Effect of spraying with Tecamin Algae and NPK fertilizer on the growth of pomegranate (Punica granatum L.) seedlings cv. California wonderful

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Abstract. This study was carried out in the lath house of the Department of Horticulture and Landscape at the Faculty of Agriculture, University of Kufa for the period from 1st March to 1st December, 2015. The experiment was conducted on six months old pomegranate seedlings (Wonderful cultivar) in order to study the effect of spraying with Tecamin Algae with four concentrations (1, 2, 3 and 4) ml.l⁻¹ and NPK fertilizer at 100, 200, 300 and 400 kg.ha⁻¹. The treatments were examined separately and overlapping. The results showed that spraying the seedlings with the treatments and their interactions caused a significant increase in seedling length and its diameter, number of leaves, leaf area, fresh and dry weight of vegetative shoot, and total chlorophyll of leaves. The treatment of interference between the marine algae extract (4 ml.l⁻¹) and NPK fertilizer (400 kg.ha⁻¹) was superior by obtaining the highest values of all traits compared with control treatment. It can be concluded from this study that a balanced fertilization management that combines the use of chemical, organic or bio-fertilizers must be developed and evaluated with different plants and under different cultivation system.

1. Introduction

Pomegranate belongs to the Punicaceae family or Lythraceae family as recent molecular studies were suggested. The Punicaceae family contains one genus (the Punica) and two species. The first species is P. protopunica which is less spread and it is a typical species for of Sumatra Island in Yemen. The second species is Punica granatum which is grown in wide areas around the world [1]. Because of the rapid increase in cultivation and production around the world, there is not an accurate data about the total area and production, but an estimated production in 2014 was around three millions [2]. In Iraq, pomegranate cultivation is successful as around 23 cultivars are cultivated and the most important one is Sulaymi cultivar [3]. The estimated number of trees in Iraq is about 5311613 with an average productivity per tree about 30.5 kg.tree⁻¹. The total production in 2013 was 161822 tonnes, which was about 27.63% of the total production of summer trees [4]. Pomegranate is cultivated for the nutritional importance of pomegranate, where the fruits contain carbohydrates,
vitamin C, protein, fat and ash in addition to water [5]. Pomegranate also has some medical and therapeutic benefits as all plant parts contain beneficial phytochemicals which are anti-oxidant and anti-microbial [6; 7; 8]. Pomegranate fruit is large in the size classified as berries fruit and range in color from light yellow to deep maroon to black filled with the arils with taste varies from sweet, tart-sweet to sour [9].

Organic agriculture is an important agricultural system that contributes to the exclusion or reduction of the use of chemical fertilizers, growth regulators and pesticides, which reduces human, and animal health damage, as well as its role in reducing environmental pollution [10]. Organic fertilizers can contribute with a positive effect in improving the growth of plants and increase their productivity because they contain many mineral elements and other compounds that are necessary for plant growth [11], thus encouraging the possibility of being used as an alternative fertilizer or supplement to chemical fertilizers.

Marine algae extracts are among the organic sources used in agricultural production and are complementary to fertilizers and not a substitute for them [12]. More than 15 million tons of them are used annually in the agricultural field around the world. These extracts are non-fertilizer materials that stimulate the growth of plants with a low concentrations and contain micro and macro nutrients and they have more than one set of growth-promoting materials such as cytokines, auxins, vitamins, amino and organic acids, compounds similar to auxins [13], and multi saccharides such as laminarin, fucoidan and alginate which have a wide range in their impact on the plant's biological activities [14]. It also contains betaines which is a source of nitrogen in the low concentrations and a regulator of osmosis in high concentrations and the role of these extracts in increasing the resistance of plants to salinity, freezing, and drought may be attributed to this compound [15; 16].

