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ORIGINAL ARTICLES

Effect of paclobutrazol and cycocel on growth, flowering, chemical composition and histological features of potted *Tabernaemontana coronaria* Stapf plant

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ABSTRACT

This study was carried out during the two successive seasons of 2011/2012 and 2012/2013 to evaluate the effect of paclobutrazol (PP_{333}) at 0.0, 50, 100 and 150ppm and cycocel (CCC) at 0.0, 1000, 1500 and 2000ppm on growth, flowering, chemical composition and histological features of potted *Tabernaemontana coronaria* plants. The obtained data showed that: All PP_{333} and CCC concentrations decreased plant height and leaf area, especially PP_{333} at 150ppm in both seasons. On contrary, all applied treatments of PP_{333} and CCC significantly increased the number of branches and leaves / plant to reach its maximum with the highest concentration for each. The heaviest fresh and dry weights of leaves/plant were gained by 100ppm pp_{333}-sprayed plants in the two seasons. Also, PP_{333} and CCC treatments delayed the flowering of Tabernaemontana plant when compared with control plants which induced the earliest flowering in both seasons. In addition, number of flowers/plant as well as their fresh and dry weights were significantly increased with PP_{333} and CCC treatments in the two seasons. However, the highest number of flowers/ plant was scored by 150ppm PP_{333}-sprayed plants, whereas the heaviest flowers fresh and dry weights/plant were gained by CCC at 2000 ppm sprayed plants. Moreover, the highest show value (plant width/ height ratio) was existed with PP_{333} at 150 and 100 ppm in the two seasons. The highest number of roots/plant as well as their fresh and dry weights were registered by 2000ppm CCC-sprayed plants in both seasons. Furthermore, PP_{333} and CCC treatments significantly increased leaf N, P, K, total carbohydrates and total chlorophylls contents as compared with un- sprayed plants in the two seasons. As for the endogenous phytohormones, all PP_{333} and CCC treatments increased cytokinins and abscisic acid (µg/g F.W), but they decreased gibberellins and auxins (µg/g F.W) of Tabernaemontana leaves. With regard to the anatomical features of leaf anatomy, most traits were increased with different applied treatments, particularly PP_{333} at 150 and 100 ppm and CCC at 2000 ppm. Among these anatomical features were the most important ones, i.e., thickness of leaf midrib, length & width of vascular bundle, phloem & 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}**,** CCC**,** growth, chemical composition, endogenous phytohormones and histological features.

Introduction

Tabernaemontana coronaria Stapf (Synonym: *Ervatamia coronaria*) is a glabrous, evergreen, dichotomously branched shrub, belonging to the Family Apocynaceae. It is distributed in upper Gangeticplain, Garhwal, East Bengal, Assam, Karnataka, Kerala and in Burma. *Tabernaemontana 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. In Ayurveda, the root is using for kapha, biliousness and the diseases of the blood. The root has a bitter taste. It is aphrodisiac; tonic, especially to the brain, liver and spleen; and purgative. The milky juice mixed with the oil and when rubbed on to the head cures pain in the eye. It is also known to kill intestinal worms and when its root part is chewed, causes the relief in the toothache (Pushpa *et al*., 2011). Also, it has been used in the folk medicine for anti-infection, anti-inflammation, analgesic, anti-tumor, ant- oxidative effect and the effect in neuronal activity (Ghani, 2003).

Controlling plant size is one of the most important aspects of ornamental plants production. Growers can control plant height genetically, environmentally, culturally, or chemically. These techniques can be effective height-suppressing strategies for some plants, but when growers are faced with ornamental plants containing large varieties of genera, species, or cultivars, these techniques may not work equally well for each crop under a common environment. An alternative, effective strategy for controlling plant height is to use chemical plant growth retardants (Chany, 2005). Application of growth retardants is a common practice for commercial growers to achieve attractive compact pot-grown plants.

The terms growth retardants is used for all chemicals that retard cell division and cell elongation in shoot tissues and regulate plant height physiologically without formative effects (PGRSA, 2007). One of the most

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widely used growth retardants is paclobutrazol (pp_{333}) [(2RS,3RS)-1-(4-chlorophenyl-4,4-dimethyl-2-(1H-1,2,4 triazol-1-yl) pentan-3-ol] is a well-known plant growth retardant (Davis and Andersen, 1989). Paclobutrazol functions by inhibiting cytochrome P-450, which mediates oxidative dimethylation reactions, including those which are necessary for the synthesis of ergosterol and the conversion of kaurene to kaurenoic acid in the gibberellins biosynthetic pathway (Fletcher *et al*., 2000). From this function, paclobutrazol has long been used to reduce plant height for potted plant production, particularly ornamental plants (Beattie *et al*., 1990; Fletcher *et al*., 2000). Paclobutrazol at concentration of 35 mg a.i./pot reduced plant foliage height and flower stem length, without affecting inflorescence length and delaying the production of potted Thai Tulip (Pinto *et al*., 2006).

Cycocel (CCC) (chlormequat; 2-chloroethyltrimethyl ammonium chloride) is a synthetic plant growth retardant used on ornamental plants for inducing dwarfism in plants and shorter internodes, stronger stems and green leaves . It is also utilized in order to produce compact, sturdy potted and bedding plants, enhance the green colour of the foliage, strengthen flower stem and promote resistance of foliage to environmental stresses. Although growth reduction effect of cycocel is common, growth reduction percentage, flowering, leaf area and chlorophyll content, flower shape and colour responses of plants to this chemical can vary depending on the dose or concentration, method, site of application, species and cultivar and also growing season (Taiz and Zeiger, 2006).

The purpose of this study was to investigate the effects of paclobutrazol and cycocel on growth, flowering, chemical composition and histological features of potted *Tabernaemontana coronaria* plants.

Materials and Methods

This work was carried out during two successive seasons of 2011/2012 and 2012/2013 in the Experimental lathe house of Horticulture Dept., Faculty of Agric., Benha university, Kalubia Governorate, Egypt to study the effect of paclobutrazol and cycocel treatments on growth , flowering , chemical composition and histological features of potted *Tabernaemontana coronaria* plants. Uniform terminal cuttings 8-10cm length and 0.4- 0.5 cm thickness were planted on November 1st 2011 and 2012 in 8 cm plastic pots containing 1:1 mixture of peat moss and sand. Then, were placed under plastic tunnel conditions at the lathe house. On February 1st 2012 and 2013, uniform well rooted cuttings producing 6-8 leaves at 16-18 cm height were repotted in 20cm diameter plastic pots filled with a mixture of 2 clay: 1 sand :1 peat moss (v:v:v). The chemical characteristics of the planting medium were shown in Table (a).
Chemical analysis was determined according to Black *et al*. (1982).

