Flowering induction of clove to reduce its yield fluctuation by using a plant growth regulator

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Abstract. The main problem in clove cultivation is yield fluctuation which occurred every 2-4 years. It is usually caused by internal factors such as photosynthates, nutrition, and plant growth regulators that control the flowering process. The study was conducted from January to December 2015 on +30 years old-cloves at a farmer's plantation in Sumedang, West Java. The trial was arranged in a randomized block design with three replications. The treatment consisted of 13 treatments of paclobutrazol application: (1) control, (2) 2500 ppm paclobutrazol, given eight weeks after harvest (WAH); (3) 2500 ppm paclobutrazol, 10 WAH, (4) Paclobutrazol 2500 ppm, 12 WAH. (5) 2500 ppm paclobutrazol, 14 WAH, (6) Paclobutrazol 2500 ppm, 16 WAH, (7) Paclobutrazol 2500 ppm, 18 WAH, (8) 5000 ppm paclobutrazol, 8 WAH, (9) Paclobutrazol 5000 ppm, 10 WAH, (10) 5000 ppm paclobutrazol, 12 WAH, (11) Paclobutrazol 5000 ppm, 14 WAH, (12) 5000 ppm paclobutrazol, 16 WAH, and (13) 5000 ppm paclobutrazol, 18 WAH. Results indicated that paclobutrazol 5000 ppm applied at 16 WAH gave a higher new shoot number and yield than control within three consecutive years (2014, 2015, and 2016). The range of chlorophyll content was 0.08-0.15%.

Keywords: Syzigium aromaticum, paclobutrazol, dosage, application time

1. Introduction
The demand for clove commodities is increasing, along with the development of the clove cigarette industry and Indonesia's clove-based pharmaceutical, cosmetic, and food industries. However, the demand increase is not followed by the stability of clove production because the national clove productivity is still low. One of the causes of the low productivity of cloves is the yield fluctuations. The main harvesting occurs every 2 - 4 years; beyond the primary harvesting time, the yield decline can be as much as 60% [1].

Clove yield fluctuations are influenced by internal factors (genetic and physiological) and external factors (climate and cultivation) [2]. Before the main harvesting period, all shoots developed into flowers. Moreover, all assimilate and nutrients are translocated to the flowers, resulting in nutrient depletion. Plants will experience stress because no new shoots are actively photosynthesized to compensate for the nutrient requirements. As a result, the number of flowers in the subsequent year will decline drastically [3]. Plants with such characteristics possess irregular-bearing properties. The plants produce flowers irregularly; in a particular year, high flower formation will be followed by low flower formation in the subsequent year. Hence the yield will have fluctuated [4].

Flowering initiation is a transition period from the vegetative to the reproductive phase, the primary key in plant development. This transition was regulated by integrating multiple environmental and
endogenous signals [5]. Clove flowering is terminal, which meant the flower primordia were only developed from the ends of the twigs in the same place as the leaf buds [2]. In addition, the reproductive shoots can turn into vegetative shoots/leaves causing yield fluctuation. This phenomenon happens due to changes in endogenous gibberellins content in plants, mainly influenced by climatic factors.

In addition to climatic factors, the application of growth regulators can also change the balance of endogenous hormones such as growth retardants. Paclobutrazol (PBZ) is a growth retardant that belongs to triazole, which inhibits gibberellin biosynthesis. Lack of gibberellin will suppress vegetative growth, induce generative growth, increase chlorophyll, dissolve protein and minerals of the leaf [6]. The vegetative growth suppression will direct the translocation of nutrient supply and photosynthetic products to meristematic areas, which will initiate flower formation [7]. PBZ application was reported very effective in suppressing vegetative growth, increasing and accelerating flowering of both annual and perennial plants [8].

Paclobutrazol can be applied by spraying the leaves, injecting the stem, and watering the soil or roots [9]. PBZ application by watering the media was more efficient at the same dose and volume than spraying the leaves [10]. Darmawan et al. (2014) reported that watering PBZ into the soil can increase the number of flowers and fruits of tangerines [11]. The effect of PBZ has been assessed on clove flowering but has not yet evaluated its impact on the yield fluctuations. PBZ application on five-year-old clove at the dosage of 0.0; 1.0 and 1.5 g, performed once a year for three years consecutively, increased 1) flower length from 3.95% to 15.70%, 2) dry flower diameter from 6.52% to 14.58%, 3) the number of flowers per bunch from 4.91% to 72.50%, 4) the weight of 1,000 dried clove flowers from 10.28% to 23.88%, and 5) dry flower yield per tree from 37.22% to 136.85% [3]. Application of PBZ 1.5-2.5 g tree⁻¹ on 8-year-old clove tree enhanced the content of chlorophyll a and b as well as the dry weight of flowers per tree [12].

