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Effect of Salicylic Acid and Arginine Spraying on Growth and Some of Its Active Compounds of Basil *Ocimum Basilicum* L

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Abstract. The study was conducted in the fields of a farmer in Baghdad Province/ Abu Ghraib district during the 2019-2020 agricultural season to study the effect of two factors. The first factor was spraying salicylic acid on the plant with four concentrations (0,100,200,300) mg. L⁻¹. The second factor was spraying amino acid Arginine on the plant with four concentrations (0,100,200,300) mg. L⁻¹. Each level was sprayed before sunset until the plant leaves become completely wet. The spraying was done twice during the growing season. Results showed that the treatments of foliar spray with Salicylic Acid, the treatment with Arginine spray, and the interaction treatments were superior in terms of improving plant height, leaf area, and ratios of volatile oils ratio, phenols, nitrogen, and proteins.

1. Introduction:

Basil is an annual plant usually producing white-purple flowers (1). It is called Rehan in Arabic (*Ocimum basilicum* L.) and it belongs to the Lamiaceae family. It is a common herb that is known for its ornamental and therapeutic characteristics (2). It can be found in the wild tropical, subtropical, and temperate regions around the world. It is a member of the Labiatae family (Lamiaceae) and contains a wide variety of constituents of medicinal importance. *Ocimum basilicum* L. is grown in many households with a broad range of therapeutic properties. It would be a blessing in disguise if this herb became medicine for ordinary people. Various plant parts such as leaves, seeds, and roots are recommended for people as folk medicines. *Ocimum basilicum* L. is believed to have medicinal uses such as antioxidant, antibacterial, antimicrobial, antifungal, antiviral, cytoprotective, anticonvulsant, hypoglycaemic, hypolipidemic, hepatoprotective, renoprotective, neuroprotective, spermicidal, dermatologic, and insecticidal. (3). Salicylic acid (2-hydroxybenzoic acid), as a natural plant hormone, has many effects on the physiological processes and growth of plants (4). Furthermore, salicylic acid has an important role in the tolerance of some environmental conditions such as heat, salts, and drought stress (5).

Growth regulators play a vital role in the physiological processes that are pertinent to the outcome of the plant. It can be considered as an agricultural tool that makes the plant use nutrients efficiently, so it exploits its inherent physiological and genetic capabilities to the highest level. Hence, they are growth modulators and not nutrition, and they are organic compounds made naturally inside the plant or artificially in the laboratory. They cause a change in the growth and development of the plant when it is added in some stages of its growth, which are either growth stimuli or inhibitors (6 and 7). The use of growth regulators



is one of the modern techniques in resisting various environmental stresses. Salicylic acid is considered a non-enzymatic growth regulator that plays an important role in increasing plant resistance to various conditions and activating some important antioxidant enzymes (8). In addition to its role in regulating many physiological processes in plants, including the opening and closing of stomata. The process of metabolism, absorption of ions, and the elimination of free radicals and their negative effects (9 and 10). Due to the exacerbation of the phenomena of contamination of food, soil, and water with the remnants of fertilizers, attention has increased on the quality of the product and food safety. The distinction of plant products free from the residual effects of fertilizers has spread recently. More importantly, the use of amino acids as leaf nutrients for plants has increased as they play a tonic role for plants. They contain nitrogen, which is the main component of these acids, and it is ready to be absorbed by the plant directly. Arginine is one of these important amino acids used in nourishing plants' leaves. It plays a role in stimulating the physiological and biochemical processes. It participates in building proteins and manufacturing carbohydrates through the nitrogen released and it is used in building chlorophyll and stimulating the process of carbonate assimilation. Released nitrogen also encourages the work of enzymes and co-enzymes in the vital processes. Moreover, it gives abundant vegetative growth, which is positively reflected in the yield per unit area (11 and 12).

This study was executed to find out the effects of salicylic acid and arginine on some active ingredients of this plant. It was conducted due to the importance of the aforementioned and the lack of agricultural and pharmaceutical studies on basil plants as well as the effects of salicylic and arginine on vegetative growth and the quantitative and qualitative outcomes on basil plants.

