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# Effect of NPK and bio-fertilizers as soil application on promoting growth of "Toffahi" olive seedlings under greenhouse condition

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This study was carried out in National Research Center, Dokki, Giza, Egypt on olive seedlings **Toffahi** 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 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.

**Keywords:** olive seedlings, **Toffahi** cv., sand soil, mineral fertilizer, bio-fertilizers, Nitrobein, Microbein and Biogein

## 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 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.

The efficiency of fertilizers used in Egypt is very low, may be due to high pH or calcium carbonate level in the soil which hamper the availability of P-

fertilizers, in addition to the leaching of nitrate or ammonia volatilization from the nitrogen fertilizers (Soliman, 2001). Thus, the application of organic fertilizer avoided these pollutions, reduced the costs of fertilization and would be safe for human, animal and environment. As a result of chemical fertilizers misuse, the natural of the agriculture land is changed and exhausted.

Biofertilizer is a natural product carrying living microorganisms derived from the root or cultivated soil. So they don't have any ill effect on soil health and environment. Besides their role in atmospheric nitrogen fixation and phosphorous solubilisation, these also help in stimulating the plant growth hormones providing better nutrient uptake and increased tolerance towards drought and moisture stress. A small dose of biofertilizer is sufficient to produce desirable results because each gram of carrier of bio-fertilizers contains at least 10 million viable cells of a specific strain (Anandaraj and Delapierre, 2010). These beneficial effects of bio-fertilizers 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 bio-fertilizers 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 N2 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).

In this respect Haggag *et al.* (1994) plant growth found that bio-fertilizers had significant effect on 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. 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. 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.

The aim of this study was to determine the best type of bio-fertilizers and the suitable rates of NPK for enhancing seedling growth and achieving the best nutrients statues of olive seedling cv. Toffahi grown under greenhouse conditions.

## Material 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 Toffahi 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 irrigated 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 biofertilizer powder with 5 cm of soil layer.

***Data recorded:***

***1. Growth parameters***

In September and October the following parameters were measured:

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

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

Data in Table (1) show the effect of different NPK rates and biofertilizer type and their 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 combined with bio-fertilizers during growing season. As for seedling height increment, data show that, use 2.5 g/ seedling of Microbein or Nitrobein individually without mineral fertilizer (Crystalon) recorded the highest plant height ( 64.50 cm ), while the lowest

plant height was recorded with the combination between 100% NPK and 2.5 gm/seedling Microbein.

Regarding lateral shoot numbers data show that, use bio-fertilizers alone recorded significant effect as compared to other treatments , specially applying Bio-gein bio-fertilizer to olive seedling without NPK recorded the maximum lateral shoot number (9.33). While the minimum values were obtained with 100 % NPK combined with 2.5 g/seedling Nitrobein (2.33).

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

| Treatment | Microbein | Nitrobein | Biogein | Mean |
|---|---|---|---|---|
| NPK | **Plant height increment %** | | | |
| 0% | 60.83 a | 64.50 a | 37.43 bc | 54.26 A |
| 25% | 31.47 d | 40.50 bc | 42.47 b | 38.14 B |
| 50% | 27.73 de | 36.77 c | 38.27 bc | 34.26 C |
| 100% | 25.73 e | 26.27 de | 30.90 de | 27.63 D |
| Mean | 36.44 B | 42.01 A | 37.27 B | |
| | **Lateral shoot numbers** | | | |
| 0% | 8.00 ab | 7.67 ab | 9.33 a | 8.33 A |
| 25% | 5.00 bc | 4.33 c | 4.33 bc | 4.22 B |
| 50% | 5.33 bc | 3.67 c | 3.00 c | 4.00 B |
| 100% | 4.33 bc | 2.33 c | 3.67 c | 3.44 B |
| Mean | 5.67 A | 4.25 A | 5.08 A | |
| | **Stem diameter (cm)** | | | |
| 0% | 6.40 ab | 6.43 ab | 5.82 bc | 6.22 AB |
| 25% | 4.45 c | 5.52 bc | 6.29 ab | 5.42 B |
| 50% | 6.66 ab | 6.19 ab | 5.81 bc | 6.22 AB |
| 100% | 5.14 bc | 7.44 a | 6.33 ab | 6.31 A |
| Mean | 5.66 A | 6.39 A | 6.07 A | |

