University of Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 13, Issue 2 (2024) PP 425-437

https://jasj.rdd.edu.iq/journals/journal/issue/18682

# Effect of pinching and spraying of Tyrosine acid on the nutrient content and inhibitory activity of Roselle oil of *Hibiscus sabdariffa* L.

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## I. Abstract

The experiment was conducted in the shade of the Department of Horticulture and Landscape Engineering, College of Agriculture / University of Basra during the two agricultural seasons (2023 and 2024) on Roselle plants, in order to know the effect of pinching and spraying with different levels of the amino acid Tyrosine at levels (0, 100, 200) mg $L^{-1}$ on the content of nutrients in the leaves and the inhibitory effectiveness of Roselle plant oil. The study followed the design of complete randomized blocks for factorial experiments with two factors pinching and spraying with Tyrosine at three concentrations for each and with three replicates for the treatment so that the number of experimental units became 27 experimental units. The least significant difference (L. S. D) test was used at a significance level of (0.05) to compare the averages. The results show that the plants that were pinched at 7-8 leaves were significantly excelled and gave the highest nitrogen content in the leaves, reaching (1.99 and 1.92)% and phosphorus reaching (0.62 and 0.59)% and potassium reached (4.98 and 4.58)% respectively for both seasons of the study, and the plants sprayed with tyrosine acid at a concentration of 100 mg $L^{-1}$ significantly excelled in giving the highest content of nutrients in their leaves, as the nitrogen content reached (2.03 and 1.94)% and phosphorus reached (0.61 and 0.59)% and potassium reached (4.89 and 4.49)%, and the results also showed the inhibitory effectiveness of Roselle oil against three types of bacteria and a significant effect of the concentration of 100 mg ml-1 which gave a 5 mm inhibition diameter for Staphylococcus aureus bacteria and a 6 mm inhibition diameter for Escherichia coli bacteria and a 13 mm inhibition diameter for Bacillus sp bacteria compared to the lowest concentration which gave the lowest area of inhibition.

***Keywords: Roselle, Tyrosine, Staphylococcus aureus, Escherichia coli, Bacillus sp***

## II. Introduction:

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 13, Issue 2 (2024) PP 425-437
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Roselle is one of the important economic plants that is characterized by a financial return and good income for the investor. In the countries that produce roselle, it is marketed locally in the markets and the other part is exported to many countries around the world due to the diversity of its products, which distinguishes it from other plants and increases its economic and medical importance (El Naim et al., 2017). Roselle is a plant native to Africa and Asia that belongs to the Malvaceae family. The plant is an erect, dense, branched shrub that reaches a length of 2.5 meters. Its stem is purple with purple-green leaves. Its flowers are solitary and consist of 5 valves that have a shape with a dense red color (Sindi et al., 2014). It lives in tropical and subtropical regions in the hemisphere of the world (Cissé et al., 2009). Roselle has great medical benefits in its various parts (such as leaves, roots, seeds, flowers, fruits, and angiosperms). The plant is used as an abortifacient, antipyretic, antibacterial, antiseptic, antispasmodic, appetite stimulant, astringent, digestive, laxative, analgesic, purgative, expectorant, diuretic, analgesic, anthelmintic, muscle, uterine, and joint pain reliever, and cholagogue (Duke et al., 2002). The most important parts of the Roselle plant are the calyx leaves, which contain active compounds, as they contain proportions of water, carbohydrates, fats, proteins, fibers, and vitamin C, in addition to minerals and amounts of calcium salts (Mahadevan et al., 2010). Roselle was selected as a potential candidate rich in anthocyanins, and it can be used as a good source for producing a red food dye (Aryanti et al., 2019). Natural dyes can be extracted by different methods, such as the Soxhlet method, traditional solvent extraction, and microwave-assisted extraction (Febriana et al., 2017).

