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# Effect of foliar spraying with Proline acid and chelated iron on vegetative growth and chlorophyll and carotene pigments of cowpea cultivated in southern Iraq *(Vigna unguiculata L. Walp)*

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**Abstract**:

The study was conducted at the Agricultural Research Station, Faculty of Agriculture, Basra University, Karma Ali region, to investigate the effects of foliar spraying of different concentrations of proline (0, 100, 200) mg. L-1 and chelated iron (0, 250, 500) mg.L-1 and their interaction on vegetative growth and chlorophyll pigments and carotenoid contents of cowpea plants. The results showed that plant height, number of leaves, leaf area, number of branches, fresh weight, dry weight, and carotenoid content in leaves increased significantly after spraying with proline. Spraying with chelated iron resulted in a significant decrease in plant height, chlorophyll and carotenoid content in leaves. On the contrary, it resulted in a significant increase in the number of branches and fresh and dry weight of the plants. The interaction between the two factors studied showed significant effects on most of the traits examined, except for total chlorophyll and carotenoid traits.

***Keywords: Cowpea plant; proline acid; chelated iron; vegetative growth; photosynthesis pigments.***

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# تأثير الرش الورقي بحمض البرولين والحديد المخلّب على النمو الخضري والأصباغ الضوئية لنباتات اللوبياء *(Vigna unguiculata L. Walp)* المزروعة في جنوب العراق

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**الخلاصة**

اجريت الدراسة في محطة الابحاث الزراعية التابعة لكلية الزراعة جامعة البصرة في منطقة كرمة علي لدراسة تأثير الرش الورقي بتراكيز مختلفة من حامض البرولين (200,100,0) ملغم لتر-1 و بتراكيز مختلفة من الحديد المخلبي (500,250,0) ملغم لتر-1 والتداخل بينهما في النمو الخضري ومحتوى الاوراق من صبغتي الكلوروفيل الكلي و الكاروتين لنباتات اللوبيا . اظهرت النتائج ان طول النبات وعدد الاوراق و المساحة الورقية و عدد الافرع و الوزن الطري و الجاف للنبات و الكاروتين في الاوراق قد ازدادت معنويا عند رشها بحامض البرولين وادى الرش بالحديد المخلبي انخفاض معنوي في طول النبات ومحتوى الاوراق من صبغتي الكلوروفيل الكلي و الكاروتين في حين سبب زيادة معنوية في عدد الافرع و الوزن الطري والجاف للنبات واظهر

التداخل بين عاملي الدراسة تاثيرا معنويا في اغلب الصفات المدروسة باستثناء صفتي الكلوروفيل الكلي و الكاروتين .
***الكلمات المفتاحية: نبات اللوبيا ,حامض البرولين ,الحديد المخلبي ,النمو الخضري ,صبغات البناء الضوئي***

## Introduction

Cowpea (*Vigna unguiculata L.Walp*) is a summer vegetable crop that belongs to the legume family Fabaceae. It is eaten as tender green pods or dry seeds, which are highly nutritious. In every 100 grams of beans, there are 11 g of water, 61 g of carbohydrates, 22.5 g of protein, 1.4 g of fat, as well as some minerals such as calcium, phosphorus, iron, and vitamins C, B1, B2, and B3 (Imungi and Potter, 1983). Cowpea also fixes atmospheric nitrogen through bacterial nodules, which increases soil fertility (Motalib, 1998). The area planted with Cowpea in Iraq in 2020 was estimated to be about 29,000 dunums, with a total production of 46,200 tons and a yield of 1.593 tons per dunum (Central Statistical Organization, 2021).

One of the ways that can lead to increased production is to increase the resistance of plants to the various environmental stresses that plants are exposed to, such as high temperatures, salinity, or drought, by using some organic compounds, including proline acid, which is an amino acid that performs important biological functions. It limits the adverse effects of environmental stresses as it is an osmotic stabilizer that allows plant cells to absorb water from the growth medium, increasing their size and stabilizing membranes and proteins, improving free radicals, cell elongation, and opening stomata, and then increasing photosynthesis (Abd El-Smed eta 2010, Haer et al., 1998). Abdelhemid et al. (2013) sprayed bean plants (Phaseolus Vulgaris L.) with proline acid at a concentration of 5 millimoles under different salinity conditions (1.81,6.03, 8.97) dS. m-2. The plants sprayed with proline at a salinity of 1.81 dS. m-2 showed significant superiority in increasing The dry weight of plants and the content of chlorophyll pigments and carotenoids in both experimental seasons compared to unsprayed plants and other salinity levels. Dawood et al. (2014) noted that when faba bean plants (Vicia Faba) were sprayed with proline at (0, 25, 50) mmol concentration, 25 mmol concentration resulted in a significant increase in plant height, number of leaves, fresh weight, dry weight of plants, and content of chlorophyll b, a, and carotenoids in leaves compared to the other two concentrations. Al-Jboory (2019) observed that when cowpea plants were sprayed with proline (0, 20, 40, 60) mg·L-1, 60 mg·L-1 concentration showed significant superiority in increasing the content of chlorophyll pigments in leaves.

