Effect of treatment with some Bio-Stimulants on the anatomical Indicators of the fruits of two cultivars of Jujube trees (*Zizphus mauritiana* Lam.)

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Abstract

The research conducted in a private orchard in Basrah Governorate In the Abu Al-Khasib area, selecting 36 jujube trees age 8years old It is irrigated using drip irrigation,five fruits were taken for each experimental unit. from the two cultivars, Tofahe and Zytone, for the agricultural season 2024-2025.The study included two factors: the first is cultivar factor,as the Tofahe and the Zytone were selected. The second factor was bio-stimulant first one BioHealth at two levels (0 and 5 mg L^{-1}) and Soli Organic at two levels (0 and 5 mg L^{-1}).aqueous solutions of bio-stimulants were sprayed three times before the flowering period, with intervals of three weeks between one spraying and the next. The results of the study demonstrated how the jujube tree cultivar significantly affected the anatomical indicators; the Tofahe cultivar was superior to the Zytone cultivar in the thickness of the epidermis, sub-epidermis, and mesocarp layer thickness (31.66, 153.67, and 586.89 µm), respectively, and that the use of bio-stimulants improved the anatomical indicators of the jujube tree fruits. The Soli Organic treatment 5 mg L^{-1} was significantly superior to the BioHealth treatment and the comparison treatment, in the highest rate of epidermis thickness,sub-epidermis, and mesocarp layer thickness (31.15, 153.85, and 547.45 µm), respectively. The interaction between the Tofahe cultivar and the bio-stimulant Soli Organic 5 mg L^{-1} also gave the highest rates of thickness of the epidermis and sub-epidermis layer, and epidermis and mesocarp layer thickness.

Keywords: bio-stimulants - anatomical Indicators - jujube fruits-Tofahe- Zytone

Introduction

the climate is warm, due to their importance in all aspects, especially medical ones, including that the aqueous and alcoholic extracts contain all the bioactive compounds that have been detected, except for the resinous compounds and furocoumarins in the aqueous extract and the resins in the alcoholic extract (13). It is believed that the original homeland of jujube is Southern Europe, the Himalayas, and Northern China, and its cultivation is widespread in North Africa, Sudan, the

The jujube plant, Zizphus mauritiana Lam. belongs to the Rhamnaceae family that includes three genera,the most important of which is the genus. *Zizphus*, which includes 170 species of evergreen trees and shrubs. Its cultivation is widespread in tropical and subtropical regions, where the two species *Z. mauritiana* and *Z. jujuba* are considered the most important from an economic standpoint (17). The cultivation of species belonging to the genus *Ziziphus* is increasing in Iraq, where

ISSN 2072-3857

study, it may also improve the plant's ability to absorb nutrients because it serves as a conduit for nutrient delivery and stimulates the expansion of the root system. The jujube tree cultivar has a notable impact on the anatomical indicators (2).

It has been proven through most recent studies that the use of chemical fertilizers in high concentrations and in large doses causes increased pollution and health damage to humans and animals. Therefore, it was necessary to search for natural alternatives that can be used to feed plants that are safer for the environment, humans, and animals (4). This resulted in obtaining a good amount of fiber.(gasson) at a concentration of 3ml L^{-1}, a significant increase in plant height, main diameter, leaf area, number of main branches, fresh and dry weight of leaves, and percentage of dry matter of leaves. As for spraying with a dry dryer at a concentration of 6g L^{-1} LED, it significantly increased in all due to the vegetative study. It interferes between the sip exchanges with the fibers (gasson) at a concentration of 3ml L^{-1} and diluted to a concentration of 6g L^{-1}, so the optical concentration increases the effect of plants on the height and diameter of the main leaves and leaves, number of branches, fresh and dry weight of leaves, and percentage of dry matter (16). Therefore, This study was done to determine the effect of biostimulants.as an alternative to chemical fertilizers and their effect on some anatomical indicators of jujube fruits.