Table a: Chemical analysis of the planting medium:

Parameters	Unit	Seasons	
		2011/2012	2012/2013
$CaCO_3$	%	1.24	1.54
Organic matter	%	1.73	1.96
Available nitrogen	%	0.91	0.84
Available phosphorus	%	0.52	0.49
Available potassium	%	0.74	0.82
E.C	ds/m	1.59	1.63
pH	--------------	6.89	6.81

After one month from repotting process (March 1st during the two seasons), the plants were received three sprays with paclobutrazol at 50, 100 and 150 ppm and cycocel at 1000, 1500 and 2000 ppm plus tap water as control at 15-day intervals. The plants were sprayed with a hand pump mister to the point of runoff. A surfactant (Tween 20) at a concentration of 0.01% was added to all tested solutions including the control. The treatments were arranged at random in three replicates with 10 pots/ each at the lathe house. After two months from replanting, the plants were fertilized every month with NPK fertilizer using ammonium sulfate (20.5% N), calcium superphosphate (15.5% P_2O5) and potassium sulfate (48% K_2O). A mixture of the three fertilizers, with a ratio of 1: 1: 1 (N: P_2O5: K_2O), was prepared and applied to the pots at the rate of 5 g/pot. Common agricultural practices (irrigation, manual weed control, . . . etc.) were carried out when needed.

Recorded data:

I- Growth measurements:

Vegetative and flowering characteristics were taken at full flowering stage (October 1st during the two seasons) included plant height (measured from surface of the potting medium to the tallest branch), number of branches/ plant, leaf area (cm^2), number of leaves/ plant, fresh and dry weights of leaves/ plant, days from planting to flowering, number of flowers/ plant, fresh and dry weights of flowers/plant and show value (as plant width / plant height ratio according to Berghage *et al*., (1989)). Whereas, roots measurements were taken at the end of experiment (November 1st during the two seasons) included roots number/ plant, fresh and dry weights of roots/plant.

II- Chemical composition determinations:

Leaves used for chemical analysis were taken just before flowering, dried at 70 C for 72 hours and used for determination of total nitrogen percentage according to A.O.A.C (1990), total phosphorus percentage was determined according to Hucker and Catroux (1980), potassium percentage was determined according to Brown and Lilleland (1946), total carbohydrates percentage was determined according to Herbert *et al*. (1971), where total chlorophylls was determined in fresh leaves according to A.O.A.C (1990).

III- Endogenous phytohormones:

Endogenous phytohormones were quantitatively determined in *Tabernaemontana coronaria* leaves just before flowering in the second season using High- Performance Liquid Chromato-graphy (HPLC) according to Koshioka *et al*. (1983) for auxin (IAA), gibberellins and abscisic acid (ABA), while cytokinins were determined according to Nicander *et al*. (1993).

IV- Anatomical study:

Leaf samples were taken from the 4^{th} leaf from top of all treated plants including control plants just before flowering. The specimens were taken then killed and fixed in FAA (5ml. formalin, 5ml. glacial acetic acid and 90ml. ethyl alchohol 70%), washed in 50% ethyl alcohol, dehydrated in series of ethyl alchohols 70,90,95 and 100%, infiltrated in xylene, embedded in paraffin wax with a melting point of 60-63 ^{o} C, sectioned to 20 microns in thickness (Sass, 1951), stained with the double stain method (fast green and safranin), cleared in xylene and mounted in Canada balsam (Johanson, 1940). Sections were read to detect histological manifestation of noticeable responses resulted from other treatments. The prepared sections were microscopically examined, counts and measurements (μ) were taken using a micrometer eye piece.

Statistical analysis:

Obtained data during the two seasons were subjected to analysis of variance as a simple experiment in a complete randomize block design. LSD method was used to difference means according to Snedecor and Cochran (1989).

Results and Discussion

Effect of paclobutrazol and cycocel on growth, flowering, chemical composition, endogenous phytohormones and histological features of Tabernaemontana coronaria plants:

1- Effect on vegetative growth:

Data recorded on vegetative growth traits i.e., plant height, branches number / plant, leaves number / plant, leaf area, fresh and dry weights of leaves/ plant as affected by paclobutrazol (PP_{333}) at 50, 100 and 150 ppm and cycocel (CCC) at 1000, 1500 and 2000 ppm are presented in Tables (1&2). Here, it could be noticed that all PP_{333} and CCC treatments were positively affected the previously mentioned vegetative growth traits of Tabernaemontana plants as compared with control in the two seasons. However, plants received the different PP_{333} and CCC treatments were shorter than the untreated control plants. Also, of the different PP_{333} and CCC treatments, the highest concentration of PP_{333} (150ppm) was the most effective one for producing the shortest plants in both seasons.

Table 1: Effect of paclobutrazol (PP_{333}) and cycocel (CCC) on plant height, branches number/plant and leaves number/plant of *Tabernaemontana coronaria* plants during 2011/2012 and 2012/2013 seasons.

Parameters / Treatments		Plant height (cm)		Branches number/plant		Leaves number/plant	
		First season	Second season	First season	Second season	First season	Second season
Control		52.24	54.16	5.13	5.62	53.34	59.46
PP_{333}	50 ppm	43.63	44.24	6.80	7.24	75.46	79.35
	100 ppm	36.75	35.89	8.14	8.36	97.62	93.64
	150 ppm	34.27	32.12	8.46	8.78	99.85	98.81
CCC	1000 ppm	47.35	49.67	6.24	6.12	64.44	68.42
	1500ppm	39.14	38.25	7.32	6.91	81.21	79.80
	2000 ppm	37.66	35.96	7.67	7.86	86.75	89.52
L.S.D at 0.05		7.34	7.16	0.83	0.94	8.16	7.96

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Table 2: Effect of paclobutrazol (PP_{333}) and cycocel (CCC) on leaf area, leaves fresh weight/plant and leaves dry weight/plant of *Tabernaemontana coronaria* plants during 2011/2012 and 2012/2013 seasons.

