

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/



Effect of Brassinolide and Chitosan on Growth and Chemical

Composition of Aglaonema commutatum plant



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Abstract

In this study, the growth rate of Aglaonema plants (*Aglaonema commutatum*) was measured by applying different levels of brassinolide at (50, 100, and 200 ppm) and chitosan at (250, 500, and 1000 ppm) using foliar spray application. The obtained results showed that brassinolide at (200 ppm) foliar application affected significantly (number of leaves and root length). However, chitosan at (250 ppm) foliar application was more effective than brassinolide foliar application on plant growth parameters (plant height, stem diameter, total fresh and dry weights, and leaf area). Furthermore, chitosan at (250 ppm) foliar application increased the chemical parameters of Aglaonema plant (total chlorophylls, carotenoids, nitrogen, potassium, phenols, and indoles), and chitosan at (500 ppm) foliar application significantly increased (posphorus, phenols, and indoles). Both brassinolide at (200 ppm) and chitosan at (500 ppm) foliar application significantly increased (roots fresh and dry weights) of Aglaonema plant. The anatomical measurements of plants treated with chitosan foliar application showed higher values compared to plants treated with brassinolide foliar application and untreated plants.

Keywords: Brassinolide; Chitosan; Aglaonema; Aglaonema commutatum.

1. Introduction

Aglaonema is an attractive, evergreen perennial species from the aroid family, Araceae. Aglaonema *commutatum* is one of the most popular indoor plants due to its attractive variegated foliage and tolerance to low light and drought conditions [1]. Natively, it was originated from tropical and subtropical climates in Asia and New Guinea. Commonly known by the names Chinese Evergreen 'Lady valentine' or Aglaonema 'Pink Anyamanee'. 'Valentine' is a new hybrid from Thailand with attractive green and pink random blotches. This cultivar is grown for the stunning, variegated pink and green, heart-shaped leaves. This plant is a good choice for growing as a houseplant. It won't cope with freezing temperatures, bright indirect light is best and moist, well-draining soil. It produces beautiful, variegated green and pink foliage. The lance-shaped leaves are mostly pink, with green margins. The variegation will be more pronounced when grown in stronger lighting. Flowers can appear, they are white in color and similar to those of Peace Lilies [2].

Brassinolide (BR) is a natural product has been extracted from a plant (e.g., pollens) or other natural

source (e.g., beeswax) usually contains several types of structural analogues. It was widely found in angiosperms, gymnosperms, and some lower plants. From the perspective of the distribution in a plant, it exists in the roots, stems, leaves, pollens, pistils, fruits and seeds for example, pollens of canola. Brassinolide is internationally recognized as an efficient, broad-spectrum and non-toxic plant growth regulator [3].

Brassinolide applied to enhance quality and quantity of productivity, cell expansion and elongation, root development, cell division and cell wall regeneration of plants. Also, enhances tolerance in plant against adverse environmental conditions such as extreme temperatures, cold, rought, salinity, pesticides. After application of brassinolide, pathogens became unable to damage the treated plants [4].

Chitosan (CS) is a unique polysaccharide has been derived from chitin. Chitosan is a natural biopolymer; biodegradable has been extracted from the marine crustacean such as crabs and shrimps or from the exoskeletons of insects under the name of chitin which can be transformed into chitosan by extracting

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EJCHEM use only: Received date 17 August 2022; revised date 29 September 2022; accepted date 02 October 2022 DOI: 10.21608/EJCHEM.2022.156931.6807

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the acetyl group and turn it into amino. Chitosan contains nitrogen in the basic unit of its formula. When the contained nitrogen in the chitosan is dissolved, it penetrates gradually and remains in the soil for a long period of time. This compound is characterized by unique properties, such as bioactivity and bio-compatibility [5].

Chitosan could stimulate plant height, leaves umbers, chlorophylls, and wider roots of *Pachira aquatic*. Chitosan application in agriculture can improves the microbial population in large numbers, even without chemical fertilizers, and transforming nutrients from organic to inorganic, also improves the plant resistance to insects and diseases [6].

The objective of this study was to investigate the effect of two natural growth regulators (brassinolide and chitosan) treatments to improve the quantitative and qualitative growth characteristics of *Aglaonema commutatum* plant including chemical composition.

