Effect of Biofertilizer and Seaweed Extract on Vegetative Growth of Sweet Orange Saplings Cv. Local

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Abstract:
The experiment was conducted in the lath house affiliated to the Dept. of Horticulture and Landscape Gardening/ College of Agriculture / Tikrit University for the season 2023 to study the effect of the mycorrhiza fungus bio-fertilizer with three levels (0,5 and 10 g. Saplings $^{-1}$) and spraying seaweed extract with three levels (0,5 and 10 ml L^{-1}) on the vegetative growth of sweet orange saplings cv.Local buded on sour orange rootstock, results obtained can be summarized as follows: soil application of biofertilizer at a concentration of 10 g. sapling^{-1} led to a significant increase in both the characteristic of the sapling height of 17.15 cm, the stem diameter of 4.34 mm and the leaf area of 29.48 cm^2 and the percentage of dry matter in the leaves of 9.20%, either at a concentration of (5 g. sapling^{-1}) the number of leaves were increased which registered 74.07 leaf, on the other hand , seaweed extract with concentration 5 ml.l^{-1} increased stem length While seaweed extract at a concentration of (10 ml L^{-1}) led to a significant increase in stem diameter of 5.14 mm, number of branches 8.94 sapling branches $^{-1}$, number of leaves 82.47 (sapling leaves^{-1}), leaf area 28.12 cm^2 and leaves relative chlorophyll content which gave(63.05 SPAD) and dry matter which gave (8.35)

Keywords: Biofertilizer, Seaweed extract, Sweet orange, Foliar application, vegetative growth.

Introduction

Among Citrus species, the sweet orange (Citrus sinensis (L.) Osbeck) is the most important in terms of production and cultivated areas. Oranges are particularly appreciated for the organoleptic characteristics and the high nutritional value of the fruits [1].

Bioorganic fertilizers are a mixture of substances derived from plant or animal organisms and living organisms that resulted during the process of their partial or complete decomposition for a long period of time [2]. mycorrhiza is one of the main microorganisms in the environment, mycorrhiza fungi provide many benefits to their host through coexistence with the plant, increase tolerance to water and salt stress of the plant by increasing the absorption of water and nutrients, resistance to diseases and salinity, the production of phytohormones, improve soil structure mycorrhiza fungi have long-term symbiotic relationships with the plant strain, as the selective mechanisms of these fungi to absorb ions improve photosynthesis by improving the absorption of nutrients and improving water absorption, and the plant's response to root fungi is equivalent to the efficiency of the fungus in improving the growth condition and absorption of plant elements for fungi to enter places where the roots can not penetrate and use a limited amount of water and nutrients. Mycorrhiza fungi are the largest group of root fungi that penetrate into the root cells and form structures called vesicles and cocci, which increase the level of metabolic content [3]. They are known to increase the efficiency of nutrient absorption, root growth and, consequently, plant growth, flowering, fruit quality, productivity, biotic and abiotic stress tolerance, regardless of their nutritional composition, the main types of biofertilizers

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consist of humic substances, silicones, protein analyzers, seaweed extracts from macroalgae, microbial biostimulants including bacterial and fungal strains [4]. Dahal et al. [5] pointed out that in an experiment to study the effect of biofertilizer fertilization on the vegetative growth of Mandarin seedlings, the experiment included five levels of mycorrhiza biofertilizer (0, 3, 6, 9 and 11 g), as the results showed that the level exceeds 9g recording the highest values of plant height 5.81 cm, stem diameter 0.33 mm, number of leaves 10.5 $leaf.plant^{-1}$. Alomar et al. [6] in an experiment to study the effect of biofertilizer fertilization in the mineral content of strawberry Saplings, to study the effect of biofertilizer (0,4 and 8ml.l^{-1}) and their interactions in the vegetative growth and qualities of strawberry Sapling gave the best values of (plant height, leaves number, leaf area). Hamza and hadi [7] reported that in an experiment to study the effect of fertilizing with biofertilizer on the vegetative growth of orange seedlings , the experiment included different levels of biofertilizer (comparison treatment, azotobacter bacteria 10 ml L^{-1}, mycorrhiza fungi 10 g, interference between azotobacter bacteria 10 ml L^{-1} + mycorrhiza fungi 10 g), the results showed the superiority of the interference treatment between Azotobacter bacteria 10 ml L^{-1} + mycorrhiza fungi 10 g recorded the highest values for the characteristic plant height 42.73 CM, stem diameter 3.94 mm, leaves relative chlorophyll content which gave 52.89 SPAD .