The pinching process is one of the mechanical processes that researchers and farmers resort to in order to obtain the largest number of green branches (Darzi and Kifaya, 2017), as the low level of auxin affects the growth of the terminal buds at the expense of the lateral buds and thus inhibits their growth. Therefore, the process of pinching the apical bud stimulates the activity of the lateral buds in the plant seedlings to grow and branch laterally, thus increasing the vegetative mass, which improves the efficiency of the photosynthesis process, which is reflected in the increase in the yield and oil. Pinching provides a wider surface area for greater photosynthesis activity, which in turn improves other growth criteria and crop components (Kumar et al., 2014). As mentioned by Sharaf-Eldien et al., (2017) in a study on Zinnia elegans L., the plant pinching recorded the highest plant height and the largest leaf area in control treatment, reaching 83.25 cm, 33.03 cm, respectively, and recorded the highest values for the number of main leaf branches at pinching, reaching 8.61 branches per plant-1. (El-Shayeb et al., (2021) confirmed that when pinching Roselle plants after 12

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 13, Issue 2 (2024) PP 425-437
https://jasj.rdd.edu.iq/journals/journal/issue/18682

weeks from the planting date, the highest increase was obtained in the number of plant branches, the dry weight of Roselle leaves, and the dry weight of the roots of the plant, while when pinching after 4 weeks from the planting date, it led to an increase in the length of hibiscus plants compared to control treatment, reaching 164.55 cm, and it also gradually led to an increase in the number of fruits per plant, and the yield of dry sepals per plant (g), as the increase in the number of fruits per plant reached about 30.70 and 40.45 fruits. Plant$^{-1}$ for pinching treatment after 12 weeks from the date of planting compared to pinching after 4 weeks from the date of planting in the first and second seasons respectively. Amino acids are particularly important for stimulating cell growth. They act as buffers that help maintain the appropriate pH value inside the plant cell because they contain both acidic and basic groups. They remove ammonia from the cell, thus protecting plants from ammonia toxicity. Amino acids have a function in the biosynthesis of other organic compounds, such as pigments, vitamins, alkaloids, enzymes, terpenoids, coenzymes, purine and pyrimidine bases. Amino acids are of great importance in plant nutrition for higher yield and quality and shortening the production cycle with better dry matter. They gave more abundant and more uniform flowering. (Massoud et al., 2020) One of the amino acids is tyrosine, which is a hydroxyphenyl amino acid used in the construction of neurotransmitters and hormones (Balbaa & El-Aziz, 2001). The biosynthesis of cinnamic acids, as the raw material for the construction of phenols is derived from the amino acid Phenylalanine and the amino acid Tyrosine (Hass, 1975). El-sherbeny et al. (2013) showed that spraying the beetroot plant Beta vulgaris with tyrosine acid at three concentrations (100, 200 and 400) mg L$^{-1}$ led to an increase in vegetative traits at a concentration of 100 mg L-1, as it led to superiority in plant height, number of leaves and plant weight, respectively (27.47 cm, 13.10 leaves.plant-1, 49.22), while the concentration of 200 mg L$^{-1}$ excelled the concentration of 400 mg L-1 and had a significant effect on chemical traits, as it led to a significant increase in chlorophyll and carbohydrates, respectively (0.74, 20.40). Karkoush (2022) explained in her study on Roselle plant that spraying with the amino acid phenylalanine at a concentration of 100 mg L-1 on the growth and yield of Roselle plant from anther leaves led to a significant increase in plant height, leaf area and fresh weight of the plant (184.8 cm, 205.5 cm and 603.2 g plant), while the concentration of 25 mg L-1 was superior in the number of branches and the total number of leaves (64.07 branches plant and 38.89 leaves plant). Therefore, the study aims to know the extent of the effect of cutting the dormant top and spraying different concentrations of tyrosine acid in improving the nutritional content of Roselle plants under environmental conditions in Basra

Governorate, and to know the extent of the effectiveness of the oil in inhibiting bacteria that are pathogenic to humans.

## III. Materials and methods:

The experiment was conducted in the fabric shade of the Department of Horticulture and Landscape Engineering, College of Agriculture / University of Basra during both seasons (2023 and 2024) on Roselle plants, in order to know the effect of pinching at three levels (without pinching - pinching from 3_4 leaves and 7_8 leaves) and spraying with different levels of tyrosine at levels (0, 100, 200) mg $L^{-1}$ on the content of nutrients and the inhibitory effectiveness of Roselle oil. Before starting, random samples were taken from the soil after mixing and for analysis Table (1) where Roselle seeds were planted in pots with 3-4 seeds and after 8 days the seedlings were completed, and the plants were thinned to one plant in the pots after 20 days of planting.

