J. Plant Production, Mansoura Univ., Vol. 10 (3): 283 - 291, 2019

Growth, as well as Leaf and Stem Anatomy in Periwinkle Plant as Affected by Certain Biotic and Abiotic Elicitors

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ABSTRACT

Catharanthus roseus (L.) G. Don is a potential source of several pharmaceutically materials used in cancer treatment. There is a growing demand for its alkaloids that are biosynthesized naturally in a very low quantity. The current study aimed to enhance plant growth and improved internal structure by using certain elicitors. The elicitors tested were calcium chloride ($CaCl_2$) at 4.4 g/ l^{-1}, sodium nitroprusside (SNP) at 0.149 g/l^{-1}, salicylic acid (SA) at 0.138 g/l^{-1} singly or in combination with yeast extract (YE) at either 5 or 10 g/L^{-1}. Pot experiments were done at the experimental farm of Agric. Bot. Dep., Mansoura Univ., Egypt; during the two growing seasons of 2015 and 2016. Results indicated that, tested elicitors positively affected on studied growth attributes, and the magnitude of effect was elicitor- dependent. Stem length as well as stem dry weight and number of leaves were increased in response to YE at 5 and 10 g/l^{-1}, respectively. Under the influence of abiotic elicitors, most growth parameters were increased due to SA and SNP. An additive effects were detected in the combination treatments between either SA or SNP and YE. Stem dry weight was highest in plants treated with SA in combination with YE at 5 g/l^{-1}. In addition, applied elicitors, generally increased proportions of the photosynthesizing tissues in the leaf and the conductive tissues in the stem. The data suggested to using a combined treatment of SA with YE at 5 g/l^{-1} to increase *C. roseus* biomass, consequently its yield from alkaloids.

Keywords: Anatomical studies; Calcium chloride; *Catharanthus roseus*; Elicitors; Salicylic acid;Sodium nitroprusside; Yeast extract.

INTRODUCTION

Periwinkle (*Catharanthus roseus* (L.) G. Don; Apocynaceae, is an important perennial tropical herb that produces over 100 monoterpenoid indole alkaloid including two commercially vital cytotoxic dimeric alkaloids used in cancer chemotherapy (Shukla *et al*., 2012). Nevertheless, tons of its materials are required to extract small quantity of the alkaloid vinblastine (VLB) or vincristine (VCR) (Loyola-Vargas *et al*. ,2007). The latter authors added that, the cost of 1 kg of VLB in the market is around one million Dollars and the world annual production in near about 12 kg. Alternately, VCR cost about 3.5 million dollars per kg and its annual production is 1 kg. The low yield of indole alkaloids, consequently their high cost have stimulated numerous efforts to develop alternative strategies for their production (Ebrahimzadeh *et al*., 1996). Accumulation of secondary metabolites in plant tissues can be enhanced by the treatments of various kind of elicitors (Angelova *et al*., 2006; Dipti *et al*., 2016). Elicitaion classified as biotic and abiotic is a reneawable alternative for secondary metabolite production (Maqsood *et al*., 2017).

Yeast extract (YE) is an autolysate of the cell of baker's yeast (*Saccharomyces cerevisiae*). It has been recognized to be a rich resource of phytohormone, vitamins, enzymes, amino acids and nutrients (Amer, 2004).These constituents elicit plant defense responses by triggering metabolite biosynthesis, (Cai *et al*., 2012), and enhance alkaloid assimilation in *C. roseus*, (Samar *et al*., 2015; Dipti *et al*., 2016). In addition, foliar YE improved growth, and productivity of several plants (Mohamed, Manal *et al*., 2018; Xi *et al*., 2019).

Exogenous application of salicylic acid (SA) enhanced *C. roseus* monomeric and dimeric alkaloids, (Khan *et al*., 2007). SA that induced plant growth under normal or stressed conditions participates in the regulation of multiple physiological processes in plant (Ghassemi-Golezani *et al*., 2017; Kareem *et al*., 2017). Furthermore, Farouk *et al*., (2018) found that salicylic acid enhance leaf area, plant dry mass and plant height in maize.

Sodium nitroprusside (SNP), is a tiny diffusible bioactive signaling molecule, plays multifunctional roles in plant develop and productivity under normal or stressed conditions (Ahmad *et al.*, 2018; Hanafy *et al.*, 2018). Treatment of SNP enhanced the production of terpenoid indole alkaloids of *C. roseus* cells (Xu *et al*., 2005). SNP can mediate stomatal movement (Garcia-Mata and Lamattina, 2007), modulate the expression of cell cycle genes (Correa-Aragunde *et al*., 2006), regulate plant maturation and senescence and the cytokinin signalling pathway (Mishina *et al*., 2007).

Calcium (Ca^{+2}) has been occupied as a second messenger of plant responses to a diversity of external stimuli (Kiegle *et al*., 2000). Supplementation with calcium improved root elongation (Kurth *et al*., 1986) and shoot growth (Amer, 2004). Additionally, Ca^{+2} increased the build- up of C. *roseus* alkaloids in cell cultures (Zhao *et al*., 2001).

The aim of the current study was to assess the enhancing effect of biotic elicitor (yeast extract 'YE' at 0, 5 or 10 g/ l^{-1}), and abiotic elicitors (salicylic acid 'SA' at 0.138 g/l^{-1}, calcium chloride '$CaCl_2$' at 4.4 g/l^{-1}, and sodium nitroprusside 'SNP' at 0.149 g/l^{-1}, beside water) as a foliar application on growth as well as internal structure of the stem and the leaf of *C. roseus*.