The use of chemicals has become limited due to their harmful effects on human health and the environment, The benefits of seaweed extract as sources of organic substances and nutrients have been known for centuries, especially in coastal areas. extracts of these seaweed have been used for decades as a spray on the vegetative aggregate and as an addition to the soil in plant production due to the fact that they contain a number of plant growth stimulating compounds. unlike chemical fertilizers, extracts derived from seaweed are biodegradable, non-toxic and non-hazardous to humans, animals and birds [8]. due to their antioxidant properties and contain various biologically active chemicals and secondary receptors and dietary fiber, vital amino acids, phytochemical elements, vitamins, polyunsaturated fatty acids and other minerals can be used as nutrients and Fertilizers [9]. Rozbiany and Ibrahim [10] explained in a study to evaluate the role of seaweed extract in the vegetative growth qualities of strawberry seedlings, the experiment included three concentrations of seaweed extract (0, 2, 4 ml L^{-1}), the results of the study showed that the concentration exceeded 4 ml L^{-1} in giving the best results for the characteristic leaf area 97.66 cm^2, leaves relative chlorophyll content which gave40.45 SPAD, dry weight of branches 21.31 g . Khalil et al. [11] conducted a study to assess the role of seaweed extract in the vegetative growth qualities of papaya seedlings, the experiment included three concentrations of seaweed extract (0, 50, 100 ml L^{-1}), the results of the study showed that the concentration exceeds 50 mg L^{-1} in giving the best results for the plant height 47.1 cm, stem diameter 53.7 mm, total leaf area 762 cm^2, dry matter 96.27 g, number of leaves 5.79

Materials and Methods

The experiment was carried out in the lath house of the Dept. of Horticulture and Landscape Gardening affiliated to the College of Agriculture / Tikrit University , 54 saplings of a local cultivar homogeneous in size were used with age of two years and grafted on the sour orange rootstock planted inside plastic bags and then transferred to a large plastic pot with dimensions (20 cm diameter and 30 cm height) with weight (15) kg and a planting medium containing mixed soil + peat moss with a ratio (2:1) The study included the following factors:

- The first factor: included soil application with bio-fertilizer (mycorrhiza fungus) as follows:
  1- H0 (with no addition)
  2- H1 (5 g. $sapling^{-1}$)
  3-H2 (10 g. $sapling^{-1}$)
- The second factor included sprayed seaweed extract with 3 concentration as follows :
  1- M0 (spray with distilled water only)
  2- M1 ($5ml.l^{-1}$)
  3- M2 ($10ml.l^{-1}$)

The saplings were sprayed with the extract in the morning until completely wet using a 5-liter manual spray on the same day as the bio-fertilizer was added, the first spray was on 12/3, the second spray was half a month after the first and third spray at the same time.

The experiment was carried out as a working experiment using the design of complete random sectors (R.C.B.D. The experimental unit should include two saplings, so that the number of experimental units will be 54

The traits were studied as follow:

1. Increment in sapling height (cm): It was measured according to this equation:
Increment in sapling height =height at the end of the experiment-height at the beginning of the experiment.

2-Increment in stem diameter (mm)
It was measured according to this equation
Increase in stem diameter= stem diameter at end of the experiment - stem diameter at the beginning of the experiment.

3-Increment in sapling branches(branch.$sapling^{-1}$):
The number of branches is measured by the equation:
Increase in the number of branches =the number of branches at the end of the experiment-the number of branches at the beginning of the experiment.

4-Increment in sapling leaves (leaf.$sapling^{-1}$) number of leaves was measured as follow:
Increase in the number of leaves=the number of leaves at the end of the experiment-the number of leaves at the beginning of the experiment.

5-leaf area (cm2)
It was calculated according to the method Patton [12]
leaf area =the average weight of the sheet shape ×the area of the reproduction sheet /the weight of the reproduction sheet .

6-the relative chlorophyll content in the leaves (SPAD)
The relative chlorophyll in the leaves was measured using a chlorophyll meter model M directly in the field, where 4 readings were taken for one leaf and for five leaves from different directions.

7-the percentage of dry matter in the leaves (g).
It was calculated according to the equation he mentioned
The ratio of dry matter in the leaves % = dry weight (g) / soft weight (g)× 100

The data were statistically analyzed as a factor experiment according to the design of complete random sectors (RCBD) [13] using the statistical program [14] and the arithmetic averages were compared using the Duncan polynomial Test at a probability level of 0.05.

