Journal of Tropical Biodiversity and Biotechnology
Volume 09, Issue 02 (2024): jtbb86238
DOI: 10.22146/jtbb.86238

Research Article

Growth and Development of Winged Bean (*Psophocarpus tetragonolobus* (L.) DC.) Treated with Paclobutrazol

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Keywords:
Development
Growth
Paclobutrazol
Psophocarpus tetragonolobus
Submitted:
27 June 2023
Accepted:
08 December 2023
Published:
31 May 2024
Editor:
Furzani Binti Pa'ee

ABSTRACT
Winged bean (*Psophocarpus tetragonolobus* (L.) DC.) is one of the tropical legumes commonly grown for vegetable in Indonesia. Winged bean is a kind of plants that growth on vine so that for cultivation it requires stakes or awnings. It is known that paclobutrazol is a growth retardant that acts by inhibiting gibberellin biosynthesis and application of paclobutrazol could make plant become semidwarf or even dwarf. This study was aimed to evaluate the effect of paclobutrazol on growth, development, some phytochemicals content and yield of winged bean plants. This study used a Completely Randomized Design (CRD) with one factor, namely paclobutrazol, which was applied at four different concentrations, namely 0 ppm (control), 25 ppm, 50 ppm, 75 ppm or 100 ppm. Three replicates were made for each treatment. The results showed that paclobutrazol significantly decreased plant height, number of leaves, leaf area, number of pods per plant, pod length, fresh weight of fruit, levels of vitamin C and protein in the pods, but increased the leaf chlorophyll content and stomata density on the abaxial (lower) leaf surfaces. Paclobutrazol showed its effect on accelerating flowering time at a concentration of 50 ppm.

INTRODUCTION
Winged bean (*Psophocarpus tetragonolobus* (L.) DC.) is a tropical legume plant belonging to the Fabaceae family. Winged beans grow as herbaceous vines, it has root tubers and pods resembling wings. This plant is widely spread in tropical countries including Indonesia, Malaysia, Thailand, Philippines, India, Bangladesh, Myanmar and Sri Lanka (Lepcha et al. 2017). Winged bean has good prospects and market potentials. The potential yield of winged bean pods varies from approximately 35.5 to 40.0 tonnes/ha, higher than the yard-long bean pods of approximately 20 tonnes/ha (Rukmana 1995; Rukmana 2000). Winged beans can reach a potential yield of approximately 4.5 tonnes/ha of dry seeds, which exceeds the average potential yield of all soybean cultivars ranging from approximately 1.8 to 2.5 tonnes/ha of dry seeds (Rukmana 2000; Kusumowarno 2015). It has been reported that the content of protein in yard long bean pods is 2.8 g/100g, which is comparable to winged bean pods, whereas vitamin C content in yard long bean pods is 18.8 mg/100g, which is less than winged bean pods (U.S. Department of Agriculture 2018b). Furthermore, the protein content of winged bean seeds is 32.8 g/100 g, which is not much different from soybean seeds

(36.5 g/100 g) (Rukmana 2000; U.S. Department of Agriculture 2018a). Winged bean pods contain variety of nutrients that are crucial as a food plant such as crude protein (1.9 g/100 g - 3.0 g/100 g) and vitamin C (21 mg/100 g - 37 mg/100 g). In addition, winged bean pods also contain carbohydrates, fiber, fat, calcium, phosphorus, sodium, potassium, iron, vitamin A, vitamin B1, vitamin B2, and niacin (Kadam et al. 1984). The winged bean parts that can be consumed as food are its green pods, young seeds, root tubers, leaves and mature seeds (Mohanty et al. 2020).