Parameters / Treatments		Leaf area(cm2)		Leaves fresh weight/plant (g)		Leaves dry weight/plant (g)	
		First season	Second season	First season	Second season	First season	Second season
Control		48.45	45.91	86.47	94.62	12.23	13.44
PP_{333}	50 ppm	39.10	40.82	98.35	104.54	14.30	14.90
	100 ppm	33.15	34.85	119.21	115.06	17.92	17.53
	150 ppm	31.45	33.15	114.35	112.37	17.14	16.94
CCC	1000 ppm	44.20	41.65	91.63	96.94	13.16	13.83
	1500 ppm	35.73	36.55	107.51	105.75	15.82	15.66
	2000 ppm	32.34	34.84	104.09	107.62	15.76	16.24
L.S.D at 0.05		5.11	4.23	8.21	8.75	1.14	1.02

Besides, PP_{333} at 100ppm and CCC at 2000 ppm gave highly significant reduction in the plant height in the two seasons. Of interest, is to note that the abovementioned results when related with the histological features of treated plants and also with their growth aspects. Since, gibberellin is known as a stimulating and individual hormone for longitudinal growth in different plants (Devlin and Witham, 1983). Hence, reduction of endogenous gibberellins level due to the use of growth retardants treatments (as will be mentioned later) led to reduction in the length of different cell types and consequently reduction in the plant height and leaf area, especially when the reduction of both gibberellins and auxins level is considered. In similar trend all tested applications of PP_{333} and CCC significantly decreased the leaf area as compared with control in both seasons. However, the lowest value of leaf area was existed by 150 ppm PP_{333} - sprayed plants, followed ascendingly by 2000 ppm CCC-sprayed plants in both seasons. On contrary, branches and leaves number / plant, fresh and dry weights of leaves/ plant were significantly increased by all treatments of PP_{333} and CCC as compared with control in both seasons. However, the increases of branches and leaves number / plant were in parallel to the increase of PP_{333} and CCC concentration in both seasons. So, the highest number of branches and leaves / plant were scored by using the highest concentration of PP_{333} (150ppm). Whereas, the heaviest fresh and dry weights of leaves/ plant were gained by 100ppm PP_{333}- sprayed plants. Also, PP_{333} at 150ppm and CCC at 2000ppm gave highly significant increments in these parameters in both seasons. Such results showed similar trend to those obtained by many investigators worked on PP_{333} and CCC on other plants. In this concern, Saker (2004) on *Hibiscus rosa sinensis and Tabernaemontana coronaria*, Youssef (2004) on *Strelitzia reginae*, Abd El-Kader (2009) on *Cestrum elegans* and *Tecoma stans*, Sibel *et al*. (2009) on *Consolida orientalis*, Gosh *et al*. (2010) on *Jatropha curcas*, Ribeiro *et al*. (2011) on sunflower, Jungklang and Saengnil (2012) on patumma cv. Chiang Mai Pink.

2- Effect on flowering growth:

Table (3) show that all PP_{333} and CCC treatments delayed the flowering (increasing the numbers of days from planting to start flowering) of Tabernaemontana plants as compared with control plants in both seasons. However, the greatest delay of Tabernaemontana flowering was gained by 150 ppm PP_{333}-sprayed plants in both seasons. On the reverse, the earliest flowering was occurred by un-treated plants in both seasons. Flowering retardation that existed with the growth retardant treatments could be attributed to the obtained stimulation of cytokinins synthesis. Thereby, the vegetative and reproductive growth periods were prolonged as cytokinin is known as a true shooting hormone (Opik and Rolf, 2005). Additionally, the highest number of flowers/ plant was gained by 150ppm PP_{333}-sprayed plants, followed by 2000 ppm CCC- sprayed plants in both seasons. Supporting for our discussion the previously mentioned note of the nature of PP_{333} and CCC effects on the prolongation of the vegetative and reproductive growth of Tabernaemontana plants. Since, increasing the endogenous level of cytokinins led to increasing the formation of leaves as well as the number of branches per plant. This effect was reflected on the increase in the formation of the number of flowers / plant.

Moreover, the heaviest fresh and dry weights of flowers/ plant were gained by 2000ppm CCC-sprayed plants, followed by 150ppm PP_{333}-sprayed plants as an average of both seasons.

Furthermore, data in the same Table (3) reveal that show value (plant width/height ratio) was significantly affected by the used growth retardants. Also, it could be noticed that in the two seasons, plant received PP_{333} at 150ppm had significantly the highest record of show value as compared with the other treatments. Furthermore, 100 ppm PP_{333} and 2000ppm CCC-sprayed plants scored high values in this respect in both seasons. The results of flowering growth traits as affected by PP_{333}, and CCC are coincided with those of Sibel *et al*. (2009) on *Consolida orientalis* L, Wilkinson and Richards (1987) on *Bouvardia humboldtii*, Wilkinson and Richards (1988) on Camellia x Williamsii, Wang and Gregg (1991) on hibiscus, De Baerdemaeker *et al*. (1994) on *Gardenia jasminoides* Ellis cultivar 'Wetchii', Matsoukis *et al*. (2001) on *Lantana camara* subsp. Camara, Banon *et al*. (2002) on *Dianthus caryophyllus* cv. Mondriaan, Karaguzel and Ortacesme (2002) on *Bougainvillea glabra* 'Sanderiana', Karaguzel *et al*. (2004) on *Lupinus varius*, Saker (2004) on *Hibiscus rosa*

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sinensis and Tabernaemontana coronaria, Youssef (2004) on *Strelitzia reginae*, Abd El-Kader (2009) on *Cestrum elegans* and *Tecoma stans,* Mansuroglu *et al*. (2009) on *Consolida orientalis* and Jungklang and Saengnil (2012) on patumma cv. Chiang Mai Pink.

Table 3: Effect of paclobutrazol (PP_{333}) and cycocel (CCC) on some flowering growth parameters of *Tabernaemontana coronaria* plants during 2011/2012 and 2012/2013 seasons.