MATERIALS AND METHODS

Two pot experiments were done in the experimental farm and laboratories of the Agric. Bot. Dept., Faculty of Agric, Mansoura University, Egypt through the two (2015 and 2016) seasons. Seeds were obtained from the Horticulture Research Institute, ARC, Giza. Egypt, disinfected with 0.01% $HgCl_2$ solution for 3 minutes, washed with distilled water and sown on 14th March in both growing seasons at the rate of 20 seeds/ pot in plastic pots (25 cm inner diameter and 30 cm depth), each pot containing 6 kg air-dried clay loam soil. The physiochemical characteristics of the experimental soil were assessment following the method of to Motsara and Roy (2008) and summarized in Table (1).

Table 1. The physiochemical characters of the experimental soil

Soil properties	1st season	2nd Season	Soil properties		1st season (meq/L)	2nd Season (meq/L)
Clay %	40.3	42.8	Cations	Calcium	1.2	1.1
Silt%	26.5	25.6		Magnesium	1.1	1.1
Fine Sand%	24.7	23.7		Sodium	2.1	2.2
Coarse Sand%	8.5	7.9		Potassium	0.2	0.3
Hygroscopic Water%	5.3	5.2	Anions	Carbonate	----	----
SSP%	56	58		Bicarbonate	1.3	1.2
EC dSm	0.54	0.56		Chloride	2.1	2.4
pH (1:1.5, soil: water)	7.53	7.45		Sulphate	1.2	1.1

Phosphorous as calcium superphosphate (15.5% P_2O_5) at the rate of 2 g/pot (333kg/fed), and 2 g/pot (333 kg/ fed) nitrogen as ammonium nitrate (33.5%N) and 1 g/pot (166 kg/ fed) potassium fertilizer as potassium sulphate (48% K_2O) were mixed into the upper 3 cm of the soil of all pots before planting. Pots were divided into three sets consisting of 20 pots for each, arranged in a randomized complete block design with five replicates. Plants were thinned to leave five plants/ pot at 30 days after sowing (DAS). Plant sprayed with YE, $CaCl_2$, SNP, and SA using hand pressure sprayer. Tween 20 (0.05%) was added as a wetting agent. Control plants were sprayed with tap water. Spraying was take place twice till dripping, the first one at 45 DAS, and whereas the second after two weeks from the first one.

Samples from each treatment were taken randomly after 75 DAS for morphological and anatomical characteristics. Root length and plant height (cm), root and shoot fresh and dry weight (g), numbers of branches and leaves per plant were recorded. For the anatomical studies specimens from the 3rd upper leaf and the 2nd upper internode were taken, killed and fixed for at least 48 hr.in F.A.A. (10 ml formalin, 5 ml glacial acetic acid, and 85 ethyl alcohol 70%). They were washed in 50% ethyl alcohol, dehydrated in a normal butyl alcohol series, embedded in paraffin wax (melting point 56 C°), sectioned by rotary microtome at a thickness of 20 μ, double stained with crystal violet- erythrosine, cleared in xylene and mounted in Canada balsam. Anatomical quantitative data were obtained using calibrate of eyepiece micrometer according to Gerlash (1977).

Statistical analysis:

Data were subjected to analysis of variance according to Norman and Streiner (2003), least significant differences (L.S.D) at 0.05, were used for comparison between the control and the other experimental treatments.

RESULTS AND DISCUSSION

Vegetative growth characters:

Treatment with YE at 5 g/l^{-1} increased plant height and stem dry weight in both seasons whereas decreased number of branches during the second season. It had no significant effect on these parameters during the first season throughout both seasons. At 10 g/l^{-1}, YE increased leaf number during the first season whereas decreased branches number during the second season. Additionally it had no significant effect on plant height (Fig. 1)

Treatment with $CaCl_2$ increased stem fresh weight during the first season whereas decreased root length, numbers of leaves and branches during the second season. Other parameters were not significantly. Treatment with SNP increased leaf fresh and dry weight throughout the experimental period as well as number of leaves, stem fresh weight and root fresh weight, only during the first season. However, SNP decreased number of branches, during the second season only.

The effect of SA on growth parameters was predominant with regard to leaf growth. Number of leaves, as well as leaf fresh and dry weight was also increased. Moreover, SA increased stem fresh weight, and stem length during the second season as well as root fresh and dry weight during the first and the second season, respectively.

The interaction between biotic and abiotic elicitors was significant for most of the studied growth parameters, only during the second season (Table 2, 3). In this case, stem length, number of leaves as well as branches, stem fresh weight, and leaf fresh weight were highest in SA treated plants with 5 g/l^{-1} YE. In addition, the maximum stem dry weight was recorded when treated with SA and combined with YE at 5 g/l^{-1}. Positive interaction effect on growth attributes resulted from the interaction between SNP and YE. Stem fresh weight and leaf fresh weight were also increased due to SNP combined with YE at 5 and 10 g/l^{-1}, respectively. On the other hand, stem dry weight was notably increased in plants received the combination between $CaCl_2$ and YE at 10 g/l^{-1}.

