

Morphological Characters and Plant Pigments Content of Three Varieties of Chrysanthemum Induced by Paclobutrazol Treatments

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ABSTRACT

Chrysanthemum is one of the favorite ornamental plants as a potted flower. Consumers currently prefer potted flowers with short stems, lush leaves, also uniform and compact flowers. It is necessary to form the potted flowers by applying paclobutrazol (PBZ). This research aimed to determine the effectiveness of paclobutrazol and find the best PBZ concentration in forming three varieties of potted Chrysanthemum based on plant morphology and plant pigments content. The study design was a factorial completely randomized design, which factors were a variation of PBZ concentration (0, 50, 100, and 150 ppm) and Chrysanthemum varieties, namely Jaguar Red (JR), Fiji White (FW), and Snow White (SW). The growth parameters were plant height, vegetative phase, flowering time, and flower diameter. Plant pigments included total leaf chlorophyll content, chlorophyll a and b levels, and flower anthocyanin content. The data analysis used ANOVA with the F test at a 5% significance level and the DMRT. The application of paclobutrazol gave different morphological characters and plant pigments content on the three varieties of Chrysanthemum. The PBZ with higher concentration resulted in slower stem growth and increased leaf chlorophyll and flower anthocyanins levels. The JR variety produced the shortest vegetative phase, the fastest flowering time, and the highest anthocyanin content. SW had the largest flower diameter and the lowest plant height. Meanwhile, the highest levels of chlorophyll content were produced by FW. The optimum concentration of PBZ to form potted Chrysanthemum was 150 ppm.

Keywords: Anthocyanin, Chrysanthemum, Giberellin, Growth retardant, Paclobutrazol

1. INTRODUCTION

Chrysanthemum is one of the favorite ornamental plants in Indonesia and even the world. The fulfillment of Chrysanthemum needs is increasing from year to year. One factor influencing the high consumer demand for ornamental plants is the diversity of plant phenotypes. Chrysanthemum has a very diverse shape, type, and color of flowers. These flowers are also classified as flowers that do not wither quickly and have various varieties. According to the Central Statistics Agency (Badan Pusat Statistik), Indonesia produced up to 751.784.043 stems of ornamental plants in 2018. There are six types of ornamental plants with higher

production plants than other types. Most of them use flowers as an attraction, such as Chrysanthemums, Roses, Jasmine, and Orchids. Two different types, namely Anthurium and Monstera as ornamental leaf plants. Chrysanthemum is the most widely produced ornamental plant. More than half of ornamental plant production in Indonesia comes from these plants. In 2018, Indonesia was able to make 387.208.754 Chrysanthemums. This amount is equivalent to 51.5% of the total production of ornamental plants in Indonesia [1].

Apart from being cut flowers, it is also used as raw materials for drinks and ornamental plants in pots. In today's modern era, potted flowers have their enthusiast.

It can be used as indoor or outdoor table decorations, and as beautiful gifts. In addition, potted flowers do not require ample space and with the various shapes and sizes of pots, potted flowers are in great demand by the public, especially in urban areas [2]. Consumers prefer potted flowers with short stems, lush leaves, and flowers growing uniformly and compactly. Special treatment is necessary to build the market taste of potted flower. One of them is the application of plant growth regulators, such as paclobutrazol. Paclobutrazol (PBZ) inhibits the biosynthesis of gibberellins so that vegetative plant growth is suppressed. The oxidative reaction between kauren and kaurenoic acid in gibberellin synthesis is inhibited by PBZ, resulting in suppression of plant stems [3]. PBZ can be applied by spraying, watering through the planting medium, or injection through the stem [2]. The application of PBZ with a certain concentration gives a different appearance effect on several types of plants [3, 4, 5, 6].

