Impact of spraying with Jasmonic acid and Ennne's nutrient solution on some anatomical characteristics and Jasmonic content of blood orange seedlings

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Abstract:-

In order to determine the effect of spraying with Jasmonates (JA) and adding the nutrient solution Ennne on the anatomical characteristics and jasmonic content of blood orange seedlings, an experiment was conducted in the wooden canopy of the College of Agriculture/ Samarra University from 1/9/2021 to 1/9/2022. The seedlings were sprayed with three Concentrations of Jasmonates (JA) (0, 5, and 10 mg.L-1) and the addition of three levels (0, 2.5 and 5 ml.L-1). The first day of spraying was September 1, 2021, the second day was 21 days later, and so on for the third and final day of spraying.

The results of the study are summarized as follows :

Spraying with the Jasmonates (JA) growth regulator, especially at the concentration of 10 mg.L-1, significantly increased the frequency of stomata, the average length and width of stomata, the average length*width of the lower surface of normal cells, the average length*width of the upper surface of normal cells, and the content of jasmonic in the leaf. The mean values for the comparison group were the lowest for length (222.11 mm), width (36.11 m), height (23.44 m), thickness (1015.4 m), width (914.3 m), and weight (66.5 mg.kg-1).

Spraying with Ennne's nutrient solution, especially at the level of 5 ml.L-1, significantly increased the frequency of stomata, the average length and width of stomata, the average length * width of lower surface of normal cells, the average length * width of upper surface of normal cells, and the jasmonic content of the leaves, which were respectively 223.44 mm, 33.89 m., 20.67 m, 980.3 There were substantial changes in all of the features tested due to the interaction of jasmonic acid and the nutritional solution Ennne, with the treatment G2M2 achieving the highest average for the aforementioned qualities and the treatment G0M0 achieving the lowest average.

Keywords: Jasmonic acid, Ennne nutrient Solution, Blood Orange

Introduction:-

Citrus fruits come from the Ponciru genus, which is part of the Rutaceae family. The three-leaf orange belongs to this genus, along with the genus Fortunella, which contains the kimquat (also known as Japanese orange or ornamental orange), and the genus Citrus, which contains the other two fundamental families of citrus. [3]

To enhance the characteristics of vegetative and anatomical growth and chemical content, the world's attention has shifted to the commercial usage of plant graft origin. All physical and chemical investigations relied on soil samples acquired from the site.

Transactions and Design of Experiment :-

First Factor : Spraying of Jasmonic acid

1. Spraying with water only (comparison treatment) and has the symbol JA_o .
2. Spraying with methyl Jasmonic at a concentration of 0.5 $ml.L^{-1}$. Its symbol is JA_1.
3. Spraying with methyl jasmonic at a concentration of 1.0 $ml.L^{-1}$. It is symbolized by the symbol JA_2.

Second Factor : Spraying with a nutrient solution (N-P-K) with high nitrogen
(30-10-10)

1. M_0 : Comparison treatment Spray with water only.
2. M_1: Spray the seedlings with Ennne's nutrient solution at a concentration of 2.5 g L^{-1} .
3. M_2: Spray the seedlings with the nutrient solution high in nitrogen at the level of 5g L^{-1} .

The spraying process took place over the course of three separate appointments, the first on 3/15/2019, the second 21 days later, and so on for the third appointment.

Two factors (Jasmonic + Nutrient solution) and three concentrations for each component were utilized in an RCBD factorial experiment, yielding nine treatments, two seedlings per experimental unit, and three replications for a total of 54 seedlings [4]. The statistical tool known as SAS (Statistical Analysis System) was used to evaluate the results. In a factorial experiment with a completely randomized design, significant differences between means were assessed using the least growth regulators like jasmonic. As such, it plays a crucial part in promoting the physiological processes that are essential to a plant's expansion and development. It is a stress hormone due to its high linolenic fatty acid content and crucial function in maintaining equilibrium under pressure. The immune defense antioxidant systems are triggered. [2]

Growth regulators like jasmonic acid improve vegetative growth, the transition of vegetative buds into flowering, fruit formation, and yield by stimulating various enzymes that are thought of as a starting point or signal to move towards increasing the plant's resistance to biotic and abiotic stresses. In addition to promoting vegetative development and increasing fruit output and quality, jasmonic acid also helps plants defend themselves from mechanical and environmental challenges. [5] [10]

