Middle East Journal of Agriculture Research, 3(3): 638-644, 2014
ISSN 2077-4605

Impact of Inorganic and Bio-Fertilizers on Growth of "Manzanillo" Olive Seedlings under Greenhouse Condition

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ABSTRACT

This study was carried out in National Research Center, Dokki, Giza, Egypt on olive seedlings Manzanillo cv. grown under greenhouse conditions and distributed in completely randomized design, included 12 treatments were 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. Using bio-fertilizers without chemical fertilizer (NPK) recorded the highest increment in plant height and lateral shoot number/ seedling. Whereas, number of leaves and dry weight of leaves/ seedling exhabted the highest values when the olive seedling treated with 100 % NPK and 2.5 g Nitrobein. Moreover, the highest contents of N and K were obtained by application of 100 % NPK plus 2.5 g Microbein.

Key words: olive seedlings, Manzanillo cv., sand soil, mineral fertilizer, NPK, biofertilizers, Nitrobein, Microbein, Biogein

Introduction

The olive , *Olea europaea*, meaning (olive from Europe") is a species of small tree in the family *Oleaceae*, found in much of Africa, the Mediterranean Basin from Portugal to the Levant, the Arabian Peninsula, and southern Asia as far east as China, as well as the Canary Islands, Mauritius and Réunion. The species is cultivated in many places and considered naturalized in France, Corsica, Crimea, Egypt, Iran, Iraq, Jordan, Syria, Java, Norfolk Island, California and Bermuda.

Its fruit, also called the olive, is of major agricultural importance in the Mediterranean region as the source of olive oil. The tree and its fruit give its name to the plant family.

The immediate ancestry of the cultivated olive is unknown. It is assumed [by whom?] that *Olea europaea* may have arisen from *O. chrysophylla* in northern tropical Africa and that it was introduced into the countries of the Mediterranean Basin via Egypt and then Crete or the Levant, Syria, Tunisia and Asia Minor.[citation needed] Fossil Olea pollen has been found in Macedonia, Greece, and other places around the Mediterranean, indicating that this genus is an original element of the Mediterranean flora. Fossilized leaves of Olea were found in the palaeosols of the volcanic Greek island of Santorini (Thera) and were dated about 37,000 BP. Imprints of larvae of olive whitefly Aleurolobus (Aleurodes) olivinus were found on the leaves. The same insect is commonly found today on olive leaves, showing that the plant-animal co-evolutionary relations have not changed since that time Friedrich W.L. (1978).

The edible olive has been cultivated for at least 5,000 to 6,000 years Vossen, (2007), with the most ancient evidence of olive cultivation having been found in Syria, Palestine and Crete, Lanza (2011).The olive tree is native to the Mediterranean region and Western Asia, and spread to nearby countries from there.

Olive has speared to all the countries around the Mediterranean basin, which is still the major region of olive production until today. 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. (Osman, 2010).

According to the International Olive Council, “The olive fruit is a drupe. It has a bitter component (oleuropein), a low sugar content (2.6–6 percent) compared with other drupes (12 percent or more) and high oil content (12–30 percent) depending on the time of year and variety.”

The unique make-up of the olive has made it a staple on the dining tables in most Mediterranean and Arab homes. According to a study conducted by the olive council, Egypt represents 14 percent of world olive production. The olive market in Egypt is considered a mass market, with an average of 13.7 percent of world consumption from 2005 to 2011. And according to the council, Egypt’s consumption was 300,000 tons during the 2011–2012 olive cycles.