Parameters / Treatments		Time to flowering (days)		Flowers number/plant		Flowers fresh weight/plant (g)		Flowers dry weight/plant (g)		Show value (plant width/height ratio)	
		First season	Second season	First season	Second season	First season	Second season	First season	Second season	First season	Second season
Control		173.2	179.7	12.34	14.62	23.41	28.77	2.57	3.24	0.39	0.36
PP_{333}	50 ppm	182.5	186.5	19.15	21.27	34.35	37.74	3.94	4.45	0.57	0.59
	100 ppm	189.4	191.1	23.45	23.14	38.61	38.95	4.59	4.59	0.77	0.80
	150 ppm	192.4	193.4	25.17	26.17	38.14	39.43	4.51	4.64	0.87	0.90
CCC	1000 pm	179.3	182.4	16.24	16.74	30.42	31.75	3.55	3.67	0.43	0.47
	1500ppm	177.6	185.6	21.28	20.11	37.43	35.78	4.41	4.21	0.63	0.70
	2000 pm	183.9	189.0	24.93	25.54	41.64	43.11	5.11	5.24	0.72	0.77
L.S.D at 0.05		8.19	6.11	4.62	5.10	7.24	6.29	0.73	0.71	0.126	0.135

3- Effect on root growth:

Data in Table (4) declare that roots number/ plant as well as fresh and dry weights of roots/ plant were greatly increased by using all $PP_{333}$and CCC treatments, with superiority of 2000ppm CCC-sprayed plants as compared with control in both seasons. Moreover, PP_{333}at 150ppm gave highly increment in this concern in both seasons. The previous mentioned findings of root traits could be interpreted on the basis of the physiological role of the nature of growth retardants action. Since, (as will be mentioned later) PP_{333} and CCC treatments alter the endogenous levels of different determined phytohormones i.e. auxin, gibberellins, ABA and cytokinins level that tended to increase the size of root system of Tabernaemontana plants. It is well established that cytokinins stimulate lateral roots initiation and thus increasing the size (number, thickness, fresh and dry weights) (Devlin and Witham, 1983). Such results are in agreement with those obtained by Youssef (2004) on *Strelitzia reginae*, Adham (2001) on *Althaea rosea,* Saker (2004) on *Hibiscus rosea sinensis* and *Tabernaemontana coronaria* plants, El-Malt *et al*. (2006) on *Hippeastrum vittatum,* Pinto *et al*. (2006) on potted Thai Tulip, Abd El-Kader (2009) on *Cestrum elegans* and *Tecoma stans,* and Ribeiro *et al*. (2011) on sunflower.

Table 4: Effect of paclobutrazol (PP_{333}) and cycocel (CCC) on roots number/plant, roots fresh weight/plant and roots dry weight/plant of *Tabernaemontana coronaria* plants during 2011/2012 and 2012/2013 seasons.

Parameters / Treatments		Roots number/plant		Roots fresh weight/plant (g)		Roots dry weight/plant (g)	
		First season	Second season	First season	Second season	First season	Second season
Control		8.64	9.17	11.50	12.27	1.98	2.13
PP_{333}	50 ppm	9.73	10.30	12.14	12.67	2.15	2.25
	100 ppm	10.88	11.26	12.91	13.65	2.32	2.47
	150 ppm	11.36	11.74	13.46	13.94	2.47	2.54
CCC	1000 ppm	9.97	11.17	11.90	13.53	2.16	2.49
	1500ppm	11.24	10.95	13.21	12.86	2.41	2.35
	2000 ppm	12.16	11.98	14.41	14.32	2.68	2.63
L.S.D at 0.05		0.72	0.86	0.76	0.72	0.13	0.11

4- Effect on chemical composition:

Results of chemical analysis of leaf samples (Table, 5) indicate that $PP_{333}$and CCC treatments resulted in an increments in N, P, K, total carbohydrates % and total chlorophylls (mg /100 g F.W) when compared with control in both seasons. However, the highest values of N, P and K contents of Tabernaemontana leaf were registered by 100ppm PP_{333}-sprayed plants, 2000ppm CCC -sprayed plants and 150ppm PP_{333}-sprayed plants, respectively. Moreover, 150 ppm PP_{333}- sprayed plants showed to be the most effective one to give the highest values of total carbohydrates and total chlorophylls of Tabernaemontana leaf in the two seasons. As for the explanation of the incremental effect of paclobutrazol and cycocel on chemical constituents in leaf of Tabernaemontana, it could be illustrated here on the basis that $PP_{333}$and CCC treatments stimulated the endogenous cytokinins synthesis as will be mentioned afterwards and there is an intimate relationship between cytokinins and chlorophylls metabolism in both excided or detached leaf disks and intact plants i.e., cytokinins retard chlorophylls degradation, preserve it and increase its synthesis (Devlin and Witham, 1983). Besides, cytokinins activate a number of enzymes participating in a wide range of metabolic reactions in the leaves. These reactions included the maturation of proplastid into chloroplasts. These enzymes could be divided into two groups according to their response to cytokinins. The first group of enzymes could be said to relate to

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chloroplast differentiation, while the second group could be related to cytokinin stimulated group (Kulaeva, 1979). Also, the increase in chlorophyll content due to growth retardants treatments might be attributed to the character of some growth retardants on depressing leaf area which lead to intensification of pigments in leaf. These results go on line with that obtained by Selim (1985) on Bougainvillea Mrs Butte, Selim (1990) on *Pelargonium zonale*, Youssef (2004) on *Strelitzia reginae*, Saker (2004) on *Hibiscus rosa sinensis* and *Tabernaemontana coronaria* shrubs, El-Malt *et al*. (2006) on *Hippeastrum vittatum*, Abd El-Kader (2009) on *Cestrum elegans* and *Tecoma stans*, and Jungklang and Saengnil (2012) on patumma cv. Chiang Mai Pink.

Table 5: Effect of paclobutrazol (PP_{333}) and cycocel (CCC) on some chemical composition of *Tabernaemontana coronaria* plants during 2011/2012 and 2012/2013 seasons.