Although the disadvantage of the chemical fertilizers and their environmental impact, these fertilizers would be very important to be added to increase plant growth especially at early growth stage. Chemical fertilizers can cause an immediate, direct and fact effect on the plant as well as their low price and they are high in nutrients content which means that small amounts are required for plant growth compared with organic fertilizers [11]. For sustainable agricultural production, using the combination of organic and chemical fertilizers has revealed to be highly recommended depending on the application rate and the nature of fertilizer [11].

The pomegranate cultivation in Iraq is neglected at present and the number of trees decreases under the prevailing circumstances. This may be due to the inefficient use of fertilizers, which is one of the most important factor affecting the success of pomegranate cultivation and its productivity. Therefore, this study was conducted to investigate the integrated use of chemical and organic fertilizers by studying the effect of adding marine algae extract and NPK fertilizer in improving the growth of pomegranate seedlings (California wonderful cultivar) and sustaining soil fertility.

2. Materials and Methods
This study was conducted at the lath house of the Department of Horticulture and Landscape at the Faculty of Agriculture, University of Kufa on 225 pomegranate seedlings cv. California wonderful. These seedlings were at 6 months old and homogeneous in size and growth and planted in 2 kg plastic bags. Seedlings were sprayed in 1st March to 1st December, 2015 with algae extract (Tecamin Algae) and mineral fertilizer (NPK) to improve some vegetative growth characteristics and content of leaves of mineral elements. In addition to the control treatment of both factors, four concentrations of algae extract were used 1, 2, 3 and 4, ml.l\(^{-1}\) with four levels of NPK fertilizer (100, 200, 300 and 400) kg.ha\(^{-1}\) and their interactions. The experiment consisted of 25 treatments with three replications for each treatment and three plants were used for each treatment. The Randomized Completed Block
Design was used to set up this experiment and the means were compared using (L.S.D) at (0.05) probability level [17].

The height of the seedling was measured using the measuring tape from the soil surface to the top of the seedling, while the diameter of the main stem was measured at a height of 5 cm from the surface of the soil and for all seedlings by the electronic vernier. The leaf area was measured using a Well-defined area borers. The total chlorophyll content in the leaves was estimated based on [18] and according to [19] by taking 100 mg of fresh leaves and cut into small pieces by scissors and crushed in a ceramic mortar with 6 ml of acetone concentration of 80% until the color of the sediment became non-green. The leachate was then separated from the precipitate using centrifuge at 1600 rpm for 10 minutes. The extract was then collected in volumetric tubes covered with darkened paper to block the light from chlorophyll to prevent oxidation of the pigment by light and the volume was completed by adding acetone. The spectral density of the extracts was then measured by Spectrophotometer at 645 and 663 nm. Using the following equations, total chlorophyll concentration was estimated in the plant leaves and calculated as mg.g\(^{-1}\) fresh plant.

\[
\text{Total Chlorophyll} = [20.2 (D_{645}) + 8.02 (D_{663})] \times V/ 1000 \times W
\]

3. Results and Discussions

3.1. The plant height (cm):

The results of Table (1) show that the highest rate of growth of seedlings was given by the treatment of marine algae extract concentration 4 ml.l\(^{-1}\), which was significantly higher than the rest of the treatments, which amounted to 87.96 cm compared with control treatment, which gave the lowest rates of seedling height, which was 61.20 cm. It is also possible to observe from the results of the same table that the mineral fertilizer (NPK) had a significant effect on the seedling height rate. The treatment of mineral fertilizer (NPK) (400) kg.ha\(^{-1}\) was significantly superior to the rest of the treatments to give the highest seeding value of 90.44 cm compared with control treatment which gave 65.14 cm. The same table indicates that the interaction between spraying with marine algae extract and NPK mineral fertilization has a significant effect on the rate of seedling height. The interaction treatment between the marine algae extract 4 ml.l\(^{-1}\) and mineral fertilizer (NPK) (400 kg.ha\(^{-1}\)) exceeded compared with other treatments to give the highest value of the seedling height of 110.30 cm compared with control treatment which gave only 51.20 cm.