Paclobutrazol plays a role in promoting flowering, pigment formation, preventing etiolation, extending cuttings, inhibiting the aging phase, and extending harvest life [7]. Previous studies have shown that PBZ concentration of 100 ppm gave the best results on the emergence of primordial flowers. In comparison, a PBZ concentration of 300 ppm could prolong the freshness of inflorescences on *Chrysanthemum* plants [6]. The applying frequency of PBZ for each species is usually 2-4 times. The previous studies showed that a one-time application of 100 mg.L-1 of paclobutrazol was as effective as a twice application of 2,500 mg.L-1 of daminozide on short and medium height of *Chrysanthemum* plants. In comparison, a 200 mg.L-1 PBZ application was needed for tall *Chrysanthemum*s accepted in the market [8]. Therefore, this research aimed to determine the effectiveness of paclobutrazol and find the best PBZ concentration in forming three varieties of potted *Chrysanthemum*, namely Jaguar Red, Snow White, and Fiji White variety, based on plant morphology and plant pigments content.

2. METHODS

2.1. Procedures

The research was carried out in the Puspita Merapi Farm greenhouse, Hargobinangun, Pakem, Yogyakarta, Indonesia. The research design used was a factorial completely randomized design (CRD). The first factor is the variation of the concentration of paclobutrazol: 0, 50, 100 and 150 ppm. The second factor is *Chrysanthemum* varieties, namely Jaguar Red (JR), Fiji White (FW) and Snow White (SW). *Chrysanthemum* seedlings are selected from healthy mother plants and free from pests. The rooted seedlings were transplanted into polybags, with five replications for each variety. Furthermore, paclobutrazol was given to plants two

weeks after planting. PBZ application by spraying all parts of the plant with concentrations of 50, 100, and 150 ppm, and distilled water as a control. PBZ was applied once a week for three times. Plants are maintained until flower buds appear. Each plant was disbudded by removing flower buds and only three flowers were left for one plant for further observations.

The growth parameters measured were plant height, length of the vegetative phase, flowering time, and flower diameter. Observations were conducted every week for eight weeks after planting. Plant physiological parameters included total leaf chlorophyll content, chlorophyll a and b levels, and flower anthocyanin content. Measurement of leaf chlorophyll content was carried out after the plant was eight weeks old following the Yoshida *et al.* [9] also Anggarwulan and Solichatun [10] method with a modification. Leaf samples were collected for each treatment, where 100 mg fresh weight (FW) of leaf was crushed using a mortar. The leaf extracts were added with 10 mL of 80 % acetone and then centrifuged. The absorbance of the supernatant was measured at a wavelength (λ) of 663 and 645 nm using a spectrophotometer (GENESYS 10 UV Scanning, Thermo Fisher Scientific). The following Equation (1), (2), (3) and (4) calculated chlorophyll content:

$$\text{Chlorophyll a (mg L}^{-1}\text{)} = (12.7 \times A_{663}) - (2.69 \times A_{645}) \quad (1)$$

$$\text{Chlorophyll b (mg L}^{-1}\text{)} = (22.9 \times A_{645}) - (4.68 \times A_{663}) \quad (2)$$

$$\text{Chlorophyll total (mg L}^{-1}\text{)} = (20.2 \times A_{645}) - (8.02 \times A_{663}) \quad (3)$$

$$\text{Conversion (mg g}^{-1}\text{)} = \left(\frac{1}{100} \times \text{chlorophyll content} \right) / \text{FW} \quad (4)$$

The determination of flower anthocyanin content for each treatment was carried out using the Prior *et al.* [11] and Sari *et al.* [12] method with modification. Twenty-five grams of corolla were crushed and dissolved in ethanol (50 ml), then stirred for 60 minutes at 27°C. The extract was centrifuged for 15 minutes at 4000 rpm to separate the filtrate and residue. The filtrate was concentrated with a rotary vacuum evaporator at a 35°C. Anthocyanin concentration was measured based on the differential pH method. The samples were put into 2 test tubes, each tube 0.05 ml. The first tube was added with 4.95 ml of potassium chloride (KCl) buffer solution (0.025 M) pH 1, and the second tube was added 4.95 ml of sodium acetate (NaCH₃COO) buffer solution (0.4 M) pH 4.5, then being left for 15 minutes. Adjustment of the pH buffer of potassium chloride and sodium acetate using concentrated HCl.

