



Influence of Salicylic Acid and Gibberellin Spraying on Some Morphological and Physiological Traits of Fenugreek Plant (*Trigonella foenum-gracenum* L.)

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Received: 11 February 2024

Accepted: 7 April 2024

DOI: <https://dx.doi.org/10.24237/ASJ.02.03.858A>

Abstract

Field experiment had conducted in the winter of 2022-2023 at a field in Baqubah, Diyala Governorate to investigate the influence of salicylic acid and GA₃ spraying on some morphological and physiological traits of the fenugreek plant. A factorial experiment was conducted using a randomized complete block design (RCBD) with three replications. The first treatment involved spraying with three different concentrations of salicylic acid (0, 10 and 20 mg L⁻¹), whereas the second treatment involved spraying with three different concentrations of GA₃ (0, 50 and 100 mg L⁻¹), so the number of experimental units area reached 27 experimental units. The data underwent statistical analysis using the Genstat program, and the least significant difference (LSD) test was utilized to compare concentrations at a significance level of 0.05. Results indicated that spraying the fenugreek plants with a concentration of 20 mg L⁻¹ of salicylic acid significantly increased height of plant and potassium content in leaves, reaching 53.20 cm and 17.99 mg g⁻¹ respectively. While, spraying with salicylic acid at a concentration of 10 mg L⁻¹ led to a significant increase in the number of branches and leaves per plant, phosphorus content in leaves, chlorophyll index, and plant dry weight which reached 13.82 branches per plant, 58.1 leaves per plant, 8.65 mg g⁻¹, 45.11 Spad and 26.71 g plant⁻¹ respectively. Spraying with GA₃ at a concentration of 100 mg L⁻¹ resulted in a notable increase in the plant height, and potassium content in leaves, reaching 55.12 cm and 17.53 mg g⁻¹ respectively. On the other hand, spraying with GA₃ at a concentration of 50 mg L⁻¹ led to a



significant increase in the number of branches and leaves per plant, phosphorus content in leaves, chlorophyll index and plant dry weight which reached 14.75 branches per plant, 61.20 leaves per plant, 7.92 mg g⁻¹, 47.45 Spad, 26.10 g per plant respectively. The interaction between salicylic acid and GA3 significantly affected all the features tested.

Keywords: Fenugreek, stimulate growth, antioxidants, plant hormones, salicylic acid, Gibberellin.

تأثير الرش بحامض السالسليك والجبرلين في بعض الصفات المظهرية والفسلجية لنبات الحلبة (*Trigonell afoenum-graceum L.*)

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الخلاصة

أجريت تجربة حقلية خلال الموسم الشتوي 2022-2023 في أحد حقول مدينة بعقوبة / محافظة ديالى لدراسة تأثير الرش بحامض السالسليك والجبرلين في بعض الصفات المظهرية والفسيولوجية لنبات الحلبة. نفذت التجربة عاملية وفق تصميم القطاعات الكاملة المعشاة بثلاثة مكررات. تضمن العامل الأول رش ثلاث تراكيز من حامض السالسليك (0، 10، 20 ملغم لتر⁻¹)، بينما تضمن العامل الثاني رش ثلاث تراكيز من الجبرلين (0، 50، 100 ملغم لتر⁻¹). بلغ عدد الوحدات التجريبية 27 وحدة تجريبية. خضعت البيانات للتحليل الإحصائي باستعمال برنامج Genstat، وتم استخدام اختبار أقل فرق معنوي (LSD) لمقارنة التراكيز عند مستوى معنوية 0.05. أظهرت النتائج أن رش نباتات الحلبة بتركيز 20 ملغم لتر⁻¹ من حامض السالسليك أدى إلى زيادة معنوية في ارتفاع النبات ومحتوى الأوراق من البوتاسيوم التي بلغت 55.20 سم و17.99 ملغم غم⁻¹ على التوالي. بينما أدى رش حامض السالسليك بتركيز 10 ملغم لتر⁻¹ إلى زيادة معنوية في عدد الفروع والأوراق لكل نبات، محتوى الأوراق من الفوسفور، مؤشر الكلوروفيل والوزن الجاف للنبات التي بلغت 13.82 فرع نبات⁻¹ و58.10 ورقة نبات⁻¹ و8.56 ملغم غم⁻¹ و45.11 سباد و26.71 غم نبات⁻¹ على التوالي. كذلك أدى الرش بالجبرلين بتركيز 100 ملغم لتر⁻¹ إلى زيادة معنوية في ارتفاع النبات ومحتوى الأوراق من البوتاسيوم التي بلغت 55.12 سم و17.53 ملغم غم⁻¹ على التوالي، في حين أدى الرش بتركيز 50 ملغم لتر⁻¹ من الجبرلين إلى زيادة معنوية في عدد الفروع للنبات وعدد الأوراق للنبات ومحتوى الأوراق من الفوسفور ودليل الكلوروفيل والوزن الجاف للنبات التي بلغت 14.75 فرع نبات⁻¹ و61.20 ورقة نبات⁻¹ و7.92 ملغم غم⁻¹ و47.45 سباد و26.10 غم نبات⁻¹ على التوالي. كان للتداخل بين حامض السالسليك والجبرلين تأثير معنوي في جميع الصفات المدروسة.