Results and Discussion

1- Increment in sapling height (cm).

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The results of Table (1) showed that the rate of increase in the length of the sapling stem was significantly affected as a result of the addition of the effect of bio-fertilizer, as the H2 transaction surpassed the rest of the transactions and recorded a higher rate of increase in sapling length of 17.15 cm compared to the rest of the transactions, which amounted to 5.94 and 12.61 cm, respectively, as a result of the effect of bio-fertilizer. While the results showed that the increase in the length of the sapling stem was significant when seaweed extract spray, noting the superiority of the M1 treatment at a concentration of 5 ml L^{-1} recorded the highest value of 14.40 cm compared to the remaining transactions, which recorded the lowest values of 10.71 and 10.59 cm, respectively. It is noted from the same table that the bilateral interference between the biofertilizer and the seaweed extract, specifically when treating the interference H2M1 and H2M2, which recorded the highest value in the length of the seedling reached 21.33 and 22.08 cm, which was significantly superior to the rest of the interventions, where the treatment gave H0M2, which gave the lowest value of 3.50 cm.

Table 1. the effect of bio-fertilizer and seaweed extract and the interaction between them in Increment in sapling height (cm) In oranges.

Bio-enriched / Marine algae extract	H0 (0) without addition	H1 5g seedling^{-1}	H2 10g seedlings $^{-1}$	Mean
M0 (0) without addition	8.83 c	15.26 b	8.05 c	10.71 b
M1 5 ml L^{-1} liter	5.50 cd	16.36 b	21.33 a	14.40 a
M2 10 ml^{-1} liter	3.50 d	6.20 cd	22.08 a	10.59 b
Mean	5.94 c	12.61 b	17.15 a	

*Averages of transactions with the same characters do not differ from each other significantly according to the Duncan polynomial Test at a probability level of 0.05.

2. Increment in stem diameter (mm)

Table (2) shows that there are significant differences in the characteristic of the increment in stem diameter, as the addition treatment H2 surpassed the highest rate of increase 4.34 mm relative to the comparison treatment H0, which recorded the lowest value of 3.77 mm under the influence of bio-enriched. While the results of the same table for the seaweed extract indicated significant differences, the M2 transaction outperformed the rest of the transactions, which recorded the highest value of 5.14 mm compared to the comparison transaction, which recorded the lowest value of 3.13 mm. Either in the bilateral interference between the bio-fertilizer and the seaweed extract, significant differences were observed, as the coefficients H2M2, which recorded the highest values of 4.75 mm, respectively, outperformed compared to the coefficients H0M0 which recorded the lowest values of 2.92 mm, respectively.

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Table 2. the effect of bio-fertilizing and seaweed extract and the interaction between them in Increment in stem diameter (mm) In oranges.

Bio-enriched / Marine algae extract	H0 (0) without addition	H1 5g seedling^{-1}	H2 10g seedlings $^{-1}$	Mean
M0 (0) without addition	2.92 b	2.99 b	3.50 b	3.13 c
M1 5 ml L^{-1} liter	3.33 b	3.51 b	4.78 a	3.87 b
M2 10 ml^{-1} liter	5.05 a	5.61 a	4.75 a	5.14 a
Mean	3.77 c	4.03 b	4.34 a	

* Averages of transactions with the same characters do not differ from each other significantly according to the Duncan polynomial Test at a probability level of 0.05.

3- Increment in sapling branches(branch.sapling^{-1}):

Table (3) shows that there are no significant differences in the number of branches (branch. sapling^{-1}) under the influence of bio-enriched. While the same table shows the presence of significant effects in the character of the increase in the number of branches (branch. sapling^{-1}), outperforming the addition coefficients M1 and M2, which recorded the highest value of 8.11 and 8.94 (branch. sapling^{-1}) respectively compared to the transaction M0, which recorded the lowest value of 5.00 branches. sapling^{-1} this was in relation to the effect of seaweed extract. As for the bilateral overlap between the effect of bio-fertilizer and seaweed extract, it is noted from the same table that there are significant differences, as the coefficients H2M2 gave the largest increase, which recorded the highest rate 9.16 (branch. Seedling^{-1}), respectively, compared to the h0m0 transaction, which recorded the lowest increase rate of 4.50 (branch. Seedling^{-1}).