Temperature, leaf properties, leaf type and age, humidity, wind, light, pH of the nutrient solution, spraying time, spray passes, and chemical composition all play a role in how efficiently leaves absorb nutrient solutions. [8]

To compensate for the loss of nutrients through fixation, precipitation, volatilization, and washing in the soil, foliar feeding is regarded as one of the most essential and quickly implementable practical applications for resolving the nutrient deficiency problems that plants face.
[4] [11]

For this study, we used Blood Orange Seedlings from the Balad region of Tikrit Governorate, and we randomly selected 54 seedlings to grow in three repetitions. These seedlings are approximately a year and a half old. based on the orange tree's lower epidermis along with the stomata.

e) Adhesive tape was placed on a clean slide and examined under an Olympus compound light microscope.

f) The samples were photographed using a modern Sony digital camera.

Determination of jasmonic acid:

Methanol (10 ml) was used to obtain standard stock solutions of jasmonic acid (100 ppm). An adequate volume of the stock solution was diluted with methanol to create the working solution. Stock and working solutions were kept in the dark at a temperature of -4 degrees Celsius.

Sample preparation:

At room temperature, liquid nitrogen was used to store all the papers. Liquid nitrogen was evaporated, and then 10 g of samples were precisely weighed into a 100 ml centrifuge tube, followed by the addition of 10 ml of cold 80% methanol (pH 3.2). After being homogenized in a mixer for three minutes at 5,000 rpm, the mixture was soaked at 4 °C for an entire night. After letting the tube sit overnight, 10 mL of ethyl acetate was added the next day, and the mixture was centrifuged at 8,000 rpm for 10 minutes after being vortexed for 10 minutes. The supernatant was discarded, and 0.2 g GCB and 0.6 g PSA were added before the mixture was cycled for 2 minutes. The supernatants were then collected, evaporated under a moderate stream of nitrogen, and dissolved in 2 mL of ethyl acetate before being analyzed further.

Anatomical characteristics :

Stomata frequency: Figures 1, 2, and 3 reveal that spraying with the growth regulator jasmonic acid significantly significant difference (LSD) at the 5% probability level.

Anatomical Study :-

1. **Epidermis Preparation:** Using forceps with two fine ends, the upper and lower epidermis were stripped off using the stripping off method, and the epidermis was spread on a glass slide with a drop of glycerine placed on it. The sample was taken from the middle of a fully developed leaf, and thus included the middle vein, as well as part of the blade and the edge. After that, a slide cover was placed on top of it, and it was ready for analysis. The models were studied with a graduated ocular lens scale and an Olympus-type compound microscope. The area of the epidermis was determined after measuring its cells (5-10) and analyzing their radial and tangential dimensions, as well as their normal epidermal cell shape, guard cell dimensions, and stomata complexes' form. frequency of stomata opening and closing as calculated by an equation
2. **Stomata frequency = number of stomata in mm^2,** According to Stace [6] **[9] .**
3. **Preparation epidermis by polish:**

The method was used according to [9]. In preparing the epidermis and stomata according to the following steps.

a) I took a ripe leaf and put a little clear polish on it .

b) Leave for 2-3 minutes until it is almost dry.

c) A transparent adhesive tape was also placed on it to cover the surface containing the paint.

d) Remove the adhesive tape forcefully to remove the upper and

M2 yielded the highest rate (223.44) while treatment M0 yielded the lowest (183.56 mm 2/28 m / 16.56 mm 2/m).

Figures 1, 2, and 3 show that the stomata frequency rate was significantly affected by the mutual interaction of the growth regulator jasmonic acid and the nutrient solution Ennne. The G2M2 treatment resulted in the highest stomata frequency rate (243 mm2, 38 m, and 26.33 m, respectively), while the treatment gave the lowest rates (123 mm2, 26.33 m, and 8.33 m). The lowest frequency is 170.67 mm, followed by 22.67 m, and then 12.67 m for the G0M0 comparator.

increased the frequency, length, and width of the stomata, with treatment G2 giving the highest rate of 222.11 mm, 2,36.11 m, and 23.44 m, respectively. This was followed by a significant difference by treatment G1, which resulted in a rate of 222.11 mm, 2,36.11 m, and 23.44 m The stomata in the experimental treatment G1 were the largest in size (207 mm 2, 31.22 m, 17.44 m) compared to the control treatment G0 (189.67 mm 2, 25.67 m, 14.67 m).

