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Botanical and Anatomical Studies On Tabernaemontana Coronaria (Crape Jasmine) Plants.

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Abstract

A pot experimental study was carried out during the season of 2019/2020. The experiment was a factorial involving 3 factors (1) H humic acid H_0 and H_1 added to soil at 0 and 2 g L^{-1}; (2) Boric acid spray B : B_0, B_1 and B_2 solutions of 0 , 2 and 4 mg L^{-1} respectively (3) PP_{333} Paclobutrazol P_0 , P_1 and P_2 foliar solution of 0, 20 and 40 mg L^{-1} respectively (18 treatments 2H x3Bx3 PP_{333}). The heaviest dry weights of leaves/plant was gained by H and 20 ppm pp_{333}-sprayed plants . Applied treatments, particularly PP_{333} at 20 , 40 ppm and B at 2 mg L^{-1} , among these anatomical features were the most important ones, i.e., thickness of leaf midrib, length and width of vascular bundle, phloem and xylem tissues and number of xylem vessels in vascular bundle as well as the leaf blade thickness.

Key words: *Tabernaemontana coronaria*, pot plant, PP_{333}, H , *B*, growth, and histological features…

Introduction

Tabernaemontana coronaria (Synonym: *Ervatamia coronaria*) is a spreading, bushy, many-branched shrub . In general, this spreading, bushy shrub grows to a height of 6 to 10 feet tall and 5 to 8 feet wide. It has oblong leaves with wavy margins that are dark green above and pale green beneath. The flowers are doubled- petaled, fragrant, white, and waxy at 1–5 cm in diameter Pushpa *et al*., 2011). Humic acid (H) exists in arable soils and is derived from soil humus; it is a microbial metabolized organic matter and constitutes over 60% of soil organic matter. It is used as a plant bio-stimulant (Peña-Méndez et al., 2005)

Boron (B) it is one of the important plant micronutrient and plays a significant role in physiological and biochemical processes. The primary function of B in plant cell wall structural integrity,Also boron is needed to the crop plants for cell division, nucleic acid synthesis, and uptake of calcium and transport of carbohydrates(Korkar, .2003). Boron also plays an important role in flowering and fruit formation (Marschner, 1995). In several plant functions, B is implicated directly and indirectly as it involves in growth of cells in newly emerging shoot and root while in some plants it is crucial for all formation, flowering, pollination, and seed development.

Paclobutrazol(PP_{333}) is a growth retardant; Suppression of growth by Paclobutrazol occurs because the compound blocks three steps in the terpenoid pathway for the production of gibberllins by binding and inhibiting the that catalyase enzymes and metabolic reaction(Ruter,1996). One of the main roles of gibberellins in plants is the stimulation of cell elongation. The morphological response to paclobutrazol is the reduction in internode length and this effect has been observed in herbaceous (Bekheta *et al*., 2003).

Material and Methods

This study aim to determine the effects of humic acid .boron and paclobutrazol on growth,flowering and anatomical studies of Tabernamontana.A pot experiment was conducted during 2019 - 2020 season to study the effect of humic acids (H), Paclobutrazol (PP_{333}) and boron (B) on *Tapernamontana* plants .The design was a randomized complete block, factorial. Factors of the experiment and their treatments were: Factor 1: humic acid (H) i.e. H_0 and H_1 : (0 and 2g $L^{-1)}$ irrigation water respectively .Factor 2: Boron(B) i.e. B_0, B_1 and B_2 : foliar spray with 0 , 2 and 4 mg L^{-1} respectively. Factor 3: Paclobutrazol (PP_{333}):i.e. P_0,P_1 and P_2 foliar spray with 0, 20 and 40 mgL^{-1} spray solution.

