*International Journal of Plant & Soil Science*

*17(6): 1-22, 2017; Article no.IJPSS.34527*
*ISSN: 2320-7035*

# Effect of Pinching and Paclobutrazol on Growth, Flowering, Anatomy and Chemical Compositions of Potted Geranium (*Pelargonium zonal* L.) Plant

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*This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/IJPSS/2017/34527
Editor(s):
(1) Abhishek Naik, Technology Development Department - Vegetable Crops, United Phosphorus Limited -Advanta, Kolkata, India.
(2) Surendra Singh Bargali, Department of Botany, DSB Campus, Kumaun University, Nainital, Uttarakhand, India.
Reviewers:
(1) Jianwei Gao, Shandong Academy of Agricultural Sciences and Shandong Key Laboratory of Greenhouse, Vegetable Biology and Shandong Branch of National Vegetable Improvement Center, China.
(2) Elizabeth Orika Ono, São Paulo State University – UNESP, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history/20269

***Original Research Article***

***Received 30th May 2017***
***Accepted 17th July 2017***
***Published 29th July 2017***

## ABSTRACT

**Aims:** A two-year field trials were carried out during the two successive seasons of 2015 and 2016 to study the effect of pinching and paclobutrazol ($PP_{333}$) at 20, 40 and 60 ppm on growth, flowering, histological characteristics and chemical compositions of potted *Pelargonium zonale* L. plant.

**Methodology:** Uniform terminal cuttings were planted in 8 cm plastic pots containing 1:1 mixture of peat moss and sand. On March 1st of 2015 and 2016, uniform well rooted cuttings were repotted in 20 cm diameter plastic pots filled with a mixture of 1 clay: 1 sand :1 peat moss (v:v:v). Pinching was applied after 21 days from transplanting by removing the apical bud of all the upcoming buds, allowed to produce side shoots. After one month, the plants were received four sprays with $PP_{333}$ at 20, 40 and 60 ppm plus tap water as control at two weeks intervals.

**Results:** Results showed that: All pinching and $PP_{333}$ concentrations decreased plant height (compacting showy plants), particularly the combined treatment of $PP_{333}$ at 60 ppm with pinching in

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both seasons. On the other hand, all applied treatments of pinching and $PP_{333}$ statistically increased number of branches / plant and stem diameter/(cm) to reach its maximum with the highest concentration for each. The heaviest fresh and dry weights of leaves/plant, the highest number of leaves/ plant, the highest number of flowers/ plant whereas the heaviest flowers fresh and dry weights/plant were gained from plants pinched and sprayed by $PP_{333}$ at 60 ppm in the two seasons. In addition, all different applied treatments of pinching and $PP_{333}$ statistically decreased leaf area / ($cm^2$) as compared with control plants (without pinching) which induced the largest leaf area / ($cm^2$) and the earliest flowers in the first and the second seasons. Moreover, the highest show value (plant width/ height ratio) was recorded by the interaction treatments of pinching and $PP_{333}$ at the highest concentration in both seasons. The highest number of roots/plant and the heaviest fresh and dry weights as well as leaf N, P, K, total carbohydrates, total chlorophylls contents and leaf total phenols content gave the significantly highest mean value for the interaction treatment of pinching and 60 ppm $PP_{333}$ -sprayed plants in the two seasons. All pinching and $PP_{333}$ treatments increased leaf total phenols content (mg/100g F.W), but they reduced total indoles (mg/100 g F.W) of Pelargonium zonale L. Leaves. Moreover, the interaction of $PP_{333}$ at 40 ppm with pinching or $PP_{333}$ at 60 ppm without pinching recorded highly significant increments of all above mention parameters in the first and the second seasons. Furthermore, the obtained results indicated that all $PP_{333}$with and without pinching treatments increased cytokinins and salicylic acid contents, but they decreased gibberellins, auxins and abscisic acid (mg/100 g F.W) of *Pelargonium zonale* L. shoot with superiority of $PP_{333}$ at 60 ppm with pinching compared with control and other used treatments. Regarding to the anatomical features of leaf and stem anatomy, most traits were increased with different applied treatments compared with the control, particularly pinching with $PP_{333}$ at 60 ppm treatment. Consequently, it is preferable, to obtain a good display of flowering pot of *Pelargonium zonale* L. plants with formative growth and flowering characteristics from the commercial point of view, treating geranium plants with $PP_{333}$ at 60 ppm applied as spray supported with pinching treatment practice four times a year.

*Keywords: Pelargonium zonale L.; pot plant; pinching; paclobutrazol; growth; flowering; anatomy and chemical composition.*

## 1. INTRODUCTION

Geraniums are considered the most species being bred for their decorative flowers and, some, liked the smell of the leaves in the family Geraniaceae [1]. *Pelargonium zonale* L. usually grows up to 1m but it can reach heights of 3m. The branches are almost succulent and are usually covered with hairs, while the older stems harden with age. The large palmately-nerved leaves are often smooth and a characteristic dark horseshoe-shaped mark is often present. The flower color ranges from rose-pink to all shades of red as well as pure white. The distinctly irregular flowers are borne in a typically umbel-like inflorescence. *P. zonale* L. flowers throughout the year with a peak in spring and autumn (September-November) [2].

Repeated application of plant growth regulators as well as some non-chemical techniques to control plant height is a common practice in the world to face the challenge of environmental stimulation of inter-node elongation. In addition, application of plant growth regulators influence flower production and can advance or delay flowering depending on the species [3]. Pinching the plants twice decreased the branch length and the dry weight of branch; meanwhile it increased the stem and branch diameter, number of branches, fresh and dry weights of shoots, chlorophyll a and carotenoids content, P% in branches as well as K% in shoots on *Cestrum nocturnum* Plants [4].

A substitute, effective strategy for controlling plant height is to use chemical plant growth retardants [5]. Application of growth retardants is a common practice for commercial growers to achieve attractive compact pot-grown plants. The terms growth retardants are used for all chemicals that retard cell division and cell elongation in shoot tissues and regulate plant height physiologically without formative effects [6]. One of the most 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 [7]. 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 [8]. From this function, paclobutrazol has long been used to reduce plant height for potted plant production, especially ornamental plants [9,8]. Paclobutrazol at concentration of 60 ppm reduced plant foliage height but it increased number of flowers / plant and delayed the production of potted *Chrysanthemum frutescens* [10]. The main target of this study was to formate growth, enhance flowering, chemical composition and histological features of potted geranium plants through pinching practice and paclobutrazol foliar spray treatments.

## 2. MATERIALS AND METHODS

This study was carried out during two successive seasons of 2015 and 2016 in the Experimental lathe house of Horticulture Dept., Faculty of Agric., Benha university, Kalubia Governorate, Egypt to study the effects of pinching and paclobutrazol treatments on growth, flowering and some chemical composition of potted *Pelargonium zonale* L. plants (local variety with red flowers).

### 2.1 Plant Material

Uniform terminal cuttings 10-12 cm tall and had 4-6 leaves were planted on January, 1st 2015 and 2016 in 8 cm plastic pots containing 1:1 mixture of peat moss and sand (v:v) and kept under plastic tunnel conditions at the lathe house. Moreover, March 1st 2015 and 2016, uniform well rooted cuttings of 14-16 cm height were repotted in 20cm diameter plastic pots filled with a mixture of 1 clay: 1 sand :1 peat moss (v:v:v).

The chemical characteristics of the planting media were shown in Table 1. Chemical analysis was determined according to [11].

### 2.2 Treatments

#### 2.2.1 Pinching practice

Pinching was conducted three weeks later of transplanting process by removing the apical bud (2cm) of all the upcoming buds, allowed to produce side shoots.

#### 2.2.2 Paclobutrazol treatments

One month later of repotting process (April 1st during the two studied seasons), the plants were received four sprays paclobutrazol i.e. 20, 40 and 60 ppm and tap water as control at two weeks intervals. The plants were sprayed in early morning with a hand pump mister up to reach the point of runoff. A surfactant (Tween 20) at 0.01% was added to all tested solutions including tap water (control).

**Table 1. Chemical analysis of the planting media**

| Chemical analysis of potted media | Unit | Seasons | |
|---|---|---|---|
| | | 2015 | 2016 |
| Organic matter | % | 1.88 | 1.92 |
| $CaCO_3$ | % | 0.85 | 0.89 |
| Total nitrogen | % | 0.77 | 0.82 |
| Total phosphorus | % | 0.36 | 0.42 |
| Total potassium | % | 0.68 | 0.72 |
| E.C | ds/m | 1.24 | 1.28 |
| pH | ---- | 6.55 | 6.69 |

The treatments were randomly arranged at random in three replicates with 15 pots/ replicate under the lathe house condition (with about 12000 - 14000 lux light intensity). One month later of replanting process, the plants were fertilized monthly with chemical NPK fertilizer using ammonium sulfate (20.5% N), calcium superphosphate (15.5% $P_2O_5$) and potassium sulfate (48% $K_2O$). A mixture of the three fertilizers 4 g/plot were applied at ratio of 1: 1: 1 (N: $P_2O_5$: $K_2O$). Common agricultural practices (irrigation, manual weed control, . . . etc.) were carried out when needed.

### 2.3 Recorded Data

#### 2.3.1 Growth parameters measurements

Vegetative and flowering characteristics were taken at full flowering stage (September 1st during the two seasons) included plant height (measured from surface of the potting medium to the tallest branch), number of branches/ plant, stem diameter/(cm) , fresh and dry weights of leaves/ plant, Leaf area / ($cm^2$), number of leaves/ plant, days to reach to flowering, number of flowers/ plant, fresh and dry weights of flowers/plant and show value (plant width / plant height ratio according to [12]. Whereas, roots measurements were taken at the end of experiment (December 1st during the two seasons) included roots number/ plant, fresh and dry weights of roots/plant.

#### 2.3.2 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 [13], total phosphorus percentage was determined according to [14], potassium percentage was determined according to [15], total carbohydrates percentage was determined according to [16], where total chlorophylls, total indoles and total phenols were determined in fresh leaves according to [13].

### 2.4 Endogenous Phytohormones

Endogenous phytohormones were quantitatively determined in *Pelargonium zonale* L. shoots 90 days later of transplanting during 2016 season using High- Performance Liquid Chromatography (HPLC) according to [17] for auxins (IAA), gibberellins, salicylic Acid and abscisic acid (ABA) while cytokinins were determined according to [18].