The spraying of plants with micronutrients also contributes to increasing the growth and yield of plants, including the essential element iron and its participation in many vital activities such as photosynthesis, chlorophyll formation, and enzymatic reactions (Al-Naemi, 2000). In addition to its role in the protein synthesis process, it contributes to the reduction of nitrates. It raises the ability of soil rehabilitation to fix biological nitrogen, in addition to the formation of RNA acid (Meugel and Kirkby, 1982). Ismail and Abou El Nour (2016) found that when cowpea plants were sprayed with different concentrations of iron (0, 150, 200) mg. L-1, both concentrations caused a significant increase in plant height, number of branches, fresh weight, and dry weight of the plant for both seasons of the experiments were compared with the control treatment. Al-Bawi (2016) found that when different concentrations of chelated iron (0, 100, 200) mg Fe L-1 were sprayed on broad bean plants, the concentration of 200 mg Fe L-1 showed significant superiority in terms of plant height, number of leaves, leaf area, vegetative body dry weight and relative chlorophyll. SPAD Fouda and Abd Elhmied (2017) observed that spraying cowpea plants with chelated iron at a concentration of

300 mg. L-1 resulted in a significant increase in plant height, number of branches and leaves, fresh weight and dry weight of the plants compared to plants that were not sprayed.

Since there are no studies on the effects of foliar spraying of proline and chelated iron on the growth of cowpea plants under the conditions of Basra Province, this study was conducted.

**Material and Methods**

The field trial was conducted on May 1, 2023 at the Karma Ali site of the Agricultural Research Station, Faculty of Agriculture, University of Basra. Planting was carried out after land preparation and soil preparation. The experiment was conducted according to a completely randomized block design (RCBD) with two factors. The experiment included two factors. The first factor was spraying three concentrations of proline: (0, 100, 200) mg. $L^{-1}$. The second factor was spraying three concentrations of chelated iron: (0, 250, 500) $mL.L^{-1}$. This resulted in nine factorial treatments, which were the interaction between three concentrations of foliar application of proline acid and three concentrations of chelated iron. The experiment was replicated three times for a total of 27 experimental units. The treatments were started three weeks after planting and repeated three times, with two weeks between each spray.

The experimental measurements included vegetative growth traits, which included plant height, number of leaves, leaf area (dm2) according to the method described by Weston and Weston (1953), number of branches per plant, fresh and dry weight of the plant, total chlorophyll (mg 100 g-1 fresh weight). Total chlorophyll pigment in green leaves was estimated according to the method described by Goodwin (1976) and carotenoids (mg 100 $g^{-1}$ fresh weight), as mentioned by Zaehrlnger et al. (1974).

The mean results were statistically analyzed using the Gestate statistical program, and the least significant differences (LSD) test was used to compare the means at a probability level of 0.05 (Al-Rawi & Khalaf Allah, 1980).

Table (1): Some chemical and physical characteristics of the study soil

| Attribute | | Value | Unit |
|---|---|---|---|
| Ph | | 7.7 | |
| ECE | | 5.22 | ds m-¹ |
| Available Phosphorus | | 38.8 | mg kg-¹ |
| Total Nitrogen | | 0.23 | g kg-¹ |
| Ready Potassium | | 101.20 | mg kg-¹ |
| solube positive ions | Calcium | 16.5 | millimoles l-¹ |
| | Magnesium | 11 | |
| | Sodium | 21.3 | |
| | Bicarbonates | 13.6 | |
| | Sulfates | 18.5 | |
| | Chlorides | 28.0 | |
| Soil separators | Sand | 593 | G kg-1 |
| | Silt | 271.5 | |
| | Clay | 135.5 | |
| Soil texture | | sandy loam | |

