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Optimizing Growth of "Picual" Olive Seedlings by Using Organic and Biofertilizers as Soil Application under Greenhouse Condition

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Abstract: The experiment was carried out in the experimental research shade house of National Research Center, Dokki, Giza, Egypt during 2014. For this purpose, healthy one years old olive and almost uniform seedlings Picual 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 sheep manure, olive seedlings irrigated were irrigated -twice weekly. Fertilization of olive seedling with 60% mineral NPK (Crystalon) and treated seedling with bio fertilizers after seedling recorded the highest plant height. Moreover application of the same rate of mineral NPK and bio-fertilizers pre sowing gave the highest values of number of leaves and roots/seedling. Application of 80 % mineral NPK only or and the mixture of bio-fertilizers pre seedling gave the highest dry weight of leaves and the tallest roots/seedling, respectively. Treated seedling with 70% mineral NPK and the mixture of bio-fertilizers pre and after seedling recorded the highest values of lateral shoot numbers and the contents of N and K in seedling, respectively.

Keywords: Olive, seeling, Biofertilizer , growth and mineral contents.

Introduction

Olive (*Olea europaea* L.) is one of the oldest cultivated tree crops in the history of the world about 8000 year's age. It was originated in the ancient times in the eastern side of the Mediterranean Sea. 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[1].

Excessive chemical fertilizers bring about severe contamination to either soil or tree. Also, mentioned fertilizer wash out and collected in ground water and rivers causing distribution in fundamental biological balance. Meanwhile, composts have favourable effects on plant growth through improving chemical and physicochemical soil properties[2], improving water deficiency[3] and providing soil with essential macro and micronutrients[4].

Recently, the use and beneficial influence of bio-fertilizers is reported for various fruit species. Biofertilizers is known to increase the availability of many nutrients in the soil by improving their uptake and utilization[5]. They increase and enhance the uptake of phosphorous and nitrogen by affecting the soil pH,

phosphorus solubility and nitrogen fixation[6]. Biofertilizers containing the Azotobacter produces many growth regulators such as IAA and GA which positively influence plant growth[7].

Now a days, clean agriculture has received more attentions by application of different compost sorts and bio-fertilizers to minimize environmental problems as well as improving structure and fertility in calcareous soil where fruit orchards[8].

Many researchers tested the effect of mineral and bio-fertilizers, in this regard, Helmy L. F. et al [9]found that application of bio-fertilizers such as Biogein, Microbein and Phosphorein enhanced growth and nutritional status of mango seedlings. Ahmed, F.F.et al[10] who found that, applying Phosphorein improved growth of Shemlali olive seedlings in comparison to the phosphate fertilizer alone. Abd El-Hameed, S.A.**[11]** 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. Osman, S. M.[1]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. Maksoud, M.A.**[12]** using FYM at 30% combined with *Azotobacter chroococcum* recorded the highest values of shoot length and number of leaves/shoot and yield / tree of olive.

El-Quesni et al**[13]** indicated that the highest values of plant height stem diameter, number of leaves/plant and leaf area in seedling of Jatropha seedlings were obtained by application algae, microbien and compost. Compost gave the highest values of fresh and dry weight of leaves and roots. Haggag, L. F. et al**[14]** found that 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 exhibited 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.

The aim of this study was to optimizing growth of "Picual" olive seedlings by using organic and bio-fertilizers as soil application under greenhouse condition.

Materials and Methods

This work was carried out in the experimental research shade house of National Research Center, Dokki, Giza, Egypt during 2014. For this purpose, healthy one years old olive and almost uniform seedlings Picual 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 sheep manure, Olive seedlings irrigated were irrigated twice weekly.

This work included 12 treatments were resulted from combination between four rates of NPK (50, 60, 70 and 80 %) equal 90, 108, 126 and 134g/seedling in the form of Cristalon 20-20-20 NPK and two application times of bio-fertilizers and sheep manure (before seedling and after seedling, beside control (without application) on growth and nutrients statues of olive seedling. These seedlings which grown under shade house conditions were distributed in completely randomized design with six replicates, each replicate included six seedlings.