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Mineral nutrition is one of the most important, factors for plant growth and yield. Mineral fertilizers, particularly mineral-nitrogen, are important means of plant nutrition; however, they are also a potential source of environmental pollution (Hartman, 1988). An attention has therefore focused on alternative fertilizers, including bio-fertilizers in Middle East. Nowadays, there is renewed interest in bio-fertilizers for nutrient supply and improve soil fertility and productivity in this region. The integrated use of bio-fertilizers and mineral fertilizers is considered as the best option not only to reduce the intensive consumption of chemical fertilizers, but also to sustain the soil with minimum undesirable impacts and to maximize fertilizer use efficiency in soil (Palm *et al*., 2001). Bio-fertilizers are considered as eco-friendly way to sustainable agriculture. They positively affect plant growth and yield, reduce the negative effects of chemical fertilizers and minimize. Some chemicals such as NO_2 and NO_3 ions in the soil and consequently in plant.

Bio-fertilizers, microbial inoculants that can promote plant growth and productivity, are internationally accepted as an alternative source of N- fertilizer. In the bio-fertilizer technology, new systems are being developed to increase the biological N -fixation with cereals and other non-legumes by establishing N -fixing bacteria within the roots (Cocking, 2000). The mechanism by which bio-fertilizers can exert a positive effect on plant growth can be through the synthesis of phytohormones, N_2-fixation, reduction in membrane potential of roots synthesis of some enzymes that modulate the level of plant hormones. Free living nitrogen-fixing bacteria such as *Azotobacter* and *Azospirilum* have the ability not only to fix nitrogen but also to release certain phytohormones i.e. GA_3, IAA and cytokinins which could stimulate plant growth and increase the availability of nutrients for plant roots by the increase in their dissolution. In addition, the increase in the capacity of photosynthesis process (Ibrahim and Abd El-Aziz, 1977).

In this regard, Haggag *et al.* (1994) found that bio-fertilizers had significant effect on plant growth, phosphorous content and dry matter of guava seedlings growing in sandy soil. Helmy and Azzazy, (1996) found that application of bio-fertilizers such as Biogein, Microbein and Phosphorein enhanced growth and nutritional status of mango seedlings. Ahmed *et al.,* (1999) who found that, applying Phosphorein improved growth of Shemlali olive seedlings in comparison to the phosphate fertilizer alone. Xiloyannis *et al.* (2000) working on mineral nutrient uptake from the soil in irrigated olive trees, cultivar Coratina, over six years after planting they recorded that, the nutrient demand was relatively steady during the different stages of the year. The results showed that demand for P and K is minimal during the first four years after planting and can be fulfilled by naturally supplied soils. Low doses of N should be applied through localized fertilization during the year.

Abdel Hameed (2002) mentioned that the interaction between 100% N and BF + BS gave the highest significant number of shoots / twigs and N, P and K contents in citrus leaves. Nawaf and Yara (2006) found that, young olive trees benefit from low levels of NPK and N alone and additional fertilizers would not be significant. However, NPK are considering to be essential element for plant growth and development. The 16 g NPK and 32 g N significantly gave the highest shoot and root dry weight, this probably due to nitrogen concentration which increased dry matter accumulation in roots and decreased shoot: root ratio. Hegazy *et al .(*2007) studied the effect of organic and bio-fertilization on vegetative growth and flowering of Picual olive trees, they recorded that, N and K contents in leaf increased significantly with applying 100% organic fertilization (poultry manure), but no significant difference was observed on leaf P content in both seasons. The same treatment gave the highest Fe leaf content in both seasons and Mn in the second season, while leaf Zn content increased in second season with using 100% mineral fertilization Fawzi *et al*.(2010) found that all treatments including bio-fertilizers used significantly increased percentage of N, P, K and Mg in the leaves of Le-Conte" pear trees as compared to the control. Osman (2010) revealed that bio and NPK fertilizer treatments significantly increase number of shoots/ branch/ meter, number of leaves per shoot, shoot length, shoot diameter, leaf area, leaf fresh and dry weights, N,P and K contents in olive leaves. El Khayat and Abdel Rehiem, (2013) applying 750 mineral N plus 200g Nitrobin and 300g Nitrobin showed an increase in leaf nitrogen as compared with 1500 g mineral N plus 100g Nitrobin on mandarin trees.

