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Effect of spraying with tryptophan and glycine on some anatomical characteristics of kumquat and local lemon seedlings

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Abstract
This study was conducted in the cloth canopy of the College of Agriculture, University of Basrah, during the growing season 2021-2022 on seedlings of kumquat and local lemon, totaling 54, 27 seedlings for each plant type, in three replications, as the seedlings were sprayed six times with three concentrations (0, 50, 100) mg l-1 of the amino acids tryptophan and glycine. The study results showed that using the two amino acids improved most of the anatomical characteristics of the seedlings of both types. The treatment with a concentration of (100 mg l-1) was recorded, and both acids had a significant superiority in the characteristics of cuticle layer thickness, epidermal layer thickness, tannin layer thickness, and columnar cell thickness at a rate of (19,000; 39.222; 39.444; 92.222) µM for tryptophan, respectively. The rates of glycine were (18.667, 33.778, 38.444, 83.889) µM, respectively, for kumquat seedlings, and the rate of tryptophan was (21.11, 44.67, 43.67, 112.33) µM, respectively, and the average of glycine was (20.33, 39.11, 42.78, 104.2) µM, respectively. For lemon seedlings, the interaction between the two acids had a significant effect, as the interaction treatment (100 mg l-1 tryptophan and 100 mg l-1 glycine) recorded the best results in the characteristics of cuticle layer thickness, epidermal layer thickness, tannins layer thickness, and columnar cell thickness (23,000; 45.000; 46.000; 99.667) µM, respectively, for kumquat seedlings, and the overlap for lemon seedlings was (25.00, 51.00, 51.00, 120.333) µM, respectively.

Keywords: local lemon,

Introduction

Citrus is one of the perennial fruit trees belonging to the Rutaceae family and includes a number of species, the most important of which is Citrus, and the species belonging to it are widely spread worldwide due to their adaptation to a wide range of environmental conditions. It includes many species with wide varieties and strains (Abbas et al. 2016; Ismail and Zhang 2004).

Citrus Limon L. belongs to the genus Citrus, which belongs to the Rutaceae family. It is its original home in northeastern India and southwestern China (Al-Khafaji et al., 1990). Lemon fruits are used for fresh consumption, making juices and flavorings for many foods, and their practical effect in treating many diseases (Forte et al., 2011, Al-Hasany, et al., 2020). The local variety is one of the most desirable varieties in Iraq because its fruits are juicy, small in size, and have thin skin, and the acidity percentage is lower than the rest of the international varieties (Al-Khafaji et al., 1990).

Kumquat, whose scientific name is *Citrus japonica* L. is considered one of the citrus fruits and belongs to the Rutaceae family. It is one of the species of the genus Fortunella, which includes many species and regions of India and East Asia, the home of its cultivation, and then its cultivation spread to all parts of the world. It is commonly used because of the vitamins and fibers it contains (Hasan et al., 2016, Noaema, et al.,2020a).

Amino acids are a source of important organic nitrogenous compounds for building proteins in ribosomes (Davies, 1982), and among the important amino acids is tryptophan, which is one of the structural units and important building blocks of enzymes and proteins and the main factor for building auxins (IAA) in some plant tissues. The amino tryptophan instead of the direct use of auxin to improve the growth and productivity of crops (Ahmad et al., 1999, Noaema, et al.,2020b) and glycin, which activates the formation of chlorophyll (Hendry and Stobart 1977) and prevents photorespiration (Taiz and Zeiger, 2002) and has an essential role in protecting the plant from environmental conditions. Different stresses (salinity, heat and drought) (Ashraf and Harris 2004).

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Date of Publication: 24-03-2023

The anatomical traits are no less critical than the phenotypic traits in our present time, and the anatomical studies of researchers were adopted in the classification of plant groups more than a hundred years ago because the anatomical traits are less exposed than the phenotypic traits to the surrounding environmental conditions (Stace, 1989). To isolate different species (Hassoun, 2011).
The current study aims to study the effect of foliar spraying with the amino acid tryptophan and the amino acid glycine on some anatomical characteristics of leaves of kumquat and local lemon seedlings.

