

Effect of Paclobutrazol on Leaf and Clove (*Syzygium aromaticum*) Flowering

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Abstract

This study aimed to determine the effect of paclobutrazol on clove leaves and flowering. The research was conducted from April 2019 – June 2020 in Talaga Jaya, South Wasile, East Halmahera. The study used a randomized block design consisting of five doses of paclobutrazol (6 ml, 8 ml, 10 ml, 12 ml, 14 ml) and control (0 ml) on 7 years old clove trees. Leaf thickness, number of blister blight, chlorophyll content, eugenol, gibberellin acid (GA), and abscisic acid (ABA) content were observed at a 1 m² area on each tree. In contrast, clove flowering was observed at primordia time. The results showed that the application of 14 ml of paclobutrazol increased the leaf thickness and chlorophyll content, while 8 ml of paclobutrazol could reduce the number of blister blight. Extending paclobutrazol at higher doses can increase ABA and accelerate generative growth. The primordial phase starts from December 2019 to June 2020 until the flowering phase. Flowering continued in paclobutrazol-treated plants except for control, which showed the effect of paclobutrazol on the flowering process.

Keywords: clove, clove leaves, flowering induction, paclobutrazol

Abstrak

Penelitian ini bertujuan untuk mengetahui pengaruh paclobutrazol terhadap daun cengkeh dan pembungaan. Penelitian dilaksanakan pada bulan April 2019 – Juni 2020 di Talaga Jaya, Wasile Selatan, Halmahera Timur. Penelitian menggunakan Rancangan Acak Kelompok yang terdiri dari lima dosis aplikasi paclobutrazol (6 ml, 8 ml, 10 ml, 12 ml, 14 ml) dan kontrol (0 ml) pada pohon cengkeh umur 7 tahun. Ketebalan daun, jumlah penyakit cacar daun cengkeh, kandungan klorofil, kandungan eugenol, asam giberelin (GA), dan asam absisat (ABA) diamati pada luas 1 m² pada setiap pohon sedangkan pembungaan cengkeh diamati pada waktu primordia. Hasil penelitian menunjukkan bahwa pemberian paclobutrazol 14 ml dapat meningkatkan ketebalan daun dan kandungan klorofil, sedangkan paclobutrazol 8 ml dapat menurunkan jumlah penyakit cacar daun cengkeh. Aplikasi paclobutrazol dengan dosis yang semakin tinggi dapat meningkatkan ABA dan mempercepat pertumbuhan generatif. Fase primordial dimulai dari Desember 2019 hingga Juni 2020 hingga fase pembungaan. Pembungaan berlanjut pada tanaman yang diberi paclobutrazol kecuali kontrol, yang menunjukkan pengaruh paclobutrazol pada proses pembungaan.

Kata kunci: cengkeh, daun cengkeh, induksi pembungaan, paclobutrazol,

Introduction

Cloves (*Syzygium aromaticum* L.) are the main plantation commodities and native Indonesian spices. One of the main causes of the low productivity of cloves from year to year is the erratic flowering time. Clove flowering that is not in unison is known as a phenomenon of yield fluctuation, namely the rhythm of relative yield fluctuations from year to year (Runtunuwu *et al.*, 2016). Cloves have a terminal flowering type in which the flowers and leaves have the same growing point. Both occur alternately and the distance between harvest and subsequent flowering is relatively short, while plant regeneration is low (Ruhnayat and Dhalimi, 1997). This poses a problem because during the harvest period photosynthetic activity decreases, all assimilate and mineral nutrients are translocated to the flower. Plants will be stressed because some new leaf buds actively photosynthesize to compensate for nutrient absorption (depletion) by growing flowers so that next year's flowering will be greatly reduced (Runtunuwu *et al.*, 2016). Photosynthetic activity must be maintained and even increased to ensure the supply of assimilate so that the quantity and quality of clove plant products is maintained. Efforts to increase photosynthesis and clove flowering through the application of paclobutrazol can stimulate the formation of chlorophyll.