Plants were grown in PVC pots of 10kg soil pot^{-1} . Soil of the pots was a mixture of a clay soil: sand: peat moss (with the ratio of 2:1:1 by volume respectively).Table 1 shows the main properties of the clay soil used in the experiment.Soil analysis was done by methods cited in(Black et al .,1965)

This experiment included the following transactions:

1-water only (control)
2-pp_{333} (20_{ppm})
3-pp_{333}(40_{ppm})
4-Boron 2 mgL^{-1}
5-pp_{333}(20_{ppm}) + Boron 2mgL^{-1}
6- pp_{333}(40_{ppm})+ Boron 2mgL^{-1}
7- Boron 4 mgL^{-1}
8- pp_{333}(20_{ppm}) + Boron 4 mgL^{-1}
9- pp_{333}(40_{ppm})+ Boron 4 mgL^{-1}
10-Humic acid 2gL^{-1}
11- pp_{333} (20_{ppm}) + Humic acid 2gL^{-1}
12-pp_{333}(40_{ppm}) + Humic acid 2gL^{-1}
13- Boron 2 mgL^{-1}+ Humic acid 2gL^{-1}
14- pp_{333}(20_{ppm}) + Boron mgL^{-1} + Humic acid 2gL^{-1}
15- pp_{333}(40_{ppm})+ Boron mgL^{-1} + Humic acid 2gL^{-1}
16- Boron 4 mgL^{-1}+ Humic acid 2gL^{-1}

17- $pp_{333}(20_{ppm})$ + Boron 4mgL^{-1}mgL^{-1} + Humic acid 2gL^{-1}

18- $pp_{333}(40_{ppm})$+ Boron 4 mgL^{-1}+ Humic acid 2gL^{-1}

Plants were grown in PVC pots of 5kg soil/ pot^{-1} . Soil of the pots was a mixture of a clay soil: sand: peat moss (at a ratio of 2:1:1 by volume).Table 1 shows the main properties of the clay soil used .Soil analysis was done by methods cited in Black et al (1965) .

Table 1: Main properties of soils of the seasons 2019 -2020.

Soil particle size distribution and texture

	Unit	Season	
		2019	2020
Coarse sand	%	14.15	15.65
Fine sand	%	13.98	13.86
Silt	%	16.65	16.84
Clay	%	55.22	53.65
Texture		Clay	Clay

Chemical properties

Parameter	Unit	Season	
		2019	2020
Organic matter	gkg^{-1}	15.7	23.3
Available N	mgkg^{-1}	65	70
Available P	,,	9.0	9.0
Available K	,,	50	50
CaCo3	gkg^{-1}	5.5	5.7
Fe	mgkg^{-1}	12.8	20.0
Zn	,,	4.3	4.8
Mn	,,	5.6	5.7
Cu	,,	2.9	2.5
B	,,	2.0	5.0
pH		7.8	7.5

Notes: Extracts (for available nutrients): KCl (K);$NaHCO_3$(P);NH_4Ac (N), hot water(B) ; DTPA(Fe,Mn.Zn,Cu)

The humic acid (obtained from Agrolink Agricultural Co., Roxy, Cairo, Egypt) was added to the irrigation water at a concentration of 2 gL^{-1} . Boron (B) (used in the form of boric acid;170 g B mg^{-1}) as well as Paclobutrazole (PP_{333}) was obtained from Sigma Aldrich .Thus there were 18 treatments combinations (2 H X 3 B X 3 PP_{333}) .Treatments were and three replicates . All pots were supplied with NPK fertilizers by means of nutrients in irrigation water of 30/24/28 mgL^{-1} NPK as salts of $(NH_4)_2SO_4$ (3 gN $^{/p1}$) and KH_2PO_4 (2 g P, 2 g K $^{/p1}$) .

Sampling and collecting data:

1- Growth characteristics:

Growth traits and characteristics were measured at 90 days after transplanting. Five plants from each pot were randomly taken for measurements. The following characteristics were inspected:

- Size of the root system according to Hanson and (Churchill ,1968). Roots were gently obtained by washing the soil round them under flow current of tap water.
- Root height(cm)
- Number of leaves /plant^{-1}.
- Dry weight of leaves (g plant$^{-1)}$.

Samples were then dried in oven at 60^{o} C for 72 hours till weight stability and dry weight was measured.

4-Anatomical study:

As a pioneer study; regarding the internal morphology of growth retardants treated plants. It was intended to carry out a comparative microscopical examination on leaves of treated plants and to compare them with the control.