Numerous investigations indicated that spraying plants with YE improved plant growth (Mohamed Manal *et al*., 2018; Xi *et al*., 2019). This enhancement may be attributed to ability of YE to increase the biosynthesis of plant growth regulators, specially, gibberellin that stimulate cell division and enlargement as well as synthesis of proteins, nucleic acid and chlorophyll (George *et al*., 2008; Sarhan 2008). Likewise, Bevilacqua *et al*. (2008) demonstrated that yeast extract contains growth factors, free amino acids which enhance vegetative growth of plants.

Calcium (Ca^{+2}) is an important nutrient that is extremely compartmentalized in plant tissues, and this partitioning is a defining constituents of its biochemical functions. In its structural role, calcium is a key component of the cell wall middle lamella where it helps to join adjacent cells together and strengthen general construction (Marschner, 1995). In addition Ca^{+2} influences membrane function, stabilizes membranes and influences permeability by birding phosphate and carboxylate groups of membrane phospholipids and protein (Davies and Monk-Talbot, 1990).

SNP plays extremely vital roles in improving plant development of several plants by modulating a diversity of metabolic pathways including: (1) scavenging ROS through activating antioxidant system (Rai *et al.*, 2018); (2) acting as a signal molecule induces alteration in the expression of stress-responsive genes (Gill *et al.*, 2013); (3) inhibition of ethylene biosynthesis (Zhu *et al.,* 2008).

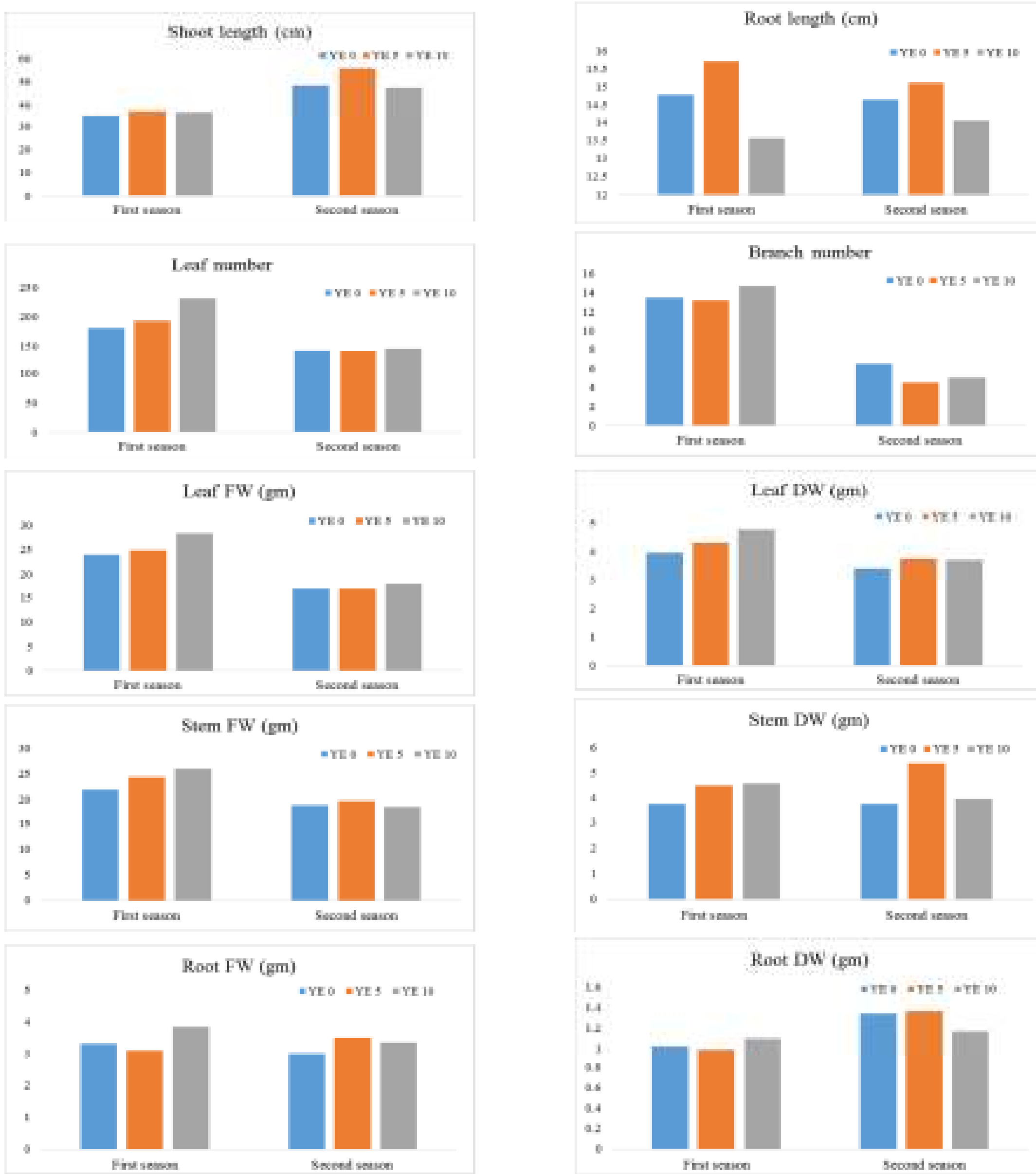

Fig .1. Growth attributes of *C. roseus* as affected by yeast extract (YE) at either 5 or 10 g/ L^{-1} at 75 days after sowing in both seasons.

Table 2. Plant height, root length as well as numbers of leaves and branches of *C. roseus* plants as affected by elicitors at 75 days after sowing during the two growing seasons (2015-2016).