The application of paclobutrazol causes inhibition of gibberellin synthesis activity which is the main component in plant vegetative growth (Chaney, 2004; Abdollahi *et al.*, 2012). The synthesis of gibberellins is inhibited by pacloburazol and diverts it towards the generative growth process so that there is an increase in chlorophyll as indicated by the greener the leaves of the plant (Mansuroglu *et al.*, 2009). The application of paclobutrazol increases the chlorophyll content and net photosynthetic rate so that it directly affects plant productivity (Tekalign, 2007; Assuero *et al.*, 2012). However, the use of paclobutrazol should pay attention to the adequacy of clove nutrients. Fertilization according to the age of the plant is very important because the vegetative growth of the plant becomes optimal if the nutrients are met (Fayaz *et al.*, 2016; Kwon *et al.*, 2009) including plants experiencing stress due to being submerged in water (Gribaldi *et al.*, 2017). The extent of the effect of paclobutrazol application on clove plants in North Maluku is the goal of this study.

Material and Method

The study of the effect of paclobutrazol application on clove plants was carried out in Talaga Jaya Village (0°51'45"N, 127°41'54"E), South Wasile District, East Halmahera Regency, North Maluku from April 2019 – June 2020. Daily temperature at the site between 21.1 – 34.2 C and the annual average rainfall is 1,991 mm (Anomin, 2020). The study used a randomized block design consisting of five doses of application of paclobutrazol (6 ml, 8

ml, 10 ml, 12 ml, 14 ml) and control (0 ml) on 7 years old clove trees. Each dose of paclobutrazol is dissolved in 240 ml of water and then poured into the soil through a hole that has previously been filled with NPK fertilizer (16: 16: 16) as much as 5 kg/tree/year (Anonymous, 2018). The number of treatment holes was 4 holes per tree with a depth of 30 cm. The holes were made using a Belgy type mineral soil drill. The clove plants observed was local superior varieties maintained by farmers with the same age (7 years) randomly selected, growing evenly on a 2 Ha plot of clove garden with a slope of 30° – 40°.

Leaf thickness and number of blister blight were observed on one side of the tree in the area of 1 m² (Tresniawati and Randriani, 2011) while the content of chlorophyll, eugenol, gibberellins (GA), and abscisic acid (ABA) was measured in the laboratory using sample leaves collected from all parts of the tree. Flower formation was observed on sample tree shoots (Tresniawati and Randriani, 2011). Leaf thickness was measured using a digital meter, and the number of blister blight was calculated manually according to their appearance on the leaf surface. Changes in both of these parameters were observed and measured 3 times. Chlorophyll content was observed in the laboratory of the Indonesian Palma Research Institute, Manado, Indonesia, while the content of eugenol, gibberellins and abscisic acid in leaves was observed at the Research Institute for Spices and Medicinal Plants, Bogor, Indonesia.

Data analysis used descriptive analysis and analysis of variance (Anova). If there is a difference between the treatments, it will be continued with Duncan's multiple distance test with level = 0.05.

Result and Discussion

The effect of paclobutrazol on leaf thickness

Cloves leaf thickness increased in three observations after paclobutrazol treatment (Figure 1) and all treatments showed a significantly different effect to control (Table 1). Paclobutrazol dose of 14 ml had the highest effect on leaf thickness than other treatments.

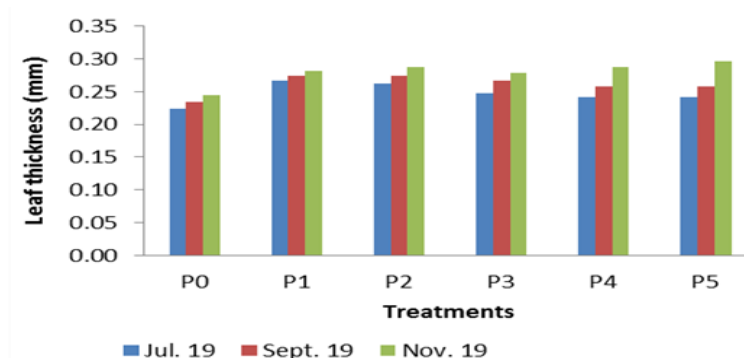


Figure 1. Cloves leaf thickness after paclobutrazol treatment

This indicates that the higher the treatment dose, the more the thickness of the cloves leaf increases. Morphological modifications of plant organs such as leaf thickening,