Research on the hormonal aspects of inhibitors to induce clove flowering was necessary to overcome yield fluctuations and stabilize every year. The study aimed to obtain appropriate concentration and application time of growth regulators to reduce flowering fluctuations in cloves.

2. Material and methods
The study was conducted from January 2013 to December 2016 in a productive clove plantation owned by farmers in Wado District, Sumedang Regency, West Java. The materials used were productive clove tree of Zanzibar variety (30-year-old), Paclobutrazol (Patrol), urea, SP-36, KCl, MgO, manure, fungicide, insecticide, and other tools for field activities. The study was arranged in Randomized Block Design, three replications, and 13 treatments as follows:

1. Control
2. PBZ 2500 ppm, applied 8 weeks after harvesting (WAH) (P1M8)
3. PBZ 2500 ppm, applied 10 WAH (P1M10)
4. PBZ 2500 ppm, applied 12 WAH (P1M12)
5. PBZ 2500 ppm, applied 14 WAH (P1M14)
6. PBZ 2500 ppm, applied 16 WAH (P1M16)
7. PBZ 2500 ppm, applied 18 WAH (P1M18)
8. PBZ 5000 ppm, applied 8 WAH (P2M8)
9. PBZ 5000 ppm, applied 10 WAH (P2M10)
10. PBZ 5000 ppm, applied 12 WAH (P2M12)
11. PBZ 5000 ppm, applied 14 WAH (P2M14)
12. PBZ 5000 ppm, applied 16 WAH (P2M16)
13. PBZ 5000 ppm, applied 18 WAH (P2M18)

Paclobutrazol was applied by watering the stems and roots around the plant base. It was given after harvesting, once per year for three years consecutively. The dosage of fertilizer based on the Standard Operational Procedure (SOP) cultivation for 30-year-old clove (N=3.96, P₂O₅=0.96, K₂O=3.06 kg
tree⁻¹ year⁻¹, manure = 30 kg tree⁻¹ year⁻¹). Each plot consisted of two plants. The parameters observed were the shoot number of secondary branches, flower production, and leaf chlorophyll content. Chlorophyll content was determined by the Spectrometric method [13]. The data were analyzed by the ANOVA test and further tested with Tukey if the significant differences occurred.

3. Result and discussion

3.1. Shoots number

The statistical analysis showed that the application of PBZ had a significant effect on the average number of clove shoots for three years. The highest number of shoots was obtained in the application of PBZ 5000 ppm, which was applied at 16 weeks after harvest (P2M16), which was 15 shoots per branch compared to the control of 3.40 shoots per branch. The other treatments of PBZ were not significantly different from the control (Table 1).

| Treatments                        | The average number of shoots for three years (2014-2016) |
|-----------------------------------|--------------------------------------------------------|
| Control                           | 3.40 b                                                 |
| PBZ 2500 ppm, 8 WAH (P1M8)        | 4.47 ab                                                |
| PBZ 2500 ppm, 10 WAH (P1M10)      | 3.32 b                                                 |
| PBZ 2500 ppm, 12 WAH (P1M12)      | 4.28 ab                                                |
| PBZ 2500 ppm, 14 WAH (P1M14)      | 11.75 ab                                               |
| PBZ 2500 ppm, 16 WAH (P1M16)      | 3.35 b                                                 |
| PBZ 2500 ppm, 18 WAH (P1M18)      | 6.13 ab                                                |
| PBZ 5000 ppm, 8 WAH (P2M8)        | 8.24 ab                                                |
| PBZ 5000 ppm, 10 WAH (P2M10)      | 7.06 ab                                                |
| PBZ 5000 ppm, 12 WAH (P2M12)      | 6.51 ab                                                |
| PBZ 5000 ppm, 14 WAH (P2M14)      | 7.35 ab                                                |
| PBZ 5000 ppm, 16 WAH (P2M16)      | **15.00 a**                                            |
| PBZ 5000 ppm, 18 WAH (P2M18)      | 8.29 ab                                                |