Treatment g/l		Plant height (cm)		Root Length (cm)		No. of leaves		No. of branches	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Interactions									
YE 0	W	29.3	39.3	12.3	14.3	102	115	10	8
	$CaCl_2$	36.3	44.0	17.0	13.0	197	91	13	4
	SNP	38.0	51.0	14.3	17.3	208	128	14	6
	SA	36.6	59.3	16.3	14.0	219	237	19	8
YE 5	W	34.3	52.6	16.6	15.3	176	118	15	4
	$CaCl_2$	36.0	51.3	14.0	12.6	166	69	12	4
	SNP	37.0	59.6	16.3	17.3	218	196	15	4
	SA	42.3	60.3	16.0	15.3	212	175	11	6
YE 10	W	37.3	51.0	13.0	15.6	164	207	12	7
	$CaCl_2$	36.3	48.3	13.0	13.3	242	108	16	4
	SNP	38.0	42.6	13.0	13.3	251	113	14	4
	SA	35.6	48.3	15.3	14.0	268	156	17	5
LSD at 5%		Ns	8.51	Ns	Ns	Ns	35.5	Ns	2.157

W=water, $CaCl_2$= calcium chloride, SNP= sodium nitroprusside, SA= salicylic acid, YE= yeast extract.

Table 3. Fresh and dry weight of *C. roseus* plant organs as affected by used elicitors at 75 days after sowing during the two growing seasons (2015-2016).

Treatment g/l		Leaf FW(g)		Stem FW(g)		Root FW(g)		Leaf DW(g)		Stem DW(g)		Root DW(g)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
		Interactions:											
YE 0	W	11.43	10.81	8.20	13.22	1.73	3.47	2.143	2.68	1.500	2.773	0.700	1.073
	$CaCl_2$	22.73	11.80	23.46	12.84	3.71	1.93	3.643	2.507	4.123	2.880	1.073	1.010
	SNP	28.07	18.32	22.49	11.98	3.92	2.79	4.930	4.083	4.207	4.190	1.193	1.673
	SA	33.40	26.83	33.58	37.80	3.89	3.81	5.163	4.397	5.170	5.097	1.090	1.603
YE 5	W	19.99	15.55	16.23	16.06	2.92	3.06	3.747	3.310	3.900	3.810	0.967	1.100
	$CaCl_2$	24.32	14.76	23.05	17.87	3.00	3.17	4.173	2.780	3.923	3.970	1.030	1.263
	SNP	27.88	18.73	29.30	22.47	3.45	3.82	4.650	4.683	4.883	6.650	0.877	1.050
	SA	27.26	18.29	29.12	22.42	3.05	3.92	4.667	4.18	5.177	7.057	1.060	2.047
YE 10	W	24.27	14.09	24.73	17.41	3.47	3.64	3.833	3.810	4.920	4.217	0.910	1.137
	$CaCl_2$	28.98	18.10	24.57	18.23	3.76	3.20	4.750	2.83	4.023	5.970	1.127	1.160
	SNP	31.29	20.62	28.51	19.64	4.11	3.92	5.427	4.853	4.587	2.613	1.133	1.117
	SA	28.76	18.55	26.49	18.74	4.05	2.65	5.027	3.300	4.720	2.953	1.21	1.243
LSD at 5%		Ns	5.78	Ns	9.437	Ns	Ns	Ns	Ns	Ns	1.963	Ns	Ns

W=water, $CaCl_2$= calcium chloride, SNP= sodium nitroprusside, SA= salicylic acid, YE= yeast extract

