leaf pigments, leaf mineral content, Flowering.

Introduction

The olive tree (*Olea europaea L.*) family Oleaceae is a widely distributed tree grown in many arid areas of the world. The Mediterranean region is its native habitat. Olive is adapted to extremely arid conditions because of its special leaf structure and ramified root system.The olive tree is an evergreen,one of the oldest cultivated tree, about 8000 years ago.

According to statistical of Food and Agriculture Organization[1] 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. In Egypt, the last statistics [2] cited that the total acreage grown with olive reached 202,743 feddans, total production reached 563,070 tons. Fayoum, Ismailia, Matrouh, South Sainai, Noubaria and desert road of Cairo/Alexandria are the most important regions of olive production.

Using biofertilizers that contain different microbial strains hassled to a decrease in the use of chemical fertilizers and hasprovided high quality products free of harmful agrochemicals for human safety[3]. Biofertilizers are products containing living cells of different types ofmicroorganisms, which have an ability to convert

nutritionally important elements from unavailable to available form through biological processes[4]. Bio-fertilization is considered an important factor in reducing the used rates of chemical fertilizers which appear to be safely for environment, improving soil fertility and increasing soil productivity[5]**.** The present study was initiated to evaluate the effect ofdifferent levels of mineral nitrogen fertilization alone or in combination with bio-fertilizer on vegetative growth, leaf mineral contents and flowering 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 analysis are given in (Table 1) and water irrigation analysis are given in (Table 2) according to procedures [6].

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[7] 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

On early January of each season, twenty healthy one year old shoots, well distributed around periphery of each tree were randomly selected and labeled (5 shoots toward each direction) for caring out the following measurements.

a. Vegetative growth

At the end of each growing season during first week of September the following characteristics were measured.

1. shoot length (cm).
2. shoot diameter (mm).
3. Number of leaves per shoot
4. Leaf area (cm^2) according to [8] using the following equilibration: Leaf area = 0.53 (length x width) + 1.66.

b. Leaf mineral content and pigments

Leaves needed were randomly sampled from the previously labeled shoots per each tree / replicate on the second week of September. Whereas, 2 - 3 leaves from every shoot (4th and 5th leaves) were picked then mixed together as a composite for carrying out the following chemical analysis:

1. Leaf mineral contents

Leaves sample from each tree / replicate was separately oven dried at 70 º C till constant weight, and then grounded for determination the following nutrient elements (Percentage as dry weight):

N – Using the modified micro – kjeldahl method [9].
P - Was estimated [10].
K – Flamephotometerically determined [11].
Fe, Zn and Mn as ppm was spectrophotometerically determined using atomic absorption (Model, spectronic 21 D) [12].

2. Pigments

Pigments i.e., chlorophyll a, b & carotene as mg/g were colormetrically determined in fresh leaf samples at wave length of 660, 640 and 440 nm for a, b & carotene respectively [13].

c. Flowering characteristics

1. Flowering density

1.1. Number of inflorescences per shoot on the labeled twenty shoots was calculated.

1.2. Number of inflorescences per meter on the labeled twenty shoots was calculated.

2.Number of total flowers per inflorescence: Sample of 20 inflorescences was taken from every tree and total number of flowers per inflorescences was counted.

3.Sex ratio: The percentage of perfect flowers to total flowers was calculated for every replicate.

4.Pollen germination (%): pollen grains were collected from inflorescences samples taken before pollen dehiscence, and kept for one night in the laboratory under room temperature. Germination of pollen grains were evaluated after incubation for 24 hrs at 25^{o}C in petri dishes with a liquid medium of 10 %sucrose, 0.01 ppm tetracycline. Three drops of the medium containing pollens per each replicate were placed on aside and numbers of germinated and ingeminated pollens were estimated then the germination percentage was calculated [14].

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. Vegetative growth

Shoot length (cm)

Data presented in the Table (3) indicted that shoot length was significantly affected with different fertilizer treatments in second and third seasons respectively. Meanwhile, in the first season no significant differences between treatments in this respect. Otherwise in the second and third seasons B+MNF75%(bio-fertilizer+75%Mineral Nitrogen Fertilization)gave the highest value in this respect since it was(26.42 and 28.22 cm) respectively . On the other side,B+MNF25% (bio-fertilizer+ 25%Mineral Nitrogen Fertilization) gave the lowest value of shoot length (24.00, and 23.30 cm)during the first and second seasons respectively. Other treatments were in between.

