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## Studies on the Effect of Bacterial Mixture and NPK Mineral Fertilizer on Vegetative and Chemical Properties of "Toffahi" Olive Seedlings under Greenhouse Condition

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**Abstract** This work was carried out in the experimental research green house of National Research Center, Dokki, Giza, Egypt during 2014. For this purpose, healthy one years old olive and almost uniform seedlings Toffahi cv. was used. The seedlings were planted in black polyethylene bags foiled with sand mixed very good with sheep manure , olive seedlings were irrigated twice weekly. Using 108 g of mineral NPK fertilizer per seedling and treated seedling with biofertilizers pre planting recorded the maximum values of lateral shoot numbers , stem diameter and number of leaves / shoot, while using the same rate of mineral NPK (108 g) and biofertilizers after seedling increased seedling P and K content. Fertilized olive seedlings Toffahi cv. With mineral fertilized by 80 % 144 g of mineral NPK only without using biofertilizers increased number of roots/ seedling and root length / seedling, while Using 144 g mineral NPK and biofertilizers pre seedling increased N content in seedling on the other side, using 144g of mineral NPK and treated seedlings with biofertilizers after planting gave the highest values of plant height increment , number of leaves/ seedling and dry weight of leaves/ seedling.

**Keyword:** Toffahi, olive seedlings, biofertilizers, sheep manure, meniral fertilizer

### Introduction

Olive (*Olea europaea* L.) is one of the oldest cultivated tree crops in the history of the world about 8000 years 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

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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 *et al.*, 2010).

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. 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 (Abd El-moniem and Radwan, 2003). They increase and enhance the uptake of phosphorous and nitrogen by affecting the soil pH, phosphorus solubility and nitrogen fixation (Frankenberger and Arshed, 1995). Biofertilizers containing the *Azotobacter* produces many growth regulators such as IAA and GA which positively influence plant growth (Sharma and Kumar, 2008).

The mechanisms by which biofertilizers can exert a positive effect on plant growth can be through the synthesis of phytohormones, N<sub>2</sub> fixation, reduction of membrane potential of the root, synthesis of some enzymes (such as ACC deaminase) that modulate the level of plant hormones as well as the solubilization of inorganic phosphate and mineralization of organic phosphate, which make phosphorus available to the plants (Rodriguez and Fraga, 1999). Free living nitrogen fixing bacteria such as *Azotobacter* and *Azospirillum* have the ability not only to fix nitrogen but also to release certain phytohormones of GA<sub>3</sub> and IAA nature which could stimulate plant growth, absorption of nutrients and photosynthesis process ( Abd el-Latif *et al.*, 2001). These beneficial effects of *biofertilizers* on plants are attributed mainly to an improvement in root development, an increase in the rate of water and mineral uptake by roots, displacement of fungi and plant pathogenic bacteria and, to a lesser extent, biological nitrogen fixation (Okon and Itzigshohn, 1995). Another important characteristic of *biofertilizers* association with crop improvement is secretion of ammonia in the rhizosphere in the presence of root exudates, which helps in modification of nutrient uptake by the plants (Narula and Gupta, 1986). The ability of *Azospirillum* to produce plant growth regulatory substances along with N<sub>2</sub> fixation stimulate plant growth and thereby productivity. The

changes that occur in the plant roots help in transport of minerals and water (Sarig *et al.*, 1988).

Many researchers tested the effect of mineral and biofertilizers, in this regard, Helmy and Azzazy (1996) found that application of biofertilizers 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. Abd el-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. Osman *et al.* (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-Quesni *et al.* (2014) 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 *et al.* (2014) 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 determine the suitable rates of NPK and the best application time of biofertilizers for increasing seedling growth and nutrients statues of olive seedling cv. Toffahi. grown under greenhouse conditions.

### **Materials and methods**

This work was carried out in the experimental research green house of National Research Center, Dokki, Giza, Egypt during 2014. For this purpose, healthy one years old olive and almost uniform seedlings Toffahi cv. was used. The seedlings were planted in black polyethylene bags with 30 cm diameter foiled 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 ( 90, 108, 126 and 144g) of mineral NPK fertilizer per seedling in the form of Cristalon (20-20-20 NPK) applied as soil application divided into 16 doses from March to October about one dose every 15 days and two application times of bio fertilizers before and after seedling, beside control ( without application) on growth and nutrients statues of olive seedling . These seedlings which grown under greenhouse conditions were distributed in completely randomized design with six replicates, each replicate included three seedlings.