The following Equation (5) and (6) measure both pH treatments in a Spectrophotometer at 520 nm and 700

nm, with a molar extinction coefficient (ϵ) of cyanidin-3-glucoside of $29.600 \text{ Lmol}^{-1}\text{cm}^{-1}$ and molecular weight (MW) of 448,8 g/mol, DF is dilution factor (5 ml/0,05 ml), A is absorbance, and b is thick of cuvette (1 cm).

Absorbance

$$= [(A_{520} - A_{700})pH_1 - (A_{510} - A_{700})pH_{4.5}] \quad (5)$$

Anthocyanin concentration (mg L^{-1})

$$= \frac{(A \times \text{MW} \times \text{DF} \times 1000)}{(\epsilon \times b)} \quad (6)$$

2.2. Data Analysis

Data analysis used analysis of variance (ANOVA) with F test at 5% significance level by MS. Excel and SPSS ver.16. If the F test has a significant effect, an intermediate test is carried out with the Duncan Multiple Range Test (DMRT) at a 5% significance level.

3. RESULTS AND DISCUSSION

3.1. Plant Morphology of Three Varieties of Chrysanthemum Induced by Paclobutrazol

3.1.1. Plant Height

This study shows that paclobutrazol inhibits plant height growth for the three varieties of Chrysanthemum. The PBZ with higher concentration decreased the stem height (Table 1). The principle of PBZ is inhibiting the oxidation reaction between kauren and kaurenoic acid in the synthesis of gibberellins, resulting in the suppression of plant stems [13]. In addition to inhibition of cell enlargement, stem shortening is also caused by inhibition of cell division and elongation of the subapical meristems. Therefore, in the previous PBZ research, the epidermal cells of the stem became shorter, marked by the inhibition of stem height in the three varieties of Chrysanthemum. Meanwhile, PBZ treatments increased the thickness of stem cortex tissue in the three varieties of Chrysanthemum [14]. SW has the shortest stem, not much different from JR (Table 1). The relationship between PBZ concentration and variety significantly affected differences in stem height of Chrysanthemums (Table 2).

Chrysanthemum with PBZ treatments had lower stems than control plant. PBZ suppressed the elongation of stem segments but did not affect the number of nodes on the stem. The number of leaves was related to the number of nodes on the stem, so the number of control leaves and PBZ treatment Chrysanthemum continued to grow well. Widaryanto *et al.* [15] showed that application time and PBZ concentration had no significant effect on the number of leaves of sunflower plants.

3.1.2. Vegetative Phase

The SW variety had the longest vegetative phase than the JR and FW varieties. In general, the length of the vegetative phase in JR was 51 days, SW was 68 days, and FW was 53 days. The application of 100 and 150 ppm paclobutrazol has not shortened the vegetative phase. Meanwhile, 50 ppm PBZ can shorten the vegetative phase of JR (Table 1). Research by Novi & Rizki [7] showed that PBZ application at a 200-1000 ppm concentration did not affect the appearance of white jasmine flower buds. Latimer [16] stated that differences in the response of plant species to the application of PBZ became an obstacle in plant flowering. PBZ also causes inhibition of the biosynthesis of active endogenous gibberellins as flower promoters. Weaver [17] suggested that PBZ can inhibit plant growth and affect flowering. There is competition for the absorption of plant nutrients for flowering, causing a delay in flower formation.

The vegetative phase of plants is different for each species. Many factors affect the length of the vegetative phase of plants, such as internal and external factors. In the cut flower of Chrysanthemum, light is usually added to extend the vegetative phase of the plant so that the length of the appropriate stems is obtained as cut flowers. However, the inappropriate concentration of PBZ application can cause a longer vegetative phase which inhibits the flower emergence. Each plant has a different sensitivity to growth inhibitors. PBZ treatment can also affect the formation of several substances needed by plants, so the formation of flower primordia is indeed inhibited [18].