Shoot diameter (mm)

As shown in Table (3) shoot diameter was statistically affected by conducted treatments in the three seasons. In this respect, B+MNF75%resulted in the highest shoot diameter (2.60, 2.50 and 2.60 mm) in the first, second and third seasons, respectively. On the other hand, the least values of shoot diameter were obtained by (B+MNF25%) treatment since it was (2.07, 1.90 and 2.13 mm) in the first, second and third seasons respectively. Other treatments were in between.

Table (3): Effect of mineral and bio-fertilizationon vegetative characteristics of "Manzanillo" olives in 2012, 2013 & 2014 seasons.

Treatments	Shoot length (cm)			Shoot diameter (mm)			no. leaves/shoot			Leaf area (cm^2)		
	2012	2013	2014	2012	2013	2014	2012	2013	2014	2012	2013	2014
MNF100%*	24.23 a	25.22 b	26.64ab	2.50 a	2.47 a	2.40 ab	29.08ab	30.46 b	30.25 b	4.88 a	4.89 a	5.36 b
B+MNF75%	24.35 a	26.42 a	28.22 a	2.60 a	2.50 a	2.60 a	30.04 a	31.87 a	32.28 a	4.45 b	4.85 a	5.71 a
B+MNF50%	25.97 a	24.83bc	25.76 b	2.50 a	2.37 ab	2.20 b	28.83 b	29.37 b	30.61 b	4.40 b	4.54 ab	5.26 b
B+MNF25%	20.19 a	24.00 c	23.30 c	2.07 b	1.90 b	2.13 b	26.41 c	25.41 c	26.33 c	4.24 c	4.20 b	4.81 c

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

Number of leaves per shoot

Data presented in Table (3) indicate that, number of leaves per shoot was significantly affected by conducted treatments in the three studied seasons. B+MNF75% (bio-fertilizer +75%Mineral Nitrogen Fertilization) resulted in significantly the highest number of leaves per shoot (30.04, 31.87 and 32.28) in the first, second and third seasonsrespectively. On the other side, the lowest number of leaves per shoot was obtained from B+MNF25% since it was (26.41, 25.41 and 26.33) in both seasons of study respectively. Other treatments were in intermediate.

Leaf area (cm^2)

Data in Table (3) showed that leaf area was affected evidently by different treatments in the three seasons of study .MNF100% treatment resulted in the largest leaf area in the first and second seasons respectively since it was (4.88 and 4.89 cm^2). However, in the third season the highest effect was attributed to the B+MNF75% it was amounting to 5.91 cm^2.). On the other contrary the lowest leaf area was found byB+MNF25% (4.24, 4.20 and 4.81 cm^2) in first, second and third seasons respectively. Other treatments however, were inbetween.

These results are in accordance with those obtained that inoculation with the two bacterial strains enhanced vegetative growth (stem length, number of new leaves and leaf area) of different plant parts of olive transplants as compared with the control[17]. Without treatment results revealed that *Azotobacter* sp. was more effective than Azospirillumon olive trees cvs. Aggizi and Picual. Also, on olive trees noticed that all measurements in vegetative growth were significantly increased with all different bio-NPK fertilizer soil applied treatments during the two seasons[18]. In this manner, [19]on olive seedling, used the combination between four rates (0, 25, 50 and 100 %) of mineral fertilizer in the form of Crystalon (20% N: 20% P: 20% K) applied as soil application and three sources of bio-fertilizers (Nitrobein, Microbein and Biogein) at the rate of 2.5 g/ seedling of each. Whereas, number of leaves / seedling exhibited the highest values when the olive seedling treated with 100 % NPK and 2.5 g Nitrobein.