For *Tabernamontana* specimen of leaves was taken from the newest mature leaf after one month from the last applied treatment. These vegetative specimens were then killed and fixed in FAA (10 ml formalin: 5ml glacial acetic acid: 85 ml ethyl alcohol 70%), washed in 50% ethyl alcohol, dehydrated in a series of ethyl alcohols 70, 90, 95 and 100%, infiltrated in xylene, embedded in paraffin wax with a melting point 60-63^{o}C, sectioned 20 microns in the thickness for the leaf (Sass, 1951), stained with the double stain method (fast green and safranin), cleared in xylene and mounted in canada-balsam (Johnson, 1940). Sections were read to detect histological manifestations of noticeable responses resulted from treating with the growth retardants under studies.

Results and discussion.

All applied materials caused effects on root lenght . The lowest weight was 14.00cm pot^{-1} it given by the $H_0B_2P_1$ treatment ,i.e. plants receiving high boric acid concentration without applying humic acid and Paclobutrazol (pp_{333}) .The highest of 35.00cm pot^{-1} was given by the $H_1B_0P_1$ treatment ; i.e. plants receiving humic acid and low $pp_{333}$with non boric acid application increase was 15% over the lowest one , pp_{333} at its low dose combined with humic acid causes a considerable increase in the root lenght.

The main effect of humic acid addition gavin an increase about 14.1% . However the positive effect of humic acid occurred only in presence of the pp_{333}material indicating that the Paclobutrazol material encourage positive effect of humic acid these obtained by (Abead et al .,2018).

Boric acid as foliar sprying decreases 33.3 and 3.8 % when applying at low and high concentration , respectively. The negative effect of boric acid was particularly marked under absence of pp_{333} Shahid et al ., (2019).

Paclobutrazol as foliar sprying showed decreases by 4.3% and 11.9 % when applying at low and high dose of pp_{333} respectively .These positive effect of pp_{333} was more pronounced when humic acid was applied .It also more marked under these conditions for low dose of boric acid(Ghatas , 2016) .

All applied treatment positive effects on leaf fresh weight. The lowest weight was 15.00 g/pot^{-1} by the $H_0B_2P_1$ treatment ,i.e. plants receiving high boric acid and low Paclobutrazol (pp_{333}) without applying humic acid .The highest was 44.50 pot^{-1} by the $H_1B_0P_2$ treatment ; i.e. plants receiving humic acid and high pp_{333} with no boric acid : this increase of 196% over the lowest one. This shows that pp_{333} at its high dose combined with humic acid causes a significant increase in the number of leaves,all these datd indicating in table(1)

Application of humic acid was increase number of leaves by 3.9 % . However the positive effect of humic acid occurred only in presence of the pp_{333}treatment indicating positive effect of humic acid(Feleafel et al ., 2019).

Boric acid as foliar sprying decreases leaves number by 36.9 and 12.9 % when applying at low and high dose ,respectively. The negative effect of boric acid was particularly marked under absence of pp_{333} (Maneesh et al ., 2018).

Paclobutrazol spray showed an increases about 27.8 % and 15.5% for leaves number when applying at low and high dose of pp_{333} respectively positive effect of pp_{333} more pronounced than humic acid when it applied Also it was more marked under conditions of the low dose of boric acid (Noor El-Deen .,2020) .

Different applied treatments caused effects on leaf fresh weight. The lowest weight was 1.31 g/ pot^{-1} by the $H_0B_1P_0$ treatment ,i.e. plants receiving low boric acid without applying humic acid and Paclobutrazol (pp_{333}) .The highest was 6.00 g/ pot^{-1} by the $H_1B_0P_2$ treatment ; i.e. plants receiving humic acid and high pp_{333} without boric acid : an increase of 358% over the lowest one. pp_{333} at high dose combined with humic acid causes a considerable increase in the weight of leaves.

Humic acid application increase by 91.2% . However the positive effect of humic acid occurred only in presence of the pp_{333}treatment indicating encourage positive effect of humic acid(Faisal et al ., 2019).

Boric acid as foliar sprying decreases by 38.9 and 40.1 % when applying at low and high dose ,respectively. The negative effect of boric acid was particularly marked under absence of pp_{333} (EL-Mahmoudy et al ., (2019).

Paclobutrazol showed an average increases by 30.2 and 30.9 % when applying at and high dose of pp_{333} respectively .This positive effect of pp_{333} was more pronounced when humic acid appliecation . Also it was also more marked at the low dose of boric acid(Sharaf-Eldien et al., 2017) .