Regarding the effect of Ennne's nutrient solution, we see from the same figure that there are substantial variations: treatment

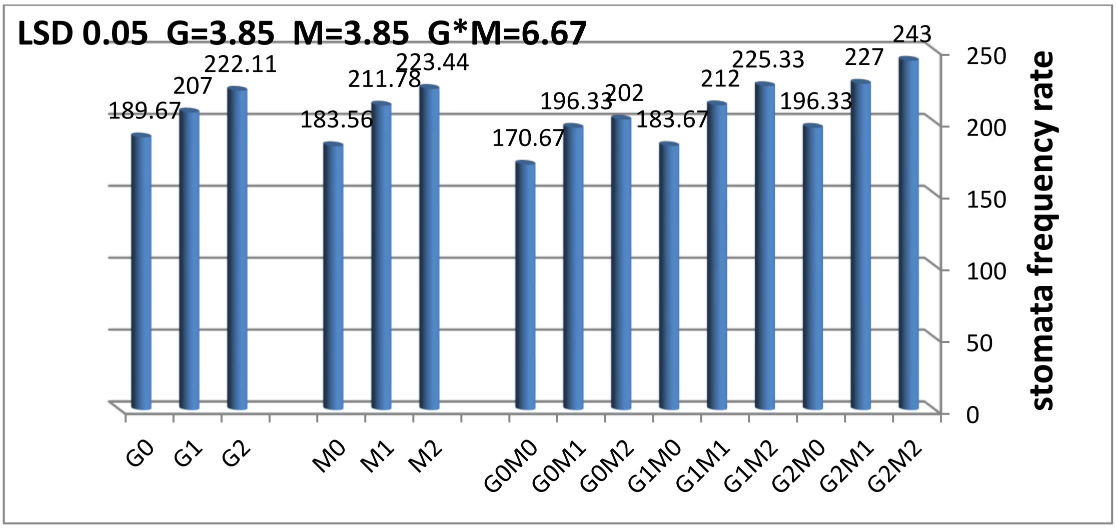

Figure (1) Impact of spraying with jasmonic acid and Ennne nutrient solution and the interaction between them on the stomata frequency of blood orange seedlings.

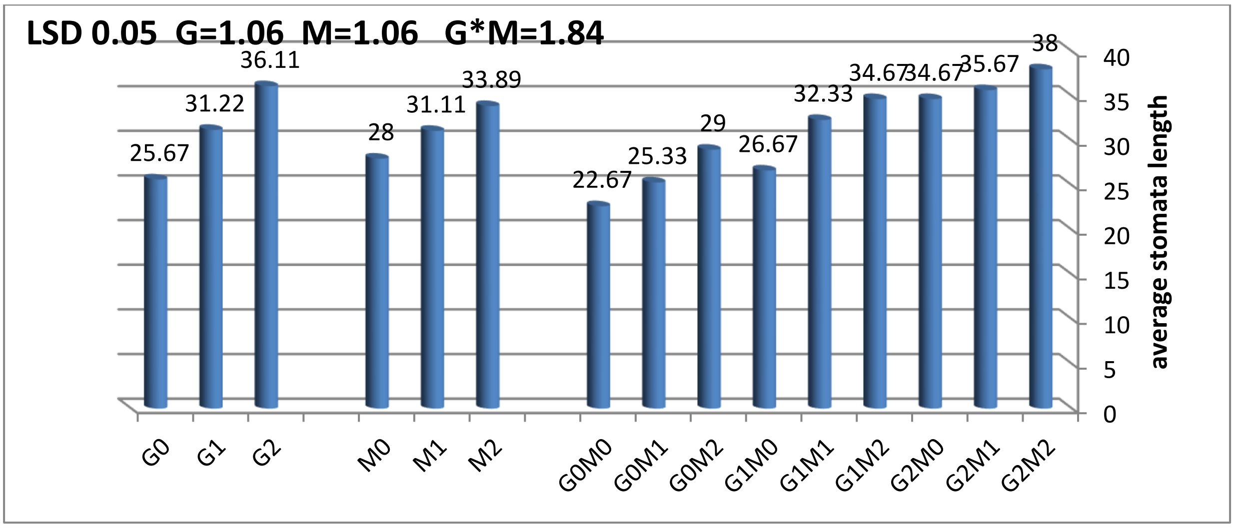

Figure (2) Impact of spraying with jasmonic acid and Ennne nutrient solution and the interaction between them on the average stomata length of blood orange seedlings.