**Table (1) Some initial traits of the soil used in the study**

| Particle size | | | Texture | PH | EC | O.M | N | P | K |
|---|---|---|---|---|---|---|---|---|---|
| Clay (%) | Silt (%) | Sand (%) | | | $dsm^{-1}$ | % | $mg\ l^{-1}$ | | |
| 14.7 | 1.5 | 83.8 | Loamy Sand | 6.90 | 1.0 | 26 | 56 | 28.5 | 61.9 |

Studied traits:

Macronutrient content of leaves:

According to the method mentioned by Cresser and Parsons (1979), the macronutrient content of leaves was estimated according to the following steps:

1. 0.2 of the dry ground sample was taken for each experimental unit and placed in the digestion tube.

2. 5 ml of concentrated sulfuric acid was added and left for 24 hours (overnight).

3. The digestion device was heated until it reached a temperature of 400 °C.

4. The samples were placed in the device for half an hour until boiling.

3 ml of the acid mixture (4 ml of concentrated perchloric acid + 96 ml of concentrated sulfuric acid) was added and then heated until the solution became clear, the solution was left to cool and the volume was completed to 50 ml with distilled water.

Nitrogen percentage in leaves %

Total nitrogen was measured in the digested samples using a Microkjeldahl steam distillation device according to the method of (Page et al., 1982).

Physphorus percentage in leaves %

It was estimated using a Spectrophotometer with a wavelength of 470 nm according to the method of (Murphy and Riley, 1962).

Potassium percentage in leaves %

Potassium was estimated in leaves using a Flame photometer type PEP 73 JEN WA 73 according to the method of Page et al., 1982)) and the results are expressed according to the standard curve in which potassium chloride is used.

Efficiency of the oil in inhibiting the growth of some types of bacteria that are pathogenic to humans.

Bacterial isolates.

Three bacterial strains were selected from the College of Science, University of Basrah, the first infects the respiratory system Staphylococcus aureus, the second infects the digestive system Escherichia coli, and the third is a positive strain Bacillus sp.

Testing the effectiveness of the oil against three types of bacteria. The effectiveness of four concentrations of oil (0, 25, 50, 100) mg on three types of selected bacteria was chosen, as stated in Smânia et al., (1999) and Vanden & Vlietinck, (1991), where Muller Hinton agar was used to grow bacteria after placing 20 ml in each Petri dish, then the dishes were planted by spreading method by placing 50 microliters of bacterial suspension prepared from bacterial isolates where they were spread by sterile cotton swabs, and incubated for 24 hours at a temperature of 37 °C. Then holes with a diameter of 7 mm were made in the agar medium and the sample to be tested for its inhibitory effectiveness (oil) was placed inside them, 20 mm apart from each other. After that, the dishes were incubated for 24 hours at 36 °C, under aerobic conditions, after which a congested bacterial growth was observed, and the result was recorded by measuring the diameter of the area Inhibition Diameters of inhibition zones in mm by means of a measuring ruler

**Statistical analysis:**

The experiment was carried out according to the Randomized Complete Block Design (R.C.B.D) and in a Factorial Experiment with two factors, the first represents pinching at three levels (no pinching, pinching at 3-4 leaves, pinching at 7-8 leaves), and the second factor represents spraying the amino acid Tyrosine at three levels (0, 100, 200) ml L-1, and the number of experimental treatments was (9) factorial treatments and the number of replicates for each treatment was 3 replicates and the total experimental units were (27) experimental units. As for the statistical analysis of the experimental data, it was done using the Genestat statistical program, and the Least Significant Differences (L.S.D) test was used at a probability level of 0.05 to compare the averages of the treatments.

## IV. Results:

**Nutrient content of Roselle leaves:**

The results in Table (2) show the significant effect of pinching on the content of Roselle leaves of nitrogen, phosphorus and potassium. The plants that were pinched at 7-8 leaves recorded the highest nitrogen content for both seasons of the study, reaching (1.99 and 1.92)%, respectively, compared to the control plants, whose average nitrogen content of leaves reached (1.72 and 1.61)%, respectively, for both seasons. It also gave the highest phosphorus content in the leaves, reaching 0.62 for the first season, compared to the control plants, whose average phosphorus content reached 0.52% for the first season, while the second season had no significant effect on the phosphorus content of the leaves, while the potassium content of the leaves reached (4.98 and 4.58)%, respectively, compared to the control plants, whose average potassium content reached (3.69 and 3.93)%, respectively, for both seasons. Both seasons.