3.1.3. Flowering times

Each plant has a different flowering time. Many factors affect flowering time. This study observed the flowering time (flower anthesis) of the three Chrysanthemum varieties from the initial formation of flower buds to bloom fully. In general, the flowering time on the JR variety was the fastest compared to the other varieties (Table 1). PBZ 150 ppm was most effective in shortening the flowering time for JR and FR varieties (Table 1). According to Weaver [17], inhibition of gibberellin biosynthesis by inhibitors in the subapical meristem will decrease cell rate. Then inhibiting vegetative growth and indirectly divert photosynthate to generative growth required for flower formation. However, the application of PBZ had less effect on the flowering time for the SW variety, indicated by the longer flowering time in the PBZ treatment than the control plants.

Table 1. Growth parameters of three varieties of Chrysanthemum with paclobutrazol treatments

Parameters	PBZ	Jaguar Red	Snow White	Fiji White	Mean
Plant height (cm)	Control	28.79 ^c	33 ^f	33.36 ^f	31.72 ± 2.54
	50 ppm	19.89 ^c	18.18 ^c	24.89 ^d	20.99 ± 3.49
	100 ppm	18.82 ^c	14.29 ^{ab}	19.46 ^c	17.52 ± 2.82
	150 ppm	14.57 ^b	11.5 ^a	19.96 ^{bc}	15.34 ± 4.28
Mean		20.52 ± 5.97	19.24 ± 9.57	24.42 ± 6.45	
Vegetative Phase (days)	Control	55 ^{bcd}	62 ^{def}	48 ^{ab}	55.00 ± 7.00
	50 ppm	46 ^a	68 ^f	51 ^{abc}	55.00 ± 11.53
	100 ppm	51 ^{abc}	65 ^{ef}	58 ^{cde}	58.00 ± 7.00
	150 ppm	54 ^{abcd}	76 ^g	61 ^{def}	63.67 ± 11.24
Mean		52 ± 4.04	68 ± 6.02	55 ± 6.03	
Flowering Time (days)	Control	31 ^{ab}	30 ^a	38 ^{bcd}	33.00 ± 4.36
	50 ppm	34 ^{abcd}	34 ^{abcd}	38 ^{bcd}	35.33 ± 2.31
	100 ppm	34 ^{abcd}	33 ^{abc}	41 ^d	36.00 ± 4.36
	150 ppm	30 ^a	40 ^{cd}	35 ^{abcd}	35.00 ± 5.00
Mean		32 ± 2.06	34 ± 4.19	38 ± 2.45	
Flower Diameters (cm)	Control	7.27 ^{ab}	7.57 ^g	6.29 ^{bcd}	7.04 ± 0.67
	50 ppm	6.36 ^{cd}	7 ^{ef}	6.43 ^{cde}	6.60 ± 0.35
	100 ppm	5.71 ^{ab}	6.79 ^{def}	5.71 ^{ab}	6.07 ± 0.62
	150 ppm	5.5 ^a	6.86 ^{def}	6 ^{abc}	6.12 ± 0.69
Mean		6.21 ± 0.80	6.12 ± 0.32	7.06 ± 0.35	

Note: Mean value followed by the same letters in the same column and rows of each parameter indicates no significant differences based on the Duncan test at P < 0.05 and two-way ANOVA. n: 5

Rugayah [2] reported that PBZ application significantly affects the flowering time of tuberose plants in pots. Tuberose plants with 375 ppm PBZ produced a more extended bloom than the control plants. The PBZ plants have thicker pseudo-stems and larger flower stalk diameters. They can store more food reserves as well as nutrients and water, so those plant organs, such as flowers, can last longer. According to Syahid [19], retardants can slow down the withering of flowers. The plants with retardants will be more resistant to water stress, hot temperatures, cold temperatures, and smoke in various room conditions. Widaryanto *et al.* [15] reported that PBZ application to the upper and lower flowers of sunflower plants was more durable when compared to controls.