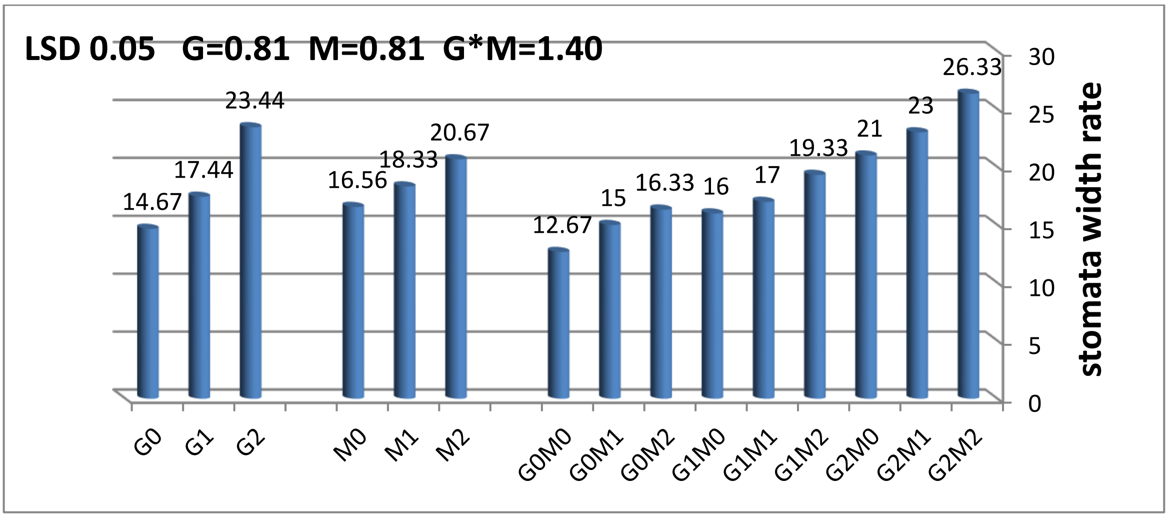

Figure (3) Impact of spraying with jasmonic acid and Ennne nutrient solution and the interaction between them on the rate of stomata width of blood orange seedlings.

producing the next highest rate (790.9 micrometers), and the M0 treatment producing the lowest rate (650.1 micrometers).

Jasmonic acid's interaction with the nutrient solution had a notable effect on the average length * width of the lower surface of normal cells, as shown in the same figure, with the G2M2 treatment yielding the highest rate, reaching 1300.7 micrometers, and the G0M0 treatment yielding the lowest rate, amounting to 566.3 micrometers.

Average length*width of the lower surface of normal cells:

Figure (4) demonstrates that there were substantial changes in the average length * breadth of the lower surface of the normal cells between the G2 and G0 treatments after jasmonic acid spraying, with the G2 treatment yielding the highest rate at 1015.4 micrometers. Its maximum dimension was 687.6 m.

There are noticeable variations in the effect of Ennne's nutrient solution, with the M2 treatment producing the highest rate (980.3 micrometers), the M1 treatment

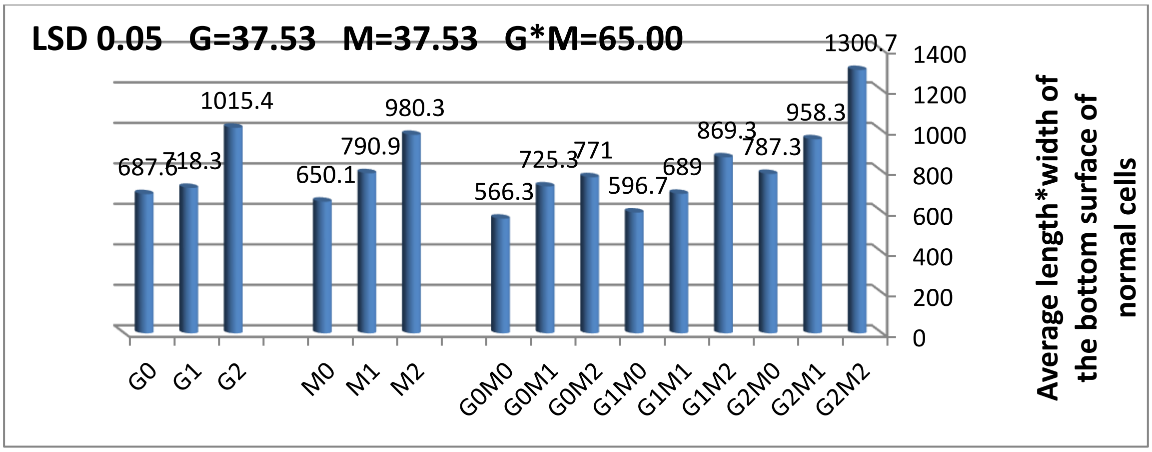

Figure (4) Impact of spraying with jasmonic acid and Ennne's nutrient solution and overlap between them on the length*width of lower surface of normal cells of Blood Orange Seedlings.