Table 3. the effect of biofertilizer and seaweed extract and the interaction between them in Increment in sapling branches(branch.sapling^{-1}) In oranges.

Bio-enriched / Marine algae extract	H0 (0) without addition	H1 5g seedling^{-1}	H2 10g seedlings $^{-1}$	Mean
M0 (0) without addition	4.50 c	5.50 ab	5.00 a	5.00 b
M1 5 ml L^{-1} liter	7.33 bc	8.33 a	8.66 a	8.11 a
M2 10 ml^{-1} liter	9.00 bc	8.66 a	9.16 a	8.94 a
Mean	6.94 a	7.50 a	7.61 a	

* Averages of transactions with the same characters do not differ from each other significantly according to the Duncan polynomial Test at a probability level of 0.05.

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4- Increment in sapling leaves (leaf.sapling^{-1})

The results of Table (4) showed the presence of significant effects in increasing the number of leaves number. sapling^{-1}, as the addition transaction H1 outperformed the rest of the transactions, which recorded the highest value of 74.07 leaf. sapling^{-1} is relative to the comparison transaction H0, which recorded the lowest value of 59.36 leaf. sapling^{-1} under the influence of bio-enriched. While the same table shows the effect of seaweed extract to the presence of significant differences, the M2 transaction outperformed the rest of the transactions, which recorded the highest average value of 82.47 plant leaves^{-1} percentage of the comparison transaction, which recorded the lowest average value of 44.21 leaves. sapling^{1}. As for the bilateral interference between the effect of the bio-fertilizer and the seaweed extract, significant effects were observed, as the addition coefficients h1m1 and H1M2, which recorded the highest rate of 87.51 and 89.03, outperformed the leaves. Seedling^{-1}, sapling^{-1} relative to the comparison coefficient H0M0, which recorded the lowest average of 38.233 leaves. S sapling^{-1}.

Table 4. the effect of biofertilizer and seaweed extract and the interaction between them in Increment in sapling leaves (leaf.sapling^{-1}) In oranges.

Bio-enriched / Marine algae extract	H0 (0) without addition	H1 5g seedling^{-1}	H2 10g seedlings $^{-1}$	Mean
M0 (0) without addition	38.23 f	45.68 d	48.72 c	44.21 c
M1 5 ml L^{-1} liter	63.70 d	87.51 a	77.26 c	76.16 b
M2 10 ml^{-1} liter	76.17 c	89.03 a	82.21 b	82.47 a
Mean	59.36 c	74.07 a	69.40 b	

* Averages of transactions with the same characters do not differ from each other significantly according to the Duncan polynomial Test at a probability level of 0.05.

5- leaf area (cm^2)

Table (5) shows that there are significant differences in the character of the rate of increase in leaf area (cm^2), as the addition treatment H2 surpassed in recording the highest rate of increase in sheet area of 29.48 cm^2 relative to the comparison treatment H0, which recorded the lowest value of 24.80 cm^2 under the influence of bio-fertilizing, and the same table shows that the addition treatment M2 was M0 the lowest value reached 25.89 cm^2. The overlap between the biofertilizer and the seaweed extract had a significant effect on the average sheet area cm^2, especially at the third level of the biofertilizer and the third level of the seaweed extract on the rest of the bilateral interferences, which gave the highest value of 30.55 cm2, while the comparison treatment gave the lowest value of 23.22 cm^2.

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Table 5. the effect of biofertilizer and seaweed extract and the overlap between them in Leaf area(cm^2) In oranges.

Bio-enriched / Marine algae extract	H0 (0) without addition	H1 5g seedling^{-1}	H2 10g seedlings $^{-1}$	Mean
M0 (0) without addition	23.22 b	25.76 ab	28.69 ab	25.89 b
M1 5 ml L^{-1} liter	24.97 ab	25.90 ab	29.20 a	26.69 ab
M2 10 ml^{-1} liter	26.21 ab	27.90 ab	30.55 a	28.22 a
Mean	24.80 c	26.52 b	29.48 a	

* Averages of transactions with the same characters do not differ from each other significantly according to the Duncan polynomial Test at a probability level of 0.05.