2. Experimental

2.1. Experimental Site, Cultivar and Cultivation

The experiment was carried out at the nursery of the Department of Ornamental Horticulture, Faculty of Agriculture, Cairo University. *Aglaonema commutatum* rooted plantlets with four leaves and 10 cm height were obtained from the Tissue Culture Lab in Agriculture Research Centre (ARC), Ministry of Agriculture (Egypt) and planted in the greenhouse in March 2019. One month later, plantlets were transplanted individually into 22 cm plastic pots filled with mixture of peat moss and sand (1:1 v:v.). Plants were harvested and data were recorded after one extended season in November 2020.

2.2. Treatments Preparation

Aglaonema commutatum plantlets were received a standard fertilization treatment N P K (2g/pot/month) with three different concentrations of brassinolide at (50, 100, and 200 ppm) which prepared by dissolving 50, 100, and 200 mg of brassinolide in 1 liter of water, respectively and 5% Tween 20 were added, and chitosan at (250, 500, and 1000 ppm) which prepared by dissolving 250, 500, and 1000 mg of chitosan in 1 liter of water, respectively (5% Tween 20 and 0.5% nitric acid were added) foliar spray and a group of control treatment. The foliar spray treatments were applied once every month.

2.3. Vegetative growth parameters and chemical content determination

Samples of *Aglaonema commutatum* plants were taken to determine vegetative growth parameters; plant height, stem diameter, total vegetative fresh and dry weights, leaf area the 3rd leaf from the top was measured (using digital leaf area meter), numbers of

leaves, and roots fresh and dry weights and chemical contents of total carbohydrates was measured on dry sample at 490 nm using spectrophotometer against a reagent blank and were calculated using standard curve of glucose [7], contents of total chlorophylls and carotenoids was measured on fresh sample at 470, 647 and 663 nm with a spectrophotometer [8] using the following equations:

Chlorophyll a (mg/L) = 9.784 E 660 - 0.99 E 640Chlorophyll b (mg/L) = 21.426 E 640 - 4.65 E 660

Carotenoids (mg/L) = 4.695 E440 - [0.268 (a+b)]Nitrogen content were determined using dry sample by the modified micro-Kjeldahl method [9], phosphorus content was measured colourimetrically using dry sample at 650 nm wave length and calculated using a standard curve of dihydrogen phosphate [10], potassium content was determined using dry sample by using Pye Unicam model Sp 1900 Atomic Absorption, Flame emission spectrophotometer with a boiling air-acetylene burner and recorded read out [11], contents of total phenols was calculated using fresh sample by using the Folin-Ciocalteu reagent with some alteration by using gallic acid as a standard curve and then assessed at 765 nm using the spectrophotometer [12], and contents of sample total indoles using fresh using spectrophotometer [13].

2.4. Anatomical observation

Anatomical data including leaf blade thickness, mesophyll thickness, upper and lower epidermis, Vascular bundle length and width, Xylem vessels diameter, Palisade tissue, and Spongy tissue was observed by digital microscope Nikon ND 600. The image was subject for analysis using imageJ for quantification. Microscopic slides for epidermis and its epicuticular wax were prepared by section paraffin method [14].

2.5. Experimental Design

The layout of the experiment was a completely randomized design included 6 treatments beside control treatment. Each treatment was replicated 3 times, with 6 pots in each replicate.

2.6. Statistical Analysis

Statistical analyses of the pooled data were performed with SPSS Software [15]. The data were analysed by one-way analysis of variance (ANOVA).

3. Results and Discussion

3.1. Effect of brassinolide and chitosan on vegetative growth parameters of *Aglaonema commutatum* plant. As shown in the following tables (1, and 2) chitosan affected significantly plant growth parameters (plant

height, stem diameter, total vegetative fresh and dry weights, leaf area, numbers of leaves, and roots fresh and dry weights). This significant effect of chitosan on plant growth may be attributed to an increase in the availability and uptake of water and essential nutrients and also synthesize plant hormones which enhances growth by some signaling pathways related to auxin biosynthesis [16]. Moreover, chitosan contains nitrogen in the basic unit of its formula which dissolves gradually and remains in the soil for a long period of time.

As shown in table (1), treatment with chitosan affected plant height significantly. The tallest plants (32.42 and 30.92 cm) were those treated with chitosan at (250 and 500 ppm), respectively. The shortest plants (23.67 and 24.50 cm) were the untreated plants and those treated with brassinolide at (50 ppm), respectively. These results are in agreement with the findings of several researchers, treatments with CS solutions significantly promoted the plant height growth rate of Origanum vulgare [17]. The effect of chitosan foliar application on basil [18], on summer squash plants [16], and on maize and soybean [19], resulted in a significant increase in significantly height compared to untreated control plants. A study was conducted on old sour orange to evaluate foliar application of chitosan, and the results showed that chitosan application enhanced the plant [20]. Moreover, chitosan treatment height significantly increased the height of the sweet basil about 12% [21], and also increased the height of the coffee seedlings up to 33.51% [22].