Parameters / Treatments		N (%) First season	N (%) Second season	P (%) First season	P (%) Second season	K (%) First season	K (%) Second season	Total carbohydrates (%) First season	Total carbohydrates (%) Second season	Total chlorophylls (mg/100g F.W) First season	Total chlorophylls (mg/100g F.W) Second season
Control		2.14	2.21	0.243	0.234	1.53	1.49	9.71	10.13	237.4	242.1
PP_{333}	50 ppm	2.64	2.59	0.266	0.272	1.61	1.64	10.82	11.92	263.2	259.3
	100 ppm	2.98	2.87	0.282	0.284	1.87	1.92	12.14	11.86	287.4	281.0
	150 ppm	2.83	2.86	0.286	0.289	1.91	1.95	12.92	12.79	294.0	287.4
CCC	1000 pm	2.34	2.42	0.262	0.269	1.59	1.73	11.20	10.94	251.4	259.7
	1500ppm	2.69	2.79	0.284	0.282	1.83	1.69	12.49	12.19	279.3	273.3
	2000ppm	2.72	2.74	0.292	0.292	1.81	1.80	12.37	12.11	286.8	279.4
L.S.D at 0.05		0.16	0.14	0.017	0.019	0.081	0.012	1.02	1.14	12.6	10.3

5- Effect on endogenous phytohormones:

Data in Table (6) show the changes in endogenous phytohoromones i.e. auxin (IAA), abscisic acid (ABA), gibberellins and cytokinins in *Tabernaemontana coronaria* leaves treated with $PP_{333}$and CCC. As for cytokinines, data clearly indicates that the level of cytokinins positively responded to the different assigned treatments. It was increased nearly to two times comparing with control. Furthermore, for auxin level, it was highly decreased with different assigned treatments compared with that of untreated plants. With regard to gibberellins, data in Table (6) also clearly show that the level of gibberellins was decreased with different applied treatments compared with control. With respect to, the growth inhibitor (abscisic acid) its level was increased with various assigned treatments compared with the control, The exception was only in case of CCC at 1000 ppm that was decreased when compared with the control.

Table 6: Effect of paclobutrazol (PP_{333}) and cycocel (CCC) treatments on endogenous phytohormones in Tabernaemontana coronaria leaves during 2013 season.

Plant hormones / Treatments		Promoters: Gibberellins µg/g F.wt.	Promoters: Auxins (IAA) µg/g F.wt.	Promoters: Cytokinins µg/g F.wt.	Total µg/g F.wt.	% relative to the control	Inhibitors: Abscisic acid (ABA) µg/g F.wt.	% relative to the control
Control		141.13	33.01	7.84	181.98	100.00	0.30	100.00
PP_{333}	50ppm	113.09	25.80	9.39	148.28	81.48	0.34	113.33
	100ppm	99.73	16.27	11.73	127.73	70.19	0.41	136.67
	150ppm	98.87	15.31	13.89	128.07	70.38	0.47	156.67
CCC	1000ppm	118.30	26.02	10.22	154.54	84.92	0.29	96.67
	1500ppm	105.41	20.72	12.87	139.00	76.38	0.33	110.00
	2000ppm	103.32	18.72	12.32	134.36	73.83	0.42	140.00

In general, increments of cytokinins as well as reduction of gibberellins and auxins obtained in the present study could be responsible for the obtained modifications in different studied histological features (Tables,7&8) and the growth (Tables,1-4) as well as chemical constituents (Table,5). For example, increasing cytokinins could be in favor of increasing the number of formed leaves, flowers and branches.

The above mentioned results are of great interest, since increment of endogenous cytokinins was on the account of reduction of other determined phytohormones (Gibberellins and Auxin) that clearly could be explained the alternations of all growth characteristics obtained in the present study. Since, cytokinin is known as shooting hormones (Salisbury and Ross, 1974) e.g. number of leaves and branches were significantly increased as well as the significant increase in flower number. Also, of interest is to note that these treatments were accompanied with significant reduction in plant height that being more expectable when related with the obtained reduction in endogenous gibberellins and auxin levels. Besides, of interest the conclusion that such treatment ornamentally is being of economic value. Since more marketable characteristics of making this plant as an attractive compact pot plant were achieved.

The above mentioned results has been previously recommended. Since, other studies reported the increase of endogenous cytokinins and reduction of endogenous gibberellins at the same time. Of these studies are Hedden and Graebe (1985), Abou El-Ghait (1993) and Youssef (2004).

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6- Effect on anatomical features:

Data in Tables (7&8) and Figure (1) clearly indicate the effect of different applied treatments on different anatomical features of *Tabernaemontana coronaria* leaves. In this respect, most of the studied features of leaf anatomy were increased with different applied treatments, particularly PP_{333} at 150 and 100ppm and CCC at 2000 ppm. Among these anatomical features were the most important ones, i.e., thickness of leaf midrib, length & width of vascular bundle, phloem & xylem tissues and number of xylem vessels in vascular bundle as well as the leaf blade thickness.

Table 7: Effect of paclobutrazol (PP_{333}) and cycocel (CCC) treatments on the mean counts and measurements of certain histological features of *Tabernaemontana coronaria* during 2013 season.

Treatments		Upper epidermal cuticle thickness.	Lower epidermal cuticle thickness.	Upper epidermis thickness	Lower epidermis thickness	palisade tissue thickness	Spongy tissue thickness	Number of spongy tissue layers	Mean of spongy layer thickness	Thickness of blade	Thickness of collenchyma layers below the upper epidermis at midrib	No. of collenchyma layers below the upper epidermis at midrib	Thickness of collenchyma layers above the lower epidermis at midrib
Control		8	4	29	18	47	142	7	20.29	221	82	4	49
PP_{333}	50 ppm	10	6	32	13	43	167	6	27.83	263	98	4	84
	100 ppm	16	7	33	22	61	193	9	21.44	298	91	3	106
	150 ppm	17	9	36	25	64	202	11	18.36	304	119	4	93
CCC	1000 ppm	7	6	35	16	63	163	8	20.38	292	71	3	51
	1500 ppm	12	5	25	19	48	179	9	19.89	263	78	4	141
	2000 ppm	11	7	31	21	59	182	8	22.75	271	83	4	64

Histological characteristics (micron)

Table 8: Effect of paclobutrazol (PP_{333}) and cycocel (CCC) treatments on the mean counts and measurements of certain histological features of *Tabernaemontana coronaria* leaf during 2013 season.