### ***Bio- fertilizers components***

#### **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  $8 \times 10^7$ ,  $7 \times 10^8$ ,  $5 \times 10^7$  and  $3 \times 10^7$  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.

#### ***1. 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: perlite (Badr El-Din *et al.* 1999). 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.

## ***Data recorded***

### ***1. 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

### ***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 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 (Duncan, 1955).

## **Results and discussion**

### ***1. Vegetative growth***

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

The data in Table (1) indicate the effect of different NPK rates 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 108 g mineral fertilizer (Crystalon) and treated seedling with biofertilizers after planting recorded the highest plant height ( 71.67 cm ), while the lowest plant height was recorded with the combination between 80 g NPK and treated seedling with biofertilizers after planting ( 50.33 cm ) .

Regarding lateral shoot numbers data show that, use 108 g mineral NPK and treated seedling with biofertilizers pre planting recorded the maximum values of lateral shoot numbers comparing other treatments (6.00). While the minimum values were obtained with 126 g of NPK combined with biofertilizers application after planting (2.33).

Data also indicate that, use of 144 g of mineral NPK without any biofertilizers gave the highest stem diameter (7.19 cm) compared to other treatments. While the minimum values (4.45 cm ) were obtained by using 144 g NPK combined with biofertilizers application pre planting (4.85 ) .

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

<i>NPK</i>	<i>90 g/ seedling</i>	<i>108g/ seedling</i>	<i>126 g/ seedling</i>	<i>144 g/ seedling</i>	<i>Mean</i>
<i>Biofertilizers</i>	Plant height increment %				
Without	70.67 a	62.00 bc	55.00 cd	57.33 bcd	61.25 A
Pre seedling	51.50 d	60.67 bc	55.00 cd	64.67 ab	57.96 A
After seedling	50.33 d	71.67 a	58.17 bcd	56.67 bcd	59.21 A
Mean	57.50 B	64.78 A	56.06 B	59.66 B	
	Lateral shoot numbers				
Without	3.00 cde	2.67 cde	3.00 cde	2.33 de	2.75 B
Pre seedling	4.00 bc	6.00 a	3.67 bcd	3.00 cdb	4.17 A
After seedling	4.67 b	2.00 e	2.00 e	3.67 bcd	3.08 B
Mean	3.89 A	3.56 AB	2.89 B	3.00 B	
	Stem diameter (cm)				
Without	6.55 ab	5.31 ab	5.22 ab	7.19 a	6.07 A
Pre seedling	5.55 ab	6.97 a	6.29 ab	4.85 b	5.92 A
After seedling	6.48 ab	5.94 ab	6.36 ab	5.61 ab	6.09 A
Mean	6.19 A	6.07 A	5.96 A	5.88 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 Samah (2002) . 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 (Abo El-Khashab, 2002].

These results are agreement with Khalil (2012) 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 El-Quesni *et al.* (2014) on *Jatropha* seedlings.

#### *1.2. Leaves number and leave dry weight*

Data in Table (2) shoed that number of leaves / seedling and dry weight were significantly increased by treated seedlings with different rates of NPK and biofertilizers through growing season. A significant increase in leaves number /seedlings recorded due to fertilization with olive seedlings with 108 g mineral NPK and using of mixture of biofertilizers when applied pre planting (194.7 leaf/ seedling) .While the lowest values were recorded by using 126 g NPK interacted without biofertilizers application ( 55.00 leaf/ seedling) .

Regarding dry weight of leaves, the same data indicate that, fertilized seedlings of olive with 144 g mineral NPK and application of the mixture of bio-fertilizers after had significant effect on dry weight of leaves in growing season and recorded the maximum of dry weight of leaves (51.61 g) The combination between 108 g NPK and application of bio-fertilizers after planting recorded the lowest value (39.39 g).

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.



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

<i>NPK</i>	<i>90 g/ seedling</i>	<i>108g/ seedling</i>	<i>126 g/ seedling</i>	<i>144 g/ seedling</i>	<i>Mean</i>
<i>Biofertilizers</i>					
	Number of leaves				
Without	141.3 abc	107.00 bc	55.00 c	114.70 abc	104.50 AB
Pre seedling	161.00 ab	194.70 a	98.67 bc	104.3 bc	139.70 A
After seedling	93.00 bc	99.00 bc	57.33 c	94.33 bc	85.92 B
Mean	131.80 A	133.60 A	70.33 B	104.40 AB	
	Dry weight of leaves (%)				
Without	44.86 g	47.42 d	48.02 c	48.88 b	47.29 A
Pre seedling	45.06 g	45.84 e	45.98 e	43.91 h	45.20 B
After seedling	45.66 ef	39.39 i	45.45 fg	51.61 a	45.53 B
Mean	45.19 C	44.22 D	46.48 B	48.13 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 144 g mineral NPK without biofertilizers recorded the number of roots/ seedling ( 8.33) and tallest root/ seedling (23.00 cm). on the other hand the lowest number of roots/ seedling were obtained by the application of 126 g mineral NPK without biofertilizers( 3.33) and fertilized seedling with 108 g mineral NPK gave the shortest root/ seedling ( 9.00 cm).