Data in Tables (4&5) clearly indicate that the effect of different applied treatments on different anatomical features of *Tabernaemontana coronaria* leaves. In this respect, most of the anatomical features measeurments of leaf anatomy were increased with different applied treatments, particularly PP_{333} at 20 ppm combined with humic acid and Boron at 4mg/L . Among these anatomical features were the most important ones, i.e., thickness of leaf midrib, length and width of vascular bundle, phloem and xylem tissues part and number of xylem vessels in the vascular bundle as well as the leaf blade thickness. It could be noticed that each of B at 4mg/L and pp_{333} at 20 ppm each separately or in combined were most pronounced treatment.

As regards, the mean thickness of collenchyma layers below the upper epidermis at midrib and the mean thickness of collenchyma layers above the upper epidermis at midrib it could be noticed that both thickness were increased in case of assigned treatments when compared with the control .These increases were slightly existed in case of pp_{333} at 20 ppm separately or in combined with B at 4mg/L treatment and more obvious in case of other assigned treatments . For the mesophyll tissue, thickness of both spongy and palisade tissues were increased with different applied treatments. Here, spongy tissue thickness was 135 µ in the control but increased to reach 200 and 185 micron with PP_{333} at 40 ppm combined with B at 4mg/L and H2g/l , respectively. Also, palisade tissue thickness was 40 for control but increased to reach 50,61 and 62µ with PP_{333} at 20 ppm separately or combined with H 2g/l , B at 4 ppm combined with H, respectively. In case of, the length and the widest of large midrib vascular bundle it could be noticed that they were increased with different

assigned treatments to reach there maximum in case of pp333 at 20 ppm +H. . Also, the thickness of each of upper and lower epidermis, were also increased with most applied treatments. For mesophyll tissue .In general, increases of vascular bundle components thickness (i.e., xylem and phloem); increases of vascular bundles number and the number of xylem vessels all of these treaties are very important for the growth of *Tabernaemontana coronaria* Plant .In which that means the different condition needed for the vigorous growth has been achieved by these applied treatments. These results of Paclobutrazol (PP_{333}) are in conformity with those obtained Youssef (2004) on *Strelitzia reginae*, Tekalign *et al*., (2005) on *Solanum tuberosum*, Kishorekumar *et al.,* (2006) on Solenostemon rotundifolius Gopi *et al.,* (2009) on *Ocimum sanctum* and Youssef & Abd El-Aal (2013) on *Tabernaemontana coronaria*. The abovementioned results of Boron are in harmony with those attained by Habiballah et al ., (2020) on(*Rosa hybrida* L) and April et al ., (2014) on the *Zea mays* .

Also, the previously mentioned and discussed results of *Tabernaemontana coronaria* leaflet anatomy of treated plants, reveal that increasing of leaf anatomy features compared with the control confirmed by vigorous growth of *Tabernaemontana coronaria* may be positively correlated with photosynthesis pigments, carbohydrates and total sugars content. This confirmed the previously discussed results of anatomy and growth, proved that the best morphological behavior of plants as affected by the applied treatments was mainly due to their inducible best morphological and anatomical performances.

Table 1 . Effect of Boric acid,Paclobutrazol and Humic acid on Root length(cm) of *Tabernaemont*ana *coronaria* (Crape jasmine) plants :

Root length(cm)					
H	B	**PP_{333}**			
		P_0	P_1	P_2	Mean
	B_0	24.50	20.00	23.50	22.66
H_0	B_1	21.50	26.00	21.00	22.83
	B_2	14.50	14.00	15.50	14.66
mean		20.16	20.00	20.00	20.05
	B_0	25.00	35.00	24.00	26.33
H_1	B_1	19.50	32.00	21.50	24.33
	B_2	22.00	16.00	16.00	18.00
mean		22.16	26.00	20.50	22.88
G mean		21.16	23.00	20.25	
			Means of B		
B_0		24.75	25.00	23.75	24.50
B_1		20.50	29.00	21.25	23.58
B_2		18.25	15.00	15.75	16.33
LSD 0.05 : H:1.63 B:1.99 P:1.99 HB:NS BP:3.45 HP:2.82 HBP:4.88					

Treatment designations: Paclobutrazol P_0 , P_1 and P_2 sprayed as solution of 0, 20 and 40 mg L-1 respectively ; B_0, B_1 and B_2 sprayed boric acid solution at 0 ,2 and 4 mg L-1 respectively ; H_0 and H_1 humic acid added to soil at 0 and 2 g /L respectively.