الكلمات المفتاحية: الحلبة، تحفيز النمو، مضادات الأكسدة، الهرمونات النباتية، حامض السالسليك، الجبرلين.



Introduction

Fenugreek (*Trigonell afoenum-graceum* L.) is a plant belongs to the leguminous family. It is an annual herbaceous plant and is classified as a medicinal crop due to its seeds containing alkaloid compounds such as choline, trigonelline and diosgenin [1, 11]; the substance contains mucilaginous protein, mineral components like phosphate and iron, and fixed oils, and certain antibiotics. The productivity of this crop in Iraq could be much higher due to various factors, such as the emphasis on strategic winter crops and the lack of specialists' attention. To improve its cultivation, it is essential to introduce advancements in agricultural technology that can enhance the plant's genetic and physiological capabilities [7].

Enhancing the crop's capacity to utilize environmental factors can be achieved by applying salicylic acid. Salicylic acid contributes to various physiological activities in plants, such as promoting cell division and differentiation, hastening the production of photosynthetic pigments, and slowing down leaf ageing. This positively impacts photosynthesis efficiency. The crop's genetic energy efficiency can be enhanced by applying gibberellic acid, an essential plant growth regulator that promotes cell division and elongation, activates enzymes to convert starch into sugar, regulates starch accumulation, and delays leaf senescence [8]. The study aimed to investigate the influence of salicylic acid and GA₃ spraying on some morphological and physiological traits of the fenugreek plant.

Materials and Methods

Field experiment had conducted during the winter of 2022-2023 in a field in Baqubah City-Diyala Governorate in a silty loam soil, shown its physical and chemical properties in Table (1), to investigate the influence of salicylic acid and GA₃ spraying on some morphological and physiological traits of the fenugreek plant.

A factorial experiment had conducted using a randomized complete block design (RCBD) with three replications. Two factors were considered: the application of three concentrations of salicylic acid (0, 10 and 20 mg/ L⁻¹) and the application of three concentrations of GA₃ (0, 50 and 100 mg L⁻¹). Both salicylic acid and GA₃ were sprayed at 6 leaf stages. Soil service



activities were carried out, followed by dividing the experimental area into 27 experimental units. Each unit consists of 4 rows, spaced 25 cm apart with 25 cm between hill. Three fenugreek seeds were planted on each hill on 10 November 2022, and after that reduced to one seedling per hill after sprouting. Crop management was conducted as necessary, and the plants were harvested at reaching maturity indicators.

Plant height, branch number, leaves number, leaves content of P and K, chlorophyll index and plant dry weight were recorded. The data underwent statistical analysis, and the least significant difference (LSD) test was utilized to compare concentrations at a significance level of 0.05 [14].