### 2.5 Anatomical Study

Leaf and stem samples were taken from the 5$^{th}$ apical internode and its corresponding leaf of all treated plants including control plants on 90 days after transplanting during 2016 season. The specimens were taken then killed and fixed in FAA (5 ml. formalin, 5 ml. glacial acetic acid and 90 ml. 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°C, sectioned to 12 microns in thickness [19], stained with the double stain method (fast green and safranin), cleared in xylene and mounted in Canada balsam [20]. 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.

### 2.6 Statistical Analysis

All obtained data during both seasons of study were subjected to analysis of variance as a factorial experiment in a complete randomize block design, according to [21]. The differences between the mean values of various treatments were compared by Duncan's multiple range test [22].

## 3. RESULTS AND DISCUSSION

### 3.1 Vegetative Growth Parameters

#### 3.1.1 Plant height (cm)

Table 2 reveals that pinching treatment decreased plant height of *Pelargonium zonale* L. as compared with un-pinched plants (control) in both seasons. In this respect, pinched plants scored (24.17 and 24.25 cm) plant height against (28.08 and 28.75 cm) for control plants in 2015 and 2016 seasons, respectively. On the other hand, all tested concentrations of paclobutrazol ($PP_{333}$) decreased plant height as compared with the un-treated plants (control) in the two seasons. Anyway, it was observed that there was a negative correlation between the values of plant height and the concentration of $PP_{333}$. Since, as the concentration of $PP_{333}$ increased plant height value decreased until reach to the lowest value at the high $PP_{333}$ concentration in the two seasons. However, 60 ppm $PP_{333}$ -sprayed plants produced the shortest plants (20.50 and 21.67 cm) against (37.50 and 37.88 cm) for the unsprayed plants in the first and second seasons, respectively. In addition, $PP_{333}$ at the medium concentration (40 ppm) recorded height reductive effect on plant height of *Pelargonium zonale L.* plants in both seasons. Moreover, data in Table 2 Showed that all pinching and $PP_{333}$ concentration interactions statistically decreased plant height values of *Pelargonium zonale* L. plants in both seasons. However, pinching combined with 60 ppm $PP_{333}$ produced the lowest plant height values (18.67 and 20.67 cm), followed in ascending order by pinched plants sprayed with 40 ppm $PP_{333}$ (20.00 and 21.67 cm), in the first and second seasons, respectively.

#### 3.1.2 Number of branches /plant and stem diameter/(cm)

Table 2 demonstrates that, there was a positive relationship between the number of branches /plant and stem diameter (cm) and $PP_{333}$ with pinching treatments. Since, as the levels of $PP_{333}$ with pinching increased, the branches number/plant and stem diameter (cm) increased to the high level. This trend was true in both seasons. In addition, all interactions treatments between pinching and paclobutrazol-sprayed plants caused remarkable increments in the branches number/plant and stem diameter (cm) in both seasons. However, the highest values of these parameters were obtained by using the combined treatment between pinching and 60 ppm paclobutrazol in both seasons. Moreover, $PP_{333}$ at 40 ppm with pinching gave highly significant increases in the number of branches/plant and stem diameter (cm) in both seasons.

#### 3.1.3 Leaf parameters

Tables 3 and 4 illustrate that pinching treatment statistically affected leaf parameter i.e., number, fresh and dry weight and leaf area/$cm^2$ in both seasons. However, the highest values of number, fresh and dry weight of leaves were scored by pinching treatment when compared with un pinched plants (control). While, the highest values of leaf area/$cm^2$ was produced with unpinched plants (control) in both seasons. In addition, all tested $PP_{333}$ treatments statistically increased leaf parameters over control in both seasons, except leaf area ($cm^2$). Whereas, $PP_{333}$ at 20, 40 and 60 ppm induced high significant decreases on leaf area/$cm^2$ in the two seasons. On the other hand, all interactions of $PP_{333}$ concentrations and pinching induced a remarkable precocity in this parameter, especially the combinations of paclobutrazol at 60 and 40 ppm with pinching in both seasons. On the reverse, the lowest value of leaf leaf area ($cm^2$) was recorded by the combination of $PP_{333}$ at 60 ppm and pinching in the two seasons.

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 [8].

Such results of $PP_{333}$ are in agreement with those obtained by [23] on sunflower, [24] on patumma cv. Chiang Mai Pink, [25] on *Tabernaemontana coronaria*, [26] on *Murraya exotica* and *Duranta repens* plants, [27] on Hibiscus plant, [28] on kumquat plants and [10] on *Chrysanthemum frutescens* plant.

With regard to overall effect of the pinching is commonly practiced promoting bushy growth of the canopy by counteracting the apical dominance. Apical dominance plays a significant role in potted plant production. It has direct relationship with plant form and subsequent potential for yield increment too [29]. However, removal of apical dominance enhances the growth of lateral branches and preliminary experiments of this study revealed that hand pruning (thumb nail pruning) is the most practicable and convenient pruning method in poinsettia production. In this experiment first pinching was done leaving 5 nodes in the stem where there was a possibility to produce 5-6 side shoots. From the second pinching, that was practiced to leave5 nodes in each lateral shoot; it was expected to number of shoots multiply again by 5-6 folds.

However, the plants sometimes produced shoots even more than the expected level (2 or more shoots from the same node). The results indicated that pinching at the right time produced more lateral shoots and make the pot plant well shaped, bushy and attractive. It was observed in the experiment that untreated plants also produced few lateral shoots during this period but did not give proper shape to the plant. According to [29], not only physical removal of bud but a number of other factors effect apical dominance and lateral branch production. Among these factors, light levels of the surrounding, inorganic nutrients and carbon dioxide are prominent. Since, this experiment was conducted under long day conditions, the light factor may have some effect on additional shoot production and taller shoot production which were obtained in the control treatment.

### 3.2 Flowering Parameters

#### 3.2.1 Number of flowers/plant

Table 4 shows that all tested pinching and $PP_{333}$ treatments increased the number of flowers/plant as compared with control treatment in both seasons. On the other hand, the increase of number of flowers/plant of *Pelargonium zonale* L. plants is proportional with the increment of paclobutrazol concentration in the two seasons. Moreover, Table 4 indicates that all the interactions between pinching and paclobutrazol concentrations statistically increased the flowers number/plant, hence the highest number of flowers/plant was recorded by pinched plants sprayed with 60 ppm $PP_{333}$ (20.67 and 19.33 flowers/plant), followed in descending order by pinched plants provided with 40 ppm $PP_{333}$ (16.33 and 17.33 flowers/plant) in the first and second seasons, respectively. Also, unpinched plants sprayed with 60 ppm $PP_{333}$ gave highly increments in this parameter in both seasons.

**Table 2. Effect of pinching, paclobutrazol and their interaction on plant height, number of branches/plant and stem diameter/(cm) of *Pelargonium zonale* L. during 2015 and 2016 seasons**

| Pinching / Paclobutrazol | Plant height (cm) | | | Number of branches /plant | | | Stem diameter/(cm) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Without pinching | With pinching | Mean | Without pinching | With pinching | Mean | Without pinching | With pinching | Mean |
| **(2015 season)** | | | | | | | | | |
| Control **(**0.0 ppm**)** | 40.33$^{a}$ | 34.67$^{b}$ | 37.50$^{A}$ | 4.33$^{f}$ | 7.00$^{c}$ | 5.67$^{C}$ | 0.72$^{e}$ | 0.79$^{d}$ | 0.76$^{D}$ |
| 20 ppm | 25.33$^{c}$ | 23.33$^{e}$ | 24.33$^{B}$ | 5.33$^{e}$ | 7.67$^{cd}$ | 6.50$^{B}$ | 0.81$^{d}$ | 0.90$^{c}$ | 0.85$^{C}$ |
| 40 ppm | 24.33$^{d}$ | 20.00$^{g}$ | 22.17$^{C}$ | 8.33$^{c}$ | 12.67$^{b}$ | 10.50$^{A}$ | 0.89$^{c}$ | 1.07$^{b}$ | 0.98$^{B}$ |
| 60 ppm | 22.33$^{f}$ | 18.67$^{h}$ | 20.50$^{D}$ | 8.33$^{c}$ | 13.67$^{a}$ | 11.00$^{A}$ | 0.91$^{c}$ | 1.13$^{a}$ | 1.02$^{A}$ |
| Mean | 28.08$^{A}$ | 24.17$^{B}$ | | 6.58$^{B}$ | 10.25$^{A}$ | | 0.84$^{B}$ | 0.97$^{A}$ | |
| **(2016 season)** | | | | | | | | | |
| Control **(**0.0 ppm**)** | 42.00$^{a}$ | 33.67$^{b}$ | 37.83$^{A}$ | 4.00$^{f}$ | 6.67$^{de}$ | 5.33$^{D}$ | 0.74$^{e}$ | 0.81$^{d}$ | 0.76$^{D}$ |
| 20 ppm | 26.67$^{c}$ | 21.67$^{f}$ | 24.17$^{B}$ | 6.00$^{e}$ | 8.67$^{c}$ | 7.33$^{C}$ | 0.81$^{d}$ | 0.91$^{c}$ | 0.86$^{C}$ |
| 40 ppm | 23.67$^{d}$ | 21.00f$^{g}$ | 22.33$^{C}$ | 7.67$^{cd}$ | 12.67$^{b}$ | 10.17$^{B}$ | 0.91$^{c}$ | 1.07$^{b}$ | 0.99$^{B}$ |
| 60 ppm | 22.67$^{e}$ | 20.67$^{g}$ | 21.67$^{D}$ | 8.67$^{c}$ | 14.67$^{a}$ | 11.67$^{A}$ | 0.91$^{c}$ | 1.17$^{a}$ | 1.03$^{A}$ |
| Mean | 28.75$^{A}$ | 24.25$^{B}$ | | 6.58$^{B}$ | 10.67$^{A}$ | | 0.84$^{B}$ | 0.99$^{A}$ | |