Cultivation problems, however, often occur in vines such as difficulties in caring for creeping plants so that efforts to limit the growth of climbing stems are necessary. Growth inhibition can be applied to shorten the stem but still maintains the quality of the winged bean organs that are used such as seeds, fruit or young leaves. With semi-dwarf or dwarf plants, it will be easier to take care of the plants and do the harvest. Paclobutrazol is one of the compounds from the triazole group which has been known as a growth retardant. This compound has a ring structure containing three nitrogen atoms, chlorophenyl and a carbon side chain (Desta & Amare 2021). Paclobutrazol works by inhibiting the process of *ent*-kaurene oxidation to *ent*-kaurenoic acid through the inactivation of cytochrome P450-dependent oxygenase so that endogenous GA3 (gibberellin) levels decrease. Paclobutrazol causes morphological side effects, namely reducing plant height, inhibiting stem internodal elongation, and reducing the number of leaves (Soumya et al. 2017). Nevertheless, paclobutrazol is one of the compounds that can make the leaf color darker. This is caused by increased levels of chlorophyll or compaction of chlorophyll in the smaller parts of the leaves (Fletcher et al. 2000). In addition, it has been reported that in mangoes plant paclobutrazol can accelerate flowering time, total fruit per tree and average fruit weight (Yeshitela et al. 2004). The application of paclobutrazol can alter the partitioning of assimilate and nutrient supply in plants (Desta & Amare 2021). Paclobutrazol was reported to be able to increase vitamin C levels in *Curcuma alismatifolia* leaves at a concentration of 1500 ppm (Jungklang et al. 2017), whereas paclobutrazol application at a dose of 125 mg/L can increase protein levels in *Camelina sativa* (Kumar et al. 2012). Paclobutrazol can increase stomatal density by 15% - 17% on the abaxial and adaxial leaf surfaces of Quinoa (*Chenopodium quinoa*) at a dose of 20 mg/L (Waqas et al. 2017).

This research was conducted to evaluate the effect of paclobutrazol application on the growth, development, some phytochemical contents from young pods, and pod yields of winged bean (*Psophocarpus tetragonolobus* (L.) DC.). Furthermore, the purpose of this study was aimed to determine the appropriate concentration of paclobutrazol for reducing vine growth but yet still maintaining the yield quality of winged beans.

MATERIALS AND METHODS

This experiment was carried out at the Sawitsari Research Station, Faculty of Biology, Universitas Gadjah Mada (UGM), Condongcatur, Depok District, Sleman Regency, Special Region of Yogyakarta. Measurement for physiological and anatomical parameters, chlorophyll and vitamin C content were carried out at the Plant Physiology Laboratory, Faculty of Biology, Universitas Gadjah Mada. Testing for protein levels in winged bean pods was carried out at the Department of Food Technology and Agricultural Products, Faculty of Agricultural Technology, Universitas Gadjah Mada. This study used Completely Randomized Design (CRD) with one factor, namely paclobutrazol, which was applied at four different concentrations, namely 0 ppm (control), 25 ppm, 50 ppm, 75 ppm or 100

ppm. Three replicates were prepared for each treatment.

Winged bean seeds were soaked in warm water for 12 hours. Seeds were then sown in a plastic germination tray filled with planting medium. The planting medium contains a mixture of manure, soil, husk charcoal, cocopeat and bamboo leaf humus. Water (as a control) or paclobutrazol was sprinkled on the media as much as 5 mL. Winged bean seedlings were then transferred to polybags that had been filled with a mixture of soil, compost, and husk charcoal at a ratio of 2:1:1 per polybag when they reached the age of 2 weeks after seed sowing. Total weight of planting medium is 5 kg per polybag and one seedlings was planted in each polybag. Bamboo stick of 1.5 meters high was prepared and plugged into the soil as a place for the vine to grow and climb. Paclobutrazol was applied again every two weeks until 8 weeks after planting (WAP) and each plant received 50 mL. Watering was carried out regularly every two days. Additional NPK fertiliser at a ratio of 15:15:15 was applied with 10 grams doses per polybag. Pests and diseases were controlled with pesticides. The parameters observed were plant height, leaf number, leaf area, flowering time, pod length, total pods per plant, fresh weight of fruit, stomatal density, chlorophyll content, vitamin C content, and protein content in fruit.

Measurement of Growth and Development Parameters

Plant height and leaf number were measured manually from 2 to 7 WAP. Plant height was measured with tape measure, from the surface of growth medium to the highest shoot tip. Flowering time was calculated from time of seed sowing to the first flower emerged. The length of the fruit (pod) was measured by marking the raffia string according to the indentation of the fruit and the length of the rope is measured with a tape measure. The number of pods was counted manually for each plant. The fruit (pod) fresh weight was determined by weighing on an analytical balance. Leaf area was measured using the gravimetric method (Irwan & Wicaksono 2017). The formula for calculating leaf area is as follows:

$$\text{Leaf area} = \frac{\text{weight of leaf replica (g)}}{\text{weight of paper cut 10 cm x 10 cm (g)}} \text{ x } 100 \text{ cm}^2$$

Stomatal Density Measurement

The 3rd leaf from the shoot was used in making this preparation. Epidermal incisions were made on the lower surface of the leaves and fixed in 70% alcohol. Preparation for sample's dyeing was carried out using safranin reagent for one minute. The sample was placed on top of the object glass and covered with a cover glass. Observations were made under a microscope with a magnification of 40 x 10. Pictures of sample 's stomata were taken with Optilab v.3.0 software. Calculation of the number of stomata and the view area was carried out with the ImageRaster v.3.0 application. Stomatal density was calculated based on Lestari (2006) with the following formula:

$$\text{Stomatal Density} = \frac{\text{Number of stomata}}{\text{Unit of view area}}$$