Table 2 : Effect of Boric acid,Paclobutrazol and Humic acid on Leaves number(plant pot^{-1}) of *Tabernaemont*ana *coronaria* (Crape jasmine) plants .

Leaves number(plant pot^{-1})					
H	B	P			
		P_0	P_1	P_2	Mean
	B_0	25.50	23.00	31.50	26.66
H_0	B_1	21.50	36.50	36.00	31.33
	B_2	21.50	15.00	22.00	19.50
Mean		22.83	24.83	29.83	25.83
	B_0	26.50	38.50	44.50	36.50
H_1	B_1	22.00	24.00	25.00	23.66
	B_2	23.00	18.00	20.00	20.33
Mean		23.83	26.83	29.83	26.83
G mean		23.33	25.83	29.83	

		Means of B		
B_0	26.00	30.75	38.00	31.58
B_1	21.75	30.25	30.50	27.50
B_2	22.25	16.60	21.00	19.91

LSD 0.05 : H:2.42 B:2.96 P:2.96 HB:4.19 BP:5.13 HP:NS HBP:7.26

Treatment designations: Paclobutrazol P_0 , P_1 and P_2 sprayed as solution of 0, 20 and 40 mg L-1 respectively ; B_0, B_1 and B_2 sprayed boric acid solution of 0 ,2 and 4 g L-1 respectively ; H_0 and H_1 humic acid added to soil at 0 and 2 g kg-1 respectively.

Table 3: Effect of of Boric acid,Paclobutrazol and Humic acid on Leaves dry weight (g pot^{-1}) of *Tabernaemont*ana *coronaria* (Crape jasmine) plants .

H	B	Leaves dry weight (g pot^{-1}) P			
		P_0	P_1	P_2	Mean
	B_0	3.14	4.29	4.40	3.94
H_0	B_1	1.31	4.58	2.99	2.96
	B_2	2.19	2.47	2.32	2.32
Mean		2.21	3.78	3.24	3.07
	B_0	3.96	4.39	6.00	4.78
H_1	B_1	1.66	2.61	2.50	2.26
	B_2	3.82	2.45	2.74	3.00
Mean		3.14	3.15	3.75	3.35
G mean		2.68	3.46	3.49	
		Means of B			
B_0		3.55	4.34	5.20	4.36
B_1		1.48	3.59	2.75	2.61
B_2		3.00	2.46	2.53	2.66

LSD 0.05 : H:NS B:0.76 P:0.76 HB:1.08 BP:1.23 HP:1.08 HBP: NS

Treatment designations: Paclobutrazol P_0 , P_1 and P_2 sprayed as solution of 0, 20 and 40 mg L-1 respectively ; B_0, B_1 and B_2 sprayed boric acid solution of 0 ,2 and 4 g L-1 respectively ; H_0 and H_1 humic acid added to soil at 0 and 2 g/L-1 respectively.

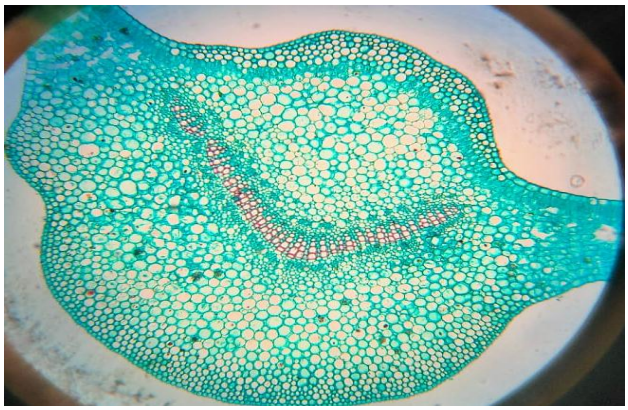
Table 4 : Effect of Boric acid , Paclobutrazol and Humic acid on the mean counts and measurements of certain histological features of *Tabernaemontana coronaria* during 2019 season.