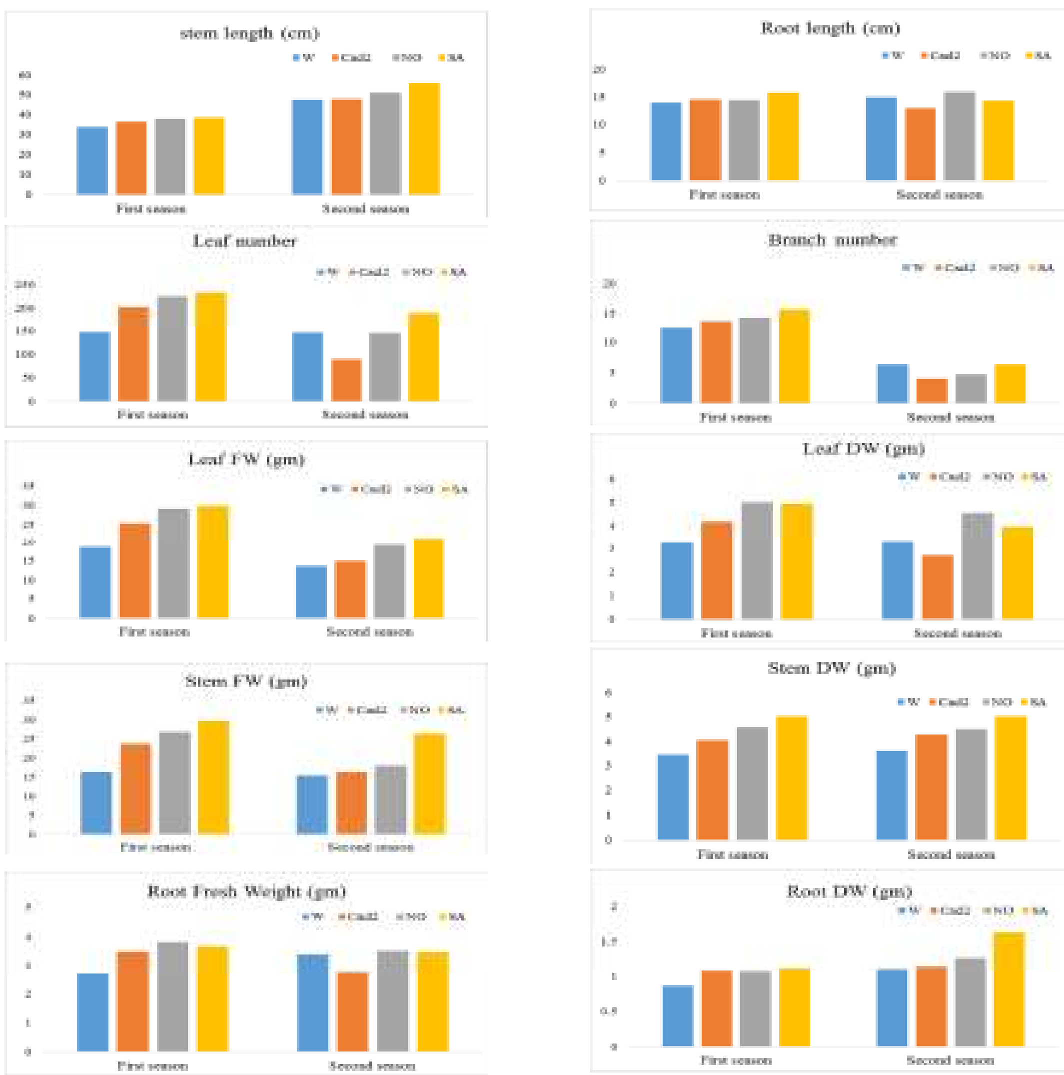

Fig .2. Growth attributes of *C. roseus* as affected by treatment with calcium chloride ($CaCl_2$), sodium nitroprusside (SNP) and salicylic acid (SA) at 75 days after sowing in both seasons

Plant growth enhancement under SA application might be due to inducing biochemical or physiological processes, including cell division, cell differentiation, and morphogenesis (Fardus *et al.*, 2017; Kareem *et al*, 2017). Additionally, the biosynthesis of many enzymes involved in metabolic pathway like glyoxylate cycle, the pentose phosphate pathway and glycolysis was strongly activated by SA, documented that SA enhances the release from a quiescence state to the establishment of a vigorous plant (Rajjou *et al.*, 2006).

The enhancing impact of SA application on plant growth may be resulted from SA- induced physiological processes i.e. improved carbon assimilates, raised biosynthesis of metabolites and maintenance of plant water status (Habibi, 2012). Mitosis and cell enlargement within the apical meristems were enhanced by SA (Sakhabutdinova *et al.*, 2003). Moreover, SA maintained RWC and photosynthetic (Mathur and Vyas, 2007). Moreover, SA spraying accelerating total soluble sugar build up that served as a substrate for enhancing initiation of leaf primordial (Munns *et al.*, 1979). It had inhibit impact on ethylene production by preventative accumulation of ethylene precursor, 1- aminocyclopropane 1- carboxylic acid (ACC) (Li *et al.*, 1992) and blocking ACC oxidase (Leslie and Romani, 1986). Salicylic acid boost leaves auxin and zeatin, in addition gibberellin (Shehata *et al.*, 2000); raised cytokinin level in plant (Sano *et al.*, 1994), additionally raised chlorophyll (Zayed, 1986).

In general, SA may be regulate plant growth by at least one or more of the following mechanisms: (1) decreasing ethylene production hence, increasing chlorophyll as well as preventing the formation of abscisson zone (Leslie and Romani, 1986); (2) protecting plant cell against hazardes of oxygen free radical under stress conditions by activating a new isoenzyme of ascorbate peroxidase, a H_2O_2 scavenging enzyme (Janda *et al.*,1999); (3) increasing the rate of photosynthetic electron transport and/or the photochemical quenching (Tari *et al.*,2002); (4) decreasing transpiration rate, as recorded in *Phaseolus vulgaris* and *Commelina communis* (Larque-Saavedra,1978).

Anatomy of the Leaf:

Without YE application, foliar application of SNP markedly increased leaf blade thickness (25%) due to increasing spongy parenchyma thickness (50%) without any alteration in palisade parenchyma thickness (Table.4 and Fig. 3). The greatest leaf dimension at midrib region was obtained under the application of SNP. Application of $CaCl_2$ gave greatest phloem tissue thickness (33%), and xylem tissue thickness (33%) relative to untreated plants or other elicitors. The same Table and Figure postulated that, application of YE at both concentration obviously declined in most cases all studied anatomical characteristics except the thickness of xylem tissue which increased by 33% and 16% due to application of YE at 5 or 10 g/l^{-1} respectively.

The interaction between YE and used abiotic elicitors, gave highest leaf blade thickness, palisade, spongy parenchyma thickness, dimension of leaf at midrib region and xylem tissue thickness. Similarly the highest palisade parenchyma thickness (50%) due to the application of SA supplemented with YE at 10 g/l^{-1}; and the xylem tissue thickness (50%) was obtained with application of SA with 5 g/l^{-1} YE. However, application of SNP with 5 or 10 g/l^{-1} YE increased phloem tissue thickness (33, 16%) respectively relative to the control.

Table 4. Anatomical characteristics of *C. roseus* leaf as affected by biotic and abiotic elicitors used as well as their interactions at 75 days from sowing during the 2nd growing season.