Measurement of Chlorophyll Levels

Winged bean leaves were weighed as much as 0.1 gram and then mashed using a mortar and pestle and added with 10 mL of 80% acetone. Then the solution was filtered using Whatmann filter paper No. 3 and the volume was adjusted to 10 mL with 80% acetone. The absorbance value of the leaf extract was measured at a wavelength of 663 nm and 646 nm using a Spectrophotometer. Chlorophyll levels were calculated based on

Harborne (1998) with the following formula:

Total chlorophyll (mg/L) = $17.3A_{646} + 7.18A_{663}$

Chlorophyll *a* (mg/L) = $12.21A_{663} - 2.81A_{646}$

Chlorophyll *b* (mg/L) = $20.13A_{646} - 5.03A_{663}$

Conversion to (mg/g) = $\frac{10/1000 \; x \; Chlorophyll \; Levels}{0.1 \; mg/g}$

Measurement of Vitamin C Levels

Vitamin C levels were measured using the iodometric titration method based on Sudarmadji et al. (1984). A young fruit sample of 10 grams was mashed and diluted by adding distilled water to a volume of 100 mL. The sample solution was filtered using Whatmann filter paper No. 3 and then 5 mL of the filtrate was taken. A 125 mL Erlemeyer flask was filled with the filtrate from the sample and 2 mL of 1% starch solution was added. The titration was carried out by the iodometric method with a standard 0.01 N iodine solution. The titration was ended when the color of the solution change to a blue color constantly. Vitamin C levels were calculated based on Sudarmadji et al. (1984) with the following formula:

$$\text{Vit. C level (mg/100 g)} = \frac{\text{Viod x 0.88 mg x fp x 100}}{\text{sample weight(w)}}$$

Notes:

V Iod = volume iodin (mL)

1 mL 0.01 N Iod = 0.88 mg ascorbic acid

w = sample weight (gram)

fp = dilution factor

Measurement of Protein Levels

The young winged bean pods were mashed using a blender. After that, the sample was weighed as much as 100 mg. Furthermore, the protein content of the sample was measured based on the micro Kjeldhal method as described in AOAC 981.10 (Latimer 2016). Protein levels were calculated based on Sudarmadji et al. (1984) with the following formula:

$$\% \text{ N} = \frac{(\text{mL NaOH blank} - \text{mL NaOH sample})}{\text{sample weight (g)x 1000}} \text{ x 100 x 14.008}$$

$$\% \text{ protein} = \% \text{ N x factor}$$

Notes:

w = sample weight (gram)

% N = nitrogen percentage

Factor = 6.25

Data Analysis

Quantitative data were analysed using Microsoft Excel and SPSS version 25 software, with a significance of 5%. If the One Way ANOVA results are significantly different (F count > F table), then the analysis was continued with the DMRT test (Duncan Multiple Range Test) at the 95% confidence level.

RESULTS

The Effect of Paclobutrazol Application on the Growth of Winged Bean (*Psophocarpus tetragonolobus* (L.) DC.)

Paclobutrazol treatment reduced plant height and leaf number of winged bean. The higher the concentration of paclobutrazol applied, the greater the reduction in plant height and leaf number were observed (Table 1).

Table 1. The effect of paclobutrazol on plant height and leaf number of winged bean (*Psophocarpus tetragonolobus* (L.) DC.) at 7 WAP.

Treatment	Plant height (cm)	Leaf number (sheet)
P0 (control)	251.17 ± 17.39^{d}	70.67 ± 4.04^{b}
P1 (25 ppm)	215.07 ± 9.86^{c}	57.33 ± 10.69^{ab}
P2 (50 ppm)	143.67 ± 29.91^{b}	54.33 ± 11.72^{a}
P3 (75 ppm)	9.53 ± 3.08^{a}	51.67 ± 3.79^{a}
P4 (100 ppm)	8.60 ± 0.53^{a}	49.67 ± 3.06^{a}

Note: The same letter shows results that are not significantly different in the DMRT test with a significance of 5%.

Figure 1. Morphology of winged bean leaves treated with paclobutrazol at various concentration: (a) 0 ppm (b) 25 ppm (c) 50 ppm (d) 75 ppm (e) 100 ppm.