**Results and Discussions**

Table 2 shows that spraying cowpea plants significantly affected plant height, leaf number and leaf area. Compared with the control treatment and 200 mg. $L^{-1}$ concentration, 100 mg. $L^{-1}$ concentration led to a significant increase in plant height by (25.53, 9.95) %, respectively. Meanwhile, 200 mg. $L^{-1}$ concentration led to a significant increase in leaf number and leaf area by (27.22, 69.03) % and (34.92, 82.51) %, respectively. The concentration of 100 mg. $L^{-1}$ showed a significant superiority in these two traits compared to the control treatment by (35.27, 32.84) %, respectively. The treatments of spraying with proline acid did not significantly affect the number of branches. The significant superiority when spraying with proline acid can be attributed to its positive role in improving the processes of respiration and photosynthesis and delaying wilting and aging of the plant (Slocum and Weinstein 1990). These results are consistent with those of Dawood et al. (2014).

The same table shows that spraying plants with chelated iron significantly affected plant height and number of branches. The concentration of 250 mg. $L^{-1}$ caused a significant decrease in plant height compared to the control treatment and the concentration of 500 mg. $L^{-1}$ by (43.30, 60.49) %, respectively. This may be attributed to the role of iron in the formation of cytokinins, which are essential for cell expansion and elongation. They are necessary for the growth of lateral buds and the formation of branches, as they work to reduce the apical dominance phenomenon and thus encourage the growth of lateral branches (Hopkins and Hiiner, 2004). These results are consistent with (Ismail and Abou El Nour,2016 and Fouda and Abd Elhmied 2017). Meanwhile, chelated iron did not significantly affect the number of leaves and leaf area.

The same table shows that the interaction significantly affected all the examined traits. Plants not sprayed with proline and chelated iron at 500 mg. $L^{-1}$ had the highest plant height, reaching 211.0 cm. Plants sprayed with proline at 200 mg L-1 and chelated iron at 250 mg. $L^{-1}$ had the highest number of leaves (60.67 leaves), leaf area (60.9 $dm^2$), and number of branches (7. 0 branches). Plants sprayed with proline at 200 mg. $L^{-1}$ and chelated iron at 500 mg. $L^{-1}$ had the lowest plant height, reaching 107.7 cm. Comparative plants not sprayed with either compound had the lowest number of leaves (15.33 leaves), leaf area (17.3 $dm^2$), and number of branches (3.0 branches).

Table (2) Effect of spraying with proline acid, iron and their interaction on some vegetative growth indicators of cowpea plants

| Treatments | | plant height cm)( | Total number of leaves (leaf. $plant^{-1}$( | Leaves area ($dm^2$) | Number branches side (branch $plant^{-1}$( |
|---|---|---|---|---|---|
| Average effect of proline acid $mg.L^{-1}$ | 0 | 166.8 | 30.11 | 32.6 | 4.78 |
| | 100 | 183.4 | 40.00 | 44.1 | 4.22 |
| | 200 | 146.1 | 50.89 | 59.5 | 4.67 |
| LSD 0.05 | | 14.3 | 4.13 | 9.8 | NS |
| The average effect of Chelated iron $m.L^{-1}$ | 0 | 175.8 | 40.89 | 45.1 | 3.67 |
| | 250 | 152.7 | 40.11 | 41.5 | 5.89 |
| | 500 | 167.9 | 40.11 | 49. 6 | 4.11 |

| | | | | | | |
|---|---|---|---|---|---|---|
| LSD 0.05 | | | 14.3 | NS | NS | 1.02 |
| Interaction between proline acid And Chelated iron | 0 | 0 | 140.7 | 15.33 | 17.3 | 3.00 |
| | | 250 | 148. 7 | 19.67 | 20.5 | 6.00 |
| | | 500 | 211. 0 | 55.33 | 60.1 | 5.33 |
| | 100 | 0 | 182.7 | 48.33 | 44.0 | 4.00 |
| | | 250 | 182.7 | 40.00 | 43.2 | 4.67 |
| | | 500 | 185. 0 | 31.67 | 45.1 | 4.00 |
| | 200 | 0 | 204.00 | 59.00 | 74.0 | 6.00 |
| | | 250 | 126.7 | 60.67 | 60.9 | 7.00 |
| | | 500 | 107.7 | 33.00 | 43.5 | 3.00 |
| LSD 0.05 | | | 24.7 | 7.16 | 17.1 | 1.77 |

