Effect of Chemical and Organic Fertilization and
*Aztobacter Chorocoocum* inoculation in IAA Acid
and Growth plant (*Lycopersicon esculentum* Mill )

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Abstract

This study was executed for the spring tomato (*Lycopersicon esculentum* Mill) for the year 2019 in a field within the Thuraya project - Qadisiyah Governorate. Mineral fertilizer NPK was used with three levels, organic fertilizer (composed poultry) with three levels, and a biological fertilizer consisting of *Aztobacter chorocoocum* bacteria after than isolating and diagnosing them. The results of the treatment of half of the fertilizer recommendation in combination with the third level of organic fertilizer and bio-fertilizer gave results that do not differ significantly from the treatment of the full fertilizer recommendation in the above triple interference in soil and plant characteristics (IAA, average plant height), and this indicates the importance of the role of organic and biological fertilizers in minimizing mineral fertilizers.

Keywords: IAA, *Aztobacter chorocoocum*, mineral fertilizer, organic fertilizer and *Lycopersicon esculentum* Mill.

1. Introduction

Showed [1,2], that growth-promoting bacteria produce many substances, including amino substances, organic acids, and IAA, which cause a change in the root system of the plant, which increases the ability of the root to absorb nutrients and water. It also works to produce IAA in good quantities [2]. As [3], showed that organic and bio fertilizers can reduce the quantities of mineral fertilizers if they are used together. The reason is attributed to the role of organic matter, as it reduces PH, which increases phosphorus available[4]. *Azotobacter* also plays an important role in increasing soil nitrogen as well as its ability to secrete growth regulators such as IAA leading to increased root system growth and increased absorption of nutrients in the plant [5]. The increase in the levels of the added mineral fertilizer led to an increase in the available phosphorus in the soil that can be absorbed by the roots, which in turn reflected on the concentration of phosphorus in the plant, where phosphorus strengthens and activates the root system of plants and thus increases the absorption of nutrients, including phosphorus, which positively affects the production of the plant. The increase in the levels of the added mineral fertilizer led to an increase in the soluble of phosphorus in the soil that can be absorbed by the roots, which in turn reflected on the concentration of phosphorus in the plant, where phosphorus strengthens and activates the root system of plants and thus increases the absorption of nutrients, including phosphorus, which positively affects the production of the plant [4]. Biological fertilization improves and increases nitrogen and phosphorous in plants as a result of nitrogen fixation and soluble phosphorous [6]. Biology also improves plant growth promoting rhizobacteria the most important of which are oxins, gibberellins and cytokines that encourage plant absorption of nutrients, including Potassium, and thus this increase was reflected in the vegetative growth indicators of the plant, including the rate of plant height [7].

2. Materials and methods

Preparation of bacterial biofertilizers: 20 soil samples were collected from different fields cultivated with tomato crops, cucumbers, watermelon, and eggplant. Samples were taken from the rhizosphere soil of crop roots to different locations within the same field and from a depth of 0-30 cm and mixed to form a compound sample and packed in clean and sterile polyethylene bags and transported to the laboratory. Physical, chemical and biological analyzes were performed as shown in Table (1) and kept in the refrigerator before use in the process of isolation.
Table 1. Physical, chemical and biological characters of the field soil.

| Unit                  | Value   | property               |
|-----------------------|---------|------------------------|
| Texture               |         |                        |
| Clay                  | 331     | gm.kg.soil -1          |
| Silt                  | 255     | gm.cm^3                |
| Sand                  | 414     |                        |
| Texture type          |         |                        |
| Clay mixtures         |         |                        |
| gm.cm^3               | 1.37    |                        |
| d. Sm^1               | 7.55    |                        |
| Cinite moul.kg^1 soil| 2.36    |                        |
| Organic matter        | 56      | EC, Electrical conductivity |
| Organic matter        | 2.9     |                        |
| Nitrogen              | 20.10   |                        |
| phosphorous           | 3.34    |                        |
| Potassium             | 138.95  |                        |
| Calcium               | 1.83    |                        |
| Sodium                | 3.32    |                        |
| Carbonate             | 2.51    |                        |
| Sulfates              | 1.40    |                        |
| Total bacteria        | 4.01 x 10^7 | Biological Estimates  |