The objective of this work was to determine the suitable levels of NPK and the best type of bio-fertilizers for improving seedling growth and mineral contents of olive seedling cv. Manzanillo under greenhouse conditions.

Materials and Methods

This study was carried out in in the experimental research green house of National Research Center, Dokki, Giza, Egypt during 2012. For this purpose, healthy one years old olive and almost uniform seedlings Manzanillo cv was used. The seedlings were planted in black polyethylene bags with 30 cm diameter fooled 10 kg washed sand mixed very good with 2.5 kg cattle manure (organic matter), olive seedlings were irrigated twice weekly. These seedlings which grown under greenhouse conditions were distributed in completely randomized design, included 12 treatments were resulted from combination between:

1. Four rates of NPK (0,25,50 and 100 %) equal 0, 45, 90 and 180 g/ seedling in the form of Crystalon (20% N: 20% P: 20% K) applied as soil application divided into 16 doses from March to October about one dose every 15 dayas.
2. Three sources of bio-fertilizers :

a) Microbein: bio-fertilizer containing N-fixing and p-dissolving bacteria as a partial substitute for mineral inorganic soluble forms of N and P (ammonium sulphate and calcium super phosphate).The nitrogen fixing bacteriaare Azospirillium braselence + Azotobacter chroccocum combined with phosphorus dissolving bacteria i.e., phosphorus dissolving bacteria of Bacillus megatherium are in a form of the commercial biofertilizer Microbin, which produced by the Egyptian Ministry of Agriculture.

b) Nitrobein: bio-fertilizer containing Azospirillum sp. (nitrogen fixing bacteria). Nitrobein was obtained from General Organization for Agriculture Equalization Fund (GOAEF) Ministry of Agriculture ,Egypt.

c) Biogein: bio-fertilizer containing Azotobacter sp. (nitrogen fixing bacteria). Biogein was obtained from General Organization for Agriculture Equalization Fund (GOAEF) Ministry of Agriculture ,Egypt.

The four rates of mineral fertilizer Crystalon (20% N: 20% P: 20% K) was applied as soil application (dissolved in the irrigation water) each rate divided into 16 equal doses from March to September during growing season, while bio-fertilizers, i.e, Nitrobein, Microbein and Biogein were added one time at the beginning of the growing season (in the first week of March) at the rate of 2.5 g per seedling in the first week of March in growing season through mixing bio-fertilizer powder with 5 cm of soil layer. In September and October the following parameters were measured:

1. Growth parameters

1. Plant height increment percentage.
2. Lateral shoot numbers.
3. Stem diameter (cm).
4. Leaves number/ seedling.
5. Leaves dry weight percentage.
6. Root numbers.
7. Root length (cm).

2. Chemical constituents:

Nitrogen and phosphorus in leaves were calorimetrically determined according to the methods described by Bremner and Mulvaney (1982) and Olsen and Sommers (1982), respectively. Potassium was determined flame photometrically according to the method advocated by Jackson (1970).

Data Analysis:

All the obtained data during the growth season of the study was statistically analyzed of variance method, differences between means were compared using Duncan's multiple range test at 0.05 level according (Duncan, 1955).

Results and Discussion

1. Vegetative growth:

There were significant differences between mineral NPK, bio-fertilizers and their combination between them due to plant height increment (Table 1). The highest seedling height increment was obtained by 50 % NPK (90 gm crystalon)combined with 2.5 g Biogein bio-fertilizer (82.67 cm) without significant differences with 25 % NPK(25 gm crystalon)+ Nitrobein or 0 NPK + 2.5 g Biogein or 25 % (45 gm crystalon) NPK without bio-fertilizers . While, the lowest was recorded by 0 NPK and 2.5 g Microbein bio-fertilizer (24.80 cm).