As for the effect of spraying with tyrosine acid, the results of Table (2) showed the significant superiority of plants sprayed with tyrosine at a concentration of 100 mg $L^{-1}$ on plants sprayed with other concentrations and for both molecules, as the highest nitrogen content in their leaves reached (2.03 and 1.94)% compared to the control plants whose leaves nitrogen content reached (1.63 and 1.52)% respectively. The plants sprayed with the same concentration also significantly excelled in their leaves phosphorus content, as it reached 0.61% for the first season compared to the control plants whose phosphorus content reached 0.51% for the first season, while there was no significant effect of this trait in the second season, and the highest potassium content reached (4.89 and 4.49)% compared to the control plants whose potassium content reached (3.87 and 3.73)% for both study seasons respectively.

The results in Table (2) show the significant effect of the interaction between pinching and spraying with tyrosine on the nitrogen, phosphorus and potassium content of leaves for both seasons of the study, as the interaction between (pinching at 7-8 leaves and a concentration of 200 mg L-1 tyrosine) was significantly excelled to the rest of the treatments and gave the highest nitrogen content in its leaves, reaching (2.17 and 2.09)% compared to the control plants, whose average nitrogen content was (1.48 and 1.34)% for both seasons of the study, respectively. This treatment also gave the highest phosphorus content in its leaves, reaching (0.66)% compared to the control plants, whose average phosphorus content was (0.47)% for the first season, while the second season had no significant effect on the phosphorus content of leaves. As for the interaction effect on the potassium content, it was significant for both seasons of the study, reaching (5.41 and 4.88)% compared to the control plants, which Its potassium content reached (3.44 and 3.31)% respectively for both seasons.

**Table (2) Effect of spraying chitosan and whey on the content of nutrients in the leaves of Doranta plants**

| pinching | Tyrosine | Nitrogen content in leaves(%) | Phosphorus content in leaves(%) | Potassium content in leaves(%) |
|---|---|---|---|---|

| | | 2023 | 2024 | 2023 | 2024 | 2023 | 2024 |
|---|---|---|---|---|---|---|---|
| **without pinching** | **0** | 1.48 | 1.34 | 0.47 | 0.62 | 3.44 | 3.31 |
| | **100** | 1.87 | 1.78 | 0.56 | 0.54 | 4.32 | 4.03 |
| | **200** | 1.81 | 1.70 | 0.54 | 0.51 | 4.02 | 3.72 |
| **4-3leaves** | **0** | 1.64 | 1.55 | 0.52 | 0.48 | 3.84 | 3.69 |
| | **100** | 2.05 | 1.95 | 0.61 | 0.58 | 4.96 | 4.58 |
| | **200** | 1.94 | 1.82 | 0.58 | 0.56 | 4.71 | 4.41 |
| **8-7leaves** | **0** | 1.76 | 1.68 | 0.55 | 0.61 | 4.34 | 4.19 |
| | **100** | 2.17 | 2.09 | 0.66 | 0.63 | 5.41 | 4.88 |
| | **200** | 2.03 | 1.98 | 0.64 | 0.53 | 5.21 | 4.67 |
| | **LSD** | **0.010** | **0.034** | **0.004** | **N.S** | **0.032** | **0.029** |
| **pinching** | **without pinching** | 1.72 | 1.61 | 0.52 | 0.56 | 3.93 | 3.69 |
| | **4-3leaves** | 1.88 | 1.77 | 0.57 | 0.55 | 4.51 | 4.23 |
| | **8-7leaves** | 1.99 | 1.92 | 0.62 | 0.59 | 4.98 | 4.58 |
| | **LSD** | **0.008** | **0.023** | **0.003** | **N.S** | **0.020** | **0.018** |
| **Tyrosine** | **0** | 1.63 | 1.52 | 0.51 | 0.49 | 3.87 | 3.73 |
| | **100** | 2.03 | 1.94 | 0.61 | 0.59 | 4.89 | 4.49 |
| | **200** | 1.93 | 1.83 | 0.58 | 0.56 | 4.65 | 4.27 |
| | **LSD** | **0.005** | **0.19** | **0.002** | **N.S** | **0.019** | **0.17** |

The effectiveness of fixed oil in inhibiting the growth of bacteria in humans.