The biggest stem diameters (10.52 and 10.22 mm) were found from the treatment with chitosan at (250 and 500 ppm), respectively. The thinnest diameters (7.78 and 8.30 mm) were obtained from control and chitosan treatment at (1000 ppm), respectively. These results are in agreement with the findings of several researchers, Chitosan foliar spray application increased the stem diameter of Solidago canadensis plant due to the adequate availability of nutrients which occurs healthy growth of the plant. Also, more carbohydrates are translocated into the phloem and can be used to enhance secondary growth, which leads to the expansion of stem cells and diameter [23]. In another research on coffee plant, chitosan increased the stem diameter up to 30.77% [22], foliar chitosan effect on old sour orange and results showed that chitosan application enhanced stem diameter [20].

The heaviest fresh weight (42.69 g) was obtained from plants treated with chitosan at (250 ppm). The least fresh weight (18.89 g) was recorded from the untreated plants. These results are in agreement with other researchers, chitosan treatment significantly increased the fresh weight of sweet basil about 17% [21]. Chitosan also increased vegetative fresh weight of strawberry [24] and enhanced the vegetative fresh weight of old sour orange [20].

The highest dry weight (5.95 g) was recorded from plants treated with chitosan at (250 ppm). The lowest dry weight (4.17 g) was obtained from the untreated plants. Similar results also show that the application of chitosan increases dry mass of Solidago canadensis plants [23]. Chitosan foliar applications affected the dry mass of maize and soybean [19], and also increased vegetative dry weights of strawberry plant [24]. Chitosan provided greater total dry weights of Mentha arvensis [25]. Chitosan foliar application effect on old sour orange and the results showed that chitosan enhanced the vegetative dry weight [20]. Foliar spray with chitosan increased total dry weight of summer squash plants as compared to the untreated plant [16]. Also sweet basil plants reached highest leaf dry weight when treated with chitosan compared to the control [26].

Brassinolide and chitosan affected significantly leaf area. The biggest leaf areas $(36.38 \text{ and } 35.27 \text{ cm}^2)$ were obtained from those plants treated with chitosan at (250 ppm) and brassinolide at (200 ppm), respectively. The smallest leaf area (17.78 cm^2) was obtained from the untreated plants. These results are in agreement with the findings of several researchers, chitosan foliar application on cordyline plant significantly improved leaf area in comparison to the control [27], on old sour orange, enhanced leaf area [20], and also chitosan increased the leaf area of coffee up to 60.53% [22]. Furthermore, increased leaf area of maize and soybean [19], leaf area of strawberry [24], and leaf area of summer squash plants compared to the untreated plant [16]. Also, brassinolide increased the leaf area of Solidago Canadensis [23].

The largest number of leaves (15.00 leaves) was obtained from those plants which treated with brassinolide at (200 ppm) and chitosan at (500 ppm). The lowest number of leaves (13.00 leaves) was obtained from the untreated plants. These results may be attributed to that both brassinolide and Chitosan affected number of leaves the same. These results are in agreement with the findings of several researchers, the number of leaves of blueberry [28], summer squash plants [16], strawberry [24], and old sour orange [20] were increased when treated with chitosan compared to the untreated plant. Also brassinolide foliar application could significantly increase number of Helianthus annuus L. leaves per plant [29].

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Treatments	Plant height (cm)		Stem diameter (mm)		Total vegetative fresh weight (g)		Total vegetative dry weight (g)		Leaf area (cm ²)		Leaves numbers	
Untreated plants	23.67	Е	7.78	С	18.89	Е	4.17	Е	17.78	F	13.00	В
Brassinolide (50 ppm)	24.50	Е	8.69	ABC	25.65	D	4.51	D	28.78	D	14.00	AB
Brassinolide (100 ppm)	29.50	BC	9.52	ABC	26.51	D	4.61	D	34.18	BC	14.00	AB
Brassinolide (200 ppm)	26.25	D	10.13	AB	29.66	С	5.12	С	35.27	AB	15.00	А
Chitosan (250 ppm)	32.42	А	10.52	А	42.69	А	5.95	А	36.38	А	14.00	AB
Chitosan (500 ppm)	30.92	AB	10.22	AB	38.94	В	5.62	В	33.29	С	15.00	А
Chitosan (1000 ppm)	27.83	CD	8.30	BC	31.32	С	5.14	С	23.72	Е	14.00	AB

Table 1

Effect of brassinolide and chitosan on vegetative growth parameters of Aglaonema commutatum plant

Means with the same letter in the same column are not significantly different.