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

As for stem diameter, data also show that, use of biofertilizers Nitrobein combined with 100 % NPK recorded the highest stem diameter (7.44 cm) compared to other treatments. While the minimum values (4.45 cm ) were obtained by using 2.5 g/seedling Microbein bio-fertilizers combined with 25 % NPK.

These results are in 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. Samah (2002) mentioned that the beneficial effect of bio-fertilizer 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.

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

Data in Table (2) cleared that leaves number and dry weight were increased significantly in treated seedlings with different rates of NPK and biofertilizers through growing season. A significant increase in leaves number /seedlings recorded due to fertilization with Nitrobein bio-fertilizers only (252.7 leaf/ seedling) or by using combination of Microbein bio-fertilizer with 50 % NPK (238.7 leaf/ seedling) or applying combination of Biogein bio-fertilizer with 100 % NPK (218.3 leaf/ seedling). While the lowest values were recorded by using 50 % NPK+ 2.5 Biogein (113 leaf/ seedling).

Concerning dry weight of leaves, such data show that, mineral fertilizer (NPK) and bio-fertilizers and combination between them had significant effect on dry weight of leaves in growing season. The highest values were recorded by application the combination between 50 % NPK and 2.5 g Nitrobein bio-fertilizer (49.44g). The combination between 100 % NPK and 2.5 g Nitrobein recorded the lowest value (35.05g).

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

| Treatment | Microbein | Nitrobein | Biogein | Mean |
|---|---|---|---|---|
| NPK | **Leaves number** | | | |
| 0% | 149.3 fg | 252.7 a | 194.0 c-e | 198.7 A |
| 25% | 208.0 b-d | 181.7 c-f | 158.0 e-g | 182.6 AB |
| 50% | 238.7 ab | 215.7 bc | 113.0 h | 189.1 AB |
| 100% | 127.7 gh | 127.3 d-f | 218.3 a-c | 174.4 B |
| Mean | 180.9 B | 206.8 A | 170.8 C | |
| | **Leaves dry weight %** | | | |
| 0% | 48.74 a | 40.69 c | 46.18 b | 45.20 A |
| 25% | 35.40 fg | 37.30 e | 38.99 d | 37.23 C |
| 50% | 37.33 e | 49.44 a | 39.39 d | 42.05 B |
| 100% | 40.81 c | 35.05 g | 36.03 f | 37.3 C |
| Mean | 40.57 A | 40.62 A | 40.15 B | |

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

### *1.3. Root number and length*

The effect of mineral 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 Using mineral fertilizer NPK with seedling treated with Nitrobein or Biogein decreasing Root numbers / seedling and with raising percentage of mineral fertilizer used from 25 to 100% Increasingly shortage Root numbers / seedling. However, using mineral fertilizer NPK at 25% with seedling treated with Microbein achieved the highest significant root numbers / seedling (8.33). Whereas, raising percentage of mineral fertilizer used up to 50 and 100% Increasingly shortage Root numbers / seedling.

Respecting root length, the same data in table (3) indicated that different combination between mineral and bio-fertilizers had significant effect on root length in growing season, the highest length of root (26.0 cm) was obtained by the combination between 25 % mineral NPK and 2.5 g Nitrobein / seedling, while the lowest length of root was recorded with the combination between 100 % NPK with 2.5 g Nitrobein/ seedling (7.67 cm).