Table (3) shows the effect of fixed oil on the growth of bacteria, where it is noted that Roselle oil has an inhibitory effect on the growth of bacteria that are pathogenic to humans, and the higher the concentration of the oil, the greater the inhibition zone, where the concentration of 100% gave the highest inhibition zone for all strains of Staphylococcus aureus, Escherichia coli and Bacillus sp., reaching 5, 6 and 13 mm, respectively, while the lowest zone was at a concentration of 20%, reaching 3, 2 and 10 mm, respectively, while control treatment had no inhibitory effect on bacteria.

**Table (3) The effect of Roselle oil in inhibiting bacteria that are pathogenic to humans.**

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 13, Issue 2 (2024) PP 425-437
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| Inhibition area (mm( | | | Fixed oil concentration |
|---|---|---|---|
| Bacillus sp. | Escherichia coli | Staphylococcus aureus | |
| 0 | 0 | 0 | 0 |
| 10 | 2 | 3 | 25 |
| 12 | 4 | 2 | 50 |
| 13 | 6 | 5 | 100 |

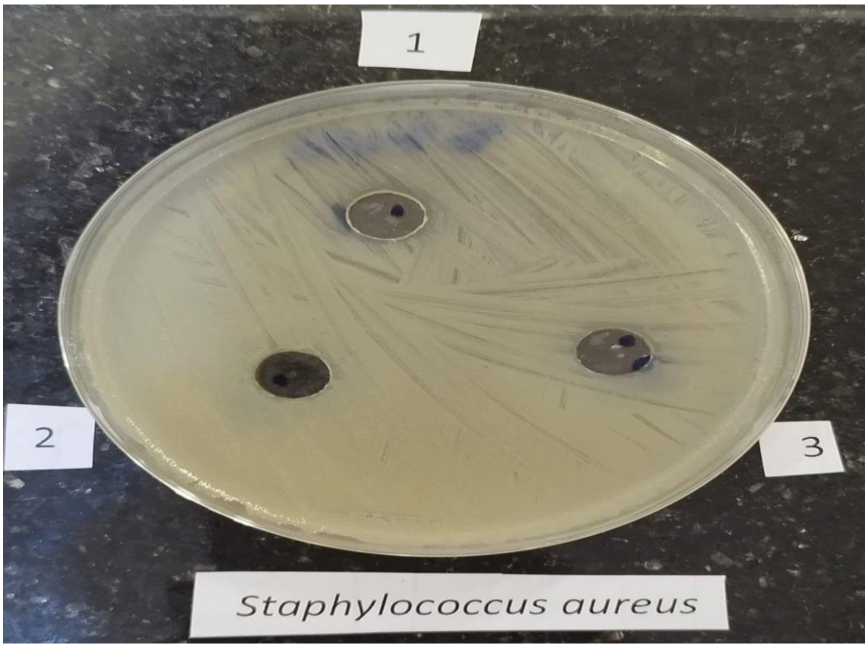


Figure (1) Bio-resistance of oil against Staphylococcus aureus bacteria

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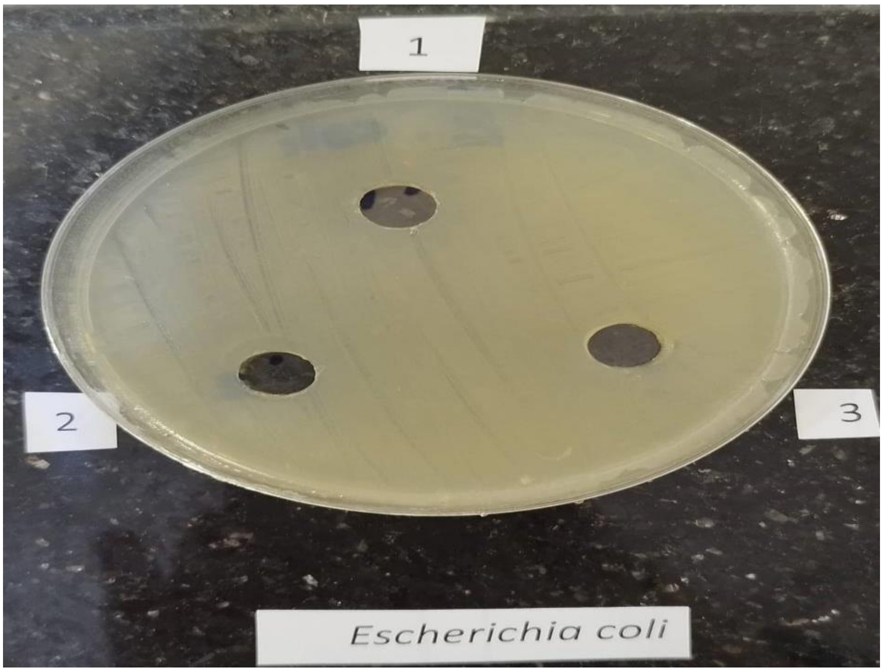