2.1. Isolation of Azotobacter spp.

Soil loosening was prepared by adding 10 g of the selected samples to 90 ml of sterile distilled water in 250-capacity flasks of liquid medium (Sucrose mineral-salts) and the test tubes were placed in the incubator at a temperature of 28 °C for 3 days and the tubes were examined by observing the brown membrane (ring Structure) which is an indicator of the growth of Azotobacter spp. I used 0.1 ml of dilution, which gave an indication of the growth of Azotobacter spp. Of them, membranes were dyed in a Gram method and examined microscopically using a light microscope to study the phenotypic characteristics. 10 pure isolates of A. chroococcum were obtained. Diagnosis of Azotobacter bacteria: - Membranes of the ten isolates diagonal cultures were prepared on concave glass slides of 48 hours and stained by the Gram method. The shapes of cells, their size and the ability of the bacteria to interact with the dye were studied using the oily lens. Morphological characteristics: The morphological (phenotypic) culture characteristics of A. chroococcum isolates were studied by observing the bacterial growth on solid culture media from inside the plates and studying the bacterial colony shape, height, edge shape, surface, size, transparency, and amount of bacterial growth. And the production of pigments according to the method [8].

Bio-tests for bacteria: -The following tests were conducted, growth at 37 °C, starch decomposition test, nitrate reduction medium, Catalase test, oxidase test, fermentation test for sugars amid movement test, isolate efficiency test for atmospheric nitrogen fixation and the amount of ammonia was estimated It was formed in the middle by taking 2 ml of it and assessing it with a Microkildahl device [8,9], and isolate No. (1) was chosen, which is (A1), as the amount of nitrogen installed was 12.43 the amount of stabilized N2 (mg. Liter^-1) A. chroococcum, which is the isolation used as a inoculation after contaminating tomato seeds by using gum arabic after removing the sterile material.

2.2. IAA extraction

A strain of A. chroococcum, isolated from plant rhizosphere, previously used as a bacterial inoculum, was grown in sucrose mineral salts supplemented with 100 μg l^-1 of DL-Tryptophan. The medium is distributed in 250 ml vials containing 100 ml of it and the bottles are inoculated with 5 ml of the vaccine isolates of different agents of A. chroococcum, whose optical density is 0.85 (this optical density is equal to the optical density of the standard turbidity that contains 1.5 x 108 bacteria. According to the method [10], then it is incubated in a vibrating incubator (100 cycles. Min^-1) at a temperature of 28 °C for 24 hours, and the growing bacterial cultures are extracted in 100 ml of the medium by centrifuging them g x 7700 in a centrifuge for 30 Accurate according to the method of [11], Then 50 mL of the filtrate was taken (the culture medium) and the pH in it was adjusted to 2.5 using 2N hydrochloric acid (HCl), after which the partitioning process was performed with a similar volume of ethyl acetate using a separating funnel. Capacity of 250 ml four times, and then collecting the organic
phase that contains plant hormones. The evaporation process was carried out using a rotary evaporator at a temperature of 35 °C, according to the [12,13], method. Then 5 ml of methanol alcohol were added to it, then the models were analyzed using a high performance (pressure) liquid chromatography (HPLC) type LC-6A with a Spd-6A-UV Spectrophotometer. The 20 μl samples were projected into the column by an Injector of the Rheodgne-7120 type, at a temperature of 40 °C, regulated by the Sil-6A thermal controller. In the analysis, a Reverse-phase-Column, type C-18, with dimensions of 250 x 4-6mm-10, was used for a mobile phase consisting of phosphoric acid and methanol at a ratio of 40:60 volume / volume. Auxins were estimated at a wavelength of (280) nanometers according to [14], standard solutions of phytohormones were prepared with concentrations (0, 2, 5, 10 and 20 mg L⁻¹) using auxin IAA, where 0.02 g L⁻¹ was taken separately to prepare Concentration 20 mg-liter-1 and take 500 ml of this concentration and continue to liter to obtain concentration 10 mg liter⁻¹, and take 500 ml of concentration 10 mg-liter-1 and continue to liter to obtain 5 mg.liter⁻¹ and then take 400 ml of concentration 5 Amalgam liter⁻¹ and continued to the liter to prepare the concentration of 2 mg liter⁻¹ and used distilled water as a concentration of zero, as standard solutions were injected and readings were taken for them, then the standard curve was drawn between the standard solutions of IAA and their readings with the device, then the readings obtained were dropped to the unknown samples to obtain The concentration of IAA in amalgam per liter.