2. Materials and Methods:

The experiment was conducted on a private farm in Baghdad province / Abu Ghraib district during the growing season of 2019-2020. The study consisted of two factors. The first factor was spraying salicylic acid on the plant in four concentrations (0,100,200,300) mg. L⁻¹. Each level was sprayed until plant leaves are completely wet. Spraying was done before sunset by using a 16-liter back sprinkler with two sprays during the growing season as follows:

The first spray: 30 days after planting, i.e. in the vegetative growth stage.

The second spray: 11 days after the first spray. A detergent (cleaning liquid) was added to the spray solution at an amount of 15 cm³ per 100 liters to reduce water surface tension and to ensure complete wetness of the leaves. This will in turn increase the efficiency of the spray solution. The control unit with normal treatment was sprayed with water only. The second factor was the amino acid arginine, which was sprayed in four concentrations (0,100,200,300) mg. L⁻¹. Each level was sprayed until complete wetness is achieved. The spraying was also done before sunset with two sprays during the growing season as follows:

The first spray: Six days after the first spray with salicylic acid

The second spray: three days after the second spray with salicylic acid

1.2 Soil analysis

The field soil was analyzed before planting by taking random samples from different locations of the field at 35 cm of depth. The samples were dried, graded, and screened through a sieve with 2 mm openings diameter. Later, the samples were mixed, and a representative sample was taken to be chemically and physically analyzed in various ways. Tests were performed in the graduate lab, department of soil, college of agricultural engineering, university of Baghdad (Table (1)).

Table 1: Some chemical and physical characteristics of the experiment field soil before planting in 2020

Characteristics	Measuring Unit	result
EC Soil	Ds.m-1	2.2
PH Soil	-----	8.8
Sand	g. kg -1	6.30
Mud	g. kg -1	54.5
Silt	g. kg -1	39.2
Soil texture		Clay Soil
Ready Nitrogen N	Mg. Kg-1	56.3
Ready Potassium K	Mg. Kg-1	281.0
Ready phosphorous P	Mg. Kg-1	12.45

The experiment was designed using(RCBD) and the average measurements were compared according to the L.S.D test (Least Significant Difference) at a probability level of 0.05 (13).

Investigated variables

1- Leaf area (dcm 2 . Plant -1)

The leaf area of the plant was calculated using the gravimetric method by taking 30 tablets for five plants with a diameter of 14 mm. The leaves and tablets were dried and weighed. Then, the total leaf area of the plants was estimated as stated in (14).

2. *Plant height(cm)* :The height of the plant was measured from the base at the ground level to the end of the main stem of the selected plants, and then the average height was measured.

3. *Estimation of nitrogen and protein ratios*:The nitrogen ratio was measured with (15) Semi-micro Kjeldahl method by taking 0.2 g of the sample and placing it in the digestion tube with the addition of 1 g of the cofactor CuSo4. Then, 5 ml of concentrated sulfuric acid (98%) was added, and the nitrogen percentage was measured by applying the following equation:

$$N\% = \text{volume of HCL X titrated acid (0.01) X 0.014 X 100 / sample weight (g)}$$

On the other hand, the protein percentage was measured by the Caldal method, which is the process of multiplying the ratio of nitrogen of one experimental unit by 6.25, because the protein contains 16% nitrogen (15).

4. Estimation of total phenol and volatile oil Volumes:

1 ml of alcoholic extract of the sample whose phenols were evaluated was taken and placed in a test tube. Standard hydrochloric acid (HCL 0.05) with 1 ml of volume was added to 1 ml of Arno's reagent, and 10 ml of distilled water was added along with 2 ml of NaOH 1 standard; a pink color appears as a result. The color absorption is measured with a Spectrophotometer at the wavelength of 515 nm. The phenols are calculated from a standard curve prepared by Catechol C6H4 (OH) (16)Volatile Oils were extracted according to what was stated in (15) using Clevenger device to extract them. 2 grams of the fresh sample were placed in the designated place for the sample, distilled water was added, and the temperature was gradually raised to the boiling point. The oils were then collected in the collection part of the device. After collecting all oils from the model within 4 hours, the oil amount was weighed and the volume ratio was measured.

3. Results and Discussion:

The results shown in Table 2 indicated that there were significant differences between the treatments for foliar spraying with Salicylic Acid. Treatment S3 outperformed other treatment types in giving the largest leaf area of 11.864 (Fat 2 Vegetable -1). On the other hand, treatment S0 recorded the lowest leaf area for the plant (9,595 dc2 Plant-1).

