The paclobutrazol application and pinching technique on Lisianthus plants (*Eustoma grandiflorum*)

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Abstract. The research concerns on the paclobutrazol application and the pinching technique on Lisianthus plants (*Eustoma grandiflorum*). This research aimed to determine the appropriate time to apply the paclobutrazol and pinching technique suitable for Lisianthus potted plants. This study was carried out using factorial experiments consisting of two factors, arranged in Randomized Complete Block Design. The first factor was the time of application of paclobutrazol that consist of A0 (Without paclobutrazol application), A1 (Application of paclobutrazol 6 days after planting), A2 (Application of paclobutrazol 12 days after planting), A3 (Application of paclobutrazol 18 days after planting). The second factor of technique pinching was P0 (Without pinching), P1 (Single pinching technique), P2 (1½ pinching technique), P3 (Double pinching technique). The data were analyzed using analysis of variance at the 5% level and mean separations with Duncan’s Multiple Range Test. The results showed that the application of paclobutrazol and pinching technique could shorten Lisianthus plants. The application of paclobutrazol decreased the number of flowers per plant and shortened the flower stalks. The pinching technique increases the number of branches, number of internodes, number of nodes, number of flowers per plant. The application of paclobutrazol 18 days after planting and double pinching techniques were able to shorten the height of lisianthus plants with dense branching and many flowers.

Keywords: *Eustoma grandiflorum*, paclobutrazol, pinching technique

1. Introduction

Ornamental plants have a significant role in the trade of agricultural commodities and will always be needed by the community. Cut flowers and potted plants are agribusiness based on ornamental plants which are widely cultivated by the community [1]. Lisianthus is commonly used as cut flowers, and many people like Lisianthus as a potted flower plant. Lisianthus has the quality as an ideal cut flower because the flower blooming age can reach 15 days [2]. The flower stalks are long and can be used as decorative potted flowers [3]. Lisianthus is a famous cut flower in Japan and the Netherlands [4], Lisianthus became the number one cut flower in Japan, and the Dutch cut flower market [5].

The Lisianthus plant (*Eustoma grandiflorum*) is one of the many flowering plant fans, but it has not been widely cultivated in Indonesia. In Indonesia, Lisianthus is increasingly known, and its demand is increasing year by year, the estimated increase in Lisianthus consumption in Indonesia...
reaches 25% per year [6]. Lisianthus seeds are challenging to obtain and are expensive, Lisianthus harvest time is relatively long, specific growing requirements, and there are still not many studies on Lisianthus cultivation technology [7]. Seeds are usually imported directly from Japan [8]. Lisianthus plants grow well at an altitude of 950-1500 m above sea level, 70-80% humidity, with soil pH ranging from 6.5-7, the optimum temperature at night ranges between 15-18ºC and during the day 18-23ºC [3]. 

In Indonesia, Lisianthus plant cultivation is only planted in a few highlands, namely in Bali, Malang, Bandung, Lembang, and Bogor. Considering that Lisianthus's demand is increasing every year, it is necessary to develop the planting area to areas that have suitable plant growth requirements for Lisianthus. Kopeng is an area located on the slopes of Mount Merbabu flanked by mountains Telomoyo and Andong with an altitude between 1200-1500 m above sea level. Furthermore, a temperature range of 19-23 ºC, which is very suitable for flower plant development [9]. Based on the conditions, it is estimated that Kopeng has good potential to be used as a place for Lisianthus cultivation.

Lisianthus, which is cultivated in Indonesia, is currently more widely used as cut flowers planted on land, when in fact, Lisianthus is also attractive when used as potted plants because of its beautiful flowers. In general, the preferred potted ornamental plants are lush, densely leafy, short stems between 25-40 cm, and many flowers. Because Lisianthus plants, which are used as cut flowers and planted on land generally have a height ranging from 60-90 cm, for planting Lisianthus in pots, arrangements need to be made so that the plants grow with short, thick stems and produce large flowers. The growth of ornamental plants in pots that are preferred and desired by consumers are small plants with well-arranged branches and large amounts of flowers. Plant growth regulation such as retardant can be used to suppress longitudinal growth to produce shorter plants and pinching treatment to form many well-arranged branches. Retardant is usually used to shorten plants' length and height, but it will not affect the absorption of light and the flowers produced [10]. Paclobutrazol is a retardant that functions to inhibit plant height growth, lengthening of internodes, and leaf area. Paclobutrazol inhibits gibberellin synthesis so that the hormonal balance in the body of the plant will be inhibited. Paclobutrazol is a retardant that is not active in the soil but is very active in plants and moves to all shoots after application. The best application time of paclobutrazol is during vegetative phase of the plants [11].