Table 1. Effect of marine algae extract and NPK fertilizer in seedling height (cm).

| kg.ha\(^{-1}\) | 0    | 1    | 2    | 3    | 4    | Mean |
|------------|------|------|------|------|------|------|
| 0          | 51.20 | 60.20 | 68.30 | 70.40 | 75.60 | 65.14 |
| 100        | 55.10 | 60.70 | 65.50 | 69.60 | 70.70 | 64.32 |
| 200        | 60.80 | 70.30 | 73.20 | 78.20 | 88.10 | 74.12 |
| 300        | 68.11 | 75.20 | 78.10 | 85.30 | 95.10 | 80.36 |
| 400        | 70.80 | 80.10 | 90.40 | 100.60 | 110.30 | 90.44 |
| Mean       | 61.20 | 69.3  | 75.1  | 80.82 | 87.96 |
| LSD\(_{0.05}\) | Tecamin Algae | Tecamin Algae \times NPK | NPK |
| | 1.80 | 3.21 | 2.10 |

These results may be due to the role of plant growth-promoting substances such as auxins, cytokinins, and betaines, which have been detected in the marine alga extracts [20]. These substances can promote shoot length and vegetative growth development [21; 22]. The present study was consistent with previous study by [23] who reported the increase of shoot length of grapevine (\textit{Vitis vinefera L.}) plants treated with the brown alga as bio-fertilizers. Moreover, this study revealed the role of NPK in promoting plant growth because of the role of the these macro nutrients cell division and elongation as well as their importance in photosynthesis [24].
3.2. The main stem diameter (cm)

From the data in Table (2), the spraying with marine algae extract (Algae Tecamin) had a significant effect on the mean of pomegranate main stem diameter. The spray treatment with marine algae extract 4 ml.l\(^{-1}\) gave the highest rate of 1.4 cm compared with the control treatment, which gave the lowest rates of 0.8 cm. The mineral fertilizer (NPK) data in the same table showed a significant effect on the rate of pomegranate main stem diameter where as the treatment with 400 kg.ha\(^{-1}\) NPK gave the highest mean (1.5 cm) while the lowest rates were 0.8 cm resulted from the control treatment. The statistical data in Table (2) indicate that the combination treatment between marine algae extract and mineral fertilization (NPK) had a significant impact on the stem diameter mean of pomegranate seedlings. The spray treatment with a concentration of 4 ml.l\(^{-1}\) combined with 400 kg.ha\(^{-1}\) NPK gave the highest diameter (1.8 cm) compared with control treatment which gave only 0.5 cm.

Table 2. Effect of marine algae extract and NPK fertilizer on diameter of pomegranate main stem (cm).

| kg.ha\(^{-1}\) | ml.l\(^{-1}\) | 0  | 1  | 2  | 3  | 4  | Mean |
|--------------|------------|----|----|----|----|----|------|
| 0            | 0.5        | 0.6| 0.8| 0.9| 1.0| 0.8|      |
| 100          | 0.7        | 0.8| 0.9| 1.0| 1.1| 0.9|      |
| 200          | 0.9        | 0.9| 1.0| 1.2| 1.3| 1.1|      |
| 300          | 0.9        | 1.0| 1.3| 1.4| 1.6| 1.2|      |
| 400          | 1.0        | 1.3| 1.5| 1.7| 1.8| 1.5|      |
| Mean         | 0.8        | 0.9| 1.1| 1.2| 1.4|    |      |
| LSD\(_{0.05}\) | Tecamin Algae | 0.1 | Tecamin Algae × NPK | 0.2 | NPK | 0.1 |      |