Table 2

Effect of brassinolide and chitosan on root growth of Aglaonema commutatum plant

Treatments	Roots fro	Roots fresh weight (g)		ry weight (g)	Root length (cm)		
Untreated plants	8.78	D	1.23	D	23.83	С	
Brassinolide (50 ppm)	17.85	С	1.26	D	24.83	BC	
Brassinolide (100 ppm)	21.48	В	1.50	С	28.83	А	
Brassinolide (200 ppm)	23.60	А	1.84	А	29.00	А	
Chitosan (250 ppm)	18.04	С	1.55	С	25.92	В	
Chitosan (500 ppm)	23.83	А	1.98	А	26.00	В	
Chitosan (1000 ppm)	20.05	В	1.79	В	24.42	BC	

Means with the same letter in the same column are not significantly different.

As shown in table (2) chitosan categorically has affected roots fresh and dry weights. However, brassinolide significantly increased roots length. These results agreed with the research that has been done on *Ipomoea* which was treated with chitosan and the results indicated increasing mean number of roots, but root elongation was decreased in the presence of chitosan [30].

Treatment with chitosan and brassinolide affected roots fresh weight significantly. The heaviest roots fresh weights (23.83 and 23.60 g) were obtained from treated plants with chitosan at (500 ppm) and brassinolide at (200 ppm), respectively. However, the lowest roots fresh weight (8.78 g) was obtained from the untreated plants. These results are in agreement with some researchers, chitosan foliar applications increased roots fresh weights of Cordyline plant [27], strawberry [24], *Serapias vomeracea* [31], and basil plant [18] compared to untreated control plants. Chitosan stimulates root growth by inducing enzymes, such as chitinases, pectinases, and glucanases, or by inducing IAA synthesis which

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increase new roots initiation [27]. Treatment of *Silene repens* plant with brassinolide led to an increase in root fresh weight [32].

Treatment with chitosan and brassinolide affected roots dry weight significantly. The heaviest roots dry weights (1.98 and 1.84 g) were obtained from plants treated with chitosan at (500 ppm) and brassinolide at (200 ppm), respectively. However, the lowest roots dry weight (1.23 g) was recorded from the untreated plants. These results are in agreement with several researchers on young plants of blueberry before transplanting and data showed that chitosan improved root dry weight [28]. Also foliar applications of chitosan increased root dry weight of strawberry [24]. Sweet basil [26], and *Serapias vomeracea* [31] plants showed greater root dry weight when treated with chitosan compared to the control treatment. Chitosan treatments induced root formation [30].

Ttreatment with brassinolide affected roots length significantly. The longest roots (29.00 and 28.83 cm) were obtained from plants treated with brassinolide at (200, 100 ppm), respectively. The shortest roots were

obtained from the untreated plants and plants were treated with brassinolide at (50 ppm), chitosan at (1000 ppm) (23.83, 24.83 and 24.42 cm), respectively. These results are in agreement with other researchers they stated that brassinolide treatment increased root length of rice [33]. Also low concentrations of brassinolide promoted root elongation in arabidopsis wild-type plants up to 50% [34].

3.2. Effect of brassinolide and chitosan on chemical composition of *Aglaonema commutatum* plant.

As shown in table (3), treatment with chitosan affected the content of total carbohydrates. The highest content of total carbohydrates was recorded from plants treated with chitosan at (250 ppm) and brassinolide at (200 ppm) (85.84 and 85.45 mg/g DW), respectively. However, the lowest content of total carbohydrates was obtained from the untreated plants (76.28 mg/g DW). These results compatible with some researchers, chitosan foliar application significantly increased carbohydrates within Phaseolus vulgaris L. plant tissues, compared with chitosan-untreated plants [35]. Treated old sour orange with chitosan foliar spray, results showed that application enhanced chitosan leaf total carbohydrates percentage [20].

Treatment with chitosan at (500 ppm) has affected the leaves content of total chlorophylls. The highest content of total chlorophylls was recorded from plants treated with chitosan at (250 ppm and 500 ppm) (4.48 and 4.47 mg/g F.W.), respectively. However, the lowest content of total chlorophylls was obtained from untreated plants (3.25 mg/g F.W.). These results are in agreement with several foliar application of researchers, chitosan significantly increased chlorophyll content of cordyline plants [27], Phaseolus vulgaris [35], and Solidago canadensis [23] within plant tissues compared with untreated plants. Application of chitosan increased content of total chlorophylls of coffee plants up to 15.36% compared to the control [22].