Materials and Methods

The research conducted in a private orchard in Basrah Governorate In the Abu Al-Khasib area, served as the study's site, where 36 jujube trees It is irrigated using drip irrigation.five fruits were taken for each experimental unit were selected and were homogeneous in terms of vegetative growth strength and age 8years old.Service operations were conducted for all the study trees.The study included two factors:

Arabian Peninsula, Iraq, and the Emirates (12). Although jujube trees are considered salt-tolerant fruit trees, as the extent of jujube trees tolerance to salinity greatly exceeds that of many other fruit trees, their productivity decreases with increasing salinity in the area where the roots spread, and their productivity decreases under extreme environmental conditions such as drought (8). The salinity of the soil and irrigation water hinders the absorption of some nutrients by the plant, and thus its need to add nutrients in larger quantities(16).

Bio-stimulants have gained importance under sustainable agricultural systems as a result of their use in activating many physiological processes that improve nutrient use efficiency, improve plant growth, and reduce fertilizer consumption. The results of the statistical analysis showed that there was a significant effect of the foliar spraying on the plant growth and yield characteristics such as plant height, stem diameter, number of leaves, leaf area, and yields (19). There are many bio-stimulants that have the ability to counter the negative effects of biotic and abiotic stress factors, and they also work to improve quality and yield by causing the plant's physiological functions to be active. These substances may promote plant development by enhancing nutrient absorption in a manner akin to that of plant hormones like auxins. They also endeavor to enhance the leaves' ability to produce more chlorophyll and to accumulate more nutrients. It may contain many active substances, which can be presented in very low concentrations, sometimes below the levels that can be detected with available techniques. Nevertheless, they can produce powerful biological effects (20). Bio-stimulants are organic plant extracts such as marine algae extracts and amino acids. They contain a wide range of biologically active compounds. These compounds are usually able to improve the efficiency of using nutrients within the plant and improve the ability to confront stress conditions, whether biological or inanimate. According to the

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The factorial experiment with two components and three replications was designed with a randomized complete block design (RCBD). There were 24 trees in the experiment, and the factor coefficients were allocated at random among them. An analysis of variance was used to examine the data and make sure that there were substantial differences between the attributes. The results were analyzed using analysis of variance (ANOVA) to confirm the presence of significant differences between the studied traits using the GenStat statistical software. Means were also analyzed, and significance was tested using the least significant difference (L.S.D) test at a probability level of 0.05 (10).

Results and Discussion

The thickness of the cuticle layer

The statistical analysis's findings, which are displayed in Table (1) and Figures (1 and 2), demonstrated how bio-stimulants and cultivar affected the jujube fruits' glycolytic layer thickness. As was the case with stimulants, the Zytone cultivar outperformed the Tofahe cultivar by a large margin, recording the greatest rate of 8.08 µm while the latter recorded The lowest rate is 5.59 µm. Vitality influenced the thickness of the cuticle layer, with the comparison treatment recording the highest value of 8.79µm and the Soli Organic 5 mg L^{-1} treatment recording the lowest value of 4.67µm. The comparison treatment was considerably superior to the other treatments.Regarding the relationship between the bio-stimulant component and the cultivar factor, the relationship of interaction between the Zytone cultivar and the comparison treatment recorded the highest rates of cuticle layer thickness, reaching 10.25 µm, while the Tofahe cultivar and the bio-stimulant Soli Organic 5 mg L^{-1} recorded the lowest rates, reaching 3.68 µm, having a notable distinction from the other exchanges.

The first factor (cultivar): The study was conducted on two types of jujube trees: Zytone and Tofahe trees.

The second factor (bio-stimulants): This factor includes the use of bio-stimulants BioHealth (an organic fertilizer consisting of 75% humic acid, 5% seaweed extracts, Trichoderma fungus, and Bacillus bacteria 10%) at two levels (0 and 5 mg L^{-1}) and Soli Organic (an organic fertilizer consisting of 0.7% gibberellin and naphthalene acetic acid 0.5%). and glycine 40%) at two levels (0 and 5 mg L^{-1}) in addition to the control treatment (without addition).Aqueous solutions of the bio-stimulants were sprayed using a diffuser three times before the flowering period, between one spraying and another for three weeks. The spraying was done early in the morning until the leaves were completely wet, and the control treatment was sprayed with distilled water only. Service operations were carried out for the plants, including irrigation and weeding for all treatments.