Materials And Methods

This study was conducted on seedlings of kumquat and local lemon during the growing season 2021-2022, which amounted to (54) seedlings for each plant type (27) seedlings, two years old, with three replications, planted in small plastic bags, and then transferred to plastic pots with a capacity of (5) kg after mixing them with sandy soil. The mixture before starting the experiment. The seedlings were placed in the cloth canopy. After mixing them well, samples were taken from the soil where the seedlings grew. The soil was analysed to find out the physical and chemical characteristics. The seedlings were sprayed six times with three concentrations (0, 50, 100) mg l-1 of the amino acid tryptophan and its formula. The molecular formula is C11H12N2O2, and its molecular weight is 23.204 g mol-1 (Al-Dawoody, 1990; Taiz and Zeiger 2002), and three concentrations (0, 50, 100) mg l-1 of the amino acid glycine, its molecular weight is 75.05 g mol-1, its molecular formula is C2H5NO2 (Ashraf and Harris 2004; Plimmer 1912).

Table (1) shows some physical and chemical properties of the seedling's soil

Analysis name	rate	unit
Ec	5.54	ds/m
PH	8.13	PPm
Na	200	PPm
K	7.25	PPm
N	233.4	PPm
P	1.62	PPm
soil texture		
sand	%78	g kg
clay	%10	g kg
silt	%12	g kg
texture	Sandy loam	

Anatomical features

The anatomical sections were prepared using paraffin technology according to the method described in Al-Najjar et al. (2021), in which molten and solid paraffin wax is used to make permanent tissue sections and the most important characteristics studied were:
1- Cuticle thickness (µm)
2- Epidermal thickness (µm)
3- The thickness of the tannins layer (µm)
4- Thickness of the columnar cell layer (µm)

Specifications of the microscope used for measurement:
Device name: dissecting microscope
Year of manufacture: 2014
Origin: Germany
Specialization: Agricultural
Device description: general
Scope of use: testing samples
Plant Anatomy Lab - 12019080003003

ISSN NO: 2771-8840
Date of Publication: 24-03-2023

Experiment design

The experiment was designed according to a randomized complete block design (R.C.B.D) as a factorial experiment with three factors (2 * 3 * 3) and three replicates for each treatment. The first factor represents the plant type (kumquat and lemon) and the second factor represents the amino acid tryptophan with three concentrations (0, 50, 100) mg l-1 and the third factor, the amino acid glycine, with three concentrations (0, 50, 100) mg l-1. Thus, the number of experimental units for each plant species is 27 experimental units, and the total number of units for the study is 54 experimental units, and the statistical analysis was conducted For the data of the experiment, the statistical program GenStat was used to analyze the variance, and the Least Significant Difference (L.S.D) test was used to compare the means at a significant level of 0.05, according to what was stated in (Al-Rawi, Khalaf Allah, 2000)

Results and Discussion

Anatomical characteristics of leaves of kumquat and lemon seedlings

The thickness of the cuticle layer

The study results shown in Table (1) and panels (1 and 2) showed the effect of treatment with the amino acid tryptophan and the amino acid glycine on the thickness of the cuticle layer of the leaves of kumquat and lemon seedlings. The highest average thickness of the cuticle layer in kumquat and lemon seedlings leaves was (19.00 and 21.11) micrometres, respectively. At the same time, the comparison treatment recorded the lowest average thickness of the cuticle layer amounted to (10.33 and 13.11) micrometers in the leaves of kumquat and lemon seedlings, respectively, as for the effect of the amino acid glycine It turns out that the treatment of glycine with a concentration of (100 mg l-1) was significantly superior to the rest of the concentrations in the thickness of the cuticle layer. The cuticle layer's highest thickness rate was recorded in the leaves of kumquat and lemon seedlings, which reached (18.667 and 20.33) micrometers, respectively.

The interaction between the treatments of the amino acid tryptophan and the amino acid claspen had a significant effect on the thickness of the cuticle layer of the leaves of kumquat and lemon seedlings, as the treatment of interaction with concentrations (100 mg l-1 of tryptophan and 100 mg l-1 of glycine) was significantly superior to the rest of the interactions and recorded the highest value. The thickness of the cuticle layer was (23.00 and 25.00) micrometers, respectively. At the same time, the treatment of interference with concentrations (0 mg l-1 of tryptophan and 0 mg l-1 of glycine) recorded the lowest values and a significant difference from the rest of the interventions, as it reached (8.667 and 12.67) micrometers sequentially.