In addition to retardants, regulation of the growth of ornamental plants in pots can also be made by pinching techniques. Pinching is removing the terminal shoots, and this is done to stop the dominance of the apical buds to stimulate the growth of lateral shoots from the armpit of the leaf. Pinching is done to stimulate the growth of lateral shoots, which are then maintained further to form buds. Several studies have shown that the number of lateral shoots that grow is related to the number of leaves left in the canopy during pinching [12]. The results of Wuryaningisih's research [13] on pinching on carnation plants with single pinching, ½ pinching, and double pinching techniques show that the double pinching treatment gives a higher number of lateral buds and stem length compared to the ½ pinching technique and single pinching technique. In the context of developing Lisianthus plants in Indonesia, Lisianthus planting studies were conducted in pots in Kopeng, by regulating plant growth using paclobutrazol and pinching technique. The study was intended to determine the exact time of paclobutrazol and pinching techniques for the growth of Lisianthus plants grown in pots.

2. Materials and Methods
The research was conducted in Pandean Lor Village, Kopeng, Central Java, with a height of 1250 m above sea level, from June to October 2017. The materials and tools used in this research were: Lisianthus plant seeds, paclobutrazol, polybags, fertilizers, growing media, bamboo, paranets, lights, and scopes sprayers, rulers, stationery, scissors.

2.1. Research Design
This research was carried out using factorial experiments consisting of two factors and was designed by a Randomized Complete Block Design. The first factor was the time of application of paclobutrazol that consist of A0 (Without paclobutrazol application), A1 (Application of paclobutrazol 6 days after planting), A2 (Application of paclobutrazol 12 days after planting), A3 (Application of paclobutrazol
18 days after planting). The second factor of technique pinching was P0 (Without pinching), P1 (Single pinching technique), P2 (1½ pinching technique), P3 (Double pinching technique). In total, there were 16 treatment combinations, namely:

- A0P0: Without paclobutrazol application + Without pinching (Control)
- A0P1: Without paclobutrazol application + Single Pinching technique
- A0P2: Without paclobutrazol application + Double pinching technique
- A0P3: Without paclobutrazol application + 1½ Pinching technique
- A1P0: Application of paclobutrazol 6 days after planting + Without pinching
- A1P1: Application of paclobutrazol 6 days after planting + Single pinching technique
- A1P2: Application of paclobutrazol 6 days after planting + Double pinching technique
- A1P3: Application of paclobutrazol 6 days after planting + 1½ pinching technique
- A2P0: Application of paclobutrazol 12 days after planting + Without pinching
- A2P1: Application of paclobutrazol 12 days after planting + Single pinching technique
- A2P2: Application of paclobutrazol 12 days after planting + Double pinching technique
- A2P3: Application of paclobutrazol 12 days after planting + 1½ pinching technique
- A3P0: Application of paclobutrazol 18 days after planting + Without pinching
- A3P1: Application of paclobutrazol 18 days after planting + Single Pinching Technique
- A3P2: Application of paclobutrazol 18 days after planting + Double pinching technique
- A3P3: Application of paclobutrazol 18 days after planting + 1½ pinching technique

Each treatment was repeated three times, each repetition consists of 3 samples, so that the total treatment unit was 144 units.

2.2. Lisanthus Plant Preparation
Lisianthus seeds used originated from the parental plant, which was planted in the experimental field in Pandean Lor Village, Kopeng, Central Java, with a height of 1250 m above sea level. The material used in this study was one-month-old lisianthus plant seeds. The planting medium used is a mixture of soil, roasted husk, cocopeat with a ratio of 1: 1: 1 and added with organic fertilizer. The medium was put into a pot with a diameter 15 cm and the seeds are planted in a pot. After planting, watering is done intensively in the morning and evening for one week, after that watering is done once a day in the afternoon and maintained until it is treated. Plants are fertilized again using NPK at the age of 15 days after planting. Also, plants were observed every day to ensure there are no pests or diseases attack. Weeding and watering were also done as a maintenance effort.