2.leaf pigments content

Leaf content of chlorophyll A, B and Carotene ($mg.g^{-1}$)

It is clearly noticed that leaf chlorophyll A content were significantly affected by different fertilizers treatments Table (4) in the second and third seasons only. The highest leaf chlorophyll A content was found under treatment B+MNF75% (1.32 and 1.52) during the second and third seasons respectively. Meanwhile in the first one there were no significant differences between treatments. On the other contrary B+MNF25% (bio-fertilizer + 25% Mineral Nitrogen Fertilization) recorded the lowest value in this respect since it was (0.82 and 0.96) in the second and third studied seasons respectively.

Table (4): Effect of mineral and bio-fertilization on chlorophyll and carotene content of "Manzanillo" olive leaves in 2012, 2013 & 2014 seasons.

Treatments	Chlorophyll A (mg.g^{-1})			Chlorophyll B (mg.g^{-1})			Carotene (mg.g^{-1})		
	2012	2013	2014	2012	2013	2014	2012	2013	2014
MNF100%*	0.950 a	1.169 ab	1.161 bc	0.685 a	0.991 ab	1.056 b	0.688 a	0.773 a	0.853 a
B+MNF75%	0.953 a	1.326 a	1.523 a	0.796 a	1.165 a	1.382 a	0.676 a	0.830 a	0.961 a
B+MNF50%	0.880 a	1.247 a	1.380 ab	0.746 a	1.045 b	1.163 b	0.648 ab	0.788 a	0.967 a
B+MNF25%	0.843 a	0.824 b	0.966 c	0.705 a	0.703 b	0.767 c	0.608 b	0.627 a	0.706 b

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

As for leaf content of chlorophyll B data in the same Table (4) reveled thatleaf content of chlorophyll Bwas significantly affected by different fertilizers treatments in the second and third seasons only. On the other hand, there was no significantly a difference between treatments in the first one .B+MNF75%recorded the highest chlorophyll B content of leaf (1.165 and 1.382) in the second and third seasons respectively. On the other hand, B+MNF25%gave the lowest value in this respect since it was (0.705, 0.703 and 0.767) in the three seasons of study respectively. Other treatments were intermediate.

Leaf content of Carotene (mg.g^{-1})

Data concerning leaf content of Carotene presented in Table (4) indicted that leaf content of Carotenewas significantly affected with different fertilizer treatments in the first and third seasons only. While in the second one there were no significantly differences between treatments. In the first season MNF100% and B+MNF75% recorded the highest value in this respect since it was (0.688 and 0.676).. Meanwhilethe highest value was recorded from B+MNF50% and B+MNF75% in the third one since it was (0.967 and 0.961) during third one.On the other contrary, in the first, second and third seasons the lowest significant leaf carotene content was observed with B+MNF25%(0.61, 0.63 and 0.71 respectively) in this respect**.**

These results are in agreement with those obtained that the combined application of three bio-fertilizers at 20 g per seedling from each biofertilizer recorded the best results for leaf chlorophyll content of Valencia orange budded on both Sour orange and Volkamer lemon rootstocks[20]. Additionally, on bitter orange, noticed that all the biofertilizer treated seedling exhibited higher chlorophyll content than those of the control[21]. Also, noticed that all treatments significantly promoted chlorophyll a, b of Jatropha seedlings than control plants due to algae, phosphorien, compost and microbien treatments[22].

On the other hand, these results disagree with [23] who found that differences in leaf chlorophyll (A&B) contents of both cultivars didn't reach level of significance. In addition, inoculation with AC + 500 g actual N as $(NH_4)_2SO_4$treatment was the superior for raising leaf chlorophyll A&B content. Also, on olive trees cv. Picual, were found that no significantly difference was observed between treatments on leaf chlorophyll A content[24].

Table (5): Effect of mineral and bio-fertilization on macro elements of "Manzanillo" olive leaves in 2012, 2013 & 2014 seasons.

Treatments	N (%)			P (%)			K (%)		
	2012	2013	2014	2012	2013	2014	2012	2013	2014
MNF100%*	1.57 a	1.68 a	1.64 b	0.247 a	0.253 a	0.267 a	1.38 a	1.29 a	1.34 a
B+MNF75%	1.58 a	1.69 a	1.71 a	0.253 a	0.267 a	0.287 a	1.37 a	1.30 a	1.36 a
B+MNF50%	1.52 a	1.59 b	1.61 b	0.233 a	0.257 a	0.267 a	1.32 b	1.25 ab	1.31 a
B+MNF25%	1.37 b	1.42 c	1.53 c	0.217 a	0.230 a	0.250 a	1.22 c	1.21 b	1.21 b

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.