**Figure (2) Bioresistance of oil against Escherichia coli bacteria**

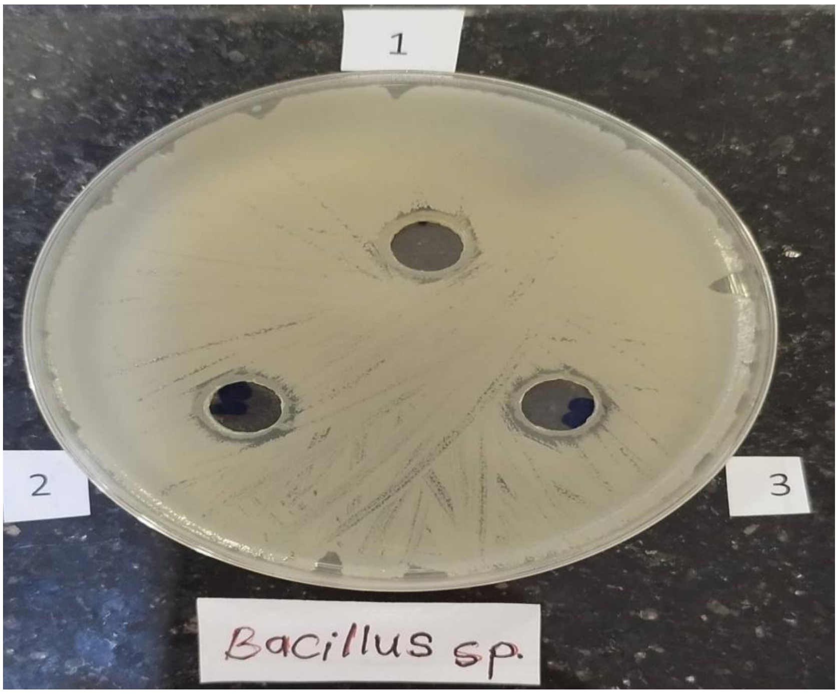


Figure (3) Bioresistance of oil against Bacillus sp.

## V. Discussion:

It is noted that excelled of the fermented plants in the content of major elements in the leaves is due to the increase in the activity of the vegetative group, because this element is one of the most important elements that enter the construction of plastids and thus increase the efficiency of the photosynthesis process, which was positively reflected in the increase in the absorption of nutrients, including nitrogen), that the percentage of total carbohydrates, nitrogen, phosphorus and potassium in Roselle increased with the increase in the rates of nutrients, Rabo (2015. Amino acids, including tyrosine acid, are important in stimulating cell growth and thus equipping the plant with carbon, nitrogen and energy to perform most of the vital activities and thus increasing nitrogen, known for its effect on the vegetative growth of the plant because it is a vital element for growth and development among all nutritional compounds and plays a fundamental role in most metabolic processes (Fatihi, 2022 & Aziz et al., 2010).

The reason for the inhibitory action of the alcoholic extract is also due to the ability of the solvents of this extract to dissolve the active substances that affect the growth of bacterial isolates through Its effect on the cell wall and its ability to penetrate it or has an effect on DNA and ribosomes and the different activity of bacteria (Al-Jariri, 2002), and is consistent with what Jassim et al. (2008) found.

## VI. Conclusions:

In the field experiment, pinching and spraying tyrosine led to an increase in the leaf content of nutrients for plant growth, and Roselle oil showed an inhibitory effect against strains of bacteria Staphylococcus aureus, Escherichia coli and Bacillus sp, and through the positive relationship between the two factors, which led to improving the cultivation of Roselle plants and their tolerance to climatic conditions in Basra province.

Acknowledgments

Many thanks to the Deanship of the College of Agriculture, professors and employees, and to the Department of Horticulture and Landscape Engineering and the Medical Unit Laboratories in the College of Agriculture, University of Basra, for their assistance in completing this experiment.

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