Parameters		Blade – Thickness		Palisade Parenchyma Thickness		Spongy – Parenchyma Thickness		Dimension of leaf at midrib region: Length		Dimension of leaf at midrib region: Width		Xylem Tissue Thickness		Phloem Tissue Thickness	
Treatments (g/l)		µm	100 % ±	µm	100 % ±	µm	100 % ±	µm	100 % ±	µm	100 % ±	µm	100 % ±	µm	100 % ±
Water		15.84	0	5.93	0	7.41	0	8.91	0	23.76	0	5.94	0	2.97	0
YE 0	$CaCl_2$	17.83	+13	5.93	0	9.26	+25	13.77	+55	32.4	+36	7.92	+33	3.96	+33
YE 0	SNP	19.81	+25	5.93	0	11.11	+50	13.77	+55	28.08	+18	5.94	0	2.97	0
YE 0	SA	13.86	- 13	4.44	- 25	7.41	0	14.17	+59	37.8	+59	8.91	+50	2.97	0
YE 5	Water	11.88	- 25	5.93	0	3.70	-50	11.34	+27	27	+14	7.92	+33	1.98	-33
YE 5	$CaCl_2$	13.86	- 13	4.44	- 25	7.41	0	8.91	0	27	+14	5.94	0	4.95	+53
YE 5	SNP	21.79	+38	7.41	+25	11.11	+50	15.79	+77	33.48	+50	7.92	+33	2.97	0
YE 5	SA	15.84	0	5.93	0	7.41	0	9.71	+9	31.32	+32	8.91	+50	3.96	+33
YE 10	Water	11.88	- 25	4.44	- 25	5.55	-25	12.55	+41	37.8	+59	6.93	+16	2.97	0
YE 10	$CaCl_2$	13.86	- 13	5.93	0	5.55	-25	8.50	-5	20.52	-14	5.94	0	3.96	+33
YE 10	SNP	19.81	+25	7.41	+25	9.26	+25	9.31	+5	23.76	0	6.93	+16	4.95	+53
YE 10	SA	17.83	+13	8.89	+50	5.55	-25	8.91	0	16.2	-32	5.94	0	4.95	+53

W = water, $CaCl_2$= calcium chloride, SNP = sodium nitroprussside, SA = salicylic acid, YE = yeast extract.

Anatomy of the Stem:

The data in Table (5) and Figure (4) recognized that, application of either 5 or 10 g/l^{-1} YE increased stem diameter, epidermis, cortex thickness, vascular cylinder dimension, and phloem thickness regarding YE- free plants. The interaction between biotic and abiotic elicitors, in most cases showed an additive elect on the studied anatomical characteristics. The greatest stem diameter (29%), epidermis, cortex thickness (75%), vascular cylinder dimension (135%), xylem thickness (20%), and phloem thickness (100%) were obtained by Ca foliar application under 5 g/l^{-1} YE, meanwhile the greatest pith diameter (8%) and the big metaxylem vessels diameter (80%) were obtained due to application of Ca with 10 g/l^{-1} YE or SA with 5 g/l^{-1} YE.

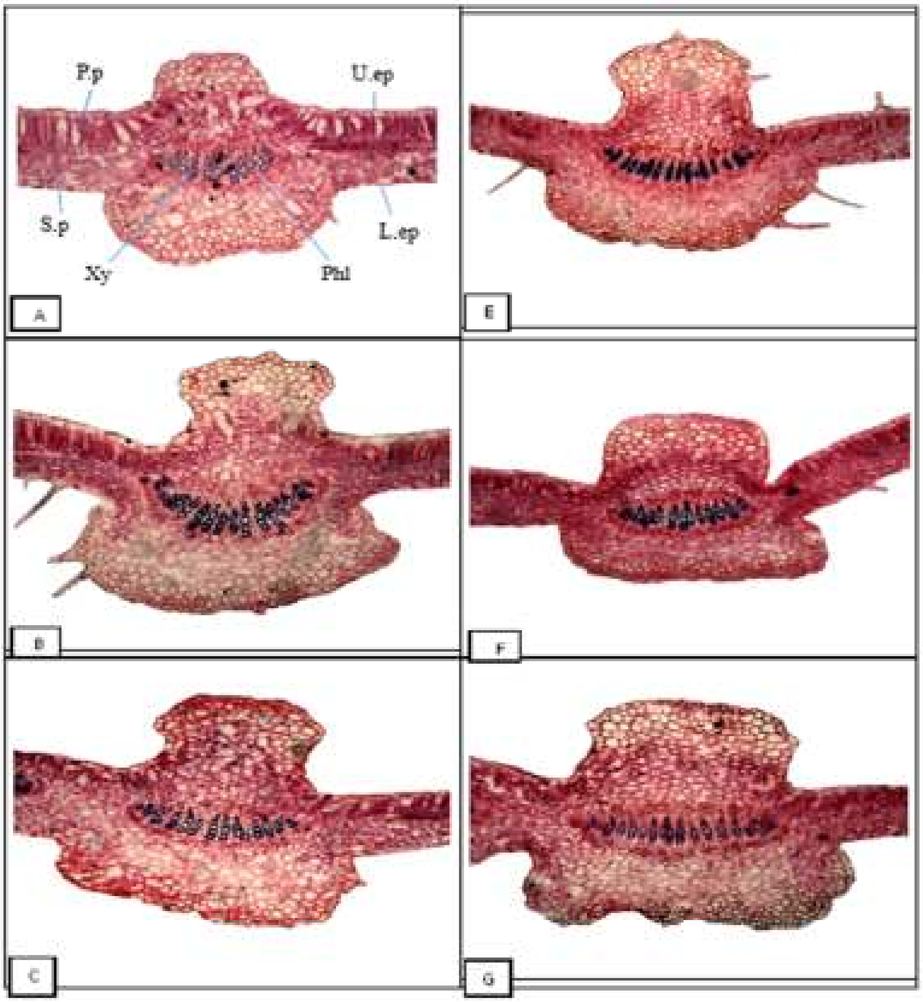

Fig. 3. Cross sections of Periwinkle leaf showing the effect of $CaCl_2$, SNP, SA, yeast extract with or without application.