The mineral NPK fertilizer was applied at fortnight intervals in 16 equal doses from March to September during growing season.

Bio- fertilizers components:

1. Bacterial preparation and inoculation techniques

Highly efficient strains of plant growth promoting rhizobacteria (PGRB) *Azotobacter chroococcum*, *Azospirillum brasilense*, phosphate solubilizing bacteria (*Bacillus megaterium* var. *phosphaticum*) potassium solubilizing bacteria (*B. cereus*) and *Pseudomonas* spp were obtained from cultural collocation of Agric. Microbiology Dep. National Research Centre, Egypt. The growth promoting rhizobacteria were independently grown in nutrient broth for 48 hours at 30°C in a rotary shaking incubator. The density of each bacterial culture in the broth was counted using a haemocytometer. Liquid broth cultures initially containing $8x10^7$, $7x10^8$, $5x10^7$ and $3x10^7$ viable cell/ml respectively. In PGPB treatments, 10 ml of either tested microorganisms suspension were added to the soil in each pot just after sowing.

2. Mycorrhizal inoculum

Mycorrhizal inocula consisted of roots, hyphae, spores and growth media from a pot culture of onion plants colonization with *Glomus mosseae* NRC31 and *G. fasciculatum* NRC15 originally isolated from Egyptian soils and multiply on peat: vermicolite: perlit[15]**.** The inoculum material contained 275 spores g^{-1} oven dry bases in addition to the colonization roots pieces (the infectivity 10^4 propagola). Mycorrhizal inoculation was done by planting the seed over a thin layer of the mycorrhizal inoculum material at the time of sowing at rate of 10 g/pot.

Growth parameters

In September and October the following parameters were measured:

Plant height increment, lateral shoot numbers, stem diameter (cm), leaves number/ seedling, leaves dry weight, root numbers and root length.

Chemical constituents

Nitrogen and phosphorus in leaves were calorimetrically determined according to the methods described by Bremner, J. M. et al[16], and Olsen, S. R. et al [17], respectively. Potassium was determined flame photometrically according to the method advocated by Jackson, M.L. et al[18].

Data Analysis:

All the obtained data during the two seasons 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 to Duncan, D.B.[19].

Results and Discussion

1. Vegetative growth

1.1. Plant height increment, lateral shoot numbers and stem diameter

The data in Table (1) showed that the effect of different NPK rates and mixture of sheep manure and bio-fertilizers as well as the combination between them on plant height increment (%), lateral shoot numbers and stem diameter during the growing season.

Seedling height increment, lateral shoot numbers and stem diameter were significantly affected by NPK rates interacted with mixture of sheep manure and bio-fertilizers during growing season. As for seedling height increment, data show that, fertilized seedlings with 60% mineral fertilizer (Crystalon) and treated seedling with bio-fertilizers after seedling recorded the highest plant height (75.17 cm), while the lowest plant height was recorded with the combination between 50% NPK and treated seedling with biofertilizers before seedling (55.20 cm) .

As for lateral shoot numbers/ seedling, data show that, use 70% mineral NPK and treated seedling with biofertilizers pre seedling recorded the highest values of lateral shoot numbers comparing other treatments (4.33). While the lowest values were obtained with 50 % NPK only (2.67).

Regarding, stem diameter, data indicate that, use of 50 % mineral NPK and treated seedling with bio-fertilizers after seedling recorded the maximum stem diameter (8.23 cm). While the minimum values (5.60 cm) were obtained by using 70% mineral NPK only.