2.3. Preparing the soil for the greenhouse

The experiment was carried out in a plastic house of dimensions (9 x 52 m) in mixed clay soil with physical, chemical and biological properties indicated in Table (1). The soil was prepared and smoothed well, soil samples were taken as representative as possible from the layer. The surface (0-30 cm) was then dried and milled with a polyethylene hammer and passed through a sieve with a hole of 2 mm in diameter and mixed well. Three ladders were established along the plastic house between each floor and another, an isolation distance of 2 meters and divided one into 18 experimental units of 2.5 m long Its width is 1.25 m, including an isolation distance of 0.75 m, to prevent interferences between experimental units in one sector [15]. The fertilizer recommendation was added according to the recommendations of the Iraqi Ministry of Agriculture.

2.4. Experimental design

An Experimental factorial was implemented according to the( R.C.B.D) design, with three replications. The experiment included [16] treatment.

- The fertilizer recommendation was added according to what was stated in 320-320-160 T. h⁻¹ (N-P205-K₂O) and in three levels (50, 0, 100)% of it.
- Levels of the chroococcum aztobacter bacterial inoculation (A₀ without inoculation and A₁ inoculation).
- Levels of organic fertilizer (2, 1, 0)T. h⁻¹.

3. Results and Discussion

Table (2) indicates that the chemical fertilization gave an increase in the concentration of IAA( mg. L⁻¹) when the full fertilizer was recommended 100%, as the value of the treatment was recorded at 23.14 (mg. L⁻¹) a significant increase compared with the control treatment, and the reason is that the chemical fertilizers Provides important nutrients to microorganisms [4], and the results are in agreement with [17]. The results of the table also confirmed that the third level of organic matter led to the highest increase, as the value of the treatment recorded 25.16 IAA (mg. L⁻¹) compared to the control treatment. The reason is that the organic matter contains the main nutrients for microorganisms in the soil, which It leads to an increase in the metabolic efficiency of the bacterial community, an increase in its numbers, and thus an increase in the secretion of auxins [18]. These results are in agreement with [5]. The inoculation with bacteria. Aztobacter chroococcum increased the concentration of IAA The inoculation treatment gave a significant increase of 23.41 (mg. L⁻¹) compared to the control treatment, due to the ability of A. chroococcum to produce auxin IAA [19]. These results are in agreement with [20].

As for the bilateral interaction between mineral and organic fertilizers, it gave an increase in all levels of fertilizer recommendation (0, 50, 100)%; the highest was in the 100% full fertilizer recommendation and the third level of organic matter, as it reached 27.02 IAA (mg. L⁻¹) with treatment the control. As for the bilateral interaction between mineral and biological fertilization, it was the highest significant increase when the 100% complete fertilizer recommendation reached 23.75 (mg. L⁻¹) compared to the control treatment, due to the fact that the fertilizer recommendation contributed with the nutrient content in increasing the root total that provides In turn, a large surface area is adsorbed by bacteria that act on the secretion of IAA [16,21]. These results are consistent with [22]. As for the double overlap between organic and biological fertilizers, it led to a significant increase in the concentration of IAA, the highest at the third level of organic matter, reaching 25.16 (mg. L⁻¹) compared to the control treatment, and the reason is attributed to the role of organic fertilizers and containing important nutrients in Increasing the numbers of the bacterial community and its secretions [23]. These results are in
agreement with [24]. As for the triple overlap between mineral, organic and biological fertilizers, it gave the highest increase at the recommendation level of 100% with the third level of organic matter, as the value of the treatment was recorded at 30.52 (mg . L⁻¹), a significant increase compared to the control treatment.

Table 2. The effect of chemical, organic and biological fertilization on the amount of IAA( mg.L⁻¹ ) produced in the soil rhizosphere.

| Interaction M*O | Levels of bio-fertilization B | Fertilization levels Organic O | Fertilization levels M |
|-----------------|-------------------------------|-------------------------------|------------------------|
|                 | inoculation                   | Without inoculation           |                        |
| 2.88            | 3.14                          | 2.88                          | 0                      |
| 3.75            | 3.81                          | 2.98                          | 1                      |
| 6.28            | 8.67                          | 6.38                          | 2                      |
| 17.03           | 17.03                         | 11.39                         | 0                      |
| 17.42           | 21.27                         | 17.52                         | 1                      |
| 22.17           | 23.69                         | 22.28                         | 2                      |
| 23.33           | 23.75                         | 23.41                         | 0                      |
| 25.09           | 25.32                         | 25.16                         | 1                      |
| 27.02           | 30.52                         | 27.02                         | 2                      |
| L.s.d 0.05      |                               |                               | 0.67                   |