shrinking of stomata pores, increasing the number and size of leaf surface appendages are caused by paclobutrazol (Chaney, 2004). Thickening of plant organs occurs because the production of cells in the cambium is stimulated, resulting in an increase in the volume of parenchymal cells in the cortex. This indicates that the administration of paclobutrazol, among others, can increase the diameter of sunflower stems (Widaryanto *et al.*, 2011) while tuberos plants tend to increase stem circumference (Rugayah *et al.*, 2020). In peanut plants, after 80 days of paclobutrazol application, leaf thickness, epidermal thickness, number of palisad, number of palisade mesophyll, number of sponge cells and number of sponges mesophyll increased compared to control (Sankar *et al.*, 2013).

This modification is beneficial for the plant because it can provide physical barriers against fungal, bacterial, or insect infections. Administration of paclobutrazol in certain doses can directly affect the growth of pathogenic bacteria *Xylella fastidiosa* (DeStefano *et al.*, 2007).

Table 1. Leaf thickness and number of blister blight on cloves after paclobutrazol treatment

Paclobutrazol treatment (ml)	Leaf thicknes (mm)	Number of blister blight
0 (P0)	0,24 ± 0,01 a	3,20 ± 2,49 a
6 (P1)	0,28 ± 0,03 b	1,40 ± 0,89 a
8 (P2)	0,29 ± 0,03 b	1,20 ± 0,45 a
10 (P3)	0,28 ± 0,02 b	2,00 ± 0,71 a
12 (P4)	0,29 ± 0,01 b	1,80 ± 0,84 a
14 (P5)	0,30 ± 0,01 b	1,40 ± 0,55 a

*) Numbers in the same column followed by the same letter mean that they are not significantly different according to the multiple-distance test Duncan at = 0.05.

The effect of paclobutrazol on number of blister blight

Paclobutrazol treatment had no effect on the number of blister blight (Table 1). The application of paclobutrazol after three times observations showed a decreasing number of blister blight compared to control (Figure 2). The highest number of decreasing blister blight occurred in two treatment 6 ml (2.6 to 1,4) and 12 ml (0.44 to 0.40). Plant protection against fungal diseases is recognized as a secondary benefit of paclobutrazol and several observations have shown a reduction in the incidence of anthracnose disease after the application of paclobutrazol. The mechanism of paclobutrazol protection against disease occurs through inhibition of steroid production in the fungal cell wall terpenoid pathway. It is suggested that the fungistatic capacity of paclobutrazol is as a steroid biosynthetic inhibitor (SBI), where steroids are an important component of the membrane (Chaney, 2004).

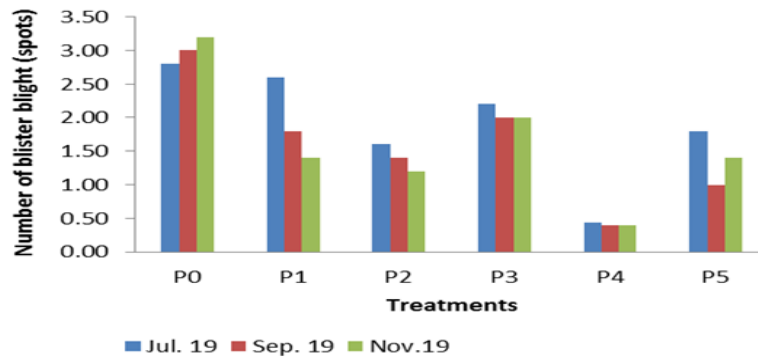


Figure 2. Number of blister blight after paclobutrazol treatment

The effect of paclobutrazol on chlorophyll content

The content of chlorophyll a and b was found to be higher in clove leaves treated with paclobutrazol than in control (Figures 3 and 4).