Table 1: Some chemical and physical properties of soil

Trait	Value	Unit
Sand	95	g Kg ⁻¹ Soil
Loam	747	
Clay	158	
Ec 1:1	1.6	ds m ⁻¹
pH	7.5	-----
O.M	5.2	g Kg ⁻¹ Soil
Total CaCO ₃	223.0	g Kg ⁻¹ Soil
Ca	7.5	Meq L ⁻¹
K	0.4	
Mg	6.2	
Na	2.1	
Cl	5.7	
HCO ₃	8.1	
CO ₃	Nil	
SO ₄	2.5	
Available N	40.0	g Kg ⁻¹ Soil
Available P	12.6	
Available K	113.9	

Results and Discussion

1. Plant height (cm)

Table (2) results indicate a significant difference in the fenugreek plant height among salicylic acid concentrations (0, 10 and 20 mg L⁻¹). The plant height was highest at 20 mg L⁻¹ salicylic acid (53.20 cm), showing a 15.02% increase compared to the control treatment, which had the lowest plant height (46.25 cm). The increase may be attributed to the synergistic interaction between salicylic acid and plant hormones that regulate cell division and elongation. [9, 12].



The results of the same table indicate that there was a significant difference between concentrations of GA₃ (0, 50 and 100 mg L⁻¹) in this trait, as the height of fenugreek plant which sprayed with GA₃ at a concentration of 100 mg L⁻¹ was significantly increased at a 19.85% compared with the plant which sprayed with distilled water (control treatment) which recorded the lowest mean of plant height (45.99 cm). The plant height grows when spraying GA₃ at high concentrations because gibberellic acid stimulates cell division and elongation by enlarging the meristematic zone and increasing the number of dividing cells [15]. Also, the reason of increasing could be due to the mutual effect of GA₃ with auxin by increasing the concentration of internal auxin as a result of its effect either on the auxin synthesis, as GA₃ stimulates the converting of tryptophan into auxin, or its effect on the preventing auxin oxidation as a result of its effect on the auxin-oxidizing enzyme (IAA-oxidase) [3, 4]. The interaction effect of the factors studied significantly impacted the trait in Table (2). The highest plant height of 59.65 cm was observed when salicylic acid was sprayed at 20 mg L⁻¹ and GA₃ at 100 mg L⁻¹, which was not significantly different from the plant height when salicylic acid was sprayed at 10 mg L⁻¹ and GA₃ at 50 mg L⁻¹. In contrast, spraying of salicylic acid at 0 mg L⁻¹ and GA₃ at 50 mg L⁻¹ resulted in the lowest plant height (41.94 cm) which was not significantly different from the plant height when spraying of distilled water.

Table 2: Influence of spraying with salicylic acid and GA₃ and their interaction on the plant height (cm)

Salicylic acid concentrations (mg L ⁻¹)	GA ₃ concentrations (mg L ⁻¹)			Mean
	0	50	100	
0	44.55	41.94	52.25	46.25
10	48.51	57.59	53.45	53.18
20	44.92	55.04	59.65	53.20
Mean	45.99	51.52	55.12	
Lsd 0.05	Salicylic acid concentrations = 2.01			
	GA ₃ concentrations = 2.01			
	Interaction = 3.49			

2. Number of branches per plant

The data from Table (3) shows that applying salicylic acid at a concentration of 10 mg L⁻¹ resulted in a significant increase in the number of branches, with 13.82 branches per plant,



representing a 23.95% increase compared to the control treatment, which had 11.15 branches per plant. The increase may be due to the synergistic interaction between salicylic acid and plant hormones that regulate cell division, elongation, and expansion. The data from the Table shows that applying GA₃ at a concentration of 50 mg L⁻¹ resulted in a considerably higher branch number of 32.29% compared to the control treatment, which had the lowest mean of 11.15 branches per plant. The increase in the number of branches may be attributed to applying gibberellic acid at a suitable concentration, which helped regulate the plant's hormone levels related to cell division, elongation, and expansion [2]. This resulted in the stimulation of dormant lateral bud growth, leading to a higher number of branches per plant. The interaction between the factors studied significantly influenced the number of branches. The highest value of 15.90 branches per plant was achieved by spraying with salicylic acid at 10 mg L⁻¹ and GA₃ at 50 mg L⁻¹, which was not significantly different from spraying with salicylic acid at 20 mg L⁻¹ and GA₃ at 50 mg L⁻¹, spraying with salicylic acid at 10 mg L⁻¹ only and spraying with GA₃ at 50 mg L⁻¹ only. However, spraying with distilled water resulted in the lowest value of 8.09 branches per plant.