Regarding lateral shoot numbers, the same data, show that, the combination between NPK with bio-fertilizers did not reflect any significant effect on lateral shoot numbers, moreover applying 2.5 g biogein to olive seedling with 25 % NPK recorded the maximum lateral shoot number (6 shoots). While the minimum values were obtained with 100 % NPK combined with 2.5 g/seedling Nitrobein (2.67).

As for stem diameter, data also show that, in general, different fertilization treatments had significant effect on stem diameter of seedling. Fertilization olive seedling with 50 % NPK (90 gm crystalon) and 2.5 g nitrobein recorded the highest stem diameter (8.14 cm) without significant differences with 100% NPK (180 gm crystalon) + Microbein or 25 %NPK (45 gm crystalon) + Biogein. On the other hand, the lowest values were recorded with 100 % NPK without biofertilizer (3.95cm).

Samah (2002) mentioned that the beneficial effect of biofertilizer effect in this respect may be attributed to its effect on increasing nitrogen fixation, production of growth promoting substances or organic acids, enhancing nutrient uptake or protecting vines against certain pathogens. Moreover, Abo El-Khashab (2002) reported that the increment of plant growth due to inoculation with N fixed bacteria could be attributed to the

capability of these organisms to produce growth regulators such as auxins, cytokinins and gibberellins which affect production of root biomass and nutrients uptake.

These results are harmony with Khalil (2012) showed that, on Flame seedless grapevines, the highest values of vegetative such as shoot length and leaf area mineral contents were obtained with microbial inoculated treatment and received 75% of recommended doses of mineral fertilizers

Table 1: Effect of mineral and bio-fertilizers on plant height, number of lateral shoot and stem diameter of olive seedling cv. Manzanillo grown under green house.

Treatment	Microben	Nitroben	Biogene	Without-bio	Mean
	Plant height increment %				
0%	24.80 e	77.20a	78.00 a	64.67 b	61.12 A
25%	27.52 e	81.00 a	66.00 b	77.67 a	63.05 A
50%	39.29 d	66.67 b	82.67 a	57.33 c	61.49 A
100%	39.09 d	56.67 c	66.33 b	52.33 c	53.61 B
Mean	32.67 D	70.33 B	73.25 A	63.00 C	
	Lateral shoot numbers				
0%	6.00 a	5.33 a	6.00a	5.00 ab	5.58 A
25%	4.67 ab	5.00 ab	6.00 a	6.00 a	5.42 A
50%	4.67 ab	4.67 ab	4.67 ab	5.67 a	4.92 A
100%	3.67 ab	2.67 b	3.67 ab	3.67 ab	3.42 B
Mean	4.75 A	4.42 A	5.08 A	5.08 A	
	Stem diameter				
0%	6.20 bc	5.22 cd	6.07 bc	5.31 bcd	5.70 AB
25%	5.61 bcd	5.78 bc	6.79 abc	6.19 bc	6.09 AB
50%	6.25 bc	8.14 a	5.63 bcd	6.02 bc	6.51 A
100%	7.15 ab	5.44 bcd	5.98 bc	3.95 d	5.63 B
Mean	6.30 A	6.14 AB	6.12 AB	5.37 B	

Means having the same letters within a column are not significantly different at 5% level.

Leaves number and leave dry weight%:

Leaves number and leaves dry weight % were increased significantly in treated seedlings with different rates of NPK and biofertilizers in growing season (Table 2). A significant increase in leaves number /seedlings due to the fertilization with mineral and bio-fertilizers was noted in growing season by different rates of mineral up to 50 % (90 gm crystalon) and 2.5 gm Nitrobein bio-fertilizer and recorded the highest number of leaves per seedling (258.30 leaf per seedling). While the lowest values were 25 % NPK (45 gm crystalon) + 2.5 Microbein (154.70 leaf/ seedling).