**Table 3. Effect of pinching, paclobutrazol and their interaction on fresh and dry weights of leaves/plant of *Pelargonium zonale* L. during 2015 and 2016 seasons**

| Pinching / Paclobutrazol | Leaves fresh weight (g) | | | Leaves dry weight (g) | | |
|---|---|---|---|---|---|---|
| | Without pinching | With pinching | Mean | Without pinching | With pinching | Mean |
| **(2015 season)** | | | | | | |
| Control (0.0 ppm) | $24.80^{h}$ | $28.80^{g}$ | $26.80^{D}$ | $1.36^{g}$ | $1.81^{f}$ | $1.59^{D}$ |
| 20 ppm | $31.50^{f}$ | $38.77^{c}$ | $35.13^{C}$ | $3.62^{e}$ | $4.26^{c}$ | $3.94^{C}$ |
| 40 ppm | $34.73^{e}$ | $40.43^{b}$ | $37.58^{B}$ | $3.97^{d}$ | $4.44^{b}$ | $4.21^{B}$ |
| 60 ppm | $35.83^{d}$ | $43.23^{a}$ | $39.53^{A}$ | $3.99^{d}$ | $4.75^{a}$ | $4.37^{A}$ |
| Mean | $31.72^{B}$ | $37.81^{A}$ | | $3.24^{B}$ | $3.82^{A}$ | |
| **(2016 season)** | | | | | | |
| Control (0.0 ppm) | $23.83^{h}$ | $29.17^{g}$ | $26.50^{D}$ | $1.24^{f}$ | $1.86^{e}$ | $1.55^{C}$ |
| 20 ppm | $31.87^{f}$ | $39.80^{c}$ | $35.83^{C}$ | $3.92^{d}$ | $4.43^{c}$ | $4.18^{B}$ |
| 40 ppm | $34.43^{e}$ | $43.70^{b}$ | $39.07^{B}$ | $3.86^{d}$ | $4.75^{b}$ | $4.31^{A}$ |
| 60 ppm | $35.47^{d}$ | $46.03^{a}$ | $40.75^{A}$ | $3.81^{d}$ | $4.95^{a}$ | $4.38^{A}$ |
| Mean | $31.40^{B}$ | $39.67^{A}$ | | $3.21^{B}$ | $4.00^{A}$ | |

**Table 4. Effect of pinching, paclobutrazol and their interaction on leaf area / ($cm^2$) and number of leaves /plant of *Pelargonium zonale* L. during 2015 and 2016 seasons**

| Pinching / Paclobutrazol | Leaf area / ($cm^2$) | | | Number of leaves /plant | | |
|---|---|---|---|---|---|---|
| | Without pinching | With pinching | Mean | Without pinching | With pinching | Mean |
| **(2015 season)** | | | | | | |
| Control (0.0 ppm) | $25.35^{a}$ | $16.53^{d}$ | $20.94^{A}$ | $25.67^{h}$ | $33.67^{g}$ | $29.67^{D}$ |
| 20 ppm | $18.90^{b}$ | $10.34^{f}$ | $14.62^{B}$ | $36.67^{f}$ | $41.67^{c}$ | $39.17^{C}$ |
| 40 ppm | $17.83^{c}$ | $9.66^{g}$ | $13.75^{C}$ | $38.67^{e}$ | $44.00^{b}$ | $41.33^{B}$ |
| 60 ppm | $13.40^{e}$ | $8.67^{h}$ | $11.03^{D}$ | $40.67^{d}$ | $45.00^{a}$ | $42.83^{A}$ |
| Mean | $18.87^{A}$ | $11.30^{B}$ | | $35.42^{B}$ | $41.08^{A}$ | |
| **(2016 season)** | | | | | | |
| Control (0.0 ppm) | $24.63^{a}$ | $17.30^{c}$ | $20.97^{A}$ | $24.00^{f}$ | $34.67^{e}$ | $29.33^{D}$ |
| 20 ppm | $18.53^{b}$ | $10.90^{f}$ | $14.72^{B}$ | $36.67^{d}$ | $43.67^{b}$ | $40.17^{C}$ |
| 40 ppm | $16.60^{d}$ | $10.50^{f}$ | $13.55^{C}$ | $37.33^{d}$ | $46.33^{a}$ | $41.83^{B}$ |
| 60 ppm | $13.73^{e}$ | $8.87^{g}$ | $11.30^{D}$ | $40.00^{c}$ | $46.67^{a}$ | $43.33^{A}$ |
| Mean | $18.38^{A}$ | $11.89^{B}$ | | $34.50^{B}$ | $42.83^{A}$ | |

#### 3.2.2 Fresh and dry weights of flowers (g)

Table 5 reveals that, fresh and dry weights of flowers per plant were increased with pinching in both seasons. Besides, all tested $PP_{333}$ concentrations statistically increased the fresh and dry weights of flowers per plant with superior for the high concentrations in both seasons. However, the heaviest flowers fresh and dry /plant were gained by the combination of 60 ppm $PP_{333}$ and pinching treatments, followed in descending order by the interaction between $PP_{333}$ at the medium concentration (40 ppm) with pinching in the two seasons. Moreover, spraying un pinched plants with 60 ppm $PP_{333}$ gave highly significant increases in flowers fresh and dry weights/plant in the two seasons. On contrary, the lowest values of flowers fresh and dry weights/plant were gained by un-treated (without pinching) plants in the two seasons.

The obtained results of the studied flowering growth traits as affected by paclobutrazol coincided with those of, [30] on *Cestrum elegans* and *Tecoma stans*, [31] on *Consolida orientalis* and Jungklang. [25] on *Tabernaemontana coronaria*, [26] on *Murraya exotica* and *Duranta repens* plants and [10] on *Chrysanthemum frutescens* plant.

#### 3.2.3 Time to flowering (days)

Table 6 shows that all tested pinching and $PP_{333}$ treatments delayed the flowering (increasing the number of days from planting to start flowering) of *Pelargonium zonale* L. plants as compared with untreated control plants in both seasons. However, the highest retardation of *Pelargonium zonale* L. flowering was gained by pinched plants sprayed with 60 ppm $PP_{333}$ (106.7 and 106.3 days), followed in descending order by pinched plants sprayed with 40 ppm $PP_{333}$ (101.0 and 101.3 days), in the first and second seasons, respectively. Moreover, the combinations of $PP_{333}$ at 20 ppm and pinching and $PP_{333}$ at 60 ppm without pinching gave high retardation of flowering process in both seasons. On the reverse, the earliest flowering was occurred by untreated plants control treatment as it registered (80.00 and 81.67) days, followed in ascending order by un pinched plants sprayed with 20 ppm $PP_{333}$ (85.67 and 88.00 days), in the first and second seasons, respectively.

#### 3.2.4 Show value (plant width / height ratio)

Table 6 demonstrates that pinching the plants recorded highly significant increases of the show value of *Pelargonium zonale* L. (0.64 and 0.65) as compared with control treatment (without pinching) (0.55 and 0.56) in the first and second seasons, respectively. On the other hand, all tested $PP_{333}$ concentrations increased the show value as compared with un-treated control in the two seasons, especially the highest concentrations of $PP_{333}$ 60 ppm. In addition, all the combinations treatments between $PP_{333}$ and pinching increased the show value of *Pelargonium zonale* L. plants when compared with control plants in both seasons. However, the highest scores of show value was recorded by pinched plants sprayed with 60 ppm $PP_{333}$ (0.71 and 0.72), followed in descending order by pinched plants sprayed with 40 ppm $PP_{333}$ (0.69 and 0.70) and finally un pinched plants sprayed with 60 ppm $PP_{333}$ (0.69 and 0.69) in the first and second seasons, respectively. On contrary, the lowest values were scored by control plants without pinching which recorded 0.42 and 0.43 followed in ascending order by $PP_{333}$ at the lowest concentration (20 ppm), without pinching which scored 0.47 and 0.48 in the two seasons, respectively.

### 3.3 Root Growth Parameters

#### 3.3.1 Number of roots / plant

Table 7 Illustrates that, the mean number of roots per plant increased progressively with increasing paclobutrazol concentration and pinching in both seasons. So, the highest $PP_{333}$ concentration (60 ppm) combined with pinching showed to be the most effective combination for inducing the highest number of roots per plant (36.33 and 37.67 roots / plant), followed in descending order by combination the high $PP_{333}$ (60 ppm) without pinching (35.67 and 34.33 roots / plant) in the first and second seasons, respectively. In addition, the medium $PP_{333}$ (40 ppm) provided with pinching recorded highly significant increments in this respect in both seasons. Irrespective control plants, the lowest values of this parameter were gained by the low $PP_{333}$ supplemented with un pinched plants in the two seasons.

#### 3.3.2 Fresh and dry weights of roots (g)

Table 7 states that all tested pinching and $PP_{333}$ statistically increased fresh and dry weights of roots per plant as compared with control in both seasons. However, the highest $PP_{333}$ (60 ppm) supported with pinching showed superiority in this concern, as it induced the heaviest fresh weight of roots/plant, followed in descending order by using the highest concentration of paclobutrazol (60 ppm) supplemented with un pinched plants in the two seasons. While, the heaviest dry weight of roots/plant, by using the highest $PP_{333}$ concentration (60 ppm) provided with un pinched plants followed in descending order was scored by the highest $PP_{333}$ concentration (60 ppm) provided with pinching in the two seasons. In addition, $PP_{333}$ at the medium concentration (40 ppm) with or without pinching recorded high significant increases in this regard in both seasons of this study. On the opposite, the lowest values of roots fresh and dry weights were scored by un-treated plants in the two seasons. Similar results were reported earlier by, [23] on sunflower [25] on *Tabernaemontana coronaria*, [26] on *Duranta repens* and *Murraya exotica* plants, [27] on *Hibiscus rosea sinensis* plant, [28] on kumquat plant and [10] on *Chrysanthemum frutescens* plant.