Table 3: Influence of spraying with salicylic acid and GA₃ and their interaction on the number of branches per plant

Salicylic acid concentrations (mg L ⁻¹)	GA ₃ concentrations (mg L ⁻¹)			Mean
	0	50	100	
0	8.09	14.37	10.99	11.15
10	13.80	15.90	11.75	13.82
20	11.55	13.98	11.86	12.46
Mean	11.15	14.75	11.53	
Lsd 0.05	Salicylic acid concentrations = 1.67			
	GA ₃ concentrations = 1.67			
	Interaction = 2.89			

3. Number of Leaves per plant

Table (4) data shows that spraying with salicylic acid at a concentration of 10 mg L⁻¹ considerably increased the number of leaves to 58.10 leaf plant⁻¹, a 26.58% increase compared to the control treatment, which was 45.9 leaf plant⁻¹. The decrease in the number of leaves per



plant sprayed with distilled water (control treatment) may be attributed to a reduction in the number of branches per plant (Table 3). The results indicate that increasing the concentration of salicylic acid up to 20 mg L⁻¹ decreased the number of leaves per plant, possibly due to a reduction in the number of branches per plant. Conversely, the increase in leaf number may be attributed to the beneficial impact of salicylic acid on enhancing cell division, photosynthetic efficiency, and metabolic product transfer to new sites (primordial). This positive effect stimulated plant growth and enhanced vegetative growth [8]. The table shows that spraying with GA₃ at a concentration of 50 mg L⁻¹ resulted in a considerably higher leaf number of 32.18% compared to the control treatment, which had the lowest mean of 46.3 leaves per plant. Increasing the number of leaves per plant after spraying with GA₃ at a concentration of 50 mg L⁻¹ may be attributed to the stimulatory effect of gibberellic acid on cell division. This is achieved by enlarging the meristematic zone and increasing the number of dividing cells. Increasing the number of branches by spraying with gibberellic acid at a concentration of 50 mg L⁻¹ (Table 3) may increase the number of leaves per plant. The interaction effect of the studied factors significantly influenced the trait. Spraying with salicylic acid at 10 mg L⁻¹ and GA₃ at 50 mg L⁻¹ resulted in the highest number of leaves per plant (65.3 leaf plant⁻¹), which was not significantly different from spraying with salicylic acid at 20 mg L⁻¹ and GA₃ at 50 mg L⁻¹, spraying with salicylic acid at 10 mg L⁻¹ alone and spraying with GA₃ at 50 mg L⁻¹ alone. In contrast, spraying with distilled water alone led to the lowest number of leaves per plant (32.1 leaf plant⁻¹).

Table 4: Influence of spraying with salicylic acid and GA₃ and their interaction on the number of leaves per plant

Salicylic acid concentrations (mg L ⁻¹)	GA ₃ concentrations (mg L ⁻¹)			Mean
	0	50	100	
0	32.1	61.8	43.9	45.9
10	60.4	65.3	48.7	58.1
20	46.5	56.5	49.3	50.8
Mean	46.3	61.2	47.3	
Lsd 0.05	Salicylic acid concentrations = 6.9			
	GA ₃ concentrations = 6.9			
	Interaction = 11.9			