Treatments	Upper epidermal thickness	Lower epidermal thickness	Palisade tissue thickness	Spongy tissue thickness	Mesophyll tissue thikness	Thickness of collenchyma layers below the upper epidermis at midrib
H (0) Control	20	25	40	135	175	110
PP_{333} at 25 ppm(1)	25	20	50	100	150	50
$PP_{333\,(1)}$+B at 2cm/L (1)	25	20	45	170	215	110
B at 4 cm/L (2)	15	20	40	200	240	100
PP_{333} at 40 ppm(2)+ B(2)	20	15	35	140	175	100
H (1)at 2g/L	21	15	40	200	240	80
PP_{333} (1) +H 1	15	25	61	175	236	100
PP_{333} (1)+B (1)+H (1)	10	20	35	165	200	50

B(2) +H(1)	20	26	62	200	262	105
PP_{333} (2) +B(2) +H(1)	16	18	40	185	225	100

Table 5 :Effect of Boric acid, Paclobutrazol and Humic acid on the mean counts and measurements of certain histological features of *Tabernaemontana coronaria* during 2019 season.

Treatments	Characters					
	Thickness of collenchyma layers above the lower epidermis at midrib	Thickness of xylem in vascular bundle	No. of xylem vessels in vascular bundle	Thickness of widest xylem vessel in vascular bundle	Thickness of phloem in v. bundle	Length of large midrib vascular bundle
H (0) Control	50	20	4	15	40	1450
PP_{333} at 25 ppm(1)	50	20	3	12	50	1430
$PP_{333\,(1\,)}$+B at 2cm/L (1)	60	30	5	20	45	1730
B at 4 cm/L (2)	40	20	4	11	65	1780
PP_{333} at 40 ppm(2)+ B(2)	80	22	5	14	40	1800
H (1)at 2g/L	30	25	4	15	55	1740
PP_{333} (1) +H 1	100	120	5	13	32	2090
PP_{333} (1)+B (1)+H (1)	49	100	4	30	52	1600
B(2) +H(1)	70	100	5	30	54	1700
PP_{333} (2) +B(2) +H(1)	45	35	5	20	50	1800



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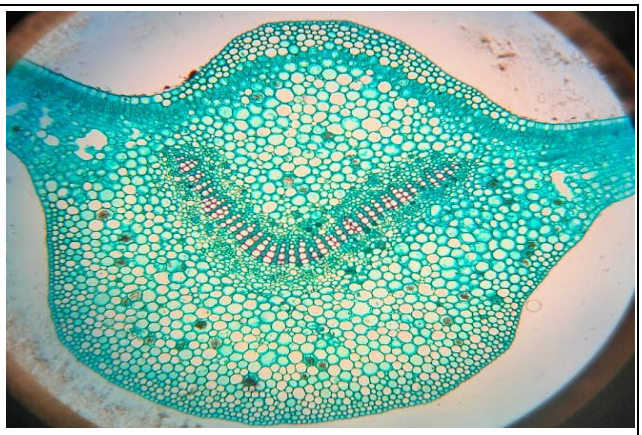

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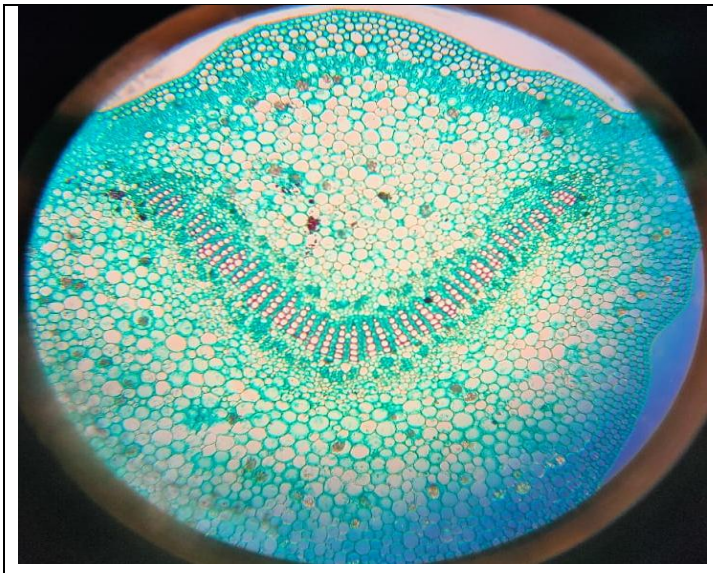