The highest content of carotenoids was obtained from plants treated with chitosan at (250 ppm) and brassinolide at (200 ppm), (1.72 and 1.46 mg/g F.W.), respectively. Whereas the lowest content of carotenoids was obtained from the untreated plants (0.99 mg/g F.W.). These results are in agreement with the results of cordyline [27], and *Solidago Canadensis* [23] plants treated with chitosan the results showed a significant increase in carotenoid content. Also coffee seedlings showed that chitosan enhanced strongly the content of carotenoid in the leaves of coffee seedlings up to 73.51% compared to the greenhouse control [22]. Carotene harvests light pigments to the reaction sites which improves photosynthesis performance and enhances organic matter accumulation [27].

Treatment with chitosan at (250 ppm) affected the percentage of nitrogen content. The highest percentage of nitrogen was found in the plants treated with chitosan at (250 ppm and 500 ppm) (1.03 and 0.83% D.W.), respectively. These results are in agreement with the findings of several researchers. Application of chitosan increased nitrogen uptake of coffee by 9.49% [22]. Chitosan also increase nutrient uptake such as nitrogen, which essential nutrient playing an important role in the biosynthesis and necessary for forming DNA and RNA [33]. The significant effect of chitosan on plant growth may be attributed to that chitosan is rich in nitrogen (6.1 -8.3%), and amino acid also Nitrogen in chitin derivatives is released to the plant by microbial degradation [27].

chitosan supplements stimulates the activity of nitrogen metabolism enzymes (glutamine synthetase, nitrate reductase, and protease) that promote plant development, growth and improved the transportation of nitrogen in the functional leaves and increased photosynthesis and forming DNA and RNA which enhanced the plant growth and development [24], [18], and [16].

The highest percentage of phosphorus was obtained from plants treated with chitosan at (500 ppm) and brassinolide at (200 ppm) (0.22 and 0.18% D.W.), respectively. These results are compatible with the findings of other researchers, application of chitosan increased phosphorus content in *Phaseolus vulgaris* L. [35], also increased phosphorus uptake of *Solidago canadensis* [23] and coffee plants by 11.76% [22]. Phosphorus is an essential nutrient playing an important role in the biosynthesis and translocation of carbohydrates, and necessary for stimulating cell division, and forming DNA and RNA [35].

The highest percentage of potassium was recorded from plants treated with chitosan at (250 ppm) and brassinolide at (200 ppm) (1.47 and 1.40% D.W.), respectively. These results are in agreement with the findings of some researchers, application of chitosan increased potassium uptake of *Solidago canadensis* [23], coffee plants by 0.98% [22]. Chitosan increases potassium uptake, playing an important role in photosynthesis and cell turgor [35].

The highest content of phenols was obtained from those plants treated with Chitosan at (500 ppm) and Brassinolide at (200 ppm) (307.63 and 225.42 mg/ 100g F.W.), respectively. These results are in agreement with the findings which indicated that the total amount of the phenolic compounds increased after the chitosan treatment in sweet basil [21], and *Solidago canadensis* [23]. Chitosan foliar application induced peroxidase and polyphenol oxidase activity, which involved in the biosynthesis of phenolic

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compounds which significantly increased in cordyline leaves compared to control [27].

The highest content of Indoles was recorded by plants treated with chitosan at (500 ppm) and brassinolide at (200 ppm) (174.04 and 155.63 mg/100g F.W.),

respectively. These results are in agreement with the results of chitosan treatments which induced higher root formation in all treatments due to its effect on indoles in *Ipomoea* plant [30].

Table 3

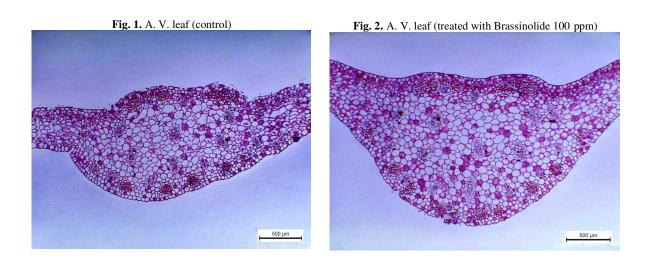
Effect of brassinolide and chitosan on chemical composition of Aglaonema commutatum plant