3. Leaf mineral content

Leaf content of N (%)

Data in Table (5) showed that leaf content of N was significantly affected by different fertilization treatments in the three seasons. In addition, leaf content of N was increased in the second and third seasons than in the first one. Bio-fertilizer +75%Mineral Nitrogen Fertilization (B+MNF75%) gave the highest leaf content of N (1.58, 1.69 and 1.71 %) in the first, second and third seasonsrespectively .While B+MNF25%recorded the lowest leaf content of N (1.37, 1.42 and 1.53 %) during both seasons of study respectively. Other treatments were intermediate.

Leaf content of P (%)

There was no significant difference in leaf content of P in the three seasons (Table 5). Leaf content of P was increased in the third season than in the first and second season. In the three seasons B+MNF75%(bio-fertilizer +75%Mineral Nitrogen Fertilization)gave the highest leaf content of P (0.253, 0.267 and 0.287 %). while the lowest leaf content of P was observed by B+MNF25% (0.217, 0.230 and 0.250 %) in the first, second and third seasons respectively.

Leaf content of K (%)

Data in Table (5) indicated that leaf content of K was significantly affected by different fertilization treatments in the three seasons. In the first season of the study MNF100% recorded the highest value in this respect (1.38 %) . Meanwhile, in the second and third one B+MNF75% gave the highest leaf content of K (1.30 and 1.36 %) respectively. On the other hand B+MNF25%gave the lowest leaf content of K in the first, second and third seasons respectively (1.22, 1.21 and 1.21) as compared with other treatments.

Table (6): Effect of mineral and bio-fertilization on micro elements of "Manzanillo" olive leaves during in 2012, 2013 & 2014 seasons.

Treatments	Fe (ppm)			Zn (ppm)			Mn (ppm)		
	2012	2013	2014	2012	2013	2014	2012	2013	2014
MNF100%*	259.4 ab	286.7 a	283.6 b	24.73 a	29.50 a	28.00 a	28.27 a	30.07 a	31.20 a
B+MNF75%	266.8 a	297.3 a	306.4 a	23.10 ab	29.51 a	28.90 a	29.90 a	32.93 a	32.33 a
B+MNF50%	253.1 b	280.7 a	270.5 c	21.93 b	26.37 b	25.13 b	27.57 a	29.07 a	27.33 b
B+MNF25%	231.2 c	263.3 b	264.8 c	22.07 ab	22.80 c	24.73 b	29.47 a	27.13 a	28.67 b

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.

These observations are in accordance with those obtained [25] who noticed that inoculation with Azotobacter or Bacillus + either NH_4NO_3 or $(NH4)_2SO_4$ treatments were significantly the most simulative for increasing leaf mineral content. However, Bacillus + either NH_4NO_3 or $(NH4)_2SO_4$ was the superior for leaf N, P, while Azotobacter + either NH_4NO_3 or $(NH4)_2SO_4$ was the superior for leaf K content. Also, [26] showed that, three-fourth dose N +full dose P + 20 g (Azotobacter) gave the highest content of K percentage. Moreover, On apricot, [27]found that different biofertilizer treatments significantly increased the leaf content of N, P and K of Canino apricot trees as compared with control treatment. In this manner on mango cv. Amrapali, [28]showed thatthe treatment NPK (100 %) + VAM + Azotobacter resulted higher nitrogen (1.56-1.88%), phosphorus (0.14-0.15%) and potassium (1.11-1.21 %) content in leaves. Also, [29] found that microbial inoculums T1 (microbial inoculated and received 100% of recommended doses of mineral fertilizers) and T2 (microbial inoculated and received 75% of recommended doses of mineral fertilizers) significantly increased N, P and K of Flame seedless grapes leaves.