### 3.4 Chemical Compositions

Results of chemical compositions of *Pelargonium zonale* L. leaf samples (Tables 8 and 9) revealed that using pinching and paclobutrazol treatments resulted in an increment in N, P, K, total carbohydrates %, total chlorophylls (mg /100 g F.W) and total phenols (mg /100 g F.W) when compared with control in both seasons. However, the highest values of N, P, K, total carbohydrates % and total chlorophylls of *Pelargonium zonale L.*eaf were registered by the combination treatments between $PP_{333}$ 60 ppm sprayed plants with pinching, followed in descending order by un pinched plants sprayed with $PP_{333}$ 60 ppm paclobutrazol in the two seasons. Meanwhile, the highest leaf total phenols (mg /100 g F.W) content was gained by 40 ppm $PP_{333}$ sprayed with un pinched plants, followed by un pinched plants sprayed with $PP_{333}$ 60 ppm in the first and second seasons. Irrespective control plants, the lowest values of above mention chemical composition were gained by the low $PP_{333}$ of (20 ppm) with or without pinching in the two seasons. As for the explanation of the incremental effect of paclobutrazol on chemical constituents in leaf of *Pelargonium zonale*, it could be illustrated here on the basis that paclobutrazol treatments stimulated the endogenous cytokinins synthesis 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 [32]. 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 chloroplast differentiation, while the second group could be related to cytokinin stimulated group [33]. 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 [30] on *Cestrum elegans* and *Tecoma stans*, [24] on patumma cv. Chiang Mai Pink, [25] on *Tabernaemontana coronaria*, [26] on *Murraya exotica* and *Duranta repens* plants, [27] on *Hibiscus rosea sinensis* plant, [28] on kumquat plant and [10] on *Chrysanthemum frutescens* plant.

### 3.5 Endogenous Phytohormones

Table 10 shows the changes in endogenous plant hormones content i.e., gibberellic acids ($GA_3$), indole acetic Acid (IAA), indole butyric Acid (IBA), cytokinins, abscisic acid (ABA), and salicylic acid of potted *Pelargonium zonale L.*. plants on 90 days after transplanting during 2016 season. It is quite evident that $PP_{333}$ sprays at 20,40 and 60 ppm with or without pinching greatly improved the morphological, metabolical and histological performances of geranium plant as obvious from the previously and after mentioned and discussed results obtained in the present study).

As for auxins level, it was highly decreased in *Pelargonium zonale* L. shoots with different assigned tested treatments compared with that of the control plants whether pinching practiced or absent. In this respect, pinching with $PP_{333}$ at 60 ppm was the most effective treatment which highly decreased auxins content compared with control and other used treatments. Also, auxins content was highly decreased in cases of pinching compared with or without pinching treatments. With regard to gibberellins level, data in Table 10 also clearly show that the level of gibberellin like-substances in *Pelargonium zonale L.* shoots was decreased with $PP_{333}$ at different used concentrations i.e., 20, 40 and 60 ppm in the two cases of pinching and without pinching treatments as compared with control plants, but it mostly decreased in case of pinching with $PP_{333}$ at 60 ppm treatment.

Furthermore, Table 10 clearly indicates that the level of cytokinins positively responded to the different assigned treatments. Since, the activity was the lowest in case of the control.

Generally, these phytohormones of promote growth aspects (i.e., growth promoters, auxins, gibberellin and cytokinin) were highly increased with different assigned $PP_{333}$ treatments. Here the treatment of pinching with $PP_{333}$ at 60 ppm gave the highest value activity of promoting phytohormones level, where the increment reached to 109.80 % relative to the control value. Also, increment of endogenous hormones in geranium plants obtained in the present study could be interpret both of the obtained modifications in different studied histological features (Tables 11 and 12) and the improvement of growth (Tables 2, 3 and 4) and flowering characteristics (Tables 5 and 6). For example, increasing cytokinins could be in favor of increasing the number of formed branches and that could also increase transverse growth. Once again, pinching with $PP_{333}$ at 60 ppm treatment showed the highest values of cytokinins , $GA_3$ and lowest value of IAA content in *Pelargonium zonale* L. shoot. In this respect, it was reported that $PP_{333}$ is a triazole compound widely used as retardant for controlling the vegetative growth of a wide range of angiosperm [34] and [35]. Paclobutrazol induce a variety of morphological, physiological and biochemical responses in plants, including delayed senescence, reducing gibberellin biosynthesis, increased cytokinins synthesis and alterations in secondary metabolite contents [25,36,37,38,39] and [40]. The main effect of paclobutrazol takes place through the alteration of hormonal balance. Since, it inhibits gibberellin biosynthesis hence, reducing cell division and cell elongation and retarding plant growth, whereas it promotes treated plants to create more cytokinins [8,41] and [37]. Several researchers have reported that paclobutrazol reduced plant growth of plant [25,39,40,42] and [43]. This may explain the increases of cytokinins and other promoting hormones in response to paclobutrazol applications compared with the control plants.

With regard to the growth inhibitor, (abscisic acid) its level was reduced with various assigned treatments compared with the control, but the reduction was more obvious with the combination of pinching and $PP_{333}$ at 60 ppm treatment.

As for, Table 10 shows that salicylic acid level was increased with the different tested treatments compared with the control and reached its maximum value in case of pinching with $PP_{333}$ at 60 mg/L treatment. In this respect, these results being of great interest for interpreting each of the obtained vigorous growth and the great flowering of *Pelargonium zonale* L. plant attained in the present study.

### 3.6 Anatomical Study

Data in (Tables 11 and 12) and Figs. 1, 2, 3 and 4 show the effect of different applied treatments on the mean counts and measurements in microns of certain histological features of the stem and leaf of (*Pelargonium zonale* L.) on 90 days after transplanting during 2016 season. In this work, *Pelargonium zonale* L. was anatomically examined to determine diagnostic characters to assess relationship between the applied treatments with the leaf and stem anatomy.

#### 3.6.1 Effect of pinching, paclobutrazol and their interaction treatments on *Pelargonium zonale L.* leaf anatomy

Data in Table 11 and (Figs. 1 and 2) clearly indicated that most of the studied features of leaf anatomy were increased with different applied treatments. Among these anatomical features were the most important ones, i.e., thickness of 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.

With regard to blade thickness, it was increased with different tested treatments to reach its maximum values (553µ) due the combination of pinching and $PP_{333}$ at 60 ppm and (456µ) in case of pinching with $PP_{333}$ at 40 ppm treatments compared with control values (313 and 267µ) with and without pinching respectively. Also, the thickness of each of upper and lower epidermis, were also increased with all applied treatments. Also, it could be noticed that increase ratio was higher of upper epidermis than that of the lower one.

As for mesophyll tissue, thickness of both spongy and palisade tissues was increased with different tested treatments. Here, $PP_{333}$ at different used concentrations as with and without pinching respectively, were the most effective treatments in this order compared with the control.

It is interest, to note that mesophyll increase belongs to that increase of each of palisade and spongy tissue thickness. Since, the two components were increased with different tested treatments but reached their maximum as other traits due to pinching combined with $PP_{333}$ at 60 ppm treatment.

With regard to midrib anatomical features, it could be noticed that increment in the midrib thickness was achieved due to different applied treatments compared with the control with and without pinching. This may have attributed to the increase in many of its histological features such as thickness of both uppermost and lower most collenchyma tissues, lower most parenchyma tissue and dimensions of main vascular bundle as well as thickness of phloem tissue, xylem tissue and also number and diameter of xylem vessels in the main vascular bundle. This increase was more obvious with of pinching and $PP_{333}$ at 60 ppm combination. The above-mentioned results especially increment of the conductive tissues (xylem and phloem) are also of great importance because they could be also involved in the interpretation about why vigorous were existed with different applied treatments especially when pinching combined with $PP_{333}$ at 60 ppm treatment. In general, these positive alterations in leaf anatomy of *Pelargonium zonale* L. plants in response to the treatments led to vigorous growth and enhance of flowering of treated plants. That as well mentioned above wards reversed upon to achieving attractive pot-grown plants.

**Table 5. Effect of pinching, paclobutrazol and their interaction on number of flowers /plant, flowers fresh and dry weights (g) of *Pelargonium zonale* L. during 2015 and 2016 seasons**

| Pinching / Paclobutrazol | Number of flowers /plant | | | Flowers fresh weight (g) | | | Flowers dry weight (g) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Without pinching | With pinching | Mean | Without pinching | With pinching | Mean | Without pinching | With pinching | Mean |
| | | | | (2015 season) | | | | | |
| Control (0.0 ppm) | 8.67$^{g}$ | 12.67$^{d}$ | 10.67$^{D}$ | 31.57$^{f}$ | 42.30$^{d}$ | 36.93$^{C}$ | 6.43$^{g}$ | 9.87$^{d}$ | 8.15$^{D}$ |
| 20 ppm | 9.67$^{f}$ | 14.67$^{c}$ | 12.17$^{C}$ | 39.83$^{e}$ | 61.27$^{b}$ | 50.55$^{B}$ | 7.78$^{f}$ | 13.17$^{c}$ | 10.48$^{C}$ |
| 40 ppm | 10.67$^{e}$ | 16.33$^{b}$ | 13.50$^{B}$ | 42.40$^{d}$ | 58.37$^{c}$ | 50.38$^{B}$ | 8.12$^{e}$ | 13.33$^{bc}$ | 10.73$^{B}$ |
| 60 ppm | 12.67$^{d}$ | 20.67$^{a}$ | 16.67$^{A}$ | 58.40$^{c}$ | 69.27$^{a}$ | 63.83$^{A}$ | 13.47$^{b}$ | 16.33$^{a}$ | 14.90$^{A}$ |
| Mean | 10.42$^{B}$ | 16.08$^{A}$ | | 43.05$^{B}$ | 57.80$^{A}$ | | 8.95$^{B}$ | 13.18$^{A}$ | |
| | | | | (2016 season) | | | | | |
| Control (0.0 ppm) | 8.00$^{f}$ | 11.67$^{d}$ | 9.83$^{D}$ | 31.23$^{g}$ | 43.30$^{e}$ | 37.27$^{D}$ | 6.50$^{h}$ | 10.02$^{e}$ | 8.26$^{D}$ |
| 20 ppm | 10.33$^{e}$ | 13.67$^{c}$ | 12.00$^{C}$ | 40.48$^{f}$ | 59.27$^{c}$ | 49.88$^{C}$ | 7.88$^{g}$ | 14.11$^{c}$ | 11.00$^{C}$ |
| 40 ppm | 10.67$^{de}$ | 17.33$^{b}$ | 14.00$^{B}$ | 43.73$^{e}$ | 61.27$^{b}$ | 52.50$^{B}$ | 8.13$^{f}$ | 14.43$^{b}$ | 11.28$^{B}$ |
| 60 ppm | 13.33$^{c}$ | 19.33$^{a}$ | 16.33$^{A}$ | 57.73$^{d}$ | 70.47$^{a}$ | 64.10$^{A}$ | 12.98$^{d}$ | 15.97$^{a}$ | 14.48$^{A}$ |
| Mean | 10.58$^{B}$ | 15.50$^{A}$ | | 43.30$^{B}$ | 58.58$^{A}$ | | 8.87$^{B}$ | 13.63$^{A}$ | |