The observations are in accordance with those obtained by Haggag *et al.* (1994) who demonstrated that, the use of multi – strain biofertilizers microbein has a significant positive effect on the vegetative growth patterns of guava seedlings, the use of this biofertilizers 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 Toffahi grown under green house

NPK	90 g/ seedling	108g/ seedling	126 g/ seedling	144 g/ seedling	Mean
Number of roots/seedling					
<i>Biofertilizers</i>					
Without	6.33 b	4.00 cd	3.33 d	8.33 a	5.5 A
Pre seedling	3.33 d	5.00 bcd	5.00 bcd	5.00 bcd	4.58 B
After seedling	4.00 cd	3.33 d	4.33 bcd	5.67 bc	4.33 B
Mean	4.56 B	4.11 B	4.22 B	6.33 A	
Root length					
Without	13.67 bcde	9.00 e	19.33 abc	23.00 a	16.25 AB
Pre seedling	21.00 ab	19.00 abc	17.00 abcde	16.33 abcde	18.33 A
After seedling	15.33 abcde	10.33 de	12.00 cde	18.00 abcd	13.92 B
Mean	16.67 AB	12.78 B	16.11 AB	19.11 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) showed that N,P and K contents were significantly affected by different NPK , biofertilizers and their combination between them , except NPK mineral with respect to P content in seedling.

As for N (%), data indicated that it was significantly increased by the combination between 108 g mineral NPK and treated seedling with biofertilizers pre planting (2.91 %) without significant differences with %108 g mineral NPK and treated with biofertilizers after planting or 144 g mineral NPK and biofertilizers pre planting , while the lowest contents was obtained with 90 g mineral NPK without bio-fertilizers ( 1.70 %).

Concerning P contents in seedling , the same data in table 4 show that, the highest value was recorded by the combination with 108 g mineral NPK and bio-fertilizers application after planting (0.11 %) . On the contrary, the lowest value was obtained with the combination between 144 g mineral NPK and bio-fertilizers application pre planting ( 0.03%).

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 108 g mineral NPK and bio-fertilizers application after planting gave the highest value of K content in seedling (2.37 %). On the other side 144 g mineral NPK without bio-fertilizers gave the lowest content of K in seedling in growing season (0.98%).

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

<i>NPK</i>	<i>90 g/ seedling</i>	<i>108g/ seedling</i>	<i>126 g/ seedling</i>	<i>144 g/ seedling</i>	<i>Mean</i>
<i>Biofertilizers</i>			<i>N (%)</i>		
Without	1.70 d	1.75 d	1.81 cd	1.89 cd	1.79 C
Pre seedling	2.55 b	2.91 a	2.04 c	2.84 a	2.59 A
After seedling	2.48 b	2.77 a	2.35 b	2.04 c	2.41 B
Mean	2.24 B	2.47 A	2.07 C	2.26 B	
			<i>P(%)</i>		
Without	0.06 ab	0.07 ab	0.07 ab	0.08 ab	0.07 A
Pre seedling	0.07 ab	0.07 b	0.06 ab	0.03 b	0.05 B
After seedling	0.08 ab	0.11 a	0.07 ab	0.06 ab	0.08 A
Mean	0.07 A	0.07 A	0.07 A	0.06 A	
			<i>K(%)</i>		
Without	2.09 b	1.21 h	1.12 i	0.98 g	1.35 C
Pre seedling	1.79 c	1.73 d	1.50 g	2.08 b	1.78 A
After seedling	1.56 f	2.37 a	1.62 e	1.10 i	1.66 B
Mean	1.81 A	1.77 B	1.41 C	1.39 C	

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. Haggag *et al.* (2014).

Similar results were obtained by Abd El-Hameed (2002) who showed that the interaction between 100%N and biofertilizers gave the highest significant leaf content of N, P and K in Manzanillo olive trees. Osman *et al.* (2010) on olive. 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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