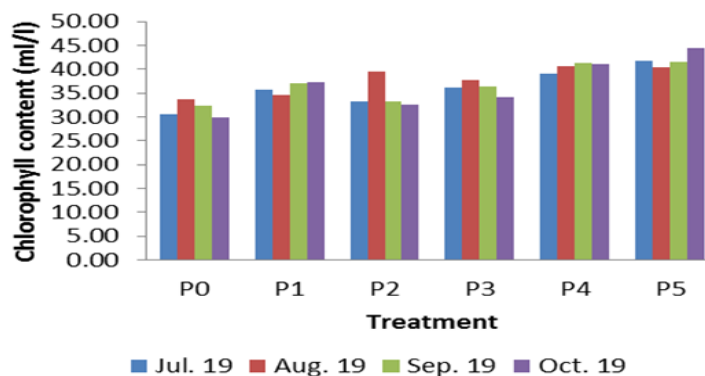


Figure 3. Chlorophyll a content on cloves leaf

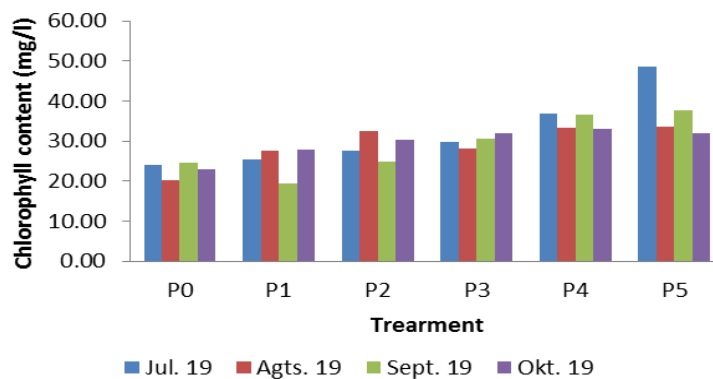


Figure 4. Chlorophyll b content on cloves leaf

The highest content of chlorophyll a and b was seen in the 12 ml and 14 ml paclobutrazol treatments, while the lowest chlorophyll a was seen in the 8 ml treatment and the lowest chlorophyll b from the 6 ml treatment. Paclobutrazol treatment caused an increase in the chlorophyll content. The generative phase of clove is about 6 months after the application of paclobutrazol. The increase in leaf chlorophyll content indicated the effect of paclobutrazol application. Plants that were given paclobutrazol had dark green leaves which showed high chlorophyll content (Chaney, 2004) and caused *Consolida orientalis* flowers to have a darker purple color compared to the control (Mansuroglu *et al.*, 2009). The leaves of

plants that were not treated with paclobutrazol had the same number of cells, but because the cells in the leaves that were given paclobutrazol became smaller, the chlorophyll was more concentrated. The increase in the amount of chlorophyll occurs due to the presence of phytyl, an important part of the chlorophyll molecule produced through the terpenoid pathway along with gibberellins. The application of paclobutrazol can inhibit the production of gibberellins, resulting in reduced intermediate compounds from the synthesis of gibberellins, resulting in more phytyl which affects the chlorophyll content (Chaney, 2004).

The effect of paclobutrazol on flowering cloves time

Clove plants do not produce flowers on a regular basis from year to year. High interest rates in a given year will be followed by low interest rates in the following year so that production will fluctuate. This fluctuation in production can be overcome by providing paclobutrazol retardant which can inhibit gibberellin biosynthesis (Darwati, 2017). In this study, the emergence of primordial in December 2019 is evidence that the formation of flowers in June 2020 begins from this phase (Figure 5). Flowering was evenly distributed in paclobutrazol-treated plants and flower buds appeared (except control) (Table 2). This indicates the induction of clove flower by paclobutrazol. The higher the dose of paclobutrazol given, the higher the yield of wet and dry flowers.