4. Leaves content of phosphorus (mg g^{-1})

The results from (Table 5) show that spraying with salicylic acid at a concentration of 10 mg L^{-1} resulted in significantly higher phosphorus content in leaves, reaching 8.19 mg g^{-1} , representing a 41.70% increase compared to the control treatment, which was the lowest phosphorus content of 5.78 mg g^{-1} . The rise may be attributed to salicylic acid's ability to stimulate plant organ growth, notably roots, by promoting cell division and enhancing nutrient absorption [10]. Table 5 indicates that spraying with GA_3 at a concentration of 50 mg L^{-1} resulted in a considerably higher phosphorus content in leaves (25.91%) compared to the control treatment, which had the lowest mean phosphorus content (6.29 mg g^{-1}). The elevated phosphorus level in leaves may be due to gibberellic acid's promotion of plant development by stimulating cell division, leading to improved absorption of essential nutrients such as phosphorus [5, 16]. The interaction between the factors studied significantly influenced the trait. Spraying with salicylic acid at 20 mg L^{-1} and GA_3 at 100 mg L^{-1} resulted in the highest phosphorus content in leaves (8.83 mg g^{-1}). This value was not significantly different from spraying with salicylic acid at 10 mg L^{-1} and GA_3 at 50 mg L^{-1} or from spraying with salicylic acid at 10 mg L^{-1} and GA_3 at 100 mg L^{-1} . Spraying with distilled water resulted in the lowest value (5.11 mg g^{-1}).

Table 5: Influence of spraying with salicylic acid and GA_3 and their interaction on the leaves content of phosphorus (mg g^{-1})

Salicylic acid concentrations (mg L^{-1})	GA_3 concentrations (mg L^{-1})			Mean
	0	50	100	
0	5.11	6.44	5.78	5.78
10	7.44	8.47	8.65	8.19
20	6.31	8.83	7.00	7.38
Mean	6.29	7.92	7.15	
Lsd 0.05	Salicylic acid concentrations = 0.56			
	GA_3 concentrations = 0.56			
	Interaction = 0.97			

5. Leaves content of potassium (mg g^{-1})

Table 6 demonstrates that applying salicylic acid at a concentration of 20 mg L^{-1} resulted in a considerable improvement, producing the highest potassium content in leaves at 17.99 mg g^{-1} ,



representing a 37.75% increase compared to the control treatment, which had the lowest potassium content in leaves at 13.06 mg g⁻¹. The results from Table 6 show that spraying with GA₃ at a concentration of 100 mg L⁻¹ resulted in a significantly higher potassium content in leaves (26.30%) compared to the control treatment, which had the lowest mean of potassium content (13.88 mg g⁻¹). The study found that higher concentrations of salicylic acid and gibberellic acid resulted in increased potassium accumulation in the fenugreek leaves. This could be attributed to plants sprayed with high levels of these growth regulators facing environmental stresses, prompting increased potassium absorption to mitigate the adverse effects of these stresses [2, 8, 13]. The interaction of the studied factors significantly impacted the trait. Spraying with salicylic acid at 20 mg L⁻¹ and GA₃ at 100 mg L⁻¹ resulted in the highest potassium content in leaves (19.97 mg g⁻¹), while spraying with distilled water led to the lowest value (11.22 mg g⁻¹).

Table 6: Influence of spraying with salicylic acid and GA₃ and their interaction on the leaves content of potassium (mg g⁻¹)

Salicylic acid concentrations (mg L ⁻¹)	GA ₃ concentrations (mg L ⁻¹)			Mean
	0	50	100	
0	11.22	13.23	14.72	13.06
10	14.04	18.31	17.89	16.74
20	16.37	17.62	19.97	17.99
Mean	13.88	16.39	17.53	
Lsd 0.05	Salicylic acid concentrations = 0.91			
	GA ₃ concentrations = 0.91			
	Interaction = 1.59			

6. Chlorophyll index (Spad)

The results from Table 7 show that spraying with salicylic acid at a concentration of 10 mg L⁻¹ significantly outperformed other treatments, resulting in the highest chlorophyll index of 45.11 Spad, representing a 19.91% increase compared to the control treatment, which was the lowest chlorophyll index of 37.62 Spad. The increase observed when spraying with salicylic acid at a concentration of 10 mg L⁻¹ may be due to its ability to regulate physiological processes related to plant growth and development, enhance the synthesis of photosynthetic pigments, protect