**Table 6. Effect of pinching, paclobutrazol and their interaction on time to flowering (days) and show value of *Pelargonium zonale* L. during 2015 and 2016 seasons**

| Pinching / Paclobutrazol | Time to flowering (days) | | | Show value | | |
|---|---|---|---|---|---|---|
| | Without pinching | With pinching | Mean | Without pinching | With pinching | Mean |
| | | | (2015 season) | | | |
| Control (0.0 ppm) | 80.00$^{g}$ | 92.67$^{d}$ | 86.83$^{D}$ | 0.42$^{e}$ | 0.49$^{d}$ | 0.45$^{D}$ |
| 20 ppm | 85.67$^{f}$ | 96.67$^{c}$ | 91.17$^{C}$ | 0.47$^{d}$ | 0.65$^{bc}$ | 0.56$^{C}$ |
| 40 ppm | 91.00$^{e}$ | 101.0$^{b}$ | 96.00$^{B}$ | 0.63$^{c}$ | 0.69$^{ab}$ | 0.66$^{B}$ |
| 60 ppm | 95.33$^{c}$ | 106.7$^{a}$ | 101.0$^{A}$ | 0.69$^{ab}$ | 0.71$^{a}$ | 0.70$^{A}$ |
| Mean | 88.00$^{B}$ | 99.50$^{A}$ | | 0.55$^{B}$ | 0.64$^{A}$ | |
| | | | (2016 season) | | | |
| Control (0.0 ppm) | 81.67$^{g}$ | 90.33$^{e}$ | 86.50$^{D}$ | 0.43$^{e}$ | 0.51$^{d}$ | 0.47$^{D}$ |
| 20 ppm | 88.00$^{f}$ | 98.67$^{c}$ | 93.33$^{C}$ | 0.48$^{d}$ | 0.67$^{b}$ | 0.58$^{C}$ |
| 40 ppm | 92.00$^{e}$ | 101.3$^{b}$ | 96.67$^{B}$ | 0.62$^{c}$ | 0.70$^{ab}$ | 0.66$^{B}$ |
| 60 ppm | 95.00$^{d}$ | 106.3$^{a}$ | 100.7$^{A}$ | 0.69$^{ab}$ | 0.72$^{a}$ | 0.71$^{A}$ |
| Mean | 89.17$^{B}$ | 99.42$^{A}$ | | 0.56$^{B}$ | 0.65$^{A}$ | |

**Table 7. Effect of pinching, paclobutrazol and their interaction on number of roots /plant, roots fresh and dry weights (g) of *Pelargonium zonale* L. during 2015 and 2016 seasons**

| Pinching / Paclobutrazol | Roots number/plant | | | Roots fresh weight(g) | | | Roots dry weight(g) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Without pinching | With pinching | Mean | Without pinching | With pinching | Mean | Without pinching | With pinching | Mean |
| | | | | **(2015 season)** | | | | | |
| Control (0.0 ppm) | 22.33$^{g}$ | 26.00$^{f}$ | 24.17$^{D}$ | 10.48$^{g}$ | 9.70$^{h}$ | 10.09$^{D}$ | 1.54$^{d}$ | 1.58$^{d}$ | 1.56$^{D}$ |
| 20 ppm | 28.33$^{e}$ | 32.33$^{c}$ | 30.33$^{C}$ | 14.17$^{f}$ | 16.23$^{e}$ | 15.20$^{C}$ | 1.91$^{c}$ | 1.88$^{c}$ | 1.90$^{C}$ |
| 40 ppm | 30.00$^{d}$ | 34.00$^{b}$ | 32.00$^{B}$ | 18.08$^{c}$ | 17.27$^{d}$ | 17.68$^{B}$ | 2.15$^{b}$ | 2.12$^{b}$ | 2.13$^{B}$ |
| 60 ppm | 35.67$^{a}$ | 36.33$^{a}$ | 36.00$^{A}$ | 19.90$^{b}$ | 22.40$^{a}$ | 21.15$^{A}$ | 2.33$^{a}$ | 2.18$^{b}$ | 2.26$^{A}$ |
| Mean | 29.08$^{B}$ | 32.17$^{A}$ | | 15.66$^{B}$ | 16.40$^{A}$ | | 1.98$^{A}$ | 1.94$^{A}$ | |
| | | | | **(2016 season)** | | | | | |
| Control (0.0 ppm) | 23.00$^{f}$ | 26.33$^{e}$ | 24.67$^{C}$ | 11.07$^{f}$ | 10.20$^{g}$ | 10.63$^{D}$ | 1.71$^{de}$ | 1.65$^{e}$ | 1.68$^{D}$ |
| 20 ppm | 28.67$^{d}$ | 33.00$^{b}$ | 30.83$^{B}$ | 16.08$^{e}$ | 16.10$^{e}$ | 16.09$^{C}$ | 1.85$^{c}$ | 1.78$^{cd}$ | 1.82$^{C}$ |
| 40 ppm | 30.67$^{c}$ | 32.67$^{b}$ | 31.67$^{B}$ | 17.67$^{d}$ | 18.40$^{c}$ | 18.03$^{B}$ | 2.18$^{b}$ | 2.09$^{b}$ | 2.14$^{B}$ |
| 60 ppm | 34.33$^{b}$ | 37.67$^{a}$ | 36.00$^{A}$ | 22.17$^{b}$ | 25.27$^{a}$ | 23.72$^{A}$ | 2.40$^{a}$ | 2.36$^{a}$ | 2.38$^{A}$ |
| Mean | 29.17$^{B}$ | 32.42$^{A}$ | | 16.74$^{B}$ | 17.49$^{A}$ | | 2.04$^{A}$ | 1.97$^{B}$ | |

**Table 8. Effect of pinching, paclobutrazol and their interaction on leaf total nitrogen percentage, leaf total phosphorus percentage and leaf potassium percentage of *Pelargonium zonale* L. contents during 2015 and 2016 seasons**

| Pinching / Paclobutrazol | Leaf total nitrogen percentage | | | Leaf total phosphorus percentage | | | Leaf potassium percentage | | |
|---|---|---|---|---|---|---|---|---|---|
| | Without pinching | With pinching | Mean | Without pinching | With pinching | Mean | Without pinching | With pinching | Mean |
| | | | | **(2015 season)** | | | | | |
| Control (0.0 ppm) | 2.09$^{d}$ | 2.14$^{d}$ | 2.12$^{D}$ | 0.217$^{c}$ | 0.221$^{bc}$ | 0.219$^{B}$ | 1.29$^{e}$ | 1.27$^{e}$ | 1.28$^{D}$ |
| 20 ppm | 2.24$^{c}$ | 2.23$^{c}$ | 2.24$^{C}$ | 0.223$^{abc}$ | 0.228$^{abc}$ | 0.226$^{AB}$ | 1.40$^{d}$ | 1.40$^{d}$ | 1.40$^{C}$ |
| 40 ppm | 2.28$^{bc}$ | 2.28$^{bc}$ | 2.28$^{B}$ | 0.228$^{abc}$ | 0.235$^{ab}$ | 0.232$^{A}$ | 1.58$^{c}$ | 1.65$^{b}$ | 1.61$^{B}$ |
| 60 ppm | 2.33$^{ab}$ | 2.36$^{a}$ | 2.35$^{A}$ | 0.233$^{abc}$ | 0.239$^{a}$ | 0.236$^{A}$ | 1.62$^{bc}$ | 1.71$^{a}$ | 1.66$^{A}$ |
| Mean | 2.24$^{A}$ | 2.25$^{A}$ | | 0.225$^{A}$ | 0.231$^{A}$ | | 1.47$^{B}$ | 1.50$^{A}$ | |
| | | | | **(2016 season)** | | | | | |
| Control (0.0 ppm) | 2.02$^{d}$ | 2.16$^{c}$ | 2.09$^{D}$ | 0.222$^{de}$ | 0.220$^{e}$ | 0.221$^{C}$ | 1.31$^{d}$ | 1.29$^{d}$ | 1.30$^{D}$ |
| 20 ppm | 2.19$^{c}$ | 2.20$^{c}$ | 2.20$^{C}$ | 0.227$^{cd}$ | 0.228$^{bc}$ | 0.228$^{B}$ | 1.45$^{bc}$ | 1.41$^{c}$ | 1.43$^{C}$ |
| 40 ppm | 2.31$^{b}$ | 2.31$^{b}$ | 2.31$^{B}$ | 0.232$^{ab}$ | 0.235$^{a}$ | 0.233$^{A}$ | 1.50$^{b}$ | 1.66$^{a}$ | 1.85$^{B}$ |
| 60 ppm | 2.34$^{ab}$ | 2.38$^{a}$ | 2.36$^{A}$ | 0.236$^{a}$ | 0.237$^{a}$ | 0.237$^{A}$ | 1.68$^{a}$ | 1.71$^{a}$ | 1.70$^{A}$ |
| Mean | 2.21$^{B}$ | 2.26$^{A}$ | | 0.229$^{A}$ | 0.230$^{A}$ | | 1.48$^{B}$ | 1.52$^{A}$ | |