A = Control B = $CaCl_2$ at 4.4 g/l^{-1} C = SNP at 0.149 g/l^{-1} D = SA at 0.138 g/l^{-1}
E = YE at 5 g/l^{-1} F = YE at 5 g/l^{-1}+ $CaCl_2$ at 4.4 g/l^{-1} G = YE at 5 g/l^{-1}+ SNP at 0.149 g/l^{-1} H = YE at 5 g/l^{-1}+ SA at 0.138 g/l^{1}
U. ep= upper epidermis, L. ep= lower epidermis, P.P= palisade parenchyma, S.P= spongy parenchyma, Xy= xylem, Phl= phloem

Table 5. Anatomical characteristics of *C. roseus* stem as affected by biotic and abiotic elicitors used as well as their interactions at 75 days from sowing during the 2nd growing season.

Parameters		Stem – diameter		Epidermis, Cortex Thickness		Vascular Cylinder-Diameter		Pith – diameter		Xylem-Thickness		Phloem Thickness		Big metaxylem Vessels diameter	
Treatments (g/l)		µm	100 % ±	µm	100 % ±	µm	100 % ±	µm	100 % ±	µm	100 % ±	µm	100 % ±	µm	100 % ±
YE 0	Water	164.50	0	52.50	0	59.50	0	47.25	0	8.75	0	3.50	0	1.265	0
	CaC_2	198.77	+21	39.37	- 25	143.50	+141	43.61	- 8	8.75	0	5.25	+50	2.024	+60
	SNP	171.35	+4	52.5	0	108.50	+82	47.25	0	7.00	-20	5.25	+50	1.265	0
	SA	260.45	+58	78.75	+ 50	159.25	+166	72.69	+ 54	10.50	+20	8.75	+150	1.265	0
YE 5	Water	198.77	+20	65.62	+ 25	106.75	+79	43.61	- 8	8.75	0	7.00	+100	2.530	+100
	$CaCl_2$	212.47	+29	91.86	+ 75	140.00	+135	47.25	0	10.50	+20	7.00	+100	1.771	+40
	SNP	198.77	+20	39.37	- 25	108.50	+82	47.25	0	7.00	-20	7.00	+100	1.518	+20
	SA	205.62	+25	52.49	0	126.00	+112	47.25	0	12.25	+40	5.25	+50	2.277	+80
YE10	Water	205.62	+25	65.61	+ 25	103.25	+74	50.88	+ 8	5.25	-40	5.25	+50	1.012	-20
	$CaCl_2$	205.62	+25	65.61	+ 25	91.00	+53	50.88	+ 8	8.75	0	3.50	0	1.518	+20
	SNP	212.47	+29	78.74	+ 50	91.00	+53	43.61	- 8	7.00	-20	7.00	+100	1.518	+20
	SA	185.06	+12	65.61	+ 25	84.00	+41	36.34	- 23	7.00	-20	5.25	+50	1.710	+35

W= water, $CaCl_2$= calcium chloride, SNP = sodium nitroprussside, SA = salicylic acid, YE = yeast extract