**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

| Treatment | Microbein | Nitrobein | Biogein | Mean |
|---|---|---|---|---|
| NPK | **Root numbers / seedling** | | | |
| 0% | 4.33 de | 5.67 b-d | 7.00 ab | 5.67 A |
| 25% | 8.33 a | 6.33 bc | 3.67 de | 6.11 A |
| 50% | 3.67 e | 5.00 c-e | 4.33 de | 4.33 B |
| 100% | 4.33 de | 3.67 e | 5.33 b-e | 4.45 B |
| Mean | 5.17 A | 5.17 A | 5.08 A | --- |
| | **Root length (cm)** | | | |
| 0% | 13.00 c-f | 15.67 b-f | 19.00 a-e | 15.89 B |
| 25% | 21.33 a-d | 26.00 a | 14.33 c-f | 20.56 AB |
| 50% | 24.33 ab | 19.00 a-e | 22.33 a-c | 21.89 A |
| 100% | 12.00 d-f | 7.67 f | 9.33 ef | 9.67 C |
| Mean | 17.67 A | 17.08 A | 16.25 A | --- |

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

The observations are in accordance with those obtained by Haggag *et al.* (1994) who demonstrated that, the use of multi – strain bio-fertilizer 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.

***2. Mineral contents in seedling leaves:***

Data in Table (4) reveals that Nitrogen (N), Phosphor (P) and potassium (K) contents were affected significantly by bio-fertilizers types and different rates of mineral fertilizer and the combination between them.

Concerning Nitrogen percentage, data shows that it was significantly increased by the combination between 100 % NPK and 2.5 g /seedling Microbein (1.93 %) or Nitrobein (1.97%), while the lowest contents was obtained with 2.5 g Nitrobein without NPK (0.95 %).

**Table (4):** Effect of mineral and bio-fertilizers on N, P and K leaves contents of olive seedling cv Toffahi grown under green house

| Treatment | Microbein | Nitrobein | Biogein | Mean |
|---|---|---|---|---|
| NPK | N (%) | | | |
| 0% | 1.17 e | 0.95 g | 1.09 f | 1.07 C |
| 25% | 1.16 e | 1.46 d | 1.82 b | 1.48 B |
| 50% | 1.46 d | 1.82 b | 1.09 f | 1.46 B |
| 100% | 1.93 a | 1.97 a | 1.75 c | 1.88 A |
| Mean | 1.43 B | 1.55 A | 1.43 B | --- |
| | P (%) | | | |
| 0% | 0.08 c | 0.15 ab | 0.12 a-c | 0.12 A |
| 25% | 0.11 a-c | 0.11 a-c | 0.11 a-c | 0.11 A |
| 50% | 0.12 a-c | 0.08 c | 0.08 c | 0.09 A |
| 100% | 0.1 bc | 0.08 c | 0.17 a | 0.12 A |
| Mean | 0.1 A | 0.1 A | 0.1 A | ---- |
| | K (%) | | | |
| 0% | 0.69 i | 0.81 h | 0.81 h | 0.77 D |
| 25% | 1.56 c | 1.69 b | 1.79 a | 1.66 A |
| 50% | 1.21 f | 1.15 g | 0.63 j | 0.99 C |
| 100% | 1.75 a | 1.37 e | 1.44 d | 1.52 B |
| Mean | 1.30 A | 1.24 B | 1.17 C | -- |

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

As for Phosphor contents in seedling leaves, the same data in table (4) show that, the highest significant was recorded by the combination with 100 % NPK combined with Biogein (0.17%). On the contrary, the lowest values were obtained by applying 2.5 g Microbein only without NPK (0.8%).

Regarding potassium leave contents, such data in the same Table, indicated that, fertilization of seedling with 100 % NPK plus 2.5 g Microbein gave the highest values of potassium content in seedling leaves (1.75 %). On the other side 50 % NPK combined with 2.5 g Biogein gave the lowest content of K in seedling leaves 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 Abd El-Hameed (2002) who 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. 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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