Anatomical Indicators of fruits

Anatomical sections of the fruits were prepared when the fruits began to discolor using the paraffin technique, based on the technique described in (5), which uses both solid and melted paraffin wax to create permanent tissue slices. and the samples were then buried in paraffin wax at a temperature of 58°C. After that, the specimens were cut with a Rotary Microtome to a thickness of 10 micrometers, mounted on slides, stained with Safranin, & placed in Fast green. stain, then mounted with the addition of drops of DPX and covered. the slides were studied and measurements were taken in micrometers (µm) using the ocular micrometer in an Olympus light microscope equipped with a camera connected to a computer.

Statistical analysis

ISSN 2072-3857

Table (1) Effect of cultivar and bio-stimulants on the thickness of the cuticle layer of jujube fruits (mµ)

Cultivar	Bio-stimulants			Cultivar effect rate
	Control (0 mg L^{-1})	BioHealth (5 mg L^{-1})	Soli Organic (5 mg L^{-1})	
Tofahe	7.32	5.78	3.68	5.59
Zytone	10.25	8.34	5.66	8.08
Effect rate of bio-stimulants	8.79	7.06	4.67	
R.L.S.D.	cultivar = 2.12 bio-stimulants = 1.11 = To overlap3.21			

measuring 31.15 mµ, with a significant difference from the other interactions as in contrast to the treatment under control, which had the lowest likelihood of a substantial difference.23.99 mµ.

Regarding the interaction between the cultivar factor and the bio-stimulant factor, the Zytone cultivar and The comparison treatment had the lowest rate of epidermis layer thickness,amounting to 20.32 mµ, while the Tofahe cultivar and the bio-stimulant Soli Organic 5 mg L^{-1} recorded the highest rate of 35.11 mµ, having by which a notable distinction from the remaining exchanges.

Thickness of epidermis layer

Table (2) and Figure (F21) present the study's findings, which demonstrate how bio-stimulants and cultivar affect the thickness of the epidermis layer. The Tofahe cultivar outperformed the Zytone cultivar by a significant margin, recording The highest rate at 31.66 mµ, while the Zytone cultivar was the lowest rate of 31.66 mµ. 23.59. The bio-stimulants exhibited a noteworthy impact on the thickness of the epidermis layer. Specifically, the Soli Organic 5 mg L^{-1} treatment demonstrated a substantially higher rate of epidermal layer thickness,

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Table (2) Effect of cultivar and bio-stimulants on the thickness of the epidermal layer of jujube fruits (mµ)

Cultivar	Bio-stimulants			Cultivar effect rate
	Control (0 mg L^{-1})	BioHealth (5 mg L^{-1})	Soli Organic (5 mg L^{-1})	
Tofahe	27.65	32.23	35.11	31.66
Zytone	20.32	23.27	27.18	23.59
Effect rate of bio-stimulants	23.99	27.75	31.15	
R.L.S.D.	cultivar = 3.11 bio-stimulants = 3.16 To overlap=4.32			

thickness, measuring 153.85 mµ, in contrast to the treatment under control, which had the lowest rate 128.88 mµ.

In terms of the interaction between the cultivar factor and the bio-stimulant factor, the Zytone cultivar and the comparison treatment recorded the lowest rates of skin layer thickness, amounting to 119.78 mµ. On the other hand, the Tofahe cultivar and the bio-stimulant Soli Organic 5 mg L^{-1} recorded the highest rate, amounting to 168.13 mµ, which is considered a notable distinction from the remaining exchanges.