Note: The numbers followed by the same letter in the same column were not significantly different at the α 5% level Tukey test.
WAH = Week After Harvest flower clove

Paclobutrazol 5000 ppm was applied at 16 weeks after harvest. The clove plant produces the most shoots, which will produce flowers in the next harvest period. Paclobutrazol is a growth inhibitor that can suppress vegetative growth, especially the elongation of plant cells. Food reserves in the form of starch are used to grow generative shoots. PBZ is a triazole compound that acts as a plant growth regulator. Growth regulation PBZ changes the balance of essential plant hormones, such as gibberellins, ABA, and cytokinin [14]. PBZ application will inhibit the process of cell elongation and division through inhibition of gibberellin biosynthesis. Gibberellins play a role in the process of cell elongation so that the application of PBZ results in shorter internodes and shoots [15, 10]. This growth inhibition will direct the translocation of nutrient supply and photosynthate products to meristematic areas, which will initiate flower formation [8].
The stability of increasing the shoots number in each harvest period is needed to reduce fluctuations in yields in each harvest period. Individual analysis was carried out by comparing significantly different treatments to see the stability value. The clove shoots were individually analyzed only on the control and P2M16 treatments (Figure 1). Based on the data for the three observations in Figure 1, it can be seen that the control had fewer shoots even though there was no sharp yield fluctuation compared to the P2M16 treatment. In the P2M16 treatment, more shoots were produced than the control, although there were quite strong fluctuations in the number of shoots between years of observation in some individual trees. Several treatments increased the number of shoots every year, namely P2M16 of the 2nd individual tree, and P2M16 of the 4th particular tree. While the rest trees had a fluctuation pattern, increasing in 2015 and decreasing in 2014 and 2016, except for P2M16 certain trees number 3 has the opposite character. This result shows that the application of PBZ 5000 ppm at 16 WAH can provide a consistently increasing in the number of shoots for three years.

3.2. Flower production
Statistical analysis showed that the PBZ application had a significant effect on the average clove flower production for three years compared to without PBZ treatment (control). The highest average flower production was obtained in the application of PBZ 5000 ppm, which was applied at 16 weeks after harvest (P2M16), which was 2.37 kg compared to control (0.80 kg per plant). The other treatments of PBZ were not significantly different from the control (Table 2). The paclobutrazol application, which was applied 16 weeks after harvest (P2M16), increased clove flower production compared to the control. Paclobutrazol is a compound to reduce vegetative growth and induce generative growth. The paclobutrazol application can accelerate flowering, increase the number of flowers, number of fruits, and weight of mangoes [17]. Paclobutrazol applications have also been shown to improve the quality of clove flowers [4]. Application of PBZ can stimulate the total phenol content in terminal shoots and inhibit the phloem/xylem ratio in stems, thereby affecting vegetative growth and promoting flowering by shifting inhibition of assimilating and nutrient transport to generative growth [18].
Table 2. The effect of PBZ on the average dry flower production per tree for three years (2014-2016).

| Treatments                       | Average flower production for three years (2014-2016) Kg |
|----------------------------------|----------------------------------------------------------|
| Control                          | 0.80 b                                                   |
| PBZ 2500 ppm, 8 WAH (P1M8)       | 1.55 ab                                                  |
| PBZ 2500 ppm, 10 WAH (P1M10)     | 1.19 b                                                   |
| PBZ 2500 ppm, 12 WAH (P1M12)     | 0.99 b                                                   |
| PBZ 2500 ppm, 14 WAH (P1M14)     | 0.82 b                                                   |
| PBZ 2500 ppm, 16 WAH (P1M16)     | 1.06 b                                                   |
| PBZ 2500 ppm, 18 WAH (P1M18)     | 1.26 b                                                   |
| PBZ 5000 ppm, 8 WAH (P2M8)       | 1.21 b                                                   |
| PBZ 5000 ppm, 10 WAH (P2M10)     | 1.15 b                                                   |
| PBZ 5000 ppm, 12 WAH (P2M12)     | 1.46 ab                                                  |
| PBZ 5000 ppm, 14 WAH (P2M14)     | 1.10 b                                                   |
| PBZ 5000 ppm, 16 WAH (P2M16)     | 2.37 a                                                   |
| PBZ 5000 ppm, 18 WAH (P2M18)     | 0.98 b                                                   |

Note: The numbers followed by the same letter in the same column were not significantly different at the 5% level Tukey test.