The results in the same table also showed that there were significant differences between the treatments of foliar spray with Arginine as treatment G3 outperformed other treatment types in giving the largest leaf area for the plant (13,186 dc2 2 plant -1). Treatment G0, on the other hand, recorded the lowest leaf area for the plant (8,648 dc2 2 plant -1). As for the interaction

coefficients between the two study factors, the results indicated that treatment S3G3 was superior by registering the highest leaf area of (15.943 dc2 plant-1) compared with the lowest value recorded in treatment S0G0 (8.427 dc2 plant-1).

Table (2) Effect of Salicylic Acid and Arginine Spray on Foliar Area (dc2 Plant-1)

S Factor Levels	G Factor Levels				S Average
	G0	G1	G2	G3	
S0	8.427	9.117	9.633	11.203	9.595
S1	8.453	9.333	9.740	11.663	9.798
S2	8.757	9.937	11.233	13.933	10.965
S3	8.957	10.367	12.190	15.943	11.864
G Average	8.648	9.688	10.699	13.186	
	G		S		GxS
L.S.D 0.05	0.335		0.335		0.669

The results of plant height displayed in Table (3) demonstrate the presence of significant differences for the treatment of spraying with Salicylic Acid (S). Treatment S3 outperformed other treatments by giving the highest value (63.918 cm), but it did not differ significantly from treatment S2 (62.488 cm). On the other hand, treatment S0 gave the lowest value (57.744 cm). As for the treatment of spraying with Arginine (G), the results (Table 3) showed that the treatment G3 was superior to other treatments registering the highest plant height (67.388 cm). The lowest value was given by treatment G0 (53.036 cm). The results illustrated in the same table showed that there were significant differences between treatments. Specifically, treatment S3G3 was superior and gave the highest value (70.117 cm). Conversely, the lowest value was given by treatment S0G0 (46.843 cm).

The results shown in Table (4) also revealed a significant increase in the percentage of nitrogen in the leaves due to an increase in the levels of spraying with Salicylic Acid. Treatment S3 recorded the highest percentage of nitrogen in the leaves (2.336%), while treatment S0 recorded the lowest percentage (2.296%). The results presented in the same table also showed a significant increase in the percentage of nitrogen in the leaves of the basil plant when increasing the concentrations of spraying with Arginine. While treatment G0 recorded the lowest percentage of nitrogen in the leaves (2.240%), treatment G3 recorded the highest percentage (2.373%).

Table (3) Effect of Arginine and Salicylic Acid Spray on Nitrogen percentage

S Factor Levels	G Factor Levels				S Average
	G0	G1	G2	G3	
S0	2.230	2.253	2.340	2.360	2.296
S1	2.237	2.260	2.353	2.367	2.304
S2	2.240	2.303	2.370	2.387	2.323
S3	2.253	2.327	2.377	2.387	2.336
G Average	2.240	2.286	2.360	2.373	
	G		S		GxS
L.S.D 0.05	0.011		0.011		0.022

The results of statistical analysis shown in table (5) indicated that there were significant differences among salicylic acid spray treatments. Treatment S3 outperformed other treatments giving the highest protein ratio (14.599%), while the lowest protein percentage was obtained when using treatment S0 with 14.349 %. The results presented in the same table showed that the treatments of Arginine amino acid spray had a significant effect on increasing the percentage of protein in the leaves. Treatment G3 recorded the highest protein content (14.833%), while treatment G0 recorded the lowest percentage of protein in the leaves (14.000%).

As for the interaction between the two study factors, the results shown in the table below demonstrate significant differences among treatments. Treatment S3G3 outperformed other treatments giving the highest protein ratio (14.917%), while the comparison treatment S0G0 recorded the lowest value (13.938%).

The results of the interaction between the two study factors shown in the table below also revealed the superiority of treatment S3G3 as it gave the highest nitrogen content in the leaves (2.387%), while the lowest value was recorded in treatment S0G0 (2.230%).