3.1.4. Flower diameters

The flower diameter of the SW variety is the largest compared to the others. The control treatment generally produced larger flower diameters than the PBZ treatment (Table 1), indicating that PBZ inhibition on gibberellins is more effective in inhibiting flower diameter than controls [6]. Rubiyanti & Rochayat [20] showed that the PBZ application with several concentrations resulted in various flower diameters. The relationship between the concentration PBZ and flower varieties showed a significant difference in the formation of flower diameter (Table 2).

The flowering process is the interaction of internal

Table 2. Statistic data of Chrysanthemums plant growth with a combination of PBZ concentrations and varieties

Parameters	PBZ Concentrations	Varieties	PBZ X Varieties
Plant height	**	**	**
Vegetative phase	**	**	ns
Flowering time	ns	**	ns
Flower diameter	**	**	**

Note: **: significant difference, ns: not significant based on Duncan Multiple Range Test (DMRT) at a 5% level of significance.

and external factors. The internal factors include hormones and genetics. Gibberellin is a growth hormone that stimulates flowering. However, gibberellins and paclobutrazol are antagonists. The flowering gene (florigen) is also activated to initiate flower formation. The external factor is light, where light affects flowering in two ways: light intensity and photoperiodicity. Light intensity is related to phytochrome. Phytochromes are pigments that play a role in receiving photoperiod stimuli that control flowering. Photoperiodicity is the ratio between the length of day and night. *Chrysanthemum* spp. is a short-day plant. It will be induced to flower if the day length is shorter than the critical value. In addition to light, external factors that affect are temperature and humidity. Low temperatures result in high humidity. It activates endogenous gibberellins to flower formation. In this study, we used *Chrysanthemum* with the standard type of flower. In cut *Chrysanthemums*, they usually only have one large flower for each stalk. Meanwhile, in this study, we grow three flowers for each stalk.

3.2. Plant Pigments of Three Varieties of *Chrysanthemum* Induced by Paclobutrazol

3.2.1. Anthocyanin Content

Chrysanthemums have many varieties of flower colors. It is due to differences in the content of dyes, one of which is anthocyanins. Anthocyanin is one of the secondary metabolites produced by plants. Anthocyanins in flowers are usually scattered in the epidermis of the corolla and the vacuole. As a secondary metabolite, anthocyanin content can be affected by the presence of stress agents. In this case, the excess PBZ concentration can be considered environmental stress. From this study, 150 ppm of PBZ significantly increased flower anthocyanin levels compared to the controls (Table 3). The PBZ treatment caused smaller flower diameters, leading to increased flower anthocyanins. The flower size causes the increased distribution of anthocyanins as phenolic compounds. From the result, the JR variety had the highest anthocyanin content compared to the other varieties (Table 3). Moreover, since the JR's flower color is red,

Table 3. Plant pigments content of three varieties of *Chrysanthemum* with paclobutrazol treatments