Treatments		No. of collenchyma layers above the lower epidermis at midrib	Thickness of phloem in the vascular bundle	Thickness of xylem tissue	Number of xylem rows in the vascular bundle	Number of vessels in the xylem row	thickness of widest xylem vessel in the vascular bundle	Length of midrib vascular bundle	Width of midrib vascular bundle	Thickness of leaf midrib
Control		3	44	108	31	4	21	161	804	1517
PP_{333}	50 ppm	4	47	113	36	4	27	169	911	1586
	100 ppm	4	67	148	39	6	28	198	994	1866
	150 ppm	4	73	154	42	6	32	204	1117	1937
CCC	1000 ppm	3	58	131	39	5	23	174	895	1692
	1500 ppm	5	51	126	34	4	21	176	967	1674
	2000 ppm	3	61	141	38	6	27	193	971	1763

Histological characteristics (micron)

With regard to blade thickness, it was increased with different used treatments to reach its maximum value (304μ) with PP_{333} at 150 ppm, (298μ) with PP_{333} at 100 ppm and (271μ) with CCC at 2000 ppm, meanwhile it

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was 221µ for the control. Also, the thickness of each of upper and lower epidermis, were also increased with most applied treatments. For mesophyll tissue, thickness of both spongy and palisade tissues were increased with different applied treatments. Here, spongy tissue thickness was 142µ in the control but increased to reach 202, 193 and 182 micron with PP_{333} at 150 ppm, PP_{333} at 100 ppm and CCC at 2000 ppm, respectively. Also, palisade tissue thickness was 47µ for control but increased to reach 64, 61, 63 and 59µ with PP_{333} at 150 and 100 ppm, CCC at 1000 and 2000 ppm, respectively.

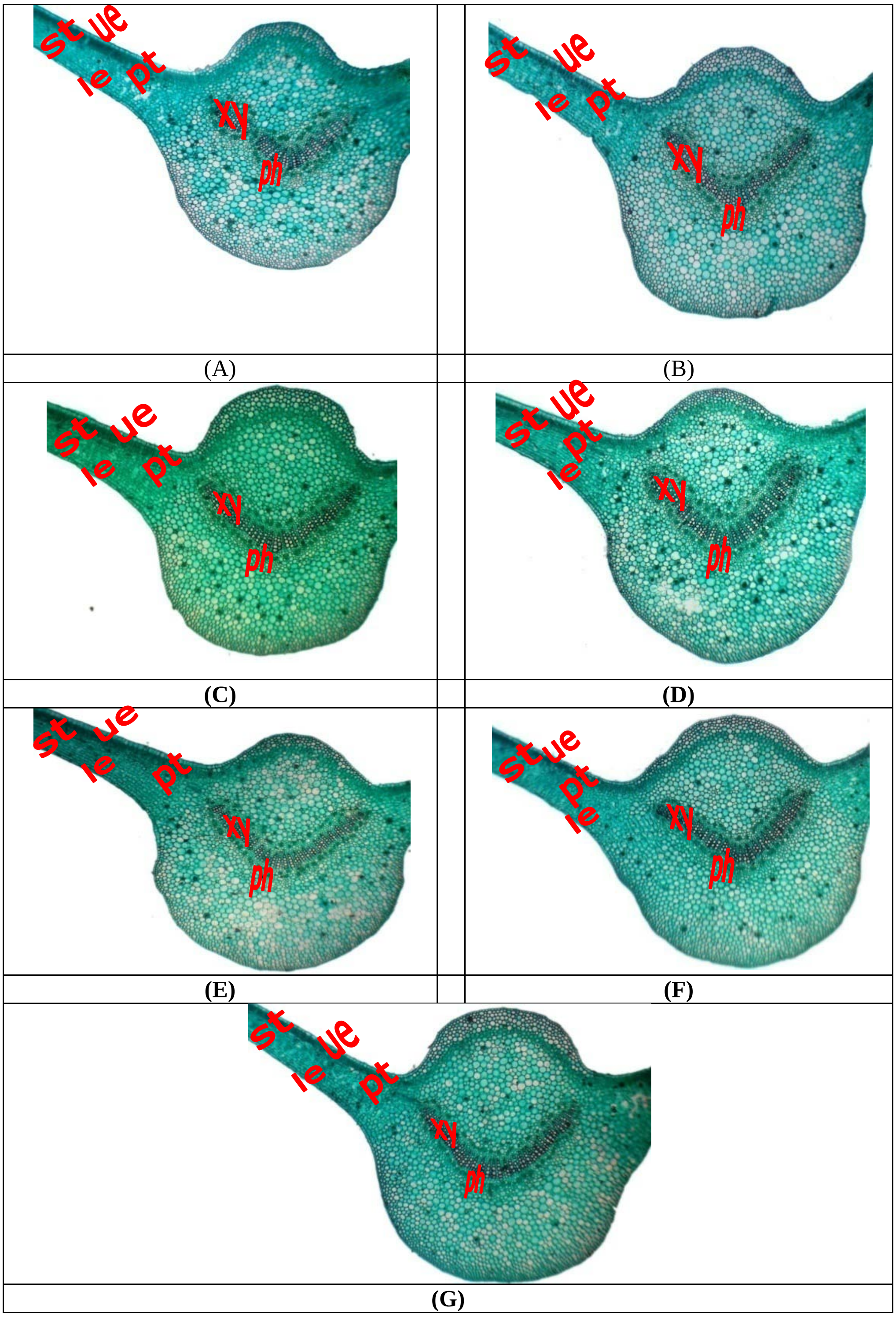

Fig. 1: Transverse sections (X = 40) through the 4 th apical leaf of *Tabernaemontana coronaria* plants as affected by different applied treatments. Where: (A): Control (B): PP_{333} at 50 ppm , (C): PP_{333} at 100 ppm, (D): PP_{333} at 1 50 ppm, (E): Cycocel at 1000 ppm , (F): Cycocel at 1500 ppm and (G): Cycocel at 2000 ppm. ue= Upper epidermis pt= Palisade tissue st= Spongy tissue xy= Xylem tissue ph= phloem tissue le= Lower epidermis

Concerning midrib anatomical features, it could be noticed that increment in its thickness with different applied treatments that is being attributed to the increase in many of its histological features such as thickness of collenchyma tissues, lower most parenchyma tissue and dimensions of the vascular bundle as well as thickness of phloem tissues, xylem tissue and also number and diameter of xylem vessels in the vascular bundle. Increasing of vascular tissues are being of great interest, because that could reversed upon improvement of translocation for nutrients and the photosynthates as well. In other meaning translocation of water and different nutrients from soil to leaves from one side and photosynthates from leaves to various plant parts from the other. In addition, the above mentioned results are being more evident when thickness of xylem vessels are considered. In general, the effects of the applied treatments upon the anatomy features of treated plants could be attributed to the effect upon cambium activity. Increment of cambium activity could mainly attributed to the increase of endogenous hormones level, especially cytokinins (Ismaeil, 1995).