Table 2. Flowering time and cloves production after paclobutrazol treatment

Paclobutrazol treatment	Application time	Primordia time of appearance	Primordia time	Number of stalks	Wet flower yield (kg/tree)	Dried flower yield (kg/tree)
0 ml (P0)	Mei 2019	-	-	0	0	0
6 ml (P1)	Mei 2019	Desember 2019	Juni 2020	17	5,1	2,2
8 ml (P2)	Mei 2019	Desember 2019	Juni 2020	19	5,3	2,5
10 ml (P3)	Mei 2019	Desember 2019	Juni 2020	16	4,5	2,0
12 ml (P4)	Mei 2019	Desember 2019	Juni 2020	22	6,5	2,8
14 ml (P5)	Mei 2019	Desember 2019	Juni 2020	34	16,4	7,4

Various research results show the effect of paclobutrazol on flowering. Paclobutrazol is a strong inhibitory compound that reduces shoot growth but increases fruit count. Paclobutrazol increased the number of crowns, fresh root weight and reproductive growth such as the number of fruits and flowering (Abdollahi *et al.*, 2012). The number of flowers in the primary chain at a concentration of 250 mg/L, stem diameter and the number of flowers in the secondary flower arrangement at a concentration of 500 mg/L paclobutrazol increased compared to the control (Mansuroglu *et al.*, 2009).

Paclobutrazol is a triazole which promotes reproductive growth by reducing vegetative growth (Abdollahi *et al.*, 2012). The application of paclobutrazol as a soil wetting agent (@ 3.0 ml/m) on the diameter of the canopy induced early flowering and harvesting period of

mango cv. Totapuri becomes faster, without changing fruit quality parameters (Upreti *et al.*, 2013). Paclobutrazol can increase flower length, diameter of dried flowers, number of flowers per bunch, weight of 1000 dried flowers, and dry flower yield per tree (Runtunuwu *et al.*, 2016). The clove plant's flowering system is terminal, where flowers form at the tips of the buds. The formation of the ovule is characterized by the formation of primordial which within 1-2 months produces branches in the form of bunches and within 6 weeks has been shaped like clove flowers.



Figure 5. Primordial formation (a) and cloves flowering (b) after paclobutrazol treatment
The effect of paclobutrazol on eugenol, gibberellin, and abscisic acid

The content of eugenol, gibberellin (GA) and abscisic acid (ABA) in clove leaves changed due to paclobutrazol treatment (Table 2). The content of gibberellins (GA) in clove leaves was lower than control. This shows that the higher the dose of paclobutrazol, the lower the gibberellin content in the clove leaves. In contrast, abscisic acid (ABA) levels were increased in plants with higher doses of paclobutrazol.

Table 3. Eugenol, gibberellin and abscisic acid content after paclobutrazol treatment

Organic content (%)	Paclobutrazol treatment (ml)		
	6	14	0
Total eugenol	83,00	78,00	80,00
Gibberellin (GA)	0,0203	0,0196	0,0247
Absciscic acid (ABA)	0,0231	0,0245	0,0225

Changes in the content of eugenol, gibberellins and abscisic acid in clove leaves is one form of plant physiological response and can provide benefits. Eugenol in clove leaves can be a compound that is able to attack bacteria and inhibit viral DNA polymerization so as to maintain the stability of plant cells and cause disruption of ion transport in cells so that bacteria will die. Changes in the content of gibberellins and abscisic acid caused inhibition of plant vegetative growth such as reduced leaf size volume increase which led to an increase in leaf thickness and an increase in chlorophyll which assisted photosynthesis which triggered the induction of clove flowering. An increase in abscisic acid and a decrease in gibberellins is an output that can induce early flowering. Paclobutrazol also increased the abscisic acid content and leaf water potential in shoots to elicit flowering responses.

Conclusion

The application of paclobutrazol will affect the thickness of the clove leaves (the higher the dose, the thicker the leaves), the chlorophyll content, and induce the formation of flowers on the clove plant. Paclobutrazol also affects the content of eugenol, abscisic acid and gibberellins in cloves. From the results of the study, it was found that the application of paclobutrazol could overcome the problem of not being able to flower on productive clove plants.

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