3.3. Leaf number per plant

Table (3) shows that the number of leaves increased significantly by increasing the concentration of Tecamin Algae extract. The treatment of the fourth level gave the highest number of leaves (81.72 leaves) compared to non-treated seedlings where the average number of leaves was 63.52 leaves. The same table shows that the increase in chemical fertilization with NPK fertilizer was reflected in the significant increase in the number of pomegranate seedlings leaves. The addition of 400 kg.ha\(^{-1}\) was associated with the highest number of leaves (90.52 leaves), while the control treatment had the lowest number of leaves (49.88 leaves). The table also shows that the interaction between spraying with marine algae extracts and chemical fertilization gave the highest number of leaves per plant (100.10 leaves) when the interaction was between concentration of 4 ml.l\(^{-1}\) and fertilization at 400 kg.ha\(^{-1}\) compared with the control treatment which gave the lowest number of leaves per plant (40.10 leaves).

Table 3. Effect of marine algae extract and NPK fertilizer on leaves number per plant.

| kg.ha\(^{-1}\) | ml.l\(^{-1}\) | 0   | 1   | 2   | 3   | 4   | Mean |
|--------------|------------|-----|-----|-----|-----|-----|------|
| 0            | 40.10      | 45.00| 48.20| 56.30| 59.80| 49.88|      |
| 100          | 55.30      | 60.90| 69.30| 70.50| 75.90| 66.38|      |
| 200          | 67.20      | 70.40| 77.60| 79.70| 82.20| 75.42|      |
| 300          | 74.60      | 79.30| 80.30| 85.60| 90.60| 82.08|      |
| 400          | 80.40      | 85.10| 90.40| 96.60| 100.10| 90.52|      |
| Mean         | 63.52      | 68.14| 73.16| 77.74| 81.72|    |      |
| LSD\(_{0.05}\) | 2.50 | 2.60Tecamin Algae | 3.10 | Tecamin Algae × NPK | NPK |
The increase in the number of leaves of the pomegranate seedlings of the (California Wonderful) cultivar may be attributed to the contents of marine algae extract from auxins, which have an effective and fundamental role in division of the cells and their expansion, which increases the leaf area and dry weight of the vegetative and root systems [20]. Studies have shown that the increase in vegetative growth in strawberry plant after spraying with marine algae extract may have been due to the content of this extract on the abundance of macro and micro elements such as iron and its importance in oxidation and reduction processes as well as zinc and its role in the synthesis of amino acid tryptophan, which is the basis of the formation of auxin IAA in addition to copper with its function in the transmission of electrons in the process of photosynthesis, which have a positive role in improving growth and yield [25].

3.4. Average of the leaf area (cm², seedling⁻¹)

The results of Table (4) indicate that spraying of pomegranate seedlings (California Wonderful cultivar) with Tecamin Algae at a concentration of 4 ml.l⁻¹ gave the highest rate of leaf area of 165.96 cm² compared to the non-treated seedlings which gave the lowest rates (149.84 cm²). It is also noted from the same table that the chemical fertilization with NPK fertilizer at a dose 400 kg.ha⁻¹ gave the highest rate of leaf area (172.30 cm²) compared to the control treatment which gave the lowest rates of leaf area reached (139.64 cm²). The interaction between the studied factors had a significant effect on the average of leaf area, where the combination between the highest concentration of the marine extract and the highest fertilizer was the reason for the highest rates (180.20 cm²) compared with the control treatment (133.10 cm²) (Table 4). This increase may be attributed to the role of marine algae extracts in increasing the size and expansion of plant cells by increasing the elasticity of cell walls, or perhaps due to the role of oxygen from the marine algae extract in cell growth and its importance in stimulating gene cloning and translation and stimulating RNA and protein synthesis [26].