Thickness of subcutaneous layer

The study findings, which are displayed in Table (3) and Figure (F21), demonstrated how bio-stimulants and cultivar affected the thickness of the sub epidermal layer. The Tofahe cultivar outperformed the Zytone cultivar by a significant margin, recording The highest rate of 153.67 mµ, while the Zytone cultivar got the lowest rate. 153.67 mµ. 128.56. The bio-stimulants exhibited a noteworthy impact on the thickness of the epidermis layer. Specifically, the Soli Organic 5 mg L^{-1} treatment demonstrated a substantially higher rate of epidermal layer

ISSN 2072-3857

Table (3) Effect of cultivar and bio-stimulants on the thickness of the endocarp layer of jujube fruits (mµ)

Cultivar	Bio-stimulants			Cultivar effect rate
	Control (0 mg L^{-1})	BioHealth (5 mg L^{-1})	Soli Organic (5 mg L^{-1})	
Tofahe	137.98	154.89	168.13	153.67
Zytone	119.78	126.34	139.56	128.56
Effect rate of bio-stimulants	128.88	140.62	153.85	
R.L.S.D.	cultivar = 9.45 bio-stimulants = 8.56 = To overlap=15.23			

The Soli Organic 5 mg L^{-1} treatment demonstrated a significantly higher rate of stone cell diameter, measuring 24.51 mµ, while the control treatment recorded the lowest rate, measuring 16.40 mµ.

The Zytone cultivar and the bio-stimulant Soli Organic 5 mg L^{-1} recorded the highest rate of stone cell diameter, amounting to 26.44 mµ, with a significant difference from the other interactions. The Tofahe cultivar and the comparison treatment recorded the lowest rates, amounting to 14.35 mµ. These results represent the interaction between the cultivar factor and the bio-stimulant factor.

Diameter of stone cells

The Zytone cultivar outperformed The Tofahe cultivar was the highest rate at 22.41 mµ, while the lowest rate was also 22.41 mµ.The study's results are displayed in Table 4 and Plate(F21),illustrating the impact of cultivar and bio-stimulants on the diameter of stone cells. 18.34

The diameter of the stone cells was also considerably influenced by the bio-stimulants.

ISSN 2072-3857

Table (4) Effect of cultivar and bio-stimulants on the diameter of the stone cells of Jujube fruits (mμ)

Cultivar	Bio-stimulants			Cultivar effect rate
	Control (0 mg L^{-1})	BioHealth (5 mg L^{-1})	Soli Organic (5 mg L^{-1})	
Tofahe	14.35	18.11	22.57	18.34
Zytone	18.45	22.34	26.44	22.41
Effect rate of bio-stimulants	16.40	20.23	24.51	
R.L.S.D.	cultivar = 2.16 = Bio-stimulants= 2.43= To overlap=3.33			

BioHealth The control treatment was a value of 27.01μm, more than the other treatments, while the Soli Organic 5 mg L^{-1} treatment got the lowest value of 17.64μm. The Tofahe cultivar and the bio-stimulant Soli Organic 5 mg L^{-1} recorded the lowest tannin cell diameter rate (15.36 μm), which differed significantly from the other interactions. In contrast, the Zytone cultivar and the control treatment recorded the highest tannin cell diameter rates, coming in at 28.58μm.

Diameter of tannin cells

The impact of cultivar and bio-stimulants on the width of the tannin cells in Jujube fruits was demonstrated by the statistical analysis results displayed in Table 5 and Figures 1 and 2. The Zytone cultivar outperformed the Tofahe cultivar by a substantial margin, recording the highest rate of 24.02 μm. The bio-stimulants and the Tofahe cultivar had the lowest rate of 20.04 μm. The diameter of tannin cells was considerably influenced by

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Table (5) Effect of cultivar and bio-stimulants on the diameter of the tannin cells of Jujube fruits (mµ)

Cultivar	Bio-stimulants			Cultivar effect rate
	Control (0 mg L^{-1})	BioHealth (5 mg L^{-1})	Soli Organic (5 mg L^{-1})	
Tofahe	25.43	19.33	15.36	20.04
Zytone	28.58	23.56	19.91	24.02
Effect rate of bio-stimulants	27.01	21.45	17.64	
R.L.S.D.	cultivar = 2.34 Bio-stimulants = 2.22 To overlap=3.18			

thickness (547.45 mµ) than the treatmentA(410.17mµ).This indicates that the bio-stimulants had a considerable impact on the epidermis layer thickness as well.