Treatment M2 achieving the maximum rate of 871.9 micrometers, Treatment M1 achieving a rate of 721.3 micrometers, and Treatment M0 achieving the lowest rate of 652.

Figure (5) shows that the growth regulator jasmonic acid interacts with the nutritional solution Ennne in a way that has a considerable impact on the average length * breadth of the upper surface of normal cells in the G2M2 treatment (1039.3 m vs. 103.3 m, respectively). This averaged out to 567.3 m
.

Length * Width of the upper surface of the regular cells:

Figure (5) shows that spraying with jasmonic acid led to statistically significant differences in the average length*width of the top surface of normal cells, with treatment G2 yielding the highest rate (914.3 micrometers) and the control treatment (G0) yielding the lowest (657.7 m).

Significant differences can be seen in the findings of the same figure for the Effect of Ennne's Nutrient Solution, with

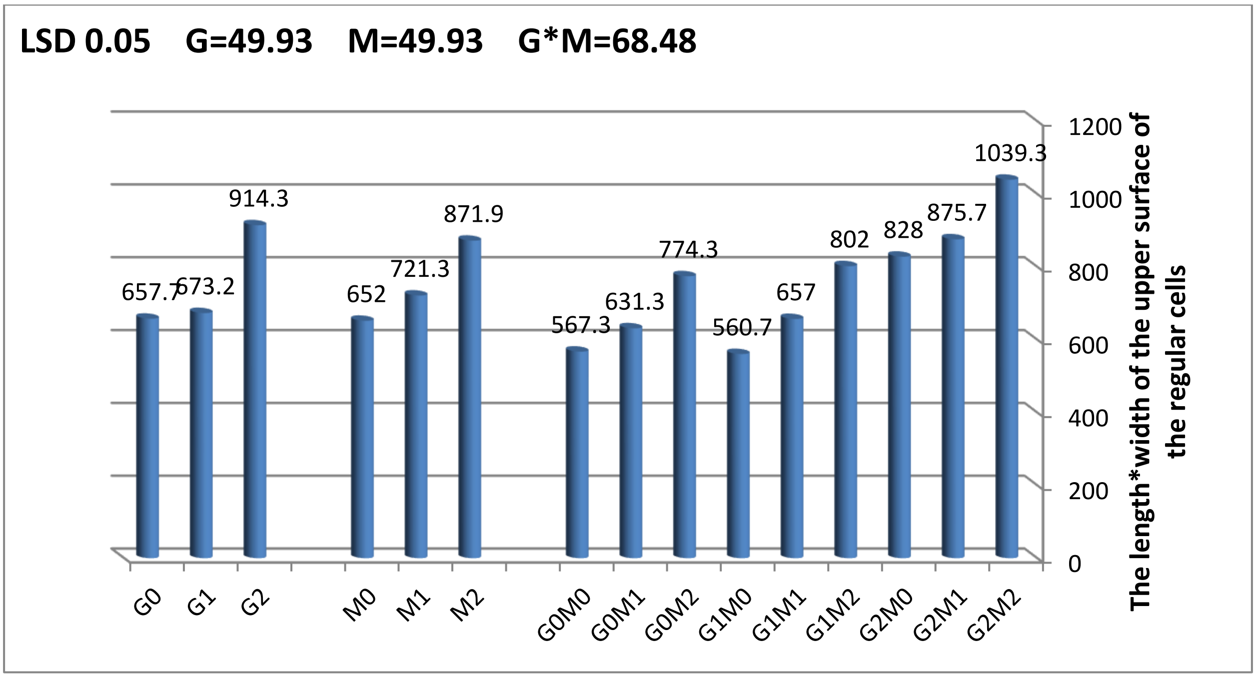

Figure (5) Impact of spraying with jasmonic acid and Ennne's nutrient solution and the interaction between the length * width of the upper surface of normal cells of blood orange seedlings.