**Table 9. Effect of pinching, paclobutrazol and their interaction on total carbohydrates percentage, total chlorophylls (mg/100 g F.W) and total phenols (mg/100g F.W) of *Pelargonium zonale* L. contents during 2015 and 2016 seasons**

| Pinching / Paclobutrazol | Leaf total carbohydrates percentage | | | Leaf total chlorophylls (mg/100g F.W) | | | Leaf total phenols (mg/100g F.W) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Without pinching | With pinching | Mean | Without pinching | With pinching | Mean | Without pinching | With pinching | Mean |
| **(2015 season)** | | | | | | | | | |
| Control **(**0.0 ppm**)** | $9.52^{e}$ | $8.49^{f}$ | $9.01^{D}$ | $151.1^{f}$ | $151.8^{f}$ | $151.4^{D}$ | $127.7^{e}$ | $125.4^{f}$ | $126.5^{D}$ |
| 20 ppm | $11.38^{c}$ | $10.88^{d}$ | $11.13^{C}$ | $161.0^{e}$ | $161.6^{e}$ | $161.3^{C}$ | $136.6^{c}$ | $129.2^{e}$ | $132.9^{C}$ |
| 40 ppm | $14.27^{b}$ | $14.11^{b}$ | $14.19^{B}$ | $180.7^{c}$ | $178.1^{d}$ | $179.4^{B}$ | $145.2^{a}$ | $135.1^{cd}$ | $140.2^{A}$ |
| 60 ppm | $15.83^{a}$ | $15.63^{a}$ | $15.73^{A}$ | $189.3^{b}$ | $191.2^{a}$ | $190.3^{A}$ | $139.2^{b}$ | $134.3^{d}$ | $136.8^{B}$ |
| Mean | $12.75^{A}$ | $12.28^{B}$ | | $170.5^{A}$ | $170.7^{A}$ | | $137.2^{A}$ | $131.0^{B}$ | |
| **(2016 season)** | | | | | | | | | |
| Control **(**0.0 ppm**)** | $8.85^{f}$ | $8.90^{f}$ | $8.88^{D}$ | $155.2^{g}$ | $152.6^{h}$ | $153.9^{D}$ | $126.2^{e}$ | 123.0f | $124.6^{D}$ |
| 20 ppm | $12.04^{d}$ | $11.20^{e}$ | $11.62^{C}$ | $170.6^{e}$ | $162.8^{f}$ | $166.7^{C}$ | $135.8^{c}$ | $130.9^{d}$ | $133.4^{C}$ |
| 40 ppm | $13.67^{c}$ | $15.07^{b}$ | $14.37^{B}$ | $178.3^{d}$ | $181.6^{c}$ | $180.0^{B}$ | $142.7^{a}$ | $136.0^{c}$ | $139.4^{A}$ |
| 60 ppm | $15.40^{b}$ | $16.27^{a}$ | $15.83^{A}$ | $189.6^{b}$ | $192.3^{a}$ | $191.0^{A}$ | $137.7^{b}$ | $135.5^{c}$ | $136.6^{B}$ |
| Mean | $12.49^{B}$ | $12.86^{A}$ | | $173.4^{A}$ | $172.3^{B}$ | | $135.6^{A}$ | $131.3^{B}$ | |

**Table 10. Effect of pinching, paclobutrazol and their interaction on endogenous phytohormones of *Pelargonium zonale* L. plant on 90 days after transplanting during 2016 season**

| Plant hormones / Treatments | | Promoters | | | | | | | Inhibitors | | Salicylic acid | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Gibberellins mg/100 g F.wt. | Auxins mg/100g F.wt. | | | Cytokinins mg/100g F.wt. | Total Promoters mg/100 g F.wt. | % relative to the control | Abscisic acid mg/100 g F.wt. | % relative to the control | mg/100 g F.wt. | % relative to the control |
| | | | indole acetic acid | Indole butyric acid | total mg/100g F.wt. | | | | | | | |
| control | Without pinching | 283.08 | 10.32 | 239.41 | 249.73 | 113.86 | 646.67 | 100.00 | 6.98 | 100.00 | 11.31 | 100.00 |
| $PP_{333}$ at 20 mg/L | | 266.89 | 14.28 | 206.77 | 221.05 | 162.16 | 650.10 | 100.53 | 2.94 | 42.12 | 12.66 | 111.94 |
| $PP_{333}$ at 40 mg/L | | 233.91 | 6.89 | 212.30 | 219.19 | 196.11 | 649.21 | 100.39 | 4.13 | 59.17 | 15.50 | 137.05 |
| $PP_{333}$ at 60 mg/L | | 144.11 | 18.01 | 196.63 | 214.64 | 296.06 | 654.81 | 101.26 | 5.14 | 73.64 | 14.40 | 127.32 |
| control | With pinching | 234.45 | 11.37 | 193.89 | 205.26 | 248.36 | 688.07 | 100.00 | 6.42 | 100.00 | 68.20 | 100.00 |
| $PP_{333}$ at 20 mg/L | | 214.93 | 16.94 | 180.43 | 197.37 | 292.63 | 704.93 | 102.45 | 4.91 | 76.48 | 101.63 | 149.02 |
| $PP_{333}$ at 40 mg/L | | 196.65 | 8.92 | 166.60 | 175.52 | 334.27 | 706.44 | 102.67 | 0.76 | 11.84 | 85.46 | 125.31 |
| $PP_{333}$ at 60 mg/L | | 212.83 | 15.95 | 108.69 | 124.64 | 418.01 | 755.48 | 109.80 | 0.13 | 2.02 | 95.67 | 140.28 |

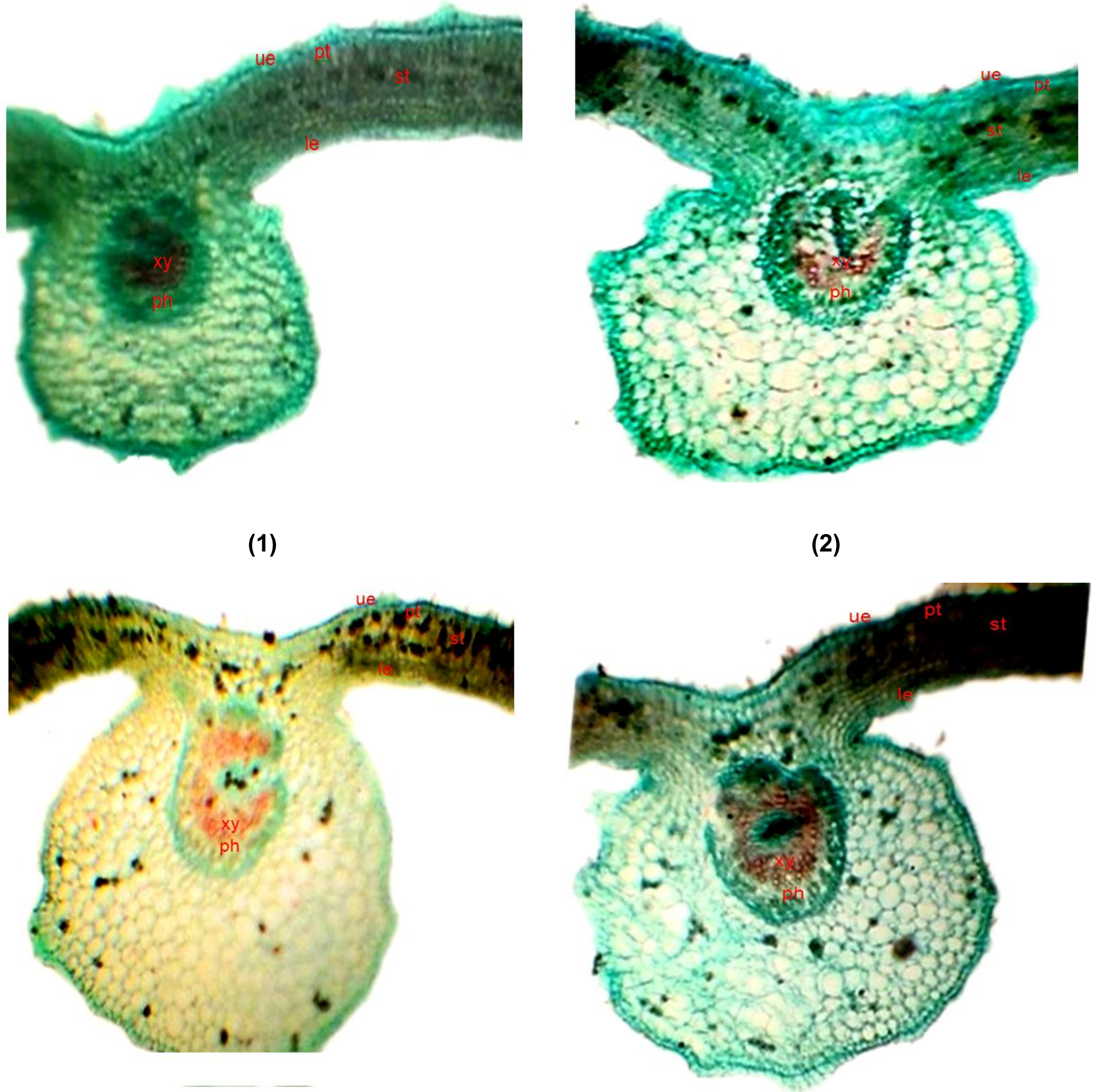
**Table 11. Effect of pinching, paclobutrazol and their interaction on the mean counts and measurements of certain histological features of *Pelargonium zonale* L. leaf on 90 days after transplanting during 2016 season**

| Treatments \ Histological characteristics (micron) | | Upper epidermis thickness | Lower epidermis thickness | Palisade tissue thickness | Spongy tissue thickness | Thickness of blade | Thickness of collenchyma layers below the upper epidermis at midrib | Thickness 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 | Without pinching | 27 | 19 | 91 | 130 | 267 | 224 | 134 | 52 | 163 | 14 | 5 | 35 | 227 | 646 | 1297 |
| $PP_{333}$ at 20 mg/L | | 29 | 15 | 122 | 141 | 307 | 251 | 131 | 74 | 182 | 18 | 8 | 41 | 288 | 824 | 1660 |
| $PP_{333}$ at 40 mg/L | | 26 | 18 | 150 | 152 | 346 | 128 | 133 | 49 | 225 | 16 | 7 | 40 | 292 | 808 | 1532 |
| $PP_{333}$ at 60 mg/L | | 28 | 17 | 151 | 185 | 381 | 165 | 155 | 162 | 211 | 22 | 7 | 42 | 381 | 835 | 1787 |
| control | With pinching | 23 | 15 | 116 | 159 | 313 | 217 | 175 | 137 | 231 | 16 | 6 | 37 | 373 | 783 | 1587 |
| $PP_{333}$ at 20 mg/L | | 30 | 20 | 136 | 162 | 348 | 235 | 101 | 126 | 237 | 23 | 7 | 44 | 395 | 982 | 1808 |
| $PP_{333}$ at 40 mg/L | | 27 | 18 | 179 | 232 | 456 | 267 | 149 | 115 | 226 | 21 | 6 | 42 | 388 | 1078 | 1577 |
| $PP_{333}$ at 60 mg/L | | 28 | 25 | 213 | 287 | 553 | 232 | 153 | 171 | 248 | 25 | 8 | 46 | 442 | 1232 | 1824 |