Table (1) Effect of treatment with tryptophan and glycine on cuticle thickness of leaves of kumquat and lemon seedlings (micrometre)

kumquat				
tryptophan concentrations **mg l-1**	glycine concentrations **mg l-1**			Tryptophan average
	0	**50**	**100**	
0	8.667	10.333	12.000	10.333
50	13.333	17.000	21.000	17.111
100	15.000	19.000	23.000	19.000
Glycine average	12.333	15.444	18.667	
.L.S.D.0.05	Tryptophan= **0.419**	Glycine = **0.419**	Interaction= 0.726	
lemon				
tryptophan	glycine concentrations **mg l-1**			Tryptophan

ISSN NO: 2771-8840
Date of Publication: 24-03-2023

concentrations **mg l-1**	**0**	**50**	**100**	average
0	12.67	13.33	13.33	13.11
50	14.67	19.00	22.67	18.78
100	17.00	21.33	25.00	21.11
Glycine average	14.78	17.89	20.33	
L.S.D.	Tryptophan=0.723	Glycine =**0.723**	Interaction= **0.940**	

The thickness of the epidermal layer

The study results shown in Table (2) and panels (1 and 2) showed the effect of treatment with the amino acid tryptophan and the amino acid glycine on the anatomical characteristics of the leaves of kumquat and lemon seedlings. It reached (39.22 and 44.67) micrometers, respectively, with a significant difference from the rest of the concentrations, while the comparison treatment recorded the lowest rate of (21.556 and 26.11) micrometers, respectively. As for the amino acid glycine effect, the treatment (100 mg l-1) was significantly superior to the rest of the concentrations. The highest thickness of the epidermal layer was recorded in the leaves of kumquat and lemon seedlings, which were (33.778 and 39.11) micrometers, respectively.

As for the effect of the interaction between the treatments of the amino acid tryptophan and the amino acid glycine, the interaction between (100 mg l-1 tryptophan and 100 mg l-1 glycine) was significant in giving the highest value of epidermal layer thickness in the leaves of kumquat and lemon seedlings, which amounted to (45.00 and 51.00) micrometers sequentially and with a significant difference from the rest of the interactions, as the interaction between the control treatment of tryptophan and the control treatment of glycine recorded the lowest average thickness of the epidermal layer (15.333 and 19.33) micrometers, respectively.

Table (2) Effect of treatment with tryptophan and glycine on the epidermal thickness of kumquat and lemon seedlings (micrometer)

kumquat				
tryptophan concentrations **mg l-1**	glycine concentrations **mg l-1**			Tryptophan average
	0	**50**	**100**	
0	15.333	22.000	27.333	21.556
50	19.000	24.000	29.000	24.000
100	34.000	38.667	45.000	39.222
Glycine average	22.778	28.222	33.778	
.L.S.D.0.05	Tryptophan= **1.347**	Glycine = **1.347**	Interaction= **2.601**	
lemon				
tryptophan concentrations	glycine concentrations **mg l-1**			Tryptophan average
	0	**50**	**100**	

ISSN NO: 2771-8840
Date of Publication: 24-03-2023

mg l-1				
0	19.33	27.00	32.00	26.11
50	23.67	29.00	34.33	29.00
100	39.00	44.00	51.00	44.67
Glycine average	27.33	33.33	39.11	
L.S.D.	Tryptophan= **1.280**	Glycine =**1.280**	Interaction= **2.948**	

The thickness of the tannin layer

The results of the study shown in Table (3) and panels (1 and 2) showed the effect of treatment with the amino acid tryptophan and the amino acid glycine on the thickness of the tannins layer in the leaves of kumquat and lemon seedlings, as the treatment of tryptophan (100 mg l-1) was significantly superior to the rest of the concentrations and recorded the highest rate of layer thickness Tannins in the leaves of kumquat and lemon seedlings reached (39.444 and 43.67) micrometers, respectively. In contrast, the comparison treatment recorded the lowest average thickness of the tannins layer (23.222 and 27.22) micrometers in the leaves of kumquat and lemon, respectively mg l-1 on the rest of the concentrations in the thickness of the layer of tannins. The highest mean of the thickness of the layer of tannins was recorded in the leaves of kumquat and lemon seedlings, as it reached (38.444 and 42.78) micrometers, respectively.