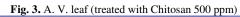
Treatments	Total carbohy- drate (mg/g D.W)	Total chloroph- ylls (mg/g F.W.)	Carotenoids content (mg/g F.W.)	Nitrogen content (% D.W.)	Phosphorus content (% D.W.)	Potassium content (% D.W.)	Phenols (mg/100g F.W.)	Indoles (mg/100g F.W.)
Untreated plants	76.28	3.25	0.99	0.29	0.10	1.35	144.38	58.21
Brassinolide (50 ppm)	78.88	3.27	1.13	0.32	0.06	1.06	162.30	65.34
Brassinolide (100 ppm)	82.44	3.77	1.35	0.33	0.12	1.33	187.81	124.74
Brassinolide (200 ppm)	85.45	4.29	1.46	0.73	0.18	1.40	225.42	155.63
Chitosan (250 ppm)	85.84	4.48	1.72	1.03	0.13	1.47	193.45	80.19
Chitosan (500 ppm)	78.49	4.47	1.20	0.83	0.22	1.13	307.63	174.04
Chitosan (1000 ppm)	73.48	3.38	1.33	0.61	0.12	1.15	209.81	146.72

3.3. Effect of brassinolide and chitosan on anatomy of *Aglaonema commutatum*.

The data in table (4) and Figs. (1, 2, and 3) show a comparison between anatomical measurements for three different treatments (control, Brassinolide at 100 ppm, and Chitosan at 500 ppm) on anatomy of *Aglaonema commutatum*, the anatomical structure of *Aglaonema c*. and the results showed that plants treated with chitosan at (500 ppm) has the highest value recorded in leaf blade thickness, mesophyll thickness, upper and lower epidermis, vascular bundle length and width, xylem vessels diameter, palisade tissue, and spongy tissue. These results are in agreement with results of cordyline Leaf thickness stimulated by chitosan spray due to nutrient

availability [27], the number of vascular bundle of Dendrobium orchid plant increased when treated with chitosan [36]. Chitosan increased thickness of blade, thickness of mesophyll tissue, thickness of the lower and upper epidermis and bundle length and width in the mid rib of wheat [37], and barley [38] compared to the control. Also chitosan application effects on stimulating physiological processes, improving vegetative growth, followed by increasing leaf-blade thickness as well as the dimensions of the vascular bundles, thicker epidermis which providing higher protection for the inner tissues [39].





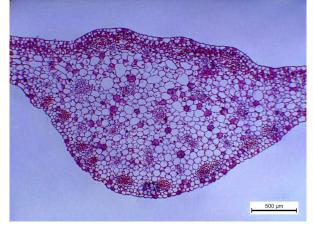


Table 4

Effect of brassinolide and chitosan on anatomy of Aglaonema commutatum

Variable	Control	Brassinolide (100 ppm)	Chitosan(500 ppm)
Mid rip thickness (µm)	1238.11	1665.79	1773.43
Leaf blade thickness (µm)	375.03	409.51	529.36
Mesophyll thickness (µm)	307.51	331.67	434.08
Upper epidermis (µm)	26.77	32.08	38.66
Lower epidermis (µm)	40.75	45.76	56.62
Vascular bundle length (µm)	153.28	167.75	199.59
Vascular bundle width (µm)	100.42	102.85	152.105
Xylem vessels diameter (µm)	29.40	32.99	35.20
Palisade tissue (µm)	68.75	84.48	84.90
Spongy tissue (µm)	238.76	247.19	349.18

4. Conclusions

In this study, foliar spray application of brassinolide and chitosan affected significantly the growth rate of Aglaonema plants. The results showed that brassinolide foliar application affected significantly (number of leaves and root length). However, chitosan foliar application affected (plant height, stem diameter, total fresh and dry weights, and leaf area) significantly more than brassinolide foliar application. Furthermore, chitosan foliar application increased the chemical parameters of Aglaonema plant (total chlorophylls, carotenoids, nitrogen, phosphorus, potassium, phenols, and indoles). Both brassinolide and chitosan foliar application increased roots fresh and dry weights of Aglaonema plant. The anatomical measurements showed that chitosan foliar application recorded higher values compared to other treatments. In the end of this study, it is recommended for Aglaonema commutatum plants to apply foliar application of chitosan at 250 ppm as a natural growth regulator.

5. Conflicts of interest

"There are no conflicts to declare".

6. Formatting of funding sources

This research did not receive any external funding

7. Acknowledgments

This study is supported by the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza, Egypt.

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