| Table 4. Effect of marine algae extract and NPK in the rate of leaf area (cm²) |
|-------------------|--------|--------|--------|--------|--------|--------|
|                   | 0      | 1      | 2      | 3      | 4      | Mean   |
| kg.ha⁻¹ ml.l⁻¹    | 0      | 100    | 200    | 300    | 400    | Mean   |
| 1                 | 133.10 | 141.40 | 150.20 | 159.10 | 165.40 | 149.84 |
| 2                 | 137.50 | 148.10 | 163.20 | 162.30 | 169.20 | 156.06 |
| 3                 | 138.60 | 160.20 | 165.10 | 167.10 | 172.40 | 162.32 |
| 4                 | 142.30 | 162.50 | 165.80 | 170.20 | 175.50 | 172.30 |
| Mean              | 146.70 | 163.20 | 167.30 | 172.40 | 180.20 | 165.96 |
| LSD₀.₀₅           | Tecamin Algae | Tecamin Algae × NPK | NPK | 1.8 | 2.60 | 2.40 |

3.5. The fresh weight of the vegetative system (g)

Table (5) shows that spraying with marine algae extract Tecamin Algae at a concentration of 4 ml.l⁻¹ gave the highest fresh weight of the vegetative system (14.70 g) compared with the control treatment which gave the lowest means reached (11.54 g). The results of the table indicate that the chemical fertilization with NPK fertilizer at the concentration 400 kg.ha⁻¹ had a significant effect on the fresh weight of the vegetative system, giving the highest rate (18.40 g) compared to the non-fertilized seedlings, which gave the lowest values (9.20 g). The interaction between spray treatment with marine algae extract at concentration 4 ml.l⁻¹ and chemical fertilization with NPK fertilizer at concentration 400 kg.ha⁻¹ gave a significant increase in the fresh weight of the vegetative system (20.90 g) compared with control treatment which gave the lowest values (8.30 g).

This may be due to an increase in the number of branches, leaves and length of branches due to the spray of marine algae extract. As a result of the interacted effects of the nutrients found in the marine algae extract, which helps in the formation of total chlorophyll and cytochromes, part of
which is involved in photosynthesis and energy production, the absorption of nutrients and the activation of vital processes in the plant, which all this affect on the nutritional status of the plant and increase the number of vegetative branches [20; 27]. The present study revealed consistent finding with previous studies by [28] who reported an increase in fresh weight of the vegetative system after treating *Ocimum sanctum* plants with seaweed extraction.

### Table 5. Effect of marine algae extract and NPK on the fresh weight (g) of the vegetative system.

| kg.ha⁻¹ | ml.l⁻¹ | 0   | 1   | 2   | 3   | 4   | Mean |
|---------|--------|-----|-----|-----|-----|-----|------|
| 0       | 8.30   | 8.40| 9.20| 9.80| 10.30| 9.20|
| 100     | 9.80   | 10.30|10.60|10.90|11.40|10.48|
| 200     | 11.50  | 12.20|12.70|13.40|14.20|12.76|
| 300     | 12.80  | 13.90|14.80|15.60|16.70|14.76|
| 400     | 15.30  | 17.40|18.60|19.80|20.90|18.40|
| Mean    | 11.54  | 12.44|13.18|13.90|14.70|      |
| LSD 0.05|        | 1.40| 0.80| 1.90|      |      |

### 3.6. The Dry weight of the vegetative system (g)

The results of Table (6) show that spraying with the Tecamin Algae extract at a concentration of 4 ml.l⁻¹ gave the highest dry weight rate of the vegetative system (7.96 g) compared with control treatment which gave the lowest dry weight of the vegetative system reached 5.84 g. The same table shows that chemical fertilization at a concentration of 400 kg.ha⁻¹ gave the highest dry weight of the vegetative system (9.00 g) compared with the control treatment, which gave the lowest rates (4.64 g). The same table also shows that the interaction between spraying with marine algae extract at a concentration of 4 ml.l⁻¹ and chemical fertilization with NPK fertilizer at 400 kg.ha⁻¹ gave the highest dry weight (10.90 g) compared with control treatment which gave the lowest values (4.10 g).