The interaction between the treatments of the amino acid tryptophan and the amino acid claspen had a significant effect on the thickness of the tannin layer of the leaves of kumquat and lemon seedlings, as the interaction treatment (100 mg l-1 of tryptophan and 100 mg l-1 of glycin) was significantly superior to the rest of the interactions and recorded the highest value for thickness. The layer of tannins reached (46,000 and 51.00) micrometers, respectively, while the interference treatment (0 mg l-1 of tryptophan and 0 mg l-1 of glycine) recorded the lowest rates and a significant difference from the rest of the interactions, as it reached (20.667 and 24.33) micrometers, respectively.

Table (3) The effect of treatment with tryptophan and glycine on the thickness of the timeline layer of the leaves of kumquat and lemon seedlings (micrometer)

kumquat				
tryptophan concentrations **mg l-1**	glycine concentrations **mg l-1**			Tryptophan average
	0	**50**	**100**	
0	20.667	23.000	26.000	23.222
50	29.667	36.667	43.333	36.556
100	33.333	39.333	46.000	39.444
Glycine average	27.778	33.000	38.444	
.L.S.D.0.05	Tryptophan= **0.347**	Glycine = **0.347**	Interaction= **2.601**	
lemon				
tryptophan concentrations	glycine concentrations **mg l-1**			Tryptophan average
	0	**50**	**100**	

ISSN NO: 2771-8840
Date of Publication: 24-03-2023

mg l-1				
0	24.33	27.33	30.00	27.22
50	33.00	40.33	47.33	40.22
100	37.33	42.67	51.00	43.67
Glycine average	31.56	36.78	42.78	
L.S.D.	Tryptophan= **0.561**	Glycine =**0.561**	Interaction= **0.971**	

The thickness of the palisade cell layer

The study results shown in Table (4) and panels (1 and 2) showed the effect of treatment with the amino acid tryptophan and the amino acid glycine on the anatomical characteristics of kumquat and lemon seedlings. (92.222 and 112.333) micrometers, respectively, with a significant difference from the rest of the concentrations, while the comparison treatment recorded the lowest rate of (61.222 and 81.111) micrometers, respectively. As for the effect of the amino acid glycine, the treatment (100 mg L-1) was significantly superior to the rest of the concentrations and recorded The highest average thickness of the palisade cell layer in the leaves of kumquat and lemon seedlings was (83.889 and 104.222) micrometers, respectively, while the control treatment recorded the lowest rates, which amounted to (62.667 and 82.778) micrometers, respectively.

As for the effect of the interaction between the treatments of the amino acid tryptophan and the amino acid glycine, the interaction between (100 mg l-1 of tryptophan and 100 mg l-1 of glycine) had a significant effect on giving the highest value for the thickness of the palisade cell layer in the leaves of kumquat and lemon seedlings, which amounted to (99.667; 120.333) micrometers, respectively, with a significant difference from the rest of the interactions, as the interference between the comparison treatment of tryptophan and the comparison treatment of cleacin recorded the lowest rate of a thickness of the palisade cell layer amounted to (48.333 and 68,000) micrometers, respectively.