In the same table, we can see that the nutritional solution also considerably affected the jasmonic acid content of the leaves, with the M2 treatment yielding the highest percentage (65.18 mg.kg-1) compared to the M0 treatment's lowest yield (45.91 mg.kg-1).

Treatment G2M2 yielded the greatest rate of 77.38 mg.kg-1 of jasmonic leaf content,

Estimation of leaves content of Jasmonic acid (mg.kg^{-1})

As can be seen in Figure (6), there was a clear effect of adding jasmonic acid on the jasmonic acid content of the leaves, with treatment G2 producing the highest percentage (66.50 mg.kg-1) and treatment G0 producing the lowest (41.57 mg.kg-1).

factors had a substantial impact on the jasmonic leaf content whereas treatment G0M0 yielded the lowest at 36.40 mg.kg-1, indicating that the interaction between the two study

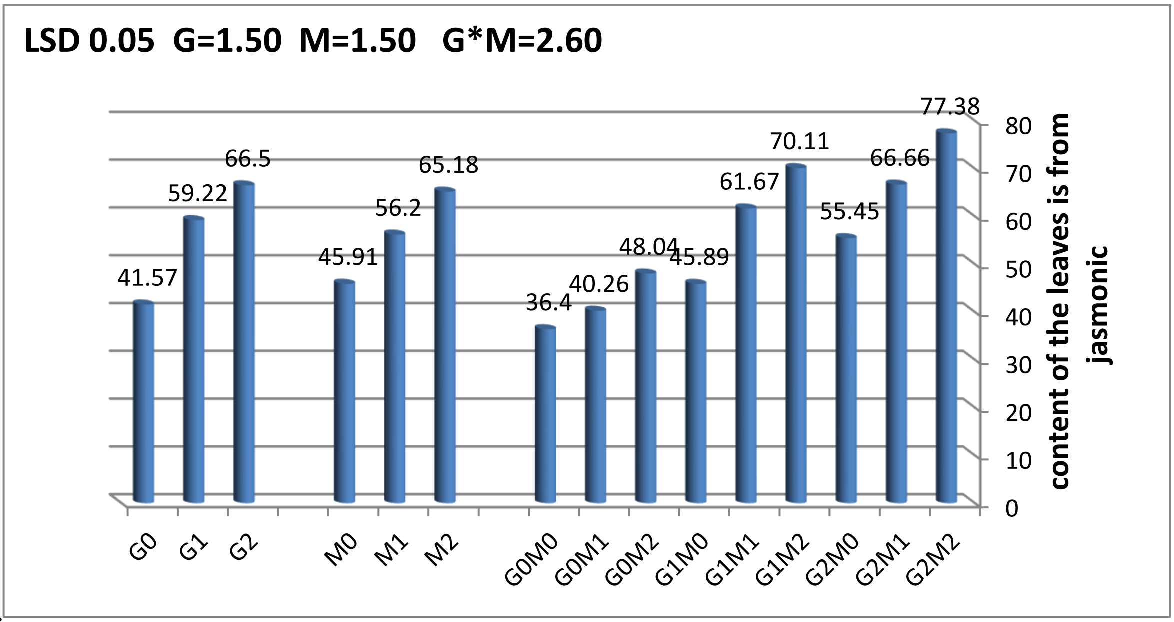

Figure (6) Impact of spraying with jasmonic acid and Ennne nutrient solution and the interaction between them on the leaves content of jasmonic acid (mg.kg^{-1}).

a part in supplying the plant with the major elements NPK, the plant's meristematic cells multiplied and expanded, the leaves became larger, and the plant produced more carbohydrates and proteins, which it then transported to where they were needed to construct plant tissues. These findings, as well as those of Al-Jasmonic [7], corroborate [1].

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Discussion :-

Treatment with jasmonic acid may have aided in the enhancement of the features under study since it boosted cell proliferation and growth. Changes in the mechanical characteristics of the cell wall, metabolic pathways, and gene expression are all factors that regulate cell elongation [2].

The bottom surface of the leaf has more stomata and has a greater impact on the absorption process due to the thickness of the cuticle layer. [8]

By participating in photosynthesis and entering energy-rich compounds, phosphorus and potassium enhance vegetative growth and, by extension, anatomical characteristics. These elements also play a role in cell division and the synthesis of nucleic acids, energy-carrying compounds, and some enzymes within the plant. Because the nutrient solution played

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