Concerning leaves dry weight %, such data show that, NPK and bio-fertilizers and combination between them had significant effect on leaves dry weight % in growing season. The highest value (70 %) was recorded by 50 % NPK (90 gm crystalon) without bio-fertilizer. The combination between 25 % NPK (45 gm crystalon) and 2.5 g Nitrobein recorded the lowest value (39.73%).

The same results are in line with the findings of Ahmed *et al.,* (1999) who found that, applying phosphorein improved leaves number and dry weight of shoot of Shemlali olive seedlings in comparison to the phosphate fertilizer alone. Also, Nawaf and Yara (2006) found that, young olive trees benefit from low levels of NPK and N alone and additional fertilizers would not be significant. However, NPK are consider to be essential element for plant growth and development. The 16 g NPK and 32 g N significantly gave the highest shoot and root dry weight, this probably due to nitrogen concentration which increased dry matter accumulation in roots and decreased shoot: root ratio.

Table 2: Effect of mineral and bio-fertilizers on number of leaves/ seedling and dry weight of leaves of olive seedling cv Manzanillo grown under green house.

Treatment	Microben	Nitroben	Biogene	Without-bio	Mean
	Leaves number/ seedling				
0%	230.00 ab	161.30 b	186.70 ab	179.00 b	189.30 A
25%	154.70 b	167.30 b	203.30 ab	206.00 ab	182.80 A
50%	169.30 b	258.30 a	184.70 ab	199.30 ab	202.90 A
100%	169.00 b	154.00 b	205.30 ab	192.70 ab	180.00 A
Mean	180.50 A	185.00 A	195.00 A	194.30 A	
	Leaves dry weight %				
0%	42.27 l	52.46 d	52.75 cd	55.53 b	50.71 B
25%	45.86 j	39.73 m	46.67 i	50.87 e	45.78 D
50%	47.58 h	45.41 jk	44.79 k	70.00 a	51.95 A
100%	49.42 fg	49.84 f	49.02 g	53.14 c	50.36 C
Mean	46.28 D	46.86 C	48.31 B	57.34 A	

Means having the same letters within a column are not significantly different at 5% level.

Root number and length:

The effect of mineral NPK and bio-fertilizers and their combinations on number of roots/ seedling and root length are shown in (Table, 3). It is clear from the data that mineral NPK, biofertilizer and their interaction between them had significant effect on root characters. As for number of roots per seedling, fertilization of seedling with 25 % NPK (45 gm crystalon) combined with 2.5 g Biogein recorded the highest root number /seedling (7.67) without significant differences with zero nor 25 % NPK + microbein or 50 %NPK without biofertilizers. On the other hand, the lowest number of roots was obtained by addition of 100 % (180 gm crystalon) without bio-fertilizers or 2.5 g Biogein bio-fertilizer (2.33 and 2.66), respectively.

Respecting root length, the same data in Table 3 indicated that mineral N,P and K as well as the combination between mineral and bio fertilizers had significant effect on root length in growing season, the highest length of root (27.0 cm) was obtained by the combination between 0 mineral NPK combined with 2.5 g/ seedling microbein , while the least length of root was recorded with the combination between 100 % NPK without bio-fertilizers (4.67 cm). The observations are in accordance with those obtained by Haggag *et al.* (1994) who demonstrated that, the use of multi – strain biofertilizer microbein has a significant positive effect on the vegetative growth patterns of guava seedlings, the use of this biofertilizer increased significantly the dry weight of roots.

Table 3: Effect of mineral and bio-fertilizers on number of roots/ seedling and root length of olive seedling cv Manzanillo grown under green house.