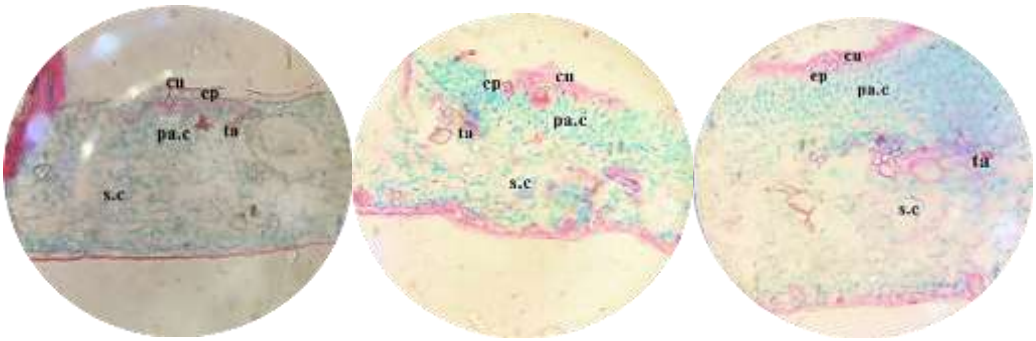
Table (4) Effect of tryptophan and glycine treatment on the thickness of the columnar cell layer of leaves of kumquat and lemon seedlings (micrometer)

kumquat				
tryptophan concentrations **mg l-1**	glycine concentrations **mg l-1**			Tryptophan average
	0	**50**	**100**	
0	48.333	62.333	73.000	61.222
50	54.667	68.000	79.000	67.222
100	85.000	92.000	99.667	92.222
Glycine average	62.667	74.111	83.889	
.L.S.D.0.05	Tryptophan= **0.408**	Glycine = **0.408**	Interaction= **0.707**	
lemon				
tryptophan	glycine concentrations **mg l-1**			Tryptophan

ISSN NO: 2771-8840
Date of Publication: 24-03-2023

concentrations **mg l-1**	**0**	**50**	**100**	average
0	68.000	82.000	93.333	81.111
50	75.333	88.333	99.000	87.556
100	105.000	111.667	120.333	112.333
Glycine average	82.778	94.000	104.222	
L.S.D.	Tryptophan= **0.441**	Glycine =**0.441**	Interaction= **0.763**	

Foliar feeding is one of the best fertilization techniques because it causes high utilization of nutrients and less environmental pollution than ground fertilizer additions to plants. Foliar feeding is the most efficient and economical method compared to other fertilization methods (Al-Shater and Al-Balkhi, 2010). It is noted from the results of the current study that the significant effect of the amino acid tryptophan and the amino acid glycine improves the anatomical characteristics of the leaves of kumquat and lemon seedlings. The reason for this may be due to the combined effect of each of the amino acid tryptophan and the amino acid glycine in increasing the absorption of water and the consequent role in the growth and development of roots and maintaining the water content of the cells compared to seedlings grown under comparison conditions, and this, in turn, may lead to an increase in the activity of cell division, which was reflected positively on the anatomical characteristics of the seedlings by increasing the concentration used through the efficiency of concentration and increasing the different physiological processes of the plant, or that these compounds may stimulate plant growth by improving the absorption of nutrients through produce phytohormone-like effects. It also works to increase the accumulation of nutrients and increase the synthesis of chlorophyll in the leaves (Muslat and Musleh, 2015), as the combined effect of the amino acid tryptophan and the amino acid glycine increases the concentration of ready-made nutrients in the soil, which increases plant absorption of them through Roots and leaves, and this may have been reflected in the improvement of the anatomical characteristics of the leaves (Chen et al., 2004). Also, such substances have a hormonal effect as they affect the cell protoplasm and the cell wall, which leads to rapid cell division and growth, thus increasing plant growth (Samavata and Malakoti 2005). Using biostimulants increases the development of chlorophyll, the accumulation of sugars, amino acids and enzymes, and helps in photosynthesis. It also leads to an increase in the strength of the growth of the root group by increasing the dry and wet weight and the lateral branches of the roots and also works to increase the absorption of nutrients from Before the plant (Hartwigson and Gvans, 2000).