Regarding the interaction between the cultivar factor and the bio-stimulant factor, the Zytone cultivar and The control treatment recorded the lowest rate of mesocarp layer thickness, amounting at 300.00 mµ, while the Tofahe cultivar and the bio-stimulant Soli Organic 5 mg L^{-1} recorded the highest rate, amounting to 650.67 mµ, having a notable distinction from the remaining exchanges.

Thickness of the mesocarp layer

The study findings, which are displayed in Table (6) and Figure (F21), demonstrated how bio-stimulants and cultivar affected the thickness of the mesocarp layer. The Tofahe cultivar outperformed the Zytone cultivar by a significant margin, recording The highest rate of 586.89 mµ, while the Zytone cultivar recorded the lowest rate of 381.20 mµ. The Soli Organic 5 mg L-1 treatment saw a significantly higher rate of mesocarp layer

ISSN 2072-3857

Table (6) Effect of cultivar and bio-stimulants on the thickness of the mesocarp layer of Jujube fruits (mμ)

cultivar	Bio-stimulants			cultivar effect rate
	Control (0 mg L^{-1})	BioHealth (5 mg L^{-1})	Soli Organic (5 mg L^{-1})	
Tofahe	520.34	589.67	650.67	586.89
Zytone	300.00	399.38	444.23	381.20
Effect rate of bio-stimulants	410.17	494.53	547.45	
R.L.S.D.	cultivar = 24.39 Bio-stimulants= 26.34 To overlap=38.48			

cell wall and protoplasm, leading to fast cell division and development. Utilizing bio-stimulants promotes the growth of chlorophyll, aids in the synthesis process, and boosts the accumulation of carbohydrates, amino acids, and enzymes. Through an increase in the dry and moist weight as well as lateral root branches, photosynthesis also strengthens the growth of the root group. Additionally, it helps the plant absorb more nutrients (15).

Bio-stimulants also play an important role in activating many enzymes. In addition to their importance in producing the plant hormone (IAA) Indol acetic acid, they're necessary for the expansion and elongation of cells (9) and thus improving the anatomical indicators of fruits. It was found (3) that spraying with salicylic acid at a concentration of 200 mg L^{-1} on Tofahe Jujube trees led to a significant increase in all vegetative and fruiting indicators, and this is consistent with what was found by (18) on the Tofahe Jujube tree.

When compared to adding fertilizer to plants from the ground, foliar feeding results in a higher nutrient uptake and less pollution to the environment, making it the best method of fertilizing. Comparing foliar feeding to other techniques of fertilization, it is also thought to be the most cost-effective and efficient technique (14&7).

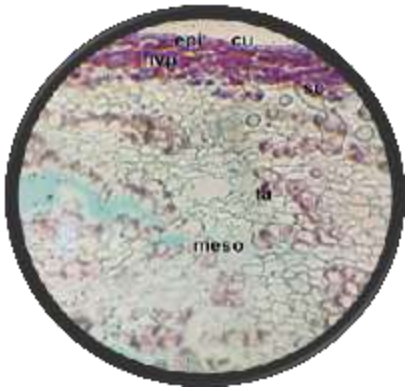
The present study's results indicate that bio-stimulants have a noteworthy impact on enhancing the anatomical features of Jujube plants' fruits. This could be because the bio-stimulants BioHealth and Soli Organic 5mg L^{-1} work together to improve the growth environment of the trees; they also increase cell division activity, which is reflected in the anatomical features of the fruits. The concentration of readily available nutrients in the soil rises in response to an increase in additional bio-stimulants, which boosts plant uptake of those nutrients. Fruits' improved anatomical features may have resulted from the use of fertilizers, which may have increased the growth of roots and leaves (11).

According to (21), these chemicals have a hormonal influence because they alter the

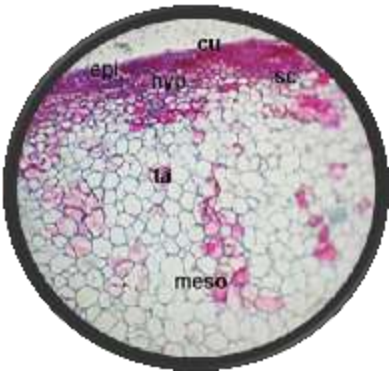
ISSN 2072-3857

weight of the seed, flesh, length, and diameter of the fruit. As so, it assisted in the weight and size of the fruit. This is involved in the synthesis of most compounds, including chlorophyll and amino acids, which are the basic elements of proteins and enzymes. As such, it is involved in all steps of protoplasmic reactions, photosynthesis, and growth. It is also an enzymatic aid. Nicotinamide Adenine Dinucleotide Phosphate (NADP+) and its role in hormone levels play an important role in elongating and increasing the size of cells (6).