Leaf content of Fe (ppm)

Data presented in Table (6) pointed out that, leaf content of Fe was significantly affected by different fertilization treatments in the three seasons of study .Leaf content of Fe was increased in the third season than in the first and second. Moreover, B+MNF75% gave the highest leaf content of Fe (266.8, 297.3 and 306.4 ppm) in the first, second and third seasons respectively. While B+MNF25%(bio-fertilizer+ 25% Mineral Nitrogen Fertilization) gave the lowest leaf content of Fe in the three seasons of study (231.2, 263.3 and 264.8 ppm) respectively.Other treatments were in between.

Leaf content of Zn (ppm)

As shown in Table (6), leaf content of Zn was significantly affected by different fertilization treatments in the three seasons. The highest leaf content of Zn was found under treatment MNF100% (24.73 ppm) during the first season .Meanwhile in the second and third seasons B+MNF75% gave the highest leaf content of Zn since it was (29.51 and 28.90 ppm) . On the other contrary, bio-fertilizer + 50% Mineral Nitrogen Fertilization (B+MNF50%) andB+MNF25% gave the lowest leaf content of Zn(21.93, 22.80 and 24.73 ppm) in the first season, second and third seasons respectively .

Leaf content of Mn (ppm)

Data in Table (6) showed that, leaf content of Mn was significantly affected by different fertilization treatments in the three seasons. Bio-fertilizer + 75% Mineral Nitrogen Fertilization (B+MNF75%) gave the highest leaf content of Mn (29.90, 32.93 and 32.33 ppm respectively) in the seasons of the study. On the other hand, in the first and third seasonsB+MNF50% gave the lowest leaf content of Mn (27.57 and 27.33 ppmrespectively). However, in the second season B+MNF25% gave the lowest value it was (27.13 ppm). Other treatments were intermediate.

The present's results are in an agreement with those found by [23]on olive trees who found that Bacillus + either NH_4NO_3 or $(NH_4)_2SO_4$was the superior as leaf Zn were concerned, while Azotobacter + either NH_4NO_3 or $(NH_4)_2SO_4$was the superior for leaf Mn contents. Moreover, on apple trees [31]indicated that all microbial bio-fertilization treatments increased leaf concentration of Fe and Zn. The recommended treatment is microbial bio-fertilization + 50% of recommended mineral fertilizers. In this respect, [31] on Anna apple orchards and reported that application of N through 50 to 75 % mineral + 25 to 50 % compost enriched with EM with or without molybdenum greatly enhanced leaf content of Zn, Mn and Fe.

4. Flowering characteristics

Flowering density (number of inflorescences per shoot and per meter)

Data presented in Table (7) showed that Flowering density as number of inflorescences per shoot were significantly affect to all tested treatments in three studied seasons. The highest number of inflorescences per shoot was recorded under the treatment B+MNF75%since it was (17.33, 12.09 and 18.67) for number of inf. per shoot in the three seasons of study respectively. On the other contrary the lowest values in this respect was recorded due to the treatment B+MNF25%since it was (15.33, 9.69 and 13.75) in the three seasons respectively . As for number of inflorescences per meter data presented in the same Table reviled that number of inflorescence per meter were significantly affected with different treatments in the first and second seasons respectively meanwhile in the third one there was no significantly difference between treatments. B+MNF25% and B+MNF75%recorded the highest values in this respect (76.03 and 66.20). Otherwise, exhibited the significant least average number of inflorescences per meter were recorded under treatments B+MNF50% and**B+MNF25%** (62.83 and 40.39) in the first and second seasons respectively. Other treatments however, were inbetween.

As for number of flowers per inflorescence data in Table (7) reveled that number of flowers per inflorescent were significantly affected with different treatments in the in the first and third seasons. Meanwhile in the second one there were no significantly differences between treatments. B+MNF75% recorded the highest number of flowers per inflorescence (29.10 and 28.38) in the first and third seasons respectively. On the other

contrary,B+MNF25%(bio-fertilizer + 25% Mineral Nitrogen Fertilization)gave the lowest values (24.97 and 24.10) in this respect in the first and third seasons. Other treatments were in between**.**

Table (7): Effect of mineral and bio-fertilization on flowering characteristics [flowering density (no. infl. /shoot and No. infl. / m) and no. total flowers/inf.] of "Manzanillo" olives in 2012, 2013 & 2014 seasons.