Parameters	PBZ	Jaguar Red	Snow White	Fiji White	Mean
Chlorophyll a (mg/g)	Control	1.04 ^a	1.04 ^a	1.25 ^{abcd}	1.11 ± 0.12
	50 ppm	1.27 ^{abcd}	1.19 ^{abc}	1.43 ^{cd}	1.30 ± 0.12
	100 ppm	1.45 ^d	1.13 ^{ab}	1.32 ^{bcd}	1.30 ± 0.16
	150 ppm	1.06 ^a	1.13 ^{ab}	1.33 ^{bcd}	1.17 ± 0.14
Mean		1.21 ± 0.19	1.12 ± 0.06	1.33 ± 0.07	
Chlorophyll b (mg/g)	Control	0.37 ^a	0.41 ^{ab}	0.43 ^{abc}	0.40 ± 0.030
	50 ppm	0.41 ^{ab}	0.41 ^{ab}	0.46 ^{abc}	0.43 ± 0.03
	100 ppm	0.55 ^c	0.43 ^{abc}	0.43 ^{abc}	0.47 ± 0.07
	150 ppm	0.39 ^{ab}	0.41 ^{ab}	0.52 ^{bc}	0.44 ± 0.07
Mean		0.43 ± 0.08	0.42 ± 0.01	0.46 ± 0.04	
Total chlorophyll (mg/g)	Control	1.57 ^{abcd}	1.30 ^a	1.74 ^{bcd}	1.54 ± 0.22
	50 ppm	1.69 ^{bcd}	1.72 ^{bcd}	1.89 ^d	1.77 ± 0.11
	100 ppm	1.78 ^{bcd}	1.46 ^{abc}	1.75 ^{bcd}	1.66 ± 0.18
	150 ppm	1.41 ^{ab}	1.62 ^{abcd}	1.85 ^{cd}	1.63 ± 0.22
Mean		1.61 ± 0.16	1.53 ± 0.18	1.81 ± 0.07	
Anthocyanin content (mg CyE/gr)	Control	9.91 ^{bcd}	0.36 ^a	3.09 ^{ab}	4.45 ± 4.92
	50 ppm	1.37 ^a	5.24 ^{ab}	7.24 ^{abc}	4.62 ± 2.98
	100 ppm	15.71 ^{de}	0.63 ^a	6.95 ^{abc}	7.76 ± 7.57
	150 ppm	13.75 ^{cde}	20.2 ^e	14.92 ^{cde}	16.29 ± 3.44
Mean		10.19 ± 6.35	6.61 ± 9.33	8.05 ± 4.96	

Note: Mean value followed by the same letters in the same column and rows of each parameter indicates no significant differences based on the Duncan test at P < 0.05 and two-way ANOVA. n: 3

Table 4. Statistic data of *Chrysanthemums* plant pigments with the combination of PBZ concentrations and varieties

Parameters	PBZ Concentrations	Varieties	PBZ X Varieties
Chlorophyll a	ns	**	**
Chlorophyll b	ns	ns	ns
Total chlorophyll	**	**	**
Anthocyanin content	**	ns	**

Note: **: significant difference, ns: not significant based on Duncan Multiple Range Test (DMRT) at a 5% level of significance.

the anthocyanin content is more abundant than the other two varieties with white flowers. In this study, anthocyanin was formed as cyanidin-3-glucoside.

Hajihashemi [21] reported that PBZ and GA treatments significantly increased the enzymatic and non-enzymatic antioxidants, which was more prominent in PBZ treated plants of *Stevia rebaudiana*. PBZ treatment significantly increased the activity of SOD, and a key enzyme involved in the early steps of the ROS detoxification system. GA and PBZ treatments induced a remarkable growth in APX, POD, and CAT activity. The content of anthocyanins, flavonoids, and phenols did not change the GA-treated plants, while PBZ significantly increased them. Shukla *et al.* [22] reported an important and linear connection between the antioxidant potential and phenolic amount, suggesting that phenolic metabolites could be the crucial contributors to the antioxidant properties of *S. rebaudiana*.

Anthocyanins belong to the group of flavonoid compounds and are one of the largest natural pigments in plants. They are soluble in water, giving color to flowers, fruits, and vegetables. Also, an important source of natural dyes in foodstuffs, cosmetics, pharmaceuticals and can substitute for artificial dyes. Other studies have also shown that anthocyanins have many beneficial properties for health, such as antioxidant activity, which can cure degenerative diseases [23]. Flower *Chrysanthemum* is usually used as a drink with many antioxidants.

The color of anthocyanin is influenced by several factors, including pigment content, pH, temperature, enzymes, metals, and copigmentation [24]. Glycosylation and methylation also affect the color of the pigment. Adding a glycoside group or increasing the number of free hydroxyl groups on the carbon chain number 5 (ring A) can increase the bluish color. In contrast, methylation can increase the reddish color [25]. This research is still limited to the determination of flower anthocyanin levels. Anthocyanin characterization can be done using spectrophotometric analysis or Thin Layer Chromatography (TLC). Determination of these anthocyanins characteristics usually involves identifying the aglycone, the presence of sugars and acyl groups, if

any, and the bonding positions of the sugar and acyl groups [26].