The role of these bio-stimulants in vegetative growth may explain the reason fruits' anatomical indicators are improving. Growth stimulants contain manganese, which is vital photosynthesis and the formation of chlorophyll molecules, amino acids, and proteins, plus potassium and zinc. Potassium and calcium set cells osmotic potential, more plant resistance to salt stress conditions (1). This activity leads a greater accumulation of nutrients such as sugars, proteins, acids, and water in the expanded cells, increasing the



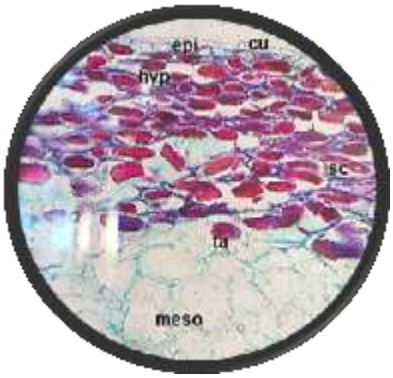
Toffahy cultivar



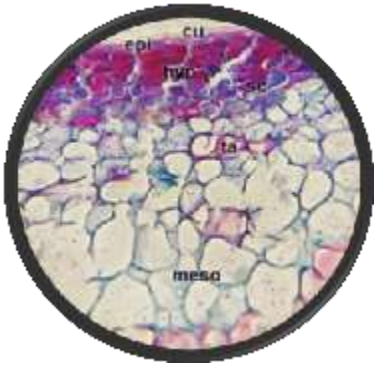
Tofahe cultivar + BioHealth

Figure (1) The effect of cultivar and bio-stimulants on some anatomical Indicators of Jujube fruits **)**
m (x40µ200

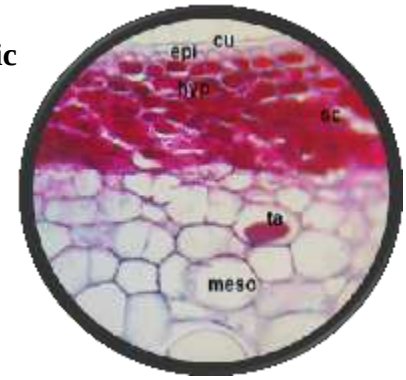
Cu =cuticle- Epi=epidermis - hyp=hypodermis- Sc= Sclereides cell- Ta= Tannin cell- Meso= Mesocarp



Toffahy cultivar + Soli Organic



Zytone cultivar + BioHealth



ISSN 2072-3857

Zytony cultivar + Soli Organic

Figure (2) The effect of cultivar and bio-stimulants on some anatomical Indicators of Jujube fruits **)** m (x40µ200

Cu =cuticle- Epi=epidermis - hyp=hypodermis- Sc= Sclereides cell- Ta= Tannin cell- Meso= Mesocarp

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Conclusion

The results of the study demonstrated that the Jujube tree cultivar had a significant effect on the anatomical indicators. The Tofahe cultivar was superior to the Zytone cultivar in the thickness of the epidermis, sub epidermis, and mesocarp layer thickness and the use of bio-stimulants improved the anatomical indicators of the Jujube tree fruits. The Soli Organic 5 mg L^{-1} treatment was significantly superior to the BioHealth 5 mg L^{-1} treatment and the control treatment, and recorded the highest rate of thickness of the epidermis, sub epidermis, and mesocarp layer thickness, and sub epidermis layer: Epidermis and mesocarp layer thickness.The interaction between the Tofahe cultivar and the bio-stimulant Soli Organic 5 mg L^{-1} also gave the highest rates of thickness of the epidermis and sub-epidermis layer, and epidermis and mesocarp layer thickness.

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