6- Relative chlorophyll content (SPAD):

Table (6) shows that there are no significant differences in the content of the leaves of total chlorophyll SPAD, this is related to the effect of the bio-fertilizer and for all transactions, while the same table shows that the seaweed extract created a significant increase in the same trait, as the treatment M2 (10 ml L-1) and treatment M1 (5 ml L-1) achieved the highest increase in this trait with a value of 63.45 and 60.75 SPAD, respectively, relative to the comparison treatment M0(0 without addition), which gave the lowest value of 56.10 SPAD. The bilateral interference between the biofertilizer and the seaweed extract had a significant effect on the total chlorophyll content of the leaves, especially at the first and third levels of the biofertilizer and the third level of the seaweed extract on the rest of the bilateral interferences, which gave, respectively, 63.30 and 64.09 SPAD, which did not differ significantly from some interferences, while the comparative treatment gave the lowest value of 55.77 SPAD.

Table 6. the effect of biofertilizer and seaweed extract and their overlap in Relative chlorophyll content (SPAD) In oranges

Bio-enriched / Marine algae extract	H0 (0) without addition	H1 5g seedling^{-1}	H2 10g seedlings $^{-1}$	Mean
M0 (0) without addition	55.77 c	56.84 bc	55.69 c	56.10 b
M1 5 ml L^{-1} liter	60.95 abc	61.29 abc	60.00 abc	60.75 a
M2 10 ml^{-1} liter	63.30 a	62.96 abc	64.09 a	63.45 a

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Mean	60.01 a	60.37 a	59.93 a	

* Averages of transactions with the same characters do not differ from each other significantly according to the Duncan polynomial Test at a probability level of 0.05.

7-Percentage of dry matter in the leaves (%)

Table (7) shows that the biofertilizer has created a significant increase in the percentage of dry matter in the leaves, as the treatment H2 (10 g seedling $^{-1}$) gave the highest increase in this trait with a value of 9.20%, relative to the comparison treatment M0 (0 without addition), which gave the latter the lowest value of 5.52%. While the same table shows that the seaweed extract created a significant increase in the same trait, as the treatment M2 (10 ml L^{-1}) achieved the highest increase in this trait with a value of 8.35% compared to the comparison treatment M0 (0 without addition), which gave the latter the lowest value of 6.50 %. The interaction between the biofertilizer and the seaweed extract had a significant effect on the percentage of dry matter in the leaves, especially at the third level of the biofertilizer and the seaweed extract on the rest of the bilateral interferences, which gave 9.57%, while the comparative treatment gave the lowest value of 4.69 %.

Table 7. the effect of biofertilizer and seaweed extract and the overlap between them in the percentage of dry matter in the leaves (%)In oranges.

Bio-enriched / Marine algae extract	H0 (0) without addition	H1 5g seedling^{-1}	H2 10g seedlings $^{-1}$	Mean
M0 (0) without addition	4.69 c	5.68 bc	9.16 ab	6.50 c
M1 5 ml L^{-1} liter	5.66 bc	8.39 abc	8.88 ab	7.64 b
M2 10 ml^{-1} liter	6.23 abc	9.26 ab	9.57 a	8.35 a
Mean	5.52 c	7.77 b	9.20 a	

* Averages of transactions with the same characters do not differ from each other significantly according to the Duncan polynomial Test at a probability level of 0.05.

The reason for the increase in the increase in vegetative growth that appeared as a result of the addition of bio-fertilizer, may be due to its role in identifying biologically active plant molecules capable of changing the metabolic pathways of the plant, which may improve the performance of seedlings in a short period of time and in an effective way [15], and may also be due to the fact that mycorrhiza fungi have an important role in improving the fertile soil qualities and increasing nutrient readiness, as their importance lies in their ability to improve the atmospheric fixation of nitrogen nitrogen-fixing microbiology and inoculation with mycorrhiza fungus is of great importance to encourage increased root growth in The first stages of sapling growth [16], all these roles of the biofertilizer combined to improve the vegetative growth process and thereby increase the stem length, stem diameter, number of leaves, number of branches,

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chlorophyll content in the leaves and the percentage of dry matter in the leaves.

As for the increase in vegetative growth resulting from spraying seaweed extract, there is an improvement in vegetative growth qualities, which may be attributed to its role in increasing the efficiency of nutrient absorption, and, consequently, sapling growth and tolerance to biotic and abiotic stress [4] as well as that it is associated with the regulation of genes and important defense-related pathways in the seedling system, and it provokes plant hormonal responses due to its specific components and interaction with the regulation of seedling growth [17]. We observe an increase in the efficiency of photosynthesis and, consequently, an increase in the length of the stem, the diameter of the stem, the number of leaves, the number of branches, the content of chlorophyll in the leaves and the percentage of dry matter in the leaves.

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