Table 3 shows that spraying with proline acid significantly affected the fresh and dry weight of the plant and the content of leaves in carotenoids. Both concentrations caused a significant increase of (102.78, 48.20) % in fresh weight, (47.20, 26.54) % in dry weight of the plant, and (11.84, 12.88) % in carotenoids compared to the control treatment, respectively. The increase can be attributed to the role of proline acid in improving the course of various physiological processes in the plant, especially cell division and elongation, and increasing leaf area (Table 2), which is effective in photosynthesis and the synthesis and accumulation of nutrients in the plant (Al-Khateeeb, 2002). These results are consistent with those of (Abdelhemid et al. 2013 and Dawood et al.2014).

The spraying with chelated iron also significantly affected all the traits under study. The concentration of 250 mg. $L^{-1}$ caused a significant increase in fresh and dry weight of the plant compared to the control treatment, with an increase of (47.20 21.58) %, respectively. The concentration of 500 mg L-1 did not differ significantly from it. The high concentration of chelated iron caused a significant decrease in total chlorophyll and carotenoid in the leaves compared to the control treatment, with a decrease of (11.92, 13.70) %, respectively. The concentration of 250 mg. $L^{-1}$ did not differ significantly from it. The increase in fresh and dry weight of the plant can be attributed to the role of chelated iron in increasing plant height, number of leaves, and leaf area, which positively impacted the increase in fresh and dry weight of the plant (Sauchella, 1969). These results are consistent with those of Dawood et al. (2014). However, the significant decrease in total chlorophyll and carotenoid pigments in the leaves can be attributed to the use of a high concentration of chelated iron (500 mg. $L^{-1}$), which negatively affected the formation of the pigments.

The same table shows that the interaction between the two factors of the study had a significant effect on the fresh and dry weight of the plant. The plants sprayed with proline acid at 200 mg L-1 and chelated iron at 250 mg L-1 gave the highest values of (81.32, 468.0) g, respectively. The comparison plants, not sprayed with either compound, gave the lowest values of (25.80, 111.0) g, respectively.

Table (3) Effect of spraying with proline acid, iron and their interaction on the fresh and dry weight and the concentrations of chlorophyll and carotene in the leaves

| Treatments | fresh | weight dry | Total | Carotene in |
|---|---|---|---|---|

| | | weight (g) | (g) | chlorophyll in leaves (mg 100g-1 fresh weight( | leaves (mg 100g-1 fresh weight( |
|---|---|---|---|---|---|
| Average effect of proline acid mg.L$^{-1}$ | 0 | 175.7 | 39.36 | 8.09 | 0.1444 |
| | 100 | 260.4 | 49.84 | 8.86 | 0.1630 |
| | 200 | 356.3 | 57.94 | 8.30 | 0.1615 |
| LSD 0.05 | | 37.5 | 3.31 | NS | 0.0112 |
| The average effect of Chelated iron m.L$^{-1}$ | 0 | 240.0 | 44.92 | 9.05 | 0.1686 |
| | 100 | 291.8 | 54.45 | 8.38 | 0.1517 |
| | 200 | 260.7 | 47.77 | 7.81 | 0.1485 |
| LSD 0.05 | | 37.5 | 3.31 | 0.95 | 0.0112 |
| Interaction between proline acid and Chelated iron | 0 | 0 | 111.0 | 25.80 | 9.37 | 0.1701 |
| | | 100 | 154.7 | 33.10 | 7.51 | 0.1316 |
| | | 200 | 261.3 | 39.17 | 7.38 | 0.1314 |
| | 100 | 0 | 295.0 | 56.30 | 9.21 | 0.1712 |
| | | 100 | 252.7 | 48.92 | 8.95 | 0.1585 |
| | | 200 | 233.7 | 44.29 | 8.40 | 0.1592 |
| | 200 | 0 | 314.0 | 52.67 | 8.57 | 0.1646 |
| | | 100 | 468.0 | 81.32 | 8.69 | 0.1649 |
| | | 200 | 287.0 | 39.83 | 7.63 | 0.1550 |
| LSD 0.05 | | | 65.0 | 5.73 | NS | NS |

## Conclusion

Can conclude from this study that in order to give good vegetative growth to cowpea plants grown in Basra Governorate, they must be sprayed with proline acid at a concentration of 200 mg. L$^{-1}$ and with chelated iron at a concentration of 250 mg. L$^{-1}$.

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