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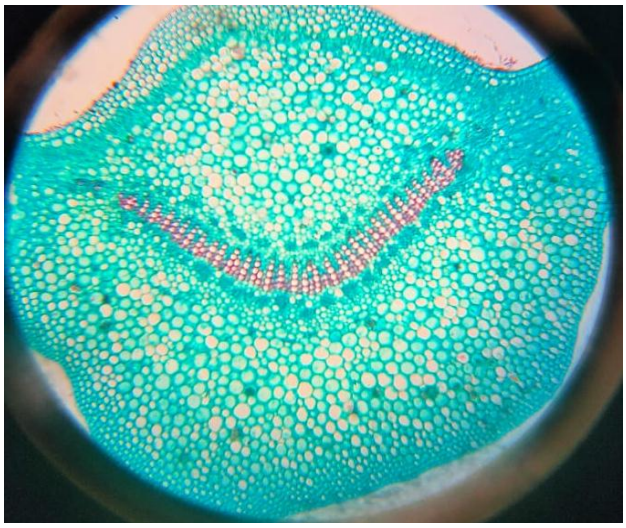

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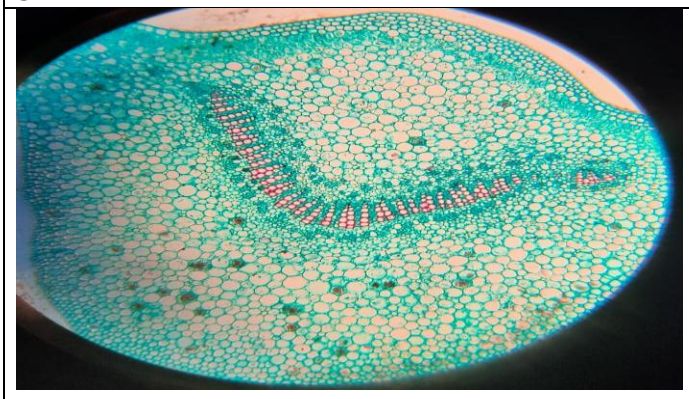

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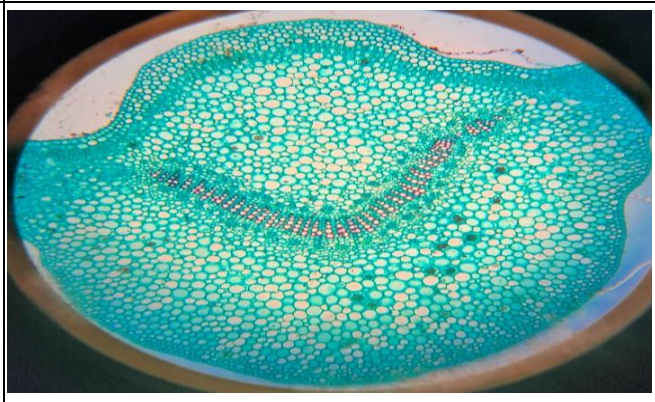

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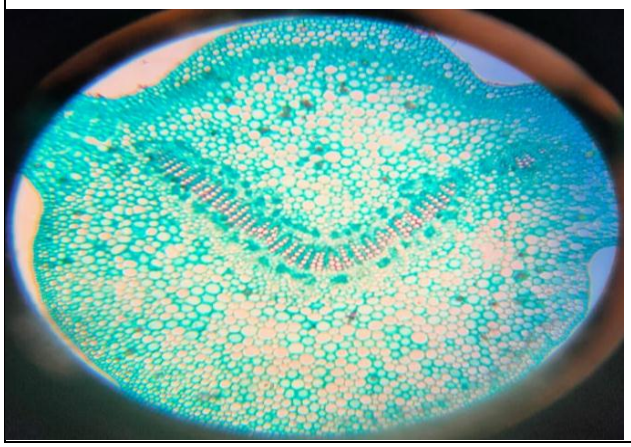