Increasing of xylem and phloem tissues means that the translocation of crude nutrients and water from soil to leaves as well as the translocation of sugar and other bioconstituents from leaves to other plant parts are being improved (Marschner, 1995). That is directly could be reflected upon the vigorous growth of such treated plants.

With regard to the nature of different applied treatments of growth retardants upon the histological features of leaf blade (lamina), data obtained are of great interest. Since, increasing the thickness of lamina at any of the applied treatments of growth retardants (e.g. was nearly one and half times more than control with spraying PP_{333} at 150 ppm); is of great economic value regarding the decorative view. That means that leaves having such characteristics could own be a great longevity comparing with other plants with low values of lamina thickness.

On the other hand, increasing of palisade thickness to reach nearly one and half times more than of control with PP_{333} at 150 ppm, also, are of great interest. Since, that means more chloroplasts are being created in such wide palisade thickness. In this respect more chloroplasts could be reflected upon the efficiency of photosynthesis itself. Thereby, more assimilates are being created and then translocated to other plant parts causing the greening of formed leaves. Here, increasing of green color with wide lamina thickness gave good decorative characteristics and long duration of such leaves. Furthermore, all of that could be reflected upon the improvement of formed flowers characteristics.

Generally, increasing each of midrib and lamina thickness as well as phloem and xylem tissues are being mainly direct response for the alteration the profile of endogenous phytohormones, i.e., cytokinins, auxin, gibberellins and abscisic acid to be in favor of tissues development and accelerating their morphogenesis. The pervious results are in conformity with those of Youssef (2004) who mentioned that treated *Strelitzia reginae* plants with PP_{333} at 300 and 200 ppm increased blade thickness, palisade thickness and phloem tissue thickness.

In general, of all obtained results; those of achieving more dwarf plants of *Tabernaemontana coronaria* with many formed flowers (Tables, 1-5) could be considered as pioneer results in this respect. Since, treatments of PP_{333} at 150 and 100 ppm or CCC at 2000ppm gave a good display (show value) of flowering pot of Tabernaemontana plant with optimum vegetative and flowering characteristics from the commercial point of view when compared with other treatments or control.

References

A.O.A.C., 1990. Official Methods of Analysis of Association of Official Analytical Chemists. Pub. A.O.A.C. INC. Suite 400, 22201 USA Fifteenth Ed. pp: 62-63, 236 and 877-878.

Abd El-Kader, S.F.A., 2009. Physiological studies on growth and flowering of some shrubs. Ph.D. Thesis, Fac. Agric. Moshtohor, Benha Univ.

Abou El-Ghait, E.M., 1993. Growth responses of *Epipremnum aureum* to kristalon and some growth retardants.3-Paclobutrazol.Annals of Agric., Sci., Moshtohor, 31(4): 2187-2207.

Adham, H.E.I., 2001. Effect of some growth retardants, pinching and planting date on growth, flowering and chemical composition of *Althaea rosea*, L. Ph.D. Thesis, Fac. Agric., Cairo Univ.

Banon, S.A., E.A. Gonzalez, J.A. Cano, J.A. Franco, and Fernandez, 2002. Growth, development and color response of potted *Dianthus caryophyllus* cv. Mondriaan to paclobutrazol reatment. Scientia Horticulturae, 94: 371-177.

Beattie, D.J., E.J. Holcomb and C.F. Deneke, 1990. Effects of uniconazole and paclobutrazol on plant height and flowering in *Physostegia virginiana* and Cheloneoblique. Plant Growth Regulator Society of America, 18(4): 187-193.

Berghage, R.D., R.D. Heins, M. Karlsson, J. Erwin and W. Carlson, 1989. Pinching technique influences lateral shoot development in poinsettia. J. Amer. Soc. Hort. Sci., 114(6): 909-914.

Black, C.D., D.O. Evans, L.E. Ensminger, J.L. White, F.E. Clark and R.C. Dinauer, 1982. Methods of soil analysis. Part 2. Chemical and microbiological properties. 2nd Ed. Soil., Soc. of Am. Inc. Publ., Madison, Wisconsin, U.S.A.

Brown, J.D. and O. Lilleland, 1946. Rapid determination of potassium and sodium in plant material and soil extract by flame photometry. Proc. Amer. Soc., Hort., Sci., 48: 341-346.

Chany, W.R., 2005. Growth retardants: A promising tool for managing urban trees. Env. Toxicol. Chem., 29: 1224-1236.

Davis, T.D. and A.S. Andersen, 1989. Growth retardants as aids in adapting new floricultural crops to pot culture. Acta Horticulturae, 252: 77-85.

De Baerdemaeker, C.I., J.M. Huylenbroeck and P.C. Debergh, 1994. Influence of paclobutrazol and photoperiod on growth and flowering of *Gardenia jasminoides* Ellis cultivar 'Wetchii'. Scientia Horticulturae, 58: 315-324.

Devlin, M. and H. Witham, 1983. Plant Physiology, 4^{th} Ed. Publishers Willard, Grant Press, Boston.

El-Malt, A.A.T., E.E. El-Maadawy, M.A. El-Khateeb and Z.H. El-Sadak, 2006. Physiological studies on *Hippeastrum vittatum* L. plants.2- Effect of NPK, CCC and BA on growth, bulblet production and flowering. Egypt.J. of Appl. Sci., (6B): 724-742.

Fletcher, R.A., A. Gill, T.D. Davis and N. Sankhla, 2000. Triazoles as plant growth regulators and stress protectants. Horticulture Review, 24: 55-138.

Ghani, A., 2003. Medicinal Plants of Bangladesh. The Asiatic Society of Bangladesh, Dhaka. 2nd Ed., 7: 176-177.

Gosh, A., J. Chikara, D.R. Chandhari, A.R. Prakashi, G. Boricha and A. Zala, 2010. Paclobutrazol arrests vegetative growth and unveils unexpressed yield potential of *Jathropa curcas*. Journal of Plant Growth Regulation, 29: 307-315.