Table (1): Effect of mineral and biofertilizers on plant height, number of lateral shoot and stem diameter of olive seedling cv Picual grown under shade house

NPK / Bio-fertilizers	50%	60%	70%	80%	Mean
	Plant height increment %				
Without	74.27 a	65.50 bc	58.50 cd	60.70 bcd	64.74 A
Pre seedling	55.20 d	64.17 bc	59.07 cd	68.20 ab	61.66 A
After seedling	53.97 d	75.17 a	61.77 bcd	60.27 bcd	62.79 A
Mean	61.14 B	68.28 A	59.78 B	63.06 B	
	Lateral shoot numbers				
Without	2.67 b	3.33 ab	3.67 ab	3.67 ab	3.33 B
Pre seedling	3.66 ab	4.00 a	4.33 a	3.67 ab	3.92 A
After seedling	3.33 ab	3.33 ab	2.67 b	2.67 b	3.00 B
Mean	3.22 A	3.56 A	3.56 A	3.33 A	
	Stem diameter (cm)				
Without	6.31 cd	5.77 d	5.60 d	7.61 ab	6.34 B
Pre seedling	6.22 cd	6.97 bc	6.29 cd	7.37 ab	6.71 AB
After seedling	8.23 a	6.03 cd	6.96 bc	7.04 bc	7.07 A
Mean	6.92 A	6.26 B	6.28 B	7.37 A	

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

The beneficial effect of bio-fertilizers 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[20]. 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[21].

These results are agreement with Khalil, H. A. et al [22]found that 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, on flame seedless grapevines and **Maksoud *et al*. (2012)** on olive. Also, on Jatropha seedlings[13].

1.2. Leaves number and leave dry weight

Data given in Table (2) illustrated that, the number of leaves / seedling and dry weight were significantly affected by treated seedlings with different rates of NPK and bio-fertilizers and their combination between them. A significant increase in leaves number /seedlings recorded due to fertilization with olive seedlings with 60% mineral NPK and using of mixture of biofertilizers when applied pre planting (183.30 leaf/ seedling) without significant differences with mineral NPK at 70% combined with the same bio-fertilizers .While the lowest values were recorded by using 50% NPK only (151 leaf/ seedling).

Regarding dry weight of leaves, the same data showed that, fertilized olive seedlings with 80 % mineral NPK only had significant effect on dry weight of leaves in growing season and gave the highest dry weight of leaves (59.25 g). The combination between 60 % NPK and application of bio-fertilizers after seedling recorded the lowest value (44.12 g).

The same results are in line with the findings of [10]who found that, applying phosphorein improved leaves number and dry weight of shoot of Shemlali olive seedlings in comparison to the phosphate fertilizer alone.

Table (2): Effect of mineral and biofertilizers on number of leaves/ seedling and dry weight of leaves of olive seedling cv Picual grown under shade house

NPK / Bio-fertilizers	50% N	60% N	70% N	80% N	Mean
	Number of leaves				
Without	151.00 f	152.00 f	160.30 de	164.00 cd	156.90 C
Pre seedling	160.30 de	164.00 cd	169.70 bc	173.00 b	166.80 B
After seedling	166.30 bd	183.30 a	180.00 a	156.30 ef	177.50 A
Mean	159.20 C	166.40 AB	170.00 A	164.60 B	
	Dry weight of leaves (%)				
Without	47.73 g	49.84 f	53.56 d	59.25 a	52.59 A
Pre seedling	48.03 g	50.39 ef	54.68 c	56.75 b	52.46 A
After seedling	45.31 h	44.12 i	49.66 f	51.12 e	47.55 B
Mean	47.02 D	48.12 C	52.63 B	55.70 A	

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

1.3. Root number and length

It is clear from the data in Table (3) that, there were significant differences regarding mineral fertilizers, biofertilizers and their interaction between them on root numbers/ seedling and root length/seedling. Fertilized olive seedling with 60 % mineral NPK and treated seedling with bio-fertilizers pre seedling and recorded the highest number of roots/ seedling (9.33). On the contrary, the lowest number of roots/ seedling were obtained by treated seedling with 70 % mineral NPK only (2.33 roots/ seedling).