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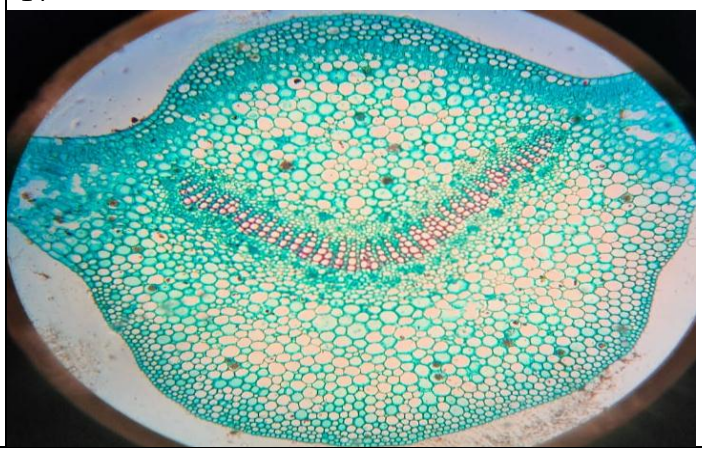

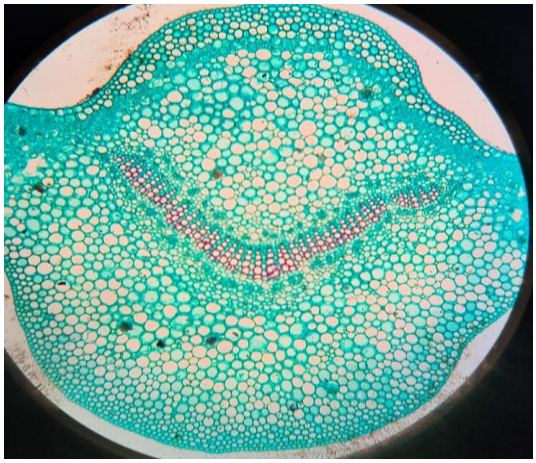

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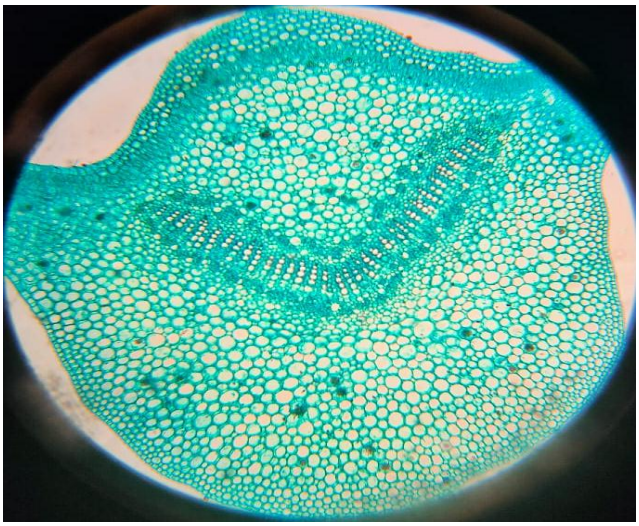

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دراسات نباتيه وتشريحيه على نبات التابرنامونتانا (كريب ياسمين)

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اجريت تجربة اصص خلال موسم 2020/2019 على نبات التابرنامونتانا .التجربه عاملية مكونه من ثلاث عوامل وهم :الهيومك اسيد مضافا للتربه بتركيز 2جم/لتر والباكلوبيوترازول بتركيزات 0,20,40 جزء فى المليون والبورون بتركيزات 0,2,4 ملجم/لتر رشا على الاوراق . وبذلك تضمنت التجربه 18 معامله (2 هيومك *3 باكلوبيوترازول*3 بورون) ولقد لوحظ زيادة فى الوزن الطازج للاوراق وكذلك القياسات الخضرية الاخرى وذلك باستخدام المعاملات ,الباكلوبيوترازول 20 و 40جزء فى المليون منفردا او مجتمعا مع البورون بتركيز 4ملجم/لتر وذلك فى وجود الهيومك اسيد وهذه المعاملات اثرت بدورها فى الصفات التشريحية للاوراق اهمها سمك النصل وطول وعرض الحزمه الوعائية وكذلك الخشب واللحاء .

الكلمات الاساسية: نبات التابرنامونتانا –ا لباكلوبيوترازول – البورون – الهيومك اسيد – الصفات التشريحية .