Table (4) Effect of spraying with Salicylic Acid and Arginine on Protein percentage

S Factor Levels	G Factor Levels				S Average
	G0	G1	G2	G3	
S0	13.938	14.083	14.625	14.750	14.349
S1	13.979	14.125	14.708	14.792	14.401
S2	14.000	14.396	14.812	14.857	14.521
S3	14.083	14.542	14.854	14.917	14.599
G Average	14.000	14.286	14.750	14.833	
	G		S		GxS
L.S.D 0.05	0.068		0.068		0.135

The spraying treatments with Salicylic Acid, as shown in Table (6), led to significant differences between treatment levels. Treatment S3 was significantly superior to all treatments with the highest percentage of phenols in the leaves of the basil plant (0.389%), while the lowest percentage of phenols was recorded in treatment S0 (0.350%). Similarly, treatment with amino acid arginine led to the emergence of significant differences among spraying levels. Treatment G3 outperformed all other spraying treatments with Arginine with (0.404%), while treatment G0 gave the lowest percentage of phenols in the leaves with (0.333%). The results of the interaction between the two study factors showed the superiority of both S3G3 and S2G3 treatments giving the highest percentage of phenols in the leaves (0.410%) for both treatments, while the lowest percentage of phenols was recorded in treatment S0G0 (0.312%).

Table (5) the effect of spraying with Salicylic Acid and Arginine on the phenolic percentage

S Factor Levels	G Factor Levels				S Average
	G0	G1	G2	G3	
S0	0.312	0.324	0.370	0.394	0.350
S1	0.316	0.342	0.375	0.404	0.359
S2	0.346	0.364	0.398	0.410	0.379
S3	0.360	0.379	0.408	0.410	
Average	0.333	0.352	0.388	0.404	
G		S		GxS	
L.S.D 0.05		0.008		0.008	
				0.016	

As for Table (6), the reported results indicate that there are significant differences between the levels of spraying with Salicylic Acid, as the two treatments S3 and S2 significantly outperformed the rest of the treatments (0.166%) and (0.165 %), respectively. On the other hand, treatment S0 recorded the lowest percentage of oils that reached (0.154%), which was not significantly different from S1 (0.156%). As for spraying with amino acid Arginine, the results of the statistical analysis, shown in the same table, revealed that treatment G3 significantly outperformed the rest of the treatments, and recorded the highest percentage of volatile oils (0.169%). However, treatment G0 recorded the lowest value (0.149%). Similarly, the results of the interaction between the two study factors showed significant superiority of treatment S3G3 over other treatments with (0.171%), while the comparison treatment S0G0 recorded the lowest rate (0.141%).

Table (6) the effect of spraying with Salicylic Acid and Arginine on the percentage of volatile oils percentage.

S Factor Levels	G Factor Levels				S Average
	G0	G1	G2	G3	
S0	0.141	0.146	0.160	0.169	0.154
S1	0.138	0.154	0.163	0.168	0.156
S2	0.157	0.164	0.167	0.170	0.165
S3	0.160	0.166	0.169	0.171	0.166
G Average	0.149	0.158	0.165	0.169	
G		S		GxS	
L.S.D 0.05		0.003		0.003	
				0.007	

The increase in plant growth characteristics resulting from spraying salicylic acid can be attributed to its stimulating role for vegetation growth as it is classified within the group of stimulating plant hormones. It also works to reduce the effect of the abiotic stress that stimulates growth and increases the level of some plant hormones such as auxins and cytokines that are proliferating in the process of cell division and elongation (17). It also has a role in regulating nutrient absorption, hormonal balance, and stomatal opening and closing (18). Physical amino acids contribute to changing the osmotic potential of the plant cell. The increase of amino acids leads to a decrease in the osmotic effort leading to a decrease in water stress of the cell, thereby increasing the cell's ability to withdraw water and dissolved nutrients in it from the growth medium. Thus, the vegetation growth will increase. Showed

(19) an increase in the properties of the yield and its components in *Foeniculum vulgare* L. when sprayed with different concentrations of amino acids and vitamins. Amino acid spraying has a role in stimulating phylogenetic and biochemical processes by sharing proteins and the carbohydrate industry by building chlorophyll, improving the properties of the logical components, and encouraging many enzymatic processes and attachments (12).

(20) and (21) concluded that exogenous application of polyamine (an end product of arginine) to several plant species have been shown to promote cell division, cell differentiation, and general growth promotion. They can also help to stabilize membrane and wall properties (22) and protect the plant against environmental stress (23) and (24).

4. Conclusion

Results showed that the treatments of foliar spray with Salicylic Acid, the treatment with Arginine spray, and the interaction treatments were superior in terms of improving plant height, leaf area, and ratios of volatile oils ratio, phenols, nitrogen, and proteins. We recommend the use of treatments agents and concentrations.

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