Fertilization levels M

| 2.88            | 3.14                          | 2.88                          | NPK 0 % |
| 11.39           | 17.01                         | 11.39                         | NPK 50 % |
| 23.14           | 23.75                         | 23.41                         | NPK 100 % |
| L.s.d 0.05      |                               |                               | 0.26     |

Fertilization levels Organic O

| 2.88            | 2.89                          | 2.88                          | NPK 0 % |
| 11.25           | 7.52                          | 11.40                         | 1        |
| 23.14 cd        | 25.16                         | 23.75                         | 2        |
| L.s.d 0.05      |                               |                               | 0.26     |
| L.s.d 0.05      |                               |                               | 0.37     |

Table (3) indicated that mineral fertilization at the level of 100% NPK gave an increase in the average plant height (cm. Plant⁻¹), if the value of the treatment (plant⁻¹ cm) gave a significant increase in comparison with the control treatment, and the reason was attributed to the increase Nutrients increase the nutrient in the plant [4,25]. The results are consistent with [5]. The table also shows that the addition of organic matter in the third level of it gave an increase in the average plant height (cm.Plant⁻¹), as the value of the treatment (cm.Plant⁻¹) gave a significant increase compared to the control treatment, and the reason is attributed to the fact that the organic substance contains many The nutrients that the plant uses in its growth, including S, NPK [5]. These results agree with[3]. The table illustrated the role of the interaction between organic and biological fertilizers, as all levels of organic fertilizer achieved an increase in plant length (cm. Plant⁻¹), as the treatment value achieved 104.35 cm (plant⁻¹) compared to the control treatment. The reason for this is attributed to the role of organic matter and it contains important nutrients in large proportions in plant nutrition, as well as the role of nutrients in mineral fertilizers [26,27]. These results agree with [5].

As for the triple interference (mineral, organic and biological fertilizers), it gave a significant increase in the value of the treatment 101.56 (cm. Plant⁻¹) in comparison with the control treatment, indicating that the second level of the fertilizer recommendation of 50% NPK with the third level of organic matter achieved a significant increase. Thus, it led to the reduction of mineral fertilizer.
Table 3. The effect of chemical, organic and biological fertilization. Average plant height (cm. Plant$^{-1}$).

| Interaction \ M$^*$O | Levels of bio-fertilization B | Inoculation | Without inoculation | Fertilization levels | Fertilization levels | M            | Levels B |
|---------------------|-------------------------------|-------------|---------------------|----------------------|----------------------|--------------|----------|
|                     |                               | 96.63       | 96.26               | 0                    | NPK %0               |              |          |
| 95.96               |                               | 97.52       | 96.99               | 1                    | NPK %50              |              |          |
| 96.89               |                               | 100.01      | 97.83               | 2                    |                      |              |          |
| 89.34               |                               | 101.01      | 99.22               | 2                    |                      |              |          |
|                     |                               | 101.47      | 100.26              | 1                    | NPK %100             |              |          |
| 103.27abcdefh      |                               | 101.56abcdefL | 100.36          | 2                    |                      |              |          |
| 101.34             |                               | 102.89      | 102.11              | 0                    |                      |              |          |
| 100.34             |                               | 104.12      | 102.15              | 1                    | NPK %100             |              |          |
| 103.00             |                               | 104.35      | 103.00              | 2                    |                      |              |          |
| **L.s.d.0.05**      |                               | 1.01        |                     |                      |                      |              |          |
| Fertilization levels |                               | **L.s.d.0.05** |                     | 0.73                 |                      |              |          |
| **M**               |                               | 89.26       | 96.63               | 96.26                | NPK %0               |              |          |
| 98.59               |                               | 99.22       | 99.07               | NPK %50              |                      |              |          |
| 101.06             |                               | 102.25      | 102.11              | NPK %100             |                      |              |          |
| **L.s.d.0.05**      |                               | 3.63        |                     |                      |                      |              |          |
| Fertilization levels |                               | **L.s.d.0.05** |                     | 1.04                 |                      |              |          |
| **Organic O**       |                               | 89.26       | 96.63               | 96.26                | 0                    | Levels O*M   |          |
| 99.54               |                               | 97.52       | 96.99               | 1                    |                      |              |          |
| 103.04             |                               | 104.35      | 102.89              | 2                    |                      |              |          |
| **L.s.d.0.05**      |                               | 5.09        |                     |                      |                      |              |          |
| **L.s.d.0.05**      |                               | 5.01        |                     |                      |                      |              |          |
| **Total bio-fertilizer levels** | **L.s.d.0.05** | 1.55        | 104.35              | 98.59                |                      |              |          |

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