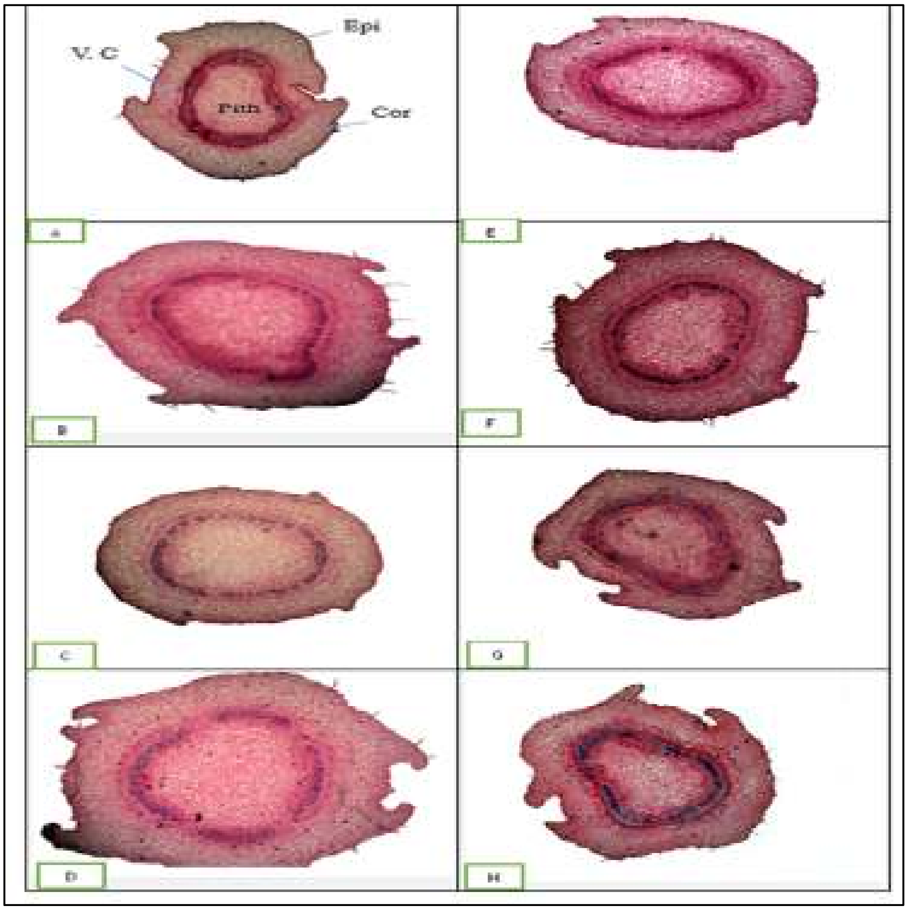

Fig. 4. Cross sections of periwinkle stem showing the effect of $CaCl_2$, SNP, SA, yeast extract with or without application.

A = Control B = $CaCl_2$ at 4.4 g/l^{-1} C = SNP at 0.149 g/l^{-1} D = SA at 0.138 g/l^{-1}
E = YE at 5 g/l^{-1} F = YE at 5 g/l^{-1} + $CaCl_2$ at 4.4 g/l^{-1} G = YE at 5 g/L^{-1} + SNP at 0.149 g/l^{-1} H = YE at 5 g/l^{-1} + SA at 0.138 g/l^{-1}
Epi= epidermis, Cor= cortex, V. C= vascular cylinder

Leaves are principle sites of essential biochemical process, like photosynthetic and transpiration. Anatomical alteration induced by elicitors in higher plants are better observed indicators; they can be directly used in agriculture and handled (Shao *et al*., 2008). Application of both elicitors used improved in most cases all anatomical characteristics of periwinkle plants. Accordingly, foliar spraying with SNP considerably raised all leaf anatomical characters of leaves, i.e. dimension of midrib region due to increasing the dimensions of main vascular bundles (Farouk and Arafa Sally, 2018). Moreover, Farouk (2005) reported that application of either calcium chloride or salicylic acid raised the thickness of the pea leaf blade, by increasing the thickness of mesophyll tissue and the main midrib main vascular bundle. All these promotive effects of elicitors, generally increased the photo assimilate production and increased plant growth.

CONCLUSION

The great periwinkle plant growth can be obtained in this investigation by foliar application of 0.138 g/l^{-1} salicylic acid with 5 g/l^{-1} yeast extract.

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النمو والتركيب التشريحي لأوراق وسيقان نبات الونكا تحت تأثير بعض المحفزات الحيوية والغير حيوية

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تعتبر الونكا مصدرا هاما لعديد من القلويدات ذات الأهمية الدوائية، وبصفة خاصة الفنكريستين والفنبلاستين اللذان يستخدمان في علاج السرطان، والتي يزداد عليهما الطلب عالميا برغم تكوينهما بكميات صغيرة جدا، وقد تم إجراء الدراسة الحالية بهدف زيادة إنتاجية النبات وتحسين نموه وتركيبه الداخلي. واستخدم لهذا الغرض بعض المحفزات الحيوية والغير حيوية مثل كلوريد الكالسيوم بتركيز ٤.٤ جم/ لتر، نيتروبروسيد الصوديوم بتركيز جم/ لتر٠.١٤٩، حامض السالسليك بتركيز ٠.١٣٨ جم/ لتر منفردة أو متداخلة مع مستخلص الخميرة بتركيز ٥ أو ١٠ جم/ لتر. وقد أجريت تجارب أصص في المزرعة البحثية ومعامل قسم النبات الزراعي، بكلية الزراعة، جامعة المنصورة، خلال موسمي نمو (٢٠١٥-٢٠١٦). وأوضحت النتائج بصفة عامة أن المحفزات المختبرة قد أثرت إيجابيا علي صفات النمو المدروسه معتمدة علي نوع المحفز حيث زاد طول الساق، الوزن الجاف له بالإضافة إلي عدد الأوراق للنبات إستجابة للمعاملة بمستخلص الخميرة بتركيز ٥ أو ١٠ جم/ لتر بالتتابع. وكانت معظم صفات النمو مرتفعة عند المعاملة بحامض السالسليك ونيتروبروسيد الصوديوم بالترتيب، وكان التأثير التحفيزي لإضافة العاملين معا واضحا خاصة عند التداخل ما بين أي من حامض السالسليك، أونيتروبروسيد الصوديوم مع مستخلص الخميرة، فقد زاد الوزن الجاف للساق في النباتات المعاملة بحامض السالسليك مع مستخلص الخميرة بتركيز ٥ جم/ لتر. ومن الناحية التشريحية وجد أن إستخدام المحفزات المستخدمة بصفة عامة أدت إلي زيادة نسبة الأنسجة الممثلة للتخليق الضوئي في أنسجة الورقة، والأنسجة الوعائية في الساق، وعليه فإنه لزيادة الكتلة الحيوية لنبات الونكا وكذلك فإنه يوصي بالمعاملة بحامض السالسليك مع مستخلص الخميرة بتركيز ٥ جم/ لتر.