**Table 12. Effect of pinching, paclobutrazol and their interaction on the mean counts and measurements of certain histological features of *Pelargonium zonale* L. stem on 90 days after transplanting during 2016 season**

| Treatments \ Histological characteristics (micron) | | Stem diameter | Epidermis thickness | Thickness of collenchyma layers | Thickness of parenchyma layers | Thickness of phloem tissue | Cambium region thickness | Xylem tissue thickness | Number of xylem rows/Vascular cylinder | No. of xylem vessels / row | diameter of the widest xylem vessel in V. cylinder | Parenchymatous pith thickness |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | Without pinching | 3634 | 25 | 97 | 481 | 71 | 34 | 252 | 64 | 4 | 88 | 1503 |
| $PP_{333}$ at 20 mg/L | | 5171 | 31 | 137 | 594 | 92 | 48 | 347 | 72 | 5 | 119 | 2498 |
| $PP_{333}$ at 40 mg/L | | 5685 | 28 | 123 | 680 | 121 | 56 | 314 | 76 | 6 | 105 | 2758 |
| $PP_{333}$ at 60 mg/L | | 6022 | 21 | 142 | 720 | 132 | 61 | 361 | 83 | 8 | 108 | 2987 |
| Control | With pinching | 4853 | 24 | 161 | 505 | 119 | 59 | 169 | 66 | 3 | 109 | 2552 |
| $PP_{333}$ at 20 mg/L | | 5927 | 19 | 111 | 683 | 123 | 81 | 383 | 78 | 5 | 122 | 2843 |
| $PP_{333}$ at 40 mg/L | | 5864 | 27 | 157 | 797 | 108 | 94 | 381 | 77 | 9 | 115 | 2640 |
| $PP_{333}$ at 60 mg/L | | 7610 | 21 | 276 | 742 | 179 | 98 | 603 | 101 | 7 | 128 | 3492 |

Also, the previously mentioned and discussed results of *Pelargonium zonale* L. leaf anatomy of treated plants, reveal that enhancing of leaf anatomy features compared with control confirmed by vigorous growth of *Pelargonium zonale* L. was positively correlated with minerals content (N, P and K), photosynthetic pigments and carbohydrates contents. This confirmed the previously discussed results of growth, proved that the best morphological behavior of




(1) (2)

(3) (4)

**Fig. 1. Transverse sections (X = 40) through 5 th apical leaf of *Pelargonium zonale* L. plants on 90 days after transplanting as affected by different applied treatments**

*Where: (1): Control without pinching (2): Paclobutrazol at 20 mg/L without pinching*
*(3): Paclobutrazol at 40 mg/L without pinching (4): Paclobutrazol at 60 mg/L without pinching*
*ue= Upper epidermis; le= Lower epidermis; pt= Palisade tissue; st= Spongy tissue*
*ph= phloem tissue; xy= Xylem tissue*

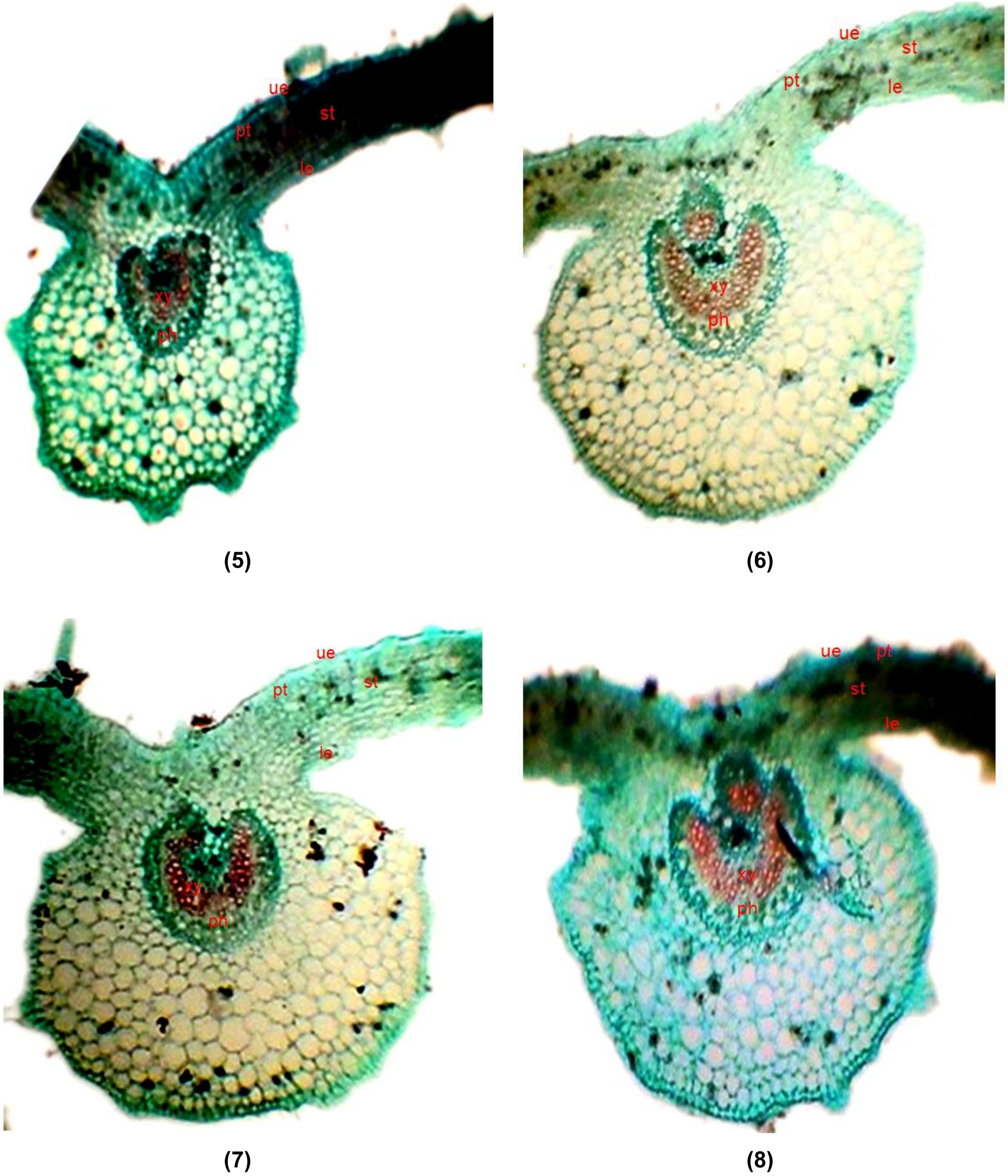



**Fig. 2. Transverse sections (X = 40) through 5 th apical leaf of *Pelargonium zonale* L. plants on 90 days after transplanting as affected by different applied treatments**

*Where: (5): Control with pinching (6): Paclobutrazol at 20mg/L with pinching*
*(7): Paclobutrazol at 40 mg/L with pinching (8): Paclobutrazol at 60 mg/L with pinching*
*ue= Upper epidermis; le= Lower epidermis; pt= Palisade tissue; st= Spongy tissue;*
*ph= phloem tissue; xy= Xylem tissue*

*Pelargonium zonale* L. plant as affected by the used treatments was mainly due to their induceable best physiological and anatomical performances. The obtained results are in agreement with those reported by [34] and [35] who mentioned that paclobutrazol is a triazole compound widely used as retardant for the controlling the vegetative growth of a wide range of angiosperm. Paclobutrazol induces a variety of morphological and anatomical responses in plants. The main effect of paclobutrazol takes place through the alteration of hormonal balance. Since, it inhibits gibberellin biosynthesis, reducing cell division and cell elongation and retarding plant growth [37,44] concluded that a reduced height and an increased stem thickness of tomato in response to paclobutrazol treatment. [8] had shown that paclobutrazol inhibits gibberellin biosynthesis, hence, reducing cell elongation and retarding plant growth. In addition, [45] stated that the cambial growth of *Liquidambar styraciflua* and *Alnus glutinosa* was reduced with paclobutrazol treatment. [46] on *Strelitzia reginae* treated plants with $PP_{333}$ at 300 and 200 ppm increased blade thickness, palisade thickness and phloem tissue thickness. [47] concluded that treating the *Solanum tuberosum* leaves with $PP_{333}$ induced increase epicuticular wax layer, elongated and thicker epidermal as well as the palisade and spongy mesophyll cells. [48] found that the number of cells per unit area in the palisade and spongy layers and number of chloroplast per cells in the leaves of *Solenostemon rotundifolius* increased by Paclobutrazol treatment when compared to the control leaves. [35] concluded that scanning electron micrographs of stems showed reduction in xylem thickness, while in the leaves, palisade and spongy parenchyma cells were more closely arranged in response to paclobutrazol. There was also an increase in palisade thickness of leaves of plants treated with 3.75 g $l^{-1}$ paclobutrazol. Paclobutrazol was found to effectively inhibit plant height and leaf expansion and alter the stem and leaf anatomy of *S. campanulatum*. [25] showed that treating *Tabernaemontana coronaria* plant with $PP_{333}$ affected on most anatomical features of leaves. 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.

Moreover, [49] concluded that paclobutrazol treatment increased thickness of leaflet midvein, length and width of midvein vascular bundle, phloem and xylem tissues thickness, number of xylem vessels in midvein vascular bundle as well as the leaflet lamina thickness of moringa plant.

#### 3.6.2 Effect of pinching, paclobutrazol and their interaction on *Pelargonium zonale* L. stem anatomy

Data in Table 12 and (Figs. 3 and 4) clearly indicate that different tested treatments increased the stem diameter compared with control. This increase reached its maximum values with the combination of pinching and $PP_{333}$ at 60 ppm treatment (7610 µ) compared with the control (4853) and (3634 µ) with and without pinching, respectively.

Also, it could be noticed that increase of the stem diameter were reversed upon different tissues comprising the whole section . Since, thickness of each cuticle layer, periderm, cortex (collenchyma and parenchyma tissues) and pith parenchyma layers as well as thickness of phloem tissues, cambial region and xylem tissue, number of xylem vessels /vascular cylinder and diameter of the widest xylem vessel were greatly increased with different applied treatments compared with the control. Also, pinching provided with $PP_{333}$ at 60 ppm treatment was the most pronounced combination in this respect.