Regarding the root length/ seedling, the same data showed that , fertilizing olive seedling with 80 % mineral NPK and treated with the mixture of bio-fertilizers pre seedling recorded the tallest roots/ seedling (23.33 cm), while the shortest root was obtained by the same rate of mineral NPK and treated seedling with mixture of bio-fertilizers after seedling (13.33 cm).

The observations are in accordance with those obtained by[23] who demonstrated that, the use of multi – strain bio-fertilizers microbein has a significant positive effect on the vegetative growth patterns of guava seedlings, the use of this bio-fertilizers increased significantly the dry weight of roots.

Table (3): Effect of mineral and biofertilizers on number of roots/ seedling and root length of olive seedling cv. Picual grown under shade house

NPK / Bio-fertilizers	50% N	60% N	70% N	80% N	Mean
	Number of roots/seedling				
Without	3.67 cde	4 bcde	2.33 e	2.67 de	3.17 C
Pre seedling	3.67 cde	9.33 a	5.33 b	3.67 cde	5.55 A
After seedling	3.33 de	3.33 de	4.33 bcd	5.00 bc	4.00 B
Mean	3.56 B	5.56 A	4.00 B	3.78 B	
	Root length (cm)				
Without	15.67 bcd	16.33 bcd	16 bcd	21 ab	17.25 A
Pre seedling	19.33 abc	15.00 cd	16.33 bcd	23.33 a	18.50 A
After seedling	17.00 bcd	17.67 bcd	19.67 abc	13.33 d	16.92 A
Mean	17.33 A	16.33 A	17.33 A	19.22 A	

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

2. Mineral contents in seedling

Data in Table (4) indicate that N and K contents were significantly affected by different NPK , biofertilizers and their combination between them , while P content had no affected by the different treatments.

As for N (%), data indicate that it was significantly increased by the combination between 70 % mineral NPK and treated seedling with biofertilizers after seedling (3.20 %) , while the lowest contents was obtained with 60 % mineral NPK without bio-fertilizers (1.16 %).

As for K contents, the same data in Table 4 , indicated that fertilization of olive seedling with 70 % mineral NPK and bio-fertilizers application after seedling gave the highest value of K content in seedling (2.25 %). On the other side 80% mineral NPK without bio-fertilizers gave the lowest content of K in seedling in growing season (1.04%).

Table (4): Effect of mineral and bio-fertilizers on N, P and K (%) number of roots/ seedling and root length of olive seedling cv. Picual grown under shade house

NPK / Bio-fertilizers	50% N	60% N	70% N	80% N	Mean
	N (%)				
Without	1.18 h	1.16 h	1.32 g	1.77 e	1.35 C
Pre seedling	1.65 f	1.32 g	1.87 d	1.75 e	1.64 B
After seedling	1.83d	2.37 c	3.20 a	2.98 b	2.59 A
Mean	1.55 D	1.61 C	2.13 B	2.16 A	
	P(%)				
Without	0.050 ab	0.020 b	0.040 ab	0.030 ab	0.035 B
Pre seedling	0.030 ab	0.050 ab	0.050 ab	0.060 ab	0.048 AB
After seedling	0.090 a	0.090 a	0.040 ab	0.050 ab	0.068 A
Mean	0.057 A	0.053 A	0.043 A	0.047 A	
	K(%)				
Without	1.56 d	1.14 g	1.12 g	1.04 h	1.21 C
Pre seedling	1.23 f	1.36 e	1.98 c	1.33 e	1.47 B
After seedling	1.56 d	1.21 f	2.25 a	2.13 b	1.78 A
Mean	1.45 C	1.23 D	1.78 A	1.50 B	

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

Bio-fertilizers contain microorganisms that help in availability of minerals as well as modification of nutrient uptake by the plant [14].

Similar results were obtained by Abd El-Hameed, S.A.[11] who showed that the interaction between 100%N and bio-fertilizers gave the highest significant leaf content of N, P and K in Manzanillo olive trees. [22]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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