Treatment	Microbein	Nitrobein	Biogein	Without-bio	Mean
	Root numbers /seedling				
0%	6.00 ab	3.33 de	5.33 bcd	5.33 bcd	5.00 A
25%	6.00 ab	3.33 de	7.67 a	5.33 bcd	5.58 A
50%	3.67 cde	3.33 de	3.33 de	5.67 abc	4.00 B
100%	3.33 de	3.33 de	2.33 e	2.67 e	2.92 C
Mean	4.75 A	3.33 B	4.67 A	4.75 A	
	Root length (cm)				
0%	27.00 a	15.33 cd	19.67 bc	19.00 bc	20.25 A
25%	17.67 bc	18.00 bc	21.33 b	19.33 bc	19.08 A
50%	10 efg	8.67 fg	11.33 def	14.33 cde	11.08 B
100%	8.33 fg	6.33 fg	6.67 fg	4.67 g	6.5 C
Mean	15.75 A	12.08 B	14.75 A	14.33 AB	

Means having the same letters within a column are not significantly different at 5% level.

2. Mineral contents in seedling:

Data in Table 4 reveals that N and K contents were affected significantly by different NPK and biofertilizers well as the combination between them, except mineral biofertilizers with respect to K content in seedling. Regarding N (%), data shows that it was significantly increased with the control treatment (without mineral or bio-fertilizers) this treatment recorded the highest concentration of N in seedling (2.21 %) without significant differences with 25 NPK+ microbein biofertilizer (2.18 %), while the lowest contents (1.20 %) was obtained with 25 % NPK (45 gm crystalon) + 2.5 g Nitrobein. As for P contents in seedling, the same data in Table 4 show that, the treatments of mineral, bio and their combination between them did not reflect any significant effect on P content in leaves of seedling.

Table 4: Effect of mineral and bio-fertilizers on N, P and K contents on olive seedling cv Manzanillo grown under green house.

Treatment	Microbein	Nitrobein	Biogein	Without-bio	Mean
	Nitrogen (%)				
0%	1.64 f	1.94 c	2.10 b	2.21 a	1.97 A
25%	2.18 a	1.20 h	1.78 e	1.86 d	1.96 A
50%	1.96 c	1.89 d	2.05 b	1.54 g	1.86 B
100%	1.84 d	2.10 b	1.60 f	2.00 c	1.88 B
Mean	1.91 B	1.98 A	1.88 B	1.90 B	
	Phosphorus (%)				
0%	0.015 a	0.017 a	0.019 a	0.027 a	0.019 A
25%	0.019 a	0.031 a	0.023 a	0.023 a	0.024 A
50%	0.025 a	0.012 a	0.028 a	0.018 a	0.021 A
100%	0.024 a	0.013 a	0.019 a	0.023 a	0.020 A
Mean	0.021 A	0.018 A	0.022 A	0.023 A	
	Potassium (%)				
0%	0.501 f	0.497 f	0.522 ef	0.564 cde	0.521 B
25%	0.587 bcd	0.654 a	0.527 def	0.565 cde	0.583 A
50%	0.630 ab	0.656 a	0.551 def	0.504 f	0.585 A
100%	0.581 bcd	0.569 cde	0.673 a	0.617 abc	0.610 A
Mean	0.575 AB	0.594 A	0.568 AB	0.563 B	

Means having the same letters within a column are not significantly different at 5% level.

Regarding K contents, such data in the same Table, indicated that , there were significant differences between treatments regarding K contents in seedling, fertilization of seedling with 100 %NPK plus 2.5 g biogein gave the highest values of K content in seedling (0.673 %) without significant differences with 25 or 50 % NPK + 2.5 g Nitrobein. On the other side 0 NPK combined with 2.5 g microbein gave the lowest content of K (0.501 %) in seedling in growing season.

Bio-fertilizers contain microorganisms that help in availability of minerals as well as modification of nutrient uptake by the plant. Haggag *et al.* (1994). Similar results were obtained by Xiloyannis *et al*. (2000) and Abd El-Hameed (2002) they found that the interaction between 100% N and bio-fertilizers gave the highest significant leaf content of N, P and K in Manzanillo olive trees. Also Khalil (2012) showed that, on Flame seedless grapevines, the highest values of mineral contents in leaves were obtained with microbial inoculated treatment and received 75% of recommended doses of mineral fertilizers.

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