Furthermore, increasing of vascular tissues is being of great interest because that could reverse 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. Also, width of the largest xylem vessel was proportional to the width of stem diameter. The aforementioned results of paclobutrazol ($PP_{333}$) are in line with those reported by [44] on *Solanum licopersicum* **,** [8] and [46] on *Strelitzia reginae*, [47] on *Solanum tuberosum*, [48] on *Solenostemon rotundifolius* [37] on *Ocimum sanctum*, [50] on Geranium, [25] on *Tabernaemontana coronaria* and [49] on moringa.

In general, the stimulatory effects of applied treatments upon the anatomy features of stem for treated plants could be attributed to the effect of tested treatments upon cambium activity. Increment of cambium activity could mainly attributed to the increase of endogenous hormones level especially cytokinins and auxins, [51] and [52] as well as the findings of the present study.

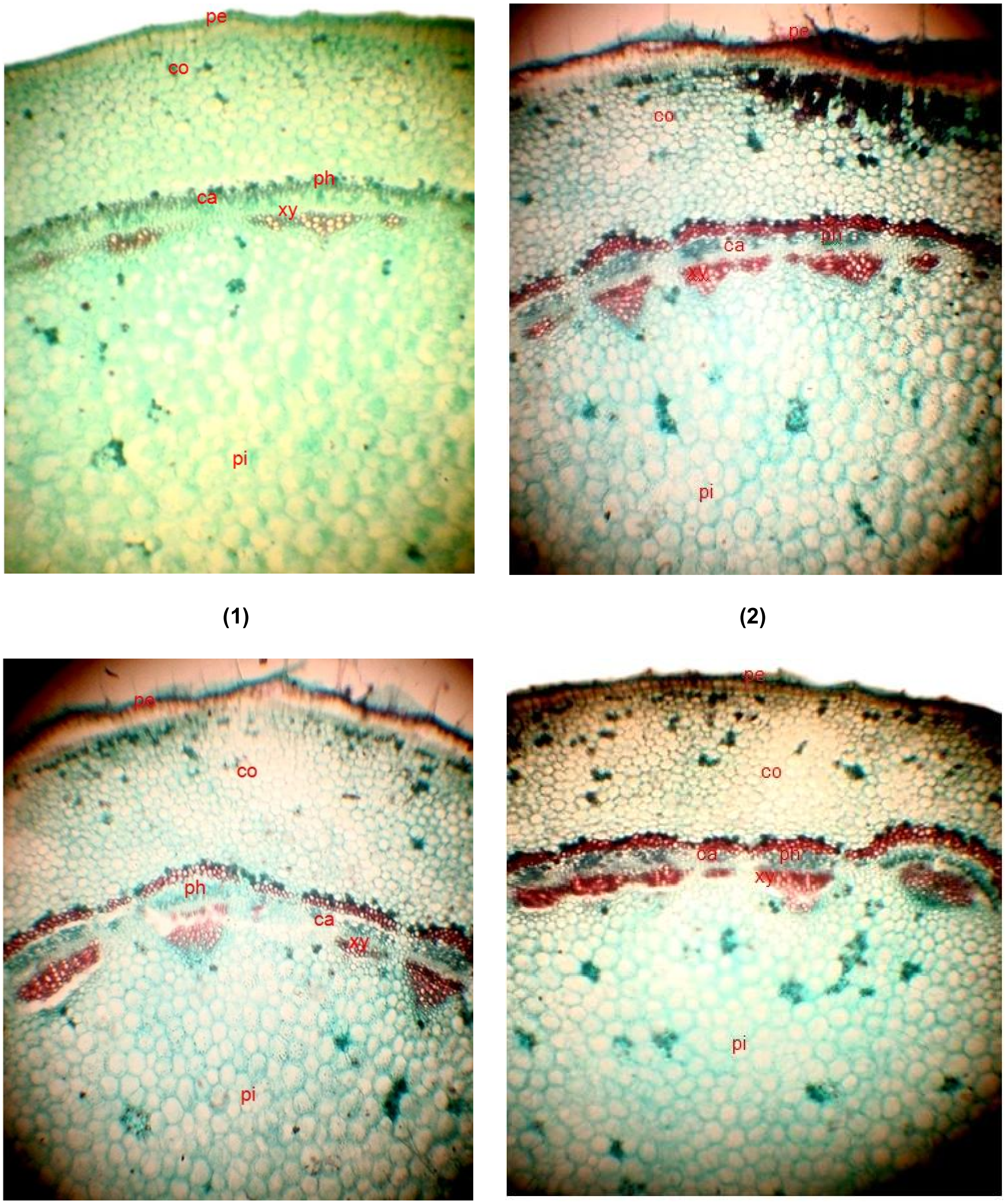
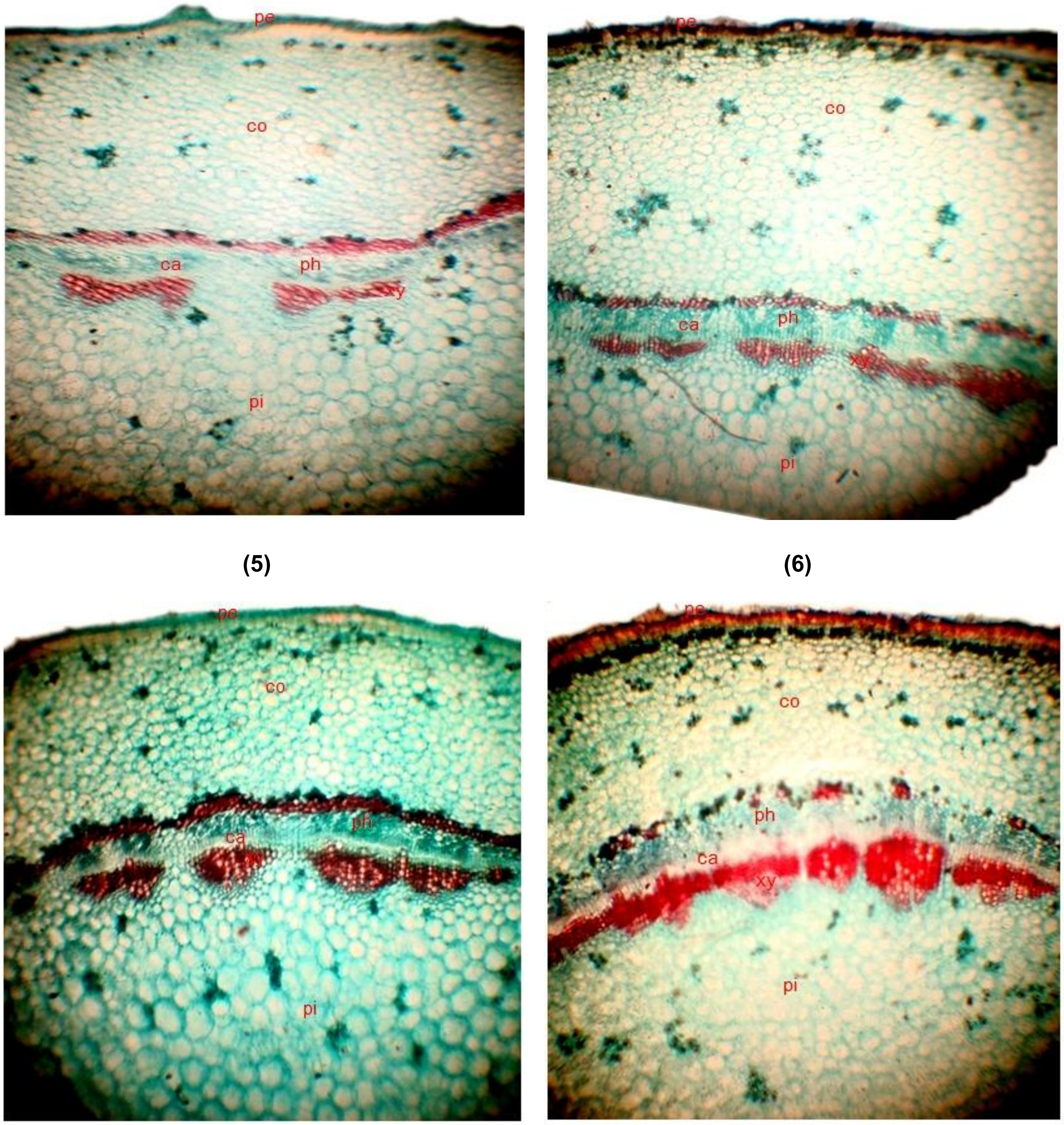



**Fig. 3. Transverse sections (X = 40) through 5 th internode of Pelargonium zonale L. stem plants on 90 days after transplanting as affected by different applied treatments.**

*Where: (1): Control without pinching (2): Paclobutrazol at 20 mg/L without pinching*
*(3): Paclobutrazol at 40 mg/L without pinching (4): Paclobutrazol at 60 mg/L without pinching*
*pe= periderm; co= Cortex; ph= phloem tissue; xy= Xylem tissue; ca= cambium; pi= pith*




**(7)** **(8)**

**Fig. 4. Transverse sections (X = 40) through 5 th internode of Pelargonium zonale L. stem plants on 90 days after transplanting as affected by different applied treatments**

*Where: (5): Control with pinching (6): Paclobutrazol at 20 mg/L with pinching*
*(7): Paclobutrazol at 40 mg/L with pinching (8): Paclobutrazol at 60 mg/L with pinching*
*pe= periderm; co= Cortex; ph= phloem tissue; xy= Xylem tissue; ca= cambium; pi= pith*

## 4. CONCLUSION

Generally, the obtained results indicated that all tested paclobutrazol concentration with or without pinching increased cytokinins and salicylic acid contents, but they decreased gibberellins, auxins and abscisic acid (mg/100g F.W) of *Pelargonium zonale* L. shoot. In this respect, paclobutrazol at 60 ppm with pinching was the most effective treatment compared with control and other used treatments. Regarding to the anatomical features of leaf and stem anatomy, most traits were increased with different applied treatments compared with the control, particularly paclobutrazol at 60 ppm with pinching treatment.

Conclusively, all gained results; those of achieving more dwarf plants of *Pelargonium zonale* L. with many formed flowers could be considered as pioneer results in this concern. Since, the combined treatments of paclobutrazol at 60 ppm with pinching gave a good display (show value) of flowering pot of *Pelargonium zonale* L. plant with optimum vegetative and flowering characteristics from the commercial point of view when compared with other treatments.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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