Hedden, P. and J.E. Graebe, 1985. Inhibition of gibberellin biosynthesis by paclobutrazol in cell-free homogenates of *Cucurbita mxima* and *Malus pumila* . J. Plant Growth Regul., 4: 111-122.

Herbert, D., P.J. Phipps and R.E. Strange, 1971. Determination of total carbohydrates, Methods in Microbiology, 5(8): 290-344.

Hucker, T. and G. Catroux, 1980. Phosphorus in sewage ridge and animals wastes slurries. Proceeding of the EEC Seminar, Haren (Gr): Gromingen Netherlands 12, 13 June.

Ismaeil, F.H., 1995. Effect of gamma radiation and paclobutrazol (P.G.R.) on *Vicia faba* from the botanical point of view. M. Sc. Thesis, Fac. Of Agric.Moshtohor, Zagazig Univ.

Johanson, D.A., 1940. Plant Microtechnique (5th edition) MC. Grow Hill, Book, Company. New York, London, 213- 236.

Jungklang, J. and K. Saengnil, 2012. Effect of paclobutrazol on patumma cv. Chiang Mai Pink under water stress. Songklanakarin J. Sci. Technol., 34(4): 361-366.

Karaguzel, O. and V. Ortacesme, 2002. Influence of paclobutrazol on the growth and flowering of *Bougainvillea glabra* 'Sanderiana'. Ziraat Fakultesi Dergisi, Akdeniz Universitesi, 15(1): 79-84.

Karaguzel, O., I. Baktir, S. Cakmakci and V. Ortacesme, 2004. Growth and flowering responses of *Lupinus varius* L. to paclobutrazol. HortScience, 39(7): 1659-1663.

Koshioka, M., J. Harda, M. Noma, T. Sassa, K. Ogiama, J.S. Taylor, S.B. Rood, R.L. Legge and R.P. Pharis, 1983. Reversed – phase C18 high performance liquid chromatography of acidic and conjugated gibbberellins. J. Chromatgr., 256: 101-115.

Kulaeva, O.N., 1979. Cytokinin action on enzyme activities in plants. In plant growth substances. (Ed.) F. Skoog, Springer-verlage Berlin, Heidelberg. New York (1980), pp: 119-128.

Mansuroglu, S., O. Karaguzel, V. Ortacesme and M.S. Sayan, 2009. Effect of paclobutrazol on flowering, leaf and flower colour of *Consolida orientalis*. Pakistan Journal of Botany, 41(5): 2323-2332.

Marschner, H., 1995. Mineral Nutrition of Higher Plants. Academic Pres Inc. San Diego, CA 92101.

Matsoukis, A.S., A. Sereli-Chronopoulou, I.D. Dimopoulus and A. Kamoutsis, 2001. Responses of *Lantana camara* subsp. camara to paclobutrazol and shading. Canadian Journal of Plant Science, 81(4): 761-764.

Nicander, B., U. Stahl, P. Bjorkman and E. Tillberg, 1993. Immyno affinity co-purification of cytokinins and analysis by high-performance liquid chromatography with ultra violet spectrum deterction. planta, 189: 312-320.

Opik, H. and S. Rolfe, 2005. The Physiology of Flowering Plants. Cambridge Univ. Press, 5: plant growth hormones, pp: 177-194.

PGRSA, 2007. Plant growth regulation handbook of the Plant Growth Regulation Society of America. 4th Edition. The Plant Growth Regulation Society of America, Athens.

Pinto, A.C.R., T.T. Graziano, J.C. Barbosa and F.B. Lasmar, 2006. Growth retardants on production of flowering potted Thai tulip. Bragantia, 65: 369-380.

Pushpa, B., K.P. Latha, V.P. Vaidya, A. Shruthi and C. Shweath, 2011. In vitro Anthelmintic activiy of leaves extracts of *Tabernaemontana coronaria*. 3(4): 1788-1790.

Ribeiro, D.M., M. Caroline, B. Jackson, B.R. Glayton and R.S. Barros, 2011. Effects of autoclaving on the physiological action of Paclobutrazol. Agricultural Sciences, 2(3): 191-197.

Saker, A.M.I., 2004. Physiological studies on growth and flowering of some ornamental shrubs. M.Sc. Thesis Fac. Agric. Moshtohor, Zagazig Univ.

Salisbury, F.B. and C.W. Ross, 1974. Plant Physiology. Publishing Inc. Belmont. California, 2^{nd} ed., pp. 422.

Sass, J. E., 1951. Botanical Microtechnique. Iowa State College Press, Ames, Iowa, pp: 228.

Selim, S.M., 1990. Effect of urea fertilization and cycocel sprays on *Pelargonium zonale* Ait. II- Effect on chemical composition. J. Agric. Res. Tanta Univ., 16(4): 757-766.

Selim, S.M., 1985. Effect of some growth regulators on growth, flowering production of Bougainvillea plants. Ph.D. Thesis, Fac. Agric., Cairo Univ.

Sibel, M., O. Karaguzel, V. Ortacesme and M.S. Sayan, 2009. Effect of paclobutrazol on flowering, leaf and flower colour of *Consolida orientalis*. Pak. J. Bot., 41(5): 2323-2332.

Snedecor, G.W. and W.G. Cochran, 1989. Statistical methods. 7^{th} Ed. Iowa state Univ. Press. Ames Iowa, USA.

Taiz, L. and E. Zeiger, 2006. Plant Physiology. 4th edition. Sinauer Associates, Inc., Publishers, Sunderland.

Wang, Y.T. and L.L. Gregg, 1991. Modification of hibiscus growth by treating unrooted cuttings and potted plants with uniconazole or paclobutrazol. Journal of Plant Growth Regulation, 10(1): 47-51.

Wilkinson, R.J. and D. Richards, 1987. Effects of paclobutrazol on growth and flowering of *Bouvardia humboldtii*. HortScience, 22: 444-445.

Wilkinson, R.J. and D. Richards, 1988. Influence of paclobutrazol on the growth and flowering of Camellia x Williamsii. HortScience, 23(2): 359-360.

Youssef, A.S.M., 2004. Physiological studies on growth and flowering of *Sterilitizia reginae* Ait. Plant. Ph.D. Thesis, Fac. of Agric., Moshtohor, Zag. Univ.