The increase in the dry weight of the vegetative system may be due to the role of marine algae extracts and chemical fertilizers in increasing vegetative growth of the plant, which led to the production and accumulation of larger quantities of carbohydrates in the plant, which led to the increase of dry matter [29]. The obtained results are consistent with [28] regarding to the dry weight of the vegetative shoots. Our results also agreed with [30] who reported a significance increased caused by spraying of marine algae extract (Kelpak) on mandarin seedlings.

### Table 6. Effect of marine algae extract and NPK on the dry weight (g) of the vegetative system.

| kg.ha⁻¹ | ml.l⁻¹ | 0   | 1   | 2   | 3   | 4   | Mean |
|---------|--------|-----|-----|-----|-----|-----|------|
| 0       | 4.10   | 4.20| 4.60| 5.10| 5.20| 4.64|
| 100     | 5.10   | 5.20| 5.30| 6.10| 6.20| 5.58|
| 200     | 6.20   | 6.60| 6.80| 7.20| 8.30| 7.02|
| 300     | 6.60   | 6.80| 7.10| 7.50| 9.20| 7.44|
| 400     | 7.20   | 8.10| 9.20| 9.60|10.90|9.00 |
| Mean    | 5.84   | 6.18| 6.60| 7.10| 7.96|      |
| LSD 0.05|        | 1.10| 0.50| 1.70|      |      |

### 3.7. The content of leaves of total chlorophyll (mg.g⁻¹)

It is noted from the data of Table (7) that spraying pomegranate seedlings (California Wonderful cultivar) with marine algae extracts at a concentration of 4 ml.l⁻¹ gave the highest rate of leaf content
of total chlorophyll (114.78 mg g⁻¹) compared with the control treatment which gave the lowest rates of leaf content of total chlorophyll. The same table indicates that NPK fertilizer gave the highest mean of leaf content of total chlorophyll in the treatment of 400 kg ha⁻¹ (123.73 mg g⁻¹) compared with the control treatment which gave the lowest mean of the total chlorophyll content of (90.22 mg g⁻¹). The interaction between the spray with Tecamin Algae at concentration 4 ml l⁻¹ and chemical fertilization with NPK at concentration 400 kg ha⁻¹ gave the highest mean of leaf content of total chlorophyll (128.10 mg g⁻¹) compared with the control treatment which gave the lowest means (80.20 mg g⁻¹).

This increase is due to the inclusion of marine algae on cytokines that promote the physiological activities and increase the plant content of total chlorophyll, which positively affects the process of photosynthesis and production of carbohydrates, which are positively reflected on the growth of vegetative [31; 32]. the results of this study is consistent with previous studies conducted on peach trees [33], Barhi dates palm trees [34], apricot [35], and olive [36].

| kg ha⁻¹ ml l⁻¹ | 0    | 1    | 2    | 3    | 4    | Mean |
|----------------|------|------|------|------|------|------|
| 0              | 80.20| 85.60| 90.70| 95.80| 98.80| 90.22|
| 100            | 90.30| 95.30| 97.20| 99.60| 100.90| 96.66|
| 200            | 110.20| 115.60| 118.10| 119.80| 120.80| 116.90|
| 300            | 115.40| 119.10| 121.30| 123.60| 125.30| 120.94|
| 400            | 120.50| 122.30| 123.30| 124.40| 128.10| 123.73|
| Mean           | 103.32| 107.58| 110.12| 112.64| 114.78|      |
| LSD 0.05       | 1.80 | 2.60 |      |      |      | 2.20 |

4. Conclusions

It can be concluded from this study that growth parameters exhibited significant increase by foliar application with marine algae extract as bio-fertilizers which could be an alternative plant growth promoting substances. This study also revealed that mineral fertilizers could not be excluded from agricultural practices to sustain soil fertility as the results indicated the significant impact on most studied traits individually or combined. Overall, the combination of organic and inorganic fertilizers can significantly enhance early stage of the plant growth. Further studies are required to examine the optimal concentration of marine algae extract and best combination of these bio-fertilizers with mineral compounds on different plants.

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