2.3. Treatment Application
Paclobutrazol application is carried out by spraying 2 ml of solution with a concentration of 5 ml / l to the plant canopy. Spraying of paclobutrazol is done once at a time according to the treatment. However, watering was not carried out after paclobutrazol application. The single pinching technique is carried out only once, namely by removing the apical shoot or main shoot leaving 5 - 6 pairs of leaves. This technique is carried out when the plants are 3-4 weeks after planting. The 1½ pinching technique is almost the same as the single pinching technique, however after growing the lateral shoots are pinched again, leaving 2-3 pairs of leaves. The double pinching technique is basically the same as single pinching and 1½ pinching, but in double pinching after the first pinching, pinching is repeated on the lateral shoots leaving 2-3 pairs of leaves.

2.4. Plant Maintenance
After application of paclobutrazol and pinching technique, the plants are then subjected to intensive maintenance until the plants flower. The supplementary fertilizer is given every two
weeks with a dose of 15-15-15 NPK fertilizer, namely as much as 2 g / m², KCl 30 g / m², Urea 4.40 g / m², MgSO₄ 2.80 g / m², and Ca(NO₃)₂ 3 g / m². Watering is done twice a day in the morning and evening or depending on plant conditions. Lisianthus plants are given additional lighting using a 7 Watt bulb lamp, the best time to add lighting is at night, which is between 10 pm – 1 am. Lisianthus plants are planted in the shade using a plastic house with an area of 3m x 6m.

2.5. Parameter Observed and Statistical Analysis
The parameters observed were plant height, number of branches, number of flowers per plant, number of internodes, length of internodes, number of nodes, and length of the flower stalks. All parameters were analyzed using analysis of variance. The means were separated with Duncan’s Multiple Range Test at the 5% level.

3. Results and Discussion
The inhibition of growth by paclobutrazol and pinching treatment was indicated by the parameters of plant height and length of internodes. The results showed an interaction between the treatment time of paclobutrazol application and pinching technique on plant height (F = 2.27, df = 9, P < 0.01) and length of internodes (F = 4.40, df = 9, P < 0.01).

| Treatment internodes | Plant height (cm) | Internode length (cm) |
|----------------------|-------------------|-----------------------|
| A0P0                 | 57.83 a           | 2.97 a                |
| A0P1                 | 46.77 b           | 1.83 bc               |
| A0P2                 | 42.27 bcd         | 1.30 def              |
| A0P3                 | 40.83 bcde        | 1.23 def              |
| A1P0                 | 44.9 bc           | 1.90 b                |
| A1P1                 | 38.67 cdef        | 1.57 bcd              |
| A1P2                 | 36.67 defg        | 1.27 def              |
| A1P3                 | 34.53 efg          | 1.00 f               |
| A2P0                 | 40.7 bcde         | 1.80 bc               |
| A2P1                 | 35.3 efgh         | 1.23 def              |
| A2P2                 | 30.13 gh          | 1.13 def              |
| A2P3                 | 33.3 fgh          | 1.07 ef               |
| A3P0                 | 32.7 fgh          | 1.47 cde              |
| A3P1                 | 33.77 fgh         | 1.17 def              |
| A3P2                 | 29.63 h           | 1.07 ef               |
| A3P3                 | 31.33 gh          | 1.07 ef               |

Means followed by same letters are non-significant (DMRT, α = 5%)

The paclobutrazol application and pinching technique could inhibit plant height and length of internodes (Table 1). Interaction of A3P2 treatment (application of paclobutrazol inhibitor 18 days after planting + 1½ pinching) showed the lowest plant height (29.63 cm) as compared to A0P0 (control, 57.83 cm), but not significantly different with A1P3 treatment (application of paclobutrazol inhibitor 6 days after planting + pinching double), A2P1 (application of paclobutrazol inhibitor 12 days after planting + single pinching), A2P2 (application of paclobutrazol inhibitor 12 days after planting + pinching 1½), A2P3 (application of paclobutrazol inhibitor 12 days after planting + double pinching), A3P0 (application of paclobutrazol inhibitor 18 days after planting + without pinching), A3P1 (application of paclobutrazol inhibitor 18 days after planting + single pinching), A3P3 (application of paclobutrazol inhibiting agent 18 days after planting + double pinching).

Lisianthus plants with paclobutrazol application and pinching can shorten the internodes, so the plant height becomes shorter. This is following Latimer's opinion [11], which states that paclobutrazol belongs to retardant that functions to inhibit plant height growth and internodes lengthening. [14]
states that paclobutrazol can inhibit gibberellin synthesis. If gibberellin synthesis is inhibited, the cells continue to divide, but the new cells do not elongate [15]. In addition, the paclobutrazol and pinching technique could inhibit plant height also showed that are given paclobutrazol and pinching technique can shorten the internodes (Figure 1).