3.2.2. Chlorophyll content

In addition to affecting anthocyanin levels, the application of PBZ could increase leaf chlorophyll levels in the three *Chrysanthemum* varieties compared to controls (Table 3). The FW variety had the highest total chlorophyll, chlorophyll a, and chlorophyll b, followed by JR and SW (Table 3). According to Anggarwulan and Solichatun [10], chlorophyll plays an important role as a device for capturing energy from sunlight which in photosynthesis will produce ATP and NADPH. Although the rate of photosynthesis is not measured in this study, the growth resulting from the photosynthesis process can be used as a benchmark. It has been proven in previous studies that the PBZ concentration of 150 ppm increased leaf thickness. Meanwhile, 100 ppm of PBZ could increase the size of the palisade and spongy tissue of leaves [14], which explains that the application of PBZ can increase the thickness of leaf tissue associated with an increase in the number of chloroplast cells and leaf chlorophyll content. The higher the chlorophyll content, cause better photosynthesis so that that plant growth will be more optimal.

The presence of leaves that were not affected by the addition of PBZ resulted in increased chlorophyll levels. Tumewu *et al.* [27] stated that paclobutrazol is one of the retardants that plays a role in reducing the rate of stem elongation without affecting leaf growth and development. Retardants suppress stem elongation by inhibiting their physiological activity, but retardants do not inhibit the production and translocation of assimilating to other organs in plants [28].

Chlorophyll is a photosynthetic pigment, an indicator of plant health, responsible for stress response mechanisms. Application of PBZ maintains structural integrity of the chloroplast and its grana with compact thylakoid under water deficit stress in chickpeas. They also preserve chloroplast structural integrity under water deficit by increasing antioxidant enzymes activity and limiting lipid peroxidation activity [29]. The increase in chlorophyll content with PBZ treatment was associated to the ability of PBZ to increase the cytokinin content

and thereby the enhancement of chlorophyll biosynthesis [30]. Paclobutrazol significantly enhanced chlorophyll a, b, and carotenoids in wheat cultivars [31]. It is aligned with the effect of PBZ in increasing the total chlorophyll content, including chlorophyll a and b.

Differences in chlorophyll content between plant species indicates a combination of physiological adaptation, especially to the shade environment and gene expressions, especially for the formation of chloroplast. The formation of chloroplast is limited by many factors such as genetics, light, Oxygen (O₂), Carbohydrates, N, Mg, Iron, other mineral elements, temperature, and water. All green plants contain chlorophyll a and chlorophyll b. Chlorophyll a composes 75% of the total chlorophyll [32]. Plants carry out the increase in chlorophyll b to adjust physiologically to optimize light capture. Chlorophyll b acts directly as a light-harvesting antenna. While chlorophyll a participates in converting radiation energy captured by chlorophyll b into chemical energy. The light that works through photosynthesis affects the growth and development of plants [33]. Adaptation of both morphological and physiological from PBZ ultimately affects leaf chlorophyll production, so PBZ effectively increased chlorophyll content in *Chrysanthemum*.

The application of paclobutrazol gave different morphological and physiological effects on the three varieties of *Chrysanthemum*. The higher PBZ concentration resulted in slower stem growth and increased levels of leaf chlorophyll and flower anthocyanins. The Jaguar Red variety produced the shortest vegetative phase, the fastest flowering time and the highest anthocyanin content. Snow White produced the largest flower diameter and the lowest plant height. Meanwhile, the highest levels of chlorophyll content were produced by Fiji White. The optimum concentration of PBZ to form potted *Chrysanthemum* was 150 ppm.

AUTHORS' CONTRIBUTIONS

IQL conceived and designed the research methodology, data collection, data analysis, literature review, manuscript writing, and final reading and approval. LHN supervised this research, designed the research methodology, data interpretation, manuscript final assignment, and acceptance.

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