Treatments	Flowering density						No. total flowers / inf.		
	No. infl. / shoot			No. infl. / m					
	2012	2013	2014	2012	2013	2014	2012	2013	2014
MNF100%*	16.56 ab	9.83 c	17.08 ab	68.39 ab	38.98 b	64.13 a	28.63 a	14.33 a	26.47 b
B+MNF75%	17.33 a	12.09 a	18.67 a	71.16 ab	45.76 a	66.20 a	29.10 a	14.50 a	28.38 a
B+MNF50%	16.13 bc	11.18 b	16.25 b	62.83 b	45.03 a	63.07 a	25.67 b	12.60 a	27.40 ab
B+MNF25%	15.33 c	9.69 c	13.75 c	76.03 a	40.39 b	59.12 a	24.97 b	12.37 a	24.10 c

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

Sex ratio (%)

Sex expression as percentage of perfect flowers to total flowers was presented in Table (8). Studding the effect of different treatments on sex expression it appeared that, B+MNF75% was the most effective treatment and resulted in the highest significant percentages since it was (73.89, 67.62 and 78.89 %) in three studied seasons respectively. On the other hand, B+MNF25% recorded the lowest sex expression during the first, second and third seasons (60.71, 58.84 and 69.15 %) respectively. Other treatments were intermediate.

Pollen germination (%)

As for pollen grains germination data presented in Table (8) indicted that, pollen grains germination was significantly affected with different fertilizer treatments. B+MNF75% recorded the highest value in this respect since it was (89.36, 73.17 and 89.45 %) during three studied seasons respectively . On the other side, B+MNF25% gave the lowest percentage of pollen grains germination (81.21, 58.65 and 85.26 %) during three studied seasons respectively. Other treatments were in between.

These results agreed with [32] who reported that adding potassium to soil, significantly enhanced the sex pression %, also, the same trend was found by [33] who found that adding N, P, K, Mg, NF and EM each at two levels to the soil significantly enhanced the sex expression % of "Aggizishami", "Manzanillo" and "Kalamata olive" trees as compared to control trees.

Also these observations are in accordance with those obtained by Chunhua, L. et al[23] who found that Manzanillo inflorescence had higher number of total flowers as compared to olive Picual cv., while the reverse was true with pollen grains viability. Referring the specific effect of bio-mineral N fertilization, inoculation with *Bacillus* + 500 g N/ tree as $(NH_4)_2SO_4$or NH_4NO_3were the most effective. Such trend was true with average inflorescence length, sex expression, pollen grain viability and a reasonable increase of total number of flowers/ inflorescence. In this manner, on olive trees, Jackson, M. L.[18] found that, the interaction effect of (olive cultivar x bio- NPK soil fertilizer treatments) showed that the highest value number of inflorescence per shoot and number of flowers per inflorescence exhibited statistically the highest values by Coronaki olive trees fertilized with the treatment 6 (kotengin + Biofertilizer + K_2So_4).

Table (8): Effect of mineral and bio-fertilization on flowering characteristics (Sex ratio and Pollen germination) of "Manzanillo" olives in 2012, 2013 & 2014 seasons.

Treatments	Sex ratio (%)			Pollen germination (%)		
	2012	2013	2014	2012	2013	2014
MNF100%*	73.27 a	62.05 b	78.20 a	89.16 a	72.28 a	89.40 a
B+MNF75%	73.89 a	67.62 a	78.89 a	89.36 a	73.17 a	89.45 a
B+MNF50%	61.23 b	66.37 a	77.46 a	88.12 a	68.48 b	88.06 b
B+MNF25%	60.71 b	58.84 c	69.15 b	81.21 b	58.65 c	85.26 c

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.

Conclusion

From the abovementioned results, we can concludethat all soil application of different levels of mineral nitrogen fertilization alone orin combination with bio-fertilizerhada positive effect on increased leaf area as well as mineralcontent, pigments contents and improved vegetative growth and flowering parameters .In addition, treatment with (B+MNF75%)was the most effectivetreatment in enhancing vegetative growth parameters as well as ; shoot length , diameter, number of leaves per shoot, leaf area, pigments content and leaf mineral contents of (N, P, K, Fe, Zn and Mn). In addition, flowering characteristics also were the best from trees treated by B+MNF75%.

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