plastids, and delay leaf senescence as an antioxidant [6]. Table 7 demonstrates that applying GA₃ at a concentration of 50 mg L⁻¹ resulted in a substantially higher chlorophyll index of 34.99% compared to the control treatment, which was the lowest mean of 35.15 Spad. The chlorophyll index increases after spraying with gibberellic acid at a concentration of 50 mg L⁻¹ due to its involvement in promoting growth and enhancing nutrient absorption, which includes ingredients necessary for chlorophyll production [3]. The interaction effect of the factors studied significantly influenced the trait. The highest chlorophyll index value of 50.83 Spad was achieved by spraying with salicylic acid at 20 mg L⁻¹ and GA₃ at 50 mg L⁻¹. This value was not significantly different from spraying with salicylic acid at 10 mg L⁻¹ and GA₃ at 50 mg L⁻¹ or from spraying with salicylic acid at 10 mg L⁻¹ and GA₃ at 100 mg L⁻¹. In contrast, spraying with distilled water resulted in the lowest value of 32.07 Spad.

Table 7: Influence of spraying with salicylic acid and GA₃ and their interaction on the leaves content of chlorophyll (mg g⁻¹)

Salicylic acid concentrations (mg L ⁻¹)	GA ₃ concentrations (mg L ⁻¹)			Mean
	0	50	100	
0	32.07	44.20	36.58	37.62
10	38.80	47.32	49.20	45.11
20	34.58	50.83	41.21	42.21
Mean	35.15	47.45	42.33	
Lsd 0.05	Salicylic acid concentrations = 2.74			
	GA ₃ concentrations = 2.74			
	Interaction = 4.75			

7. Plant dry weight (g plant⁻¹)

Table 8 shows that spraying with salicylic acid at a concentration of 10 mg L⁻¹ resulted in significantly higher plant dry weight (26.71 g plant⁻¹), representing a 37.40% increase compared to spraying with distilled water (control treatment), which resulted in the lowest plant dry weight (19.44 g plant⁻¹). The increase observed after spraying with salicylic acid at a dosage of 10 mg L⁻¹ may be due to its effectiveness in promoting more branch and leaf growth per plant, as well as increasing phosphorus content in the leaves and chlorophyll index (Tables 3, 4, 5, 7). The data from Table 8 show that spraying with GA₃ at a concentration of 50 mg L⁻¹



resulted in a substantial increase of 30.83% in plant dry weight compared to spraying with distilled water (control treatment), which was the lowest mean of 19.95 g plant⁻¹. The rise in plant dry weight may be attributed to the higher concentration of branches and leaves per plant, phosphorus content in leaves and chlorophyll index (Tables 3, 4, 5, 7). The interaction effect of salicylic acid and GA₃ on plant dry weight was significant. The highest plant dry weight of 28.45 g plant⁻¹ was observed when salicylic acid was sprayed at 10 mg L⁻¹ and GA₃ at 100 mg L⁻¹. This value was not significantly different when salicylic acid was sprayed at 10 mg L⁻¹ and GA₃ at 50 mg L⁻¹ or when salicylic acid was sprayed at 20 mg L⁻¹ and GA₃ at 50 mg L⁻¹. The lowest dry weight of 16.18 g plant⁻¹ was recorded when sprayed with distilled water (16.18 g plant⁻¹).

Table 8: Influence of spraying with salicylic acid and GA₃ and their interaction on the plant dry weight (g plant⁻¹)

Salicylic acid concentrations (mg L ⁻¹)	GA ₃ concentrations (mg L ⁻¹)			Mean
	0	50	100	
0	16.18	22.33	19.80	19.44
10	24.11	27.56	28.45	26.71
20	19.55	28.41	23.54	23.83
Mean	19.95	26.10	23.93	
Lsd 0.05	Salicylic acid concentrations = 1.46			
	GA ₃ concentrations = 1.46			
	Interaction = 2.54			

Conclusion

Applying salicylic acid at 10 mg L⁻¹ and GA₃ at a concentration of 50 mg L⁻¹ improved the morphological and physiological characteristics of fenugreek plants, as indicated by the results. Increasing the amounts of salicylic acid and gibberellic acid had a detrimental effect on most of the tested features.



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