Tryptophan100 mgl Tryptophan50 mgl-1 Control

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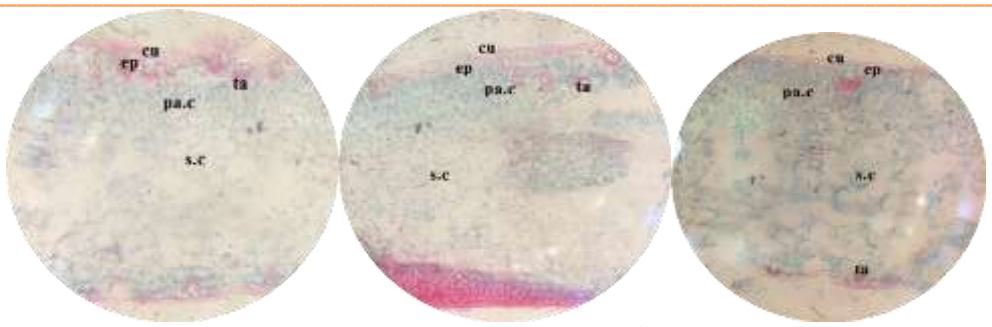
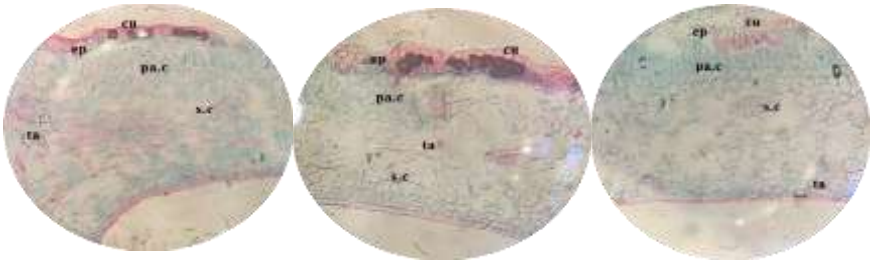
ISSN NO: 2771-8840
Date of Publication: 24-03-2023



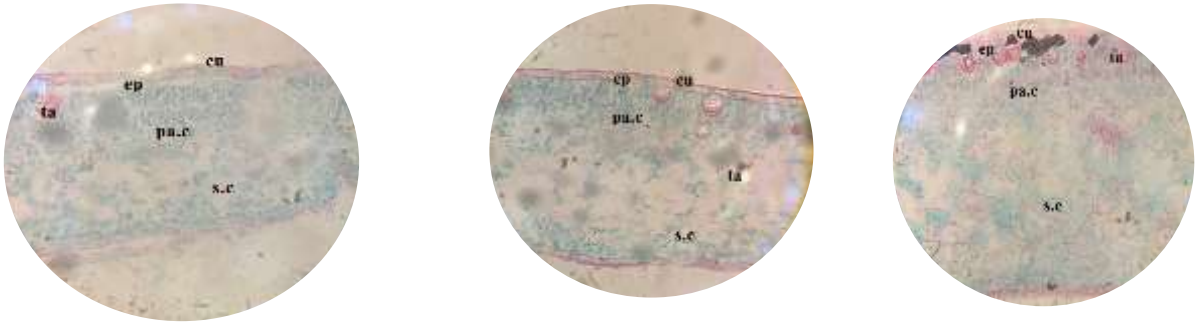
Tryptophan 50XClycine **50 Clycine 100 mg l^{-1}** **Clycine 50 mg l^{-1}**

Panel (1a-) Effect of treatment with tryptophan and glycine on some anatomical characteristics of leaves of kumquat seedlings.
It shows (Cu=cuticle , Ep=epidermis, Ta=tannin cell , Pa.c=palisade cell) 10x
Cu = cuticle layer, Ep = epidermis layer, Ta = tannins, Pa.c = bast cell layer