WAH = Week After Harvest flower clove

In Figure 2 can be seen that the clove individual has different characteristics to the treatment given. In 2014, 2015, and 2016, clove trees treated with P2M16 had higher yields than control. The second and sixth individual trees produced more stable dry flower production than the control, with annual production ranging from 1.7 to 4 kg tree⁻¹. Among individuals treated with P2M16, the second tree individual was the one that corresponded to the number of shoots and the weight of dry flowers produced. Clove individual analysis was performed on the control and P2M16 treatments only (Figure 2).

![Figure 2](image_url)

Figure 2. Graph of individual analysis of the flower production.

3.3. Chlorophyll content of leaves

After PBZ treatment in year II (2015), the chlorophyll content ranged from 0.09–0.16% (Table 3). Chlorophyll content decreased again in year II, both in control and PBZ treatments compared to year I.
(0.16–0.22%), but some PBZ treatments had higher chlorophyll content than control, especially in PBZ treatment of 2500 ppm (8 WAH) and 5000 ppm (8, 14 and 16 WAH). Chlorophyll content in year III (2016) was almost the same in year II (2015), ranging from 0.08-0.15%.

Table 3. Chlorophyll content of clove leaves before and after PBZ treatment.

| Treatments          | Chlorophyll content (%) | Before treatments (2013) | After treatments |
|---------------------|-------------------------|--------------------------|-----------------|
| Control             |                         | 0.16                     | 0.21            | 0.14 | 0.10 |
| PBZ 2500 ppm, 8 WAH |                         | 0.19                     | 0.16            | 0.15 | 0.15 |
| PBZ 2500 ppm,10 WAH |                         | 0.13                     | 0.18            | 0.10 | 0.12 |
| PBZ 2500 ppm,12 WAH |                         | 0.15                     | 0.19            | 0.11 | 0.08 |
| PBZ 2500 ppm,14 WAH |                         | 0.18                     | 0.22            | 0.14 | 0.10 |
| PBZ 2500 ppm,16 WAH |                         | 0.17                     | 0.22            | 0.14 | 0.12 |
| PBZ 2500 ppm,18 WAH |                         | 0.16                     | 0.17            | 0.09 | 0.11 |
| PBZ 5000 ppm, 8 WAH |                         | 0.11                     | 0.22            | 0.15 | 0.15 |
| PBZ 5000 ppm,10 WAH |                         | 0.15                     | 0.17            | 0.12 | 0.12 |
| PBZ 5000 ppm,12 WAH |                         | 0.16                     | 0.19            | 0.13 | 0.10 |
| PBZ 5000 ppm,14 WAH |                         | 0.14                     | 0.22            | 0.16 | 0.10 |
| PBZ 5000 ppm,16 WAH |                         | 0.14                     | 0.21            | 0.15 | 0.11 |
| PBZ 5000 ppm,18 WAH |                         | 0.19                     | 0.16            | 0.09 | 0.11 |

Note: WAH = Week After Harvest flower clove

The blocking of gibberellin biosynthesis by PBZ in the terpenoid pathway caused an increase in the content of chlorophyll. The increase in chlorophyll is an essential part of the chlorophyll molecule through the rise in phytol compounds. This compound is produced through the terpenoid pathway as well as gibberellins. Inhibition of gibberellin biosynthesis by PBZ application will produce more phytol compounds so that the chlorophyll content increases [10, 15]. Increased chlorophyll content will increase photosynthetic activity so that clove flower production increases. Application of paclobutrazol can increase chlorophyll content of leaves, therefore leaves look greener such as in potato [14, 15, 16], Jatropha curcas [17], Amorphophallus campanulatus [18], and ginger [19]. Applying PBZ 1.5-2.5 g per tree on clove plants aged 8 years can increase the a and b chlorophyll content and flower dry weight per tree [12].

4. Conclusion
The application of 5000 ppm paclobutrazol at16 weeks after harvest resulted in better shoots and clove production per tree for three years (2014, 2015, and 2016) than the control.

Acknowledgments
We would like to thank Susi Noor Syamsiah and Siti Rohmah as assistant in the research both at the laboratory and field activities.

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