The results showed significant in the treatment time of paclobutrazol application on the number of flowers per plant ($F = 2.55$, $df = 3$, $P < 0.01$) and the length of flower stalks ($F = 13.37$, $df = 3$, $P < 0.01$); non-significant on the number of branches ($F = 1.41$, $df = 3$, $P > 0.01$), the number of internodes ($F = 0.61$, $df = 3$, $P > 0.01$), and the number of nodes ($F = 0.40$, $df = 3$, $P > 0.01$).

Table 2. Effect of paclobutrazol application time on the growth of Lisianthus plants

| Paclobutrazol Application | Number of Flowers per plant | Branches | Internodes | Nodes | Length (cm) Flower stalk |
|---------------------------|-----------------------------|---------|------------|-------|-------------------------|
| Without paclobutrazol     | 8.92 a                      | 9.75 a  | 27.92 a    | 25.58 a| 8.60 a                  |
| Paclobutrazol application 6 days planting | 7.92 ab                   | 10.00 a | 28.17 a   | 25.50 a| 7.73 b                  |
| Paclobutrazol application 12 after planting | 8.08 ab                 | 8.67 a  | 27.58 a    | 27.00 a| 7.53 b                  |
| Paclobutrazol application 18 after planting | 7.67 b                     | 9.08 a  | 26.58 a    | 26.58 a| 6.78 c                  |

Means followed by same letters are non-significant (DMRT, $\alpha = 5\%$)

The results showed significant in the treatment of the pinching technique on the number of internodes ($F = 25.06$, $df = 3$, $P < 0.01$), the number of branches ($F = 17.33$, $df = 3$, $P < 0.01$), the
number of nodes (F = 18.86, df = 3, P < 0.01), the number of flowers per plant (F = 3.59, df = 3, P < 0.01), non-significant on the length of flower stalks (F = 0.31, df = 3, P > 0.01).

Table 3. Effect of pinching techniques on the growth of Lisianthus plants

| Pinching techniques | Internodes | Branches | Nodes | Flowers per plant | Flower stalk |
|---------------------|------------|----------|-------|-------------------|--------------|
| Without pinching    | 21.75 c    | 6.67 c   | 19.00 c | 7.25 b            | 7.65 a       |
| Single pinching     | 27.17 b    | 8.75 b   | 26.75 b | 8.17 ab           | 7.50 a       |
| ½ pinching          | 28.92 b    | 10.67 ab | 27.92 ab| 8.42 a            | 7.72 a       |
| Double pinching     | 32.42 a    | 11.5 a   | 31.00 a | 9.33 a            | 7.76 a       |

Means followed by same letters are non-significant (DMRT, α= 5%)

Pinching techniques increases the number of branches, number of internodes, number of nodes, number of flowers per plant and non-significant on length of the flower stalks (Table 3). The treatment of double pinching technique produces the most significant number of internodes (32.42 internodes). Treatment of the double pinching technique (11.5 branches) was non-significant from the ½ pinching technique (10.67 branches), resulting in the number of branches and the number of nodes more than the application without pinching (6.67 branches) and single pinching (8.75 branches). The pinching technique application also increases the number of flowers per plant. Plants have a growing point at the tip of the plant, known as apical dominance. This apical dominance can inhibit the growth of lateral shoots. Pinching removes the apical dominance of the shoots to promote the appearance of lateral branches. This is because the meristem cells that are present in the shoots of the plant are removed. As a result, the plants whose stem tips are trimmed switch to branch growth or lateral shoots. According to [18], defoliation (cutting or taking parts of the plant) will stimulate the formation of more shoots. This is reinforced by [13] that plant pinching can trigger the growth of secondary shoots, automatically increasing the number of internodes in each plant. The pinching technique can suppress plant growth because growth is focused on the formation of new shoots or branching so that plant growth will be stunted and, consequently, the length of the resulting internodes is also small [19].

4. Conclusion
The application of paclobutrazol and pinching technique could shorten Lisianthus plants. The application of paclobutrazol decreased the number of flowers per plant and shortened the flower stalks. The pinching technique increases the number of branches, number of internodes, number of nodes, number of flowers per plant. The application of paclobutrazol 18 days after planting and double pinching techniques were able to shorten the height of lisianthus plants with dense branching and many flowers.

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