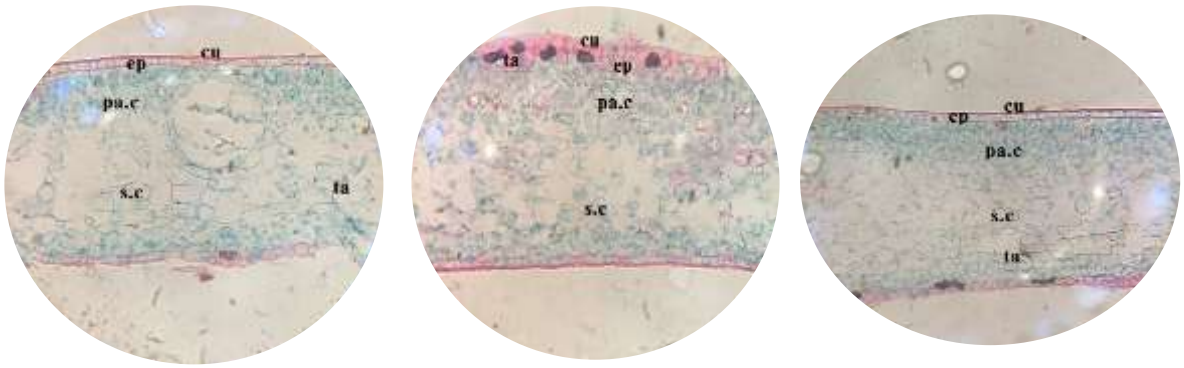


Tryptophan 100 x Clycine 100; Tryptophan 50 x Clycine 100; Tryptophan 100 x Clycine 50

Panel (1b-) Effect of treatment with tryptophan and glycine on some anatomical characteristics of leaves of kumquat seedlings. It shows (Cu=cuticle , Ep=epidermis, Ta=tannin cell , Pa.c=palisade cell) 10x
Cu = cuticle layer, Ep = epidermis layer, Ta = tannins, Pa.c = bast cell layer



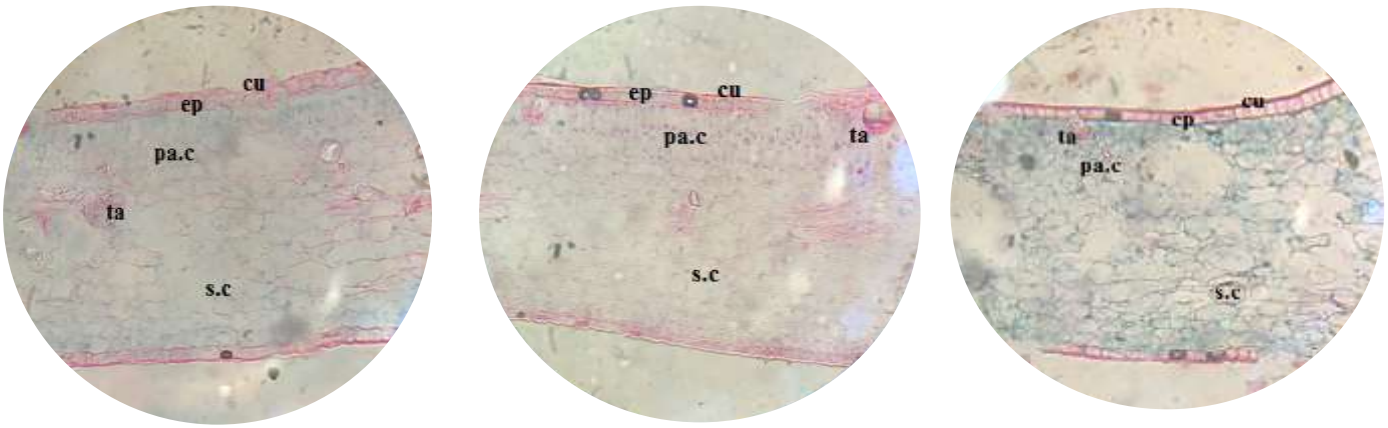
glycine **50 mg l-1** tryptophan **50 mg l-1** Control



Tryptophan 50 x Clycine 50; Clycine 100 mg l-1; Tryptophan 100 mg l-1

ISSN NO: 2771-8840
Date of Publication: 24-03-2023

Panel (2a-) Effect of treatment with tryptophan and glycine on some anatomical characteristics of leaves of lemon seedlings. It shows (Cu=cuticle , Ep=epidermis, Ta=tannin cell, Pa.c=palisade cell) 10x
Cu = cuticle layer, Ep = epidermal layer, Ta = tannins, Pa.c = bast cell layer



Tryptophan 100 x Clycine 100; Tryptophan 100 x Clycine 50; Tryptophan 100 x Clycine 100

Panel (2b-) Effect of treatment with tryptophan and glycine on some anatomical characteristics of leaves of lemon seedlings.
It shows (Cu=cuticle , Ep=epidermis, Ta=tannin cell, Pa.c=palisade cell) 10x
Cu = cuticle layer, Ep = epidermal layer, Ta = tannins, Pa.c = bast cell layer

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