From Figure 1 it is clear that the trifoliate leaf of winged bean treated with paclobutrazol also become smaller compared to control. The measurement of leaf area showed that the higher concentration of paclobutrazol applied, the greater reduction in leaf area were observed (Table 2).

Table 2. Effect of paclobutrazol on winged bean (*Psophocarpus tetragonolobus* (L.) DC.) leaf area.

Treatment	Leaf Area (cm^2)
P0 (control)	116.56 ± 6.71^{c}
P1 (25 ppm)	95.40 ± 11.60^{b}
P2 (50 ppm)	89.81 ± 11.15^{b}
P3 (75 ppm)	70.70 ± 8.09^{a}
P4 (100 ppm)	68.66 ± 7.00^{a}

Note: The same letter shows results that are not significantly different in the DMRT test with a significance of 5%.

Further ANOVA and DMRT tests, showed that there were significant effect due to the application of various paclobutrazol concentrations on reducing plant height and leaf number of winged bean plants (α<0.05) (Table 1). The leaf area of plants treated with paclobutrazol were significantly smaller compared to control (α<0.05) (Table 2).

Effect of Paclobutrazol on the Development of Winged Bean (*Psophocarpus tetragonolobus* (L.) DC.)

Based on ANOVA and DMRT tests, there was a significant effect of paclobutrazol on the initiation of flowering ($\alpha<0.05$) (Table 3). The fastest flowering time occurred in those plants treated with 50 ppm paclobutrazol, which was 45.33 days (Table 3).

Table 3. Effect of paclobutrazol on winged bean (*Psophocarpus tetragonolobus* (L.) DC.) flowering time.

Treatment	Flowering Time (DAP)
P0 (control)	50.00 ± 1.00^{b}
P1 (25 ppm)	51.33 ± 1.53^{bc}
P2 (50 ppm)	45.33 ± 0.58^{a}
P3 (75 ppm)	52.00 ± 1.73^{bc}
P4 (100 ppm)	52.33 ± 0.58^{c}

Note: The same letter shows results that are not significantly different in the DMRT test with a significance of 5%

The application of various concentrations of paclobutrazol gave a significant decrease on the number of pods per plant according to statistical tests, ANOVA and DMRT tests ($\alpha<0.05$) (Table 4).

Table 4. Effect of paclobutrazol on the number of winged bean (*Psophocarpus tetragonolobus* (L.) DC.) pods per plant.

Treatment	Number of Pod (fruit)
P0 (control)	15.00 ± 5.69^{c}
P1 (25 ppm)	9.00 ± 4.00^{b}
P2 (50 ppm)	8.33 ± 1.53^{b}
P3 (75 ppm)	7.33 ± 2.52^{b}
P4 (100 ppm)	2.33 ± 0.58^{a}

Note: The same letter shows results that are not significantly different in the DMRT test with a significance of 5%.

From Figure 2, it can be seen that the application of paclobutrazol of 25 ppm, 50 ppm or 75 ppm only slightly affected pod length but paclobutrazol of 100 ppm decreased pod length. It is shown that pod length of those plants treated with paclobutrazol up to 75 ppm are similar to control but pod length of plants treated with 100 ppm decreased significantly compared to control (Table 5). However, the fresh weight of pod decreased according to the increase in the concentration of paclobutrazol applied (Table 5).

Table 5. Effect of paclobutrazol on winged bean (*Psophocarpus tetragonolobus* (L.) DC.) pod size.

Treatment	Length of pod (cm)	Fresh weight per fruit (gram)
P0 (control)	17.83 ± 1.48^{b}	21.47 ± 1.13^{c}
P1 (25 ppm)	16.80 ± 1.22^{b}	14.13 ± 1.01^{ab}
P2 (50 ppm)	17.50 ± 0.36^{b}	16.33 ± 2.10^{b}
P3 (75 ppm)	15.20 ± 3.15^{ab}	9.58 ± 5.08^{a}
P4 (100 ppm)	12.47 ± 1.27^{a}	9.26 ± 1.38^{a}

Note: The same letter shows results that are not significantly different in the DMRT test with a significance of 5%.

Figure 2. Morphology of winged bean pods treated with paclobutrazol at various concentration: (a) 0 ppm (b) 25 ppm (c) 50 ppm (d) 75 ppm (e) 100 ppm.

Pod length and fruit fresh weight of winged bean plants decreased significantly with increasing application of paclobutrazol concentration according to ANOVA and DMRT test results ($\alpha<0.05$) (Table 5).

Effect of Paclobutrazol on Stomatal Density of Winged Bean (*Psophocarpus tetragonolobus* (L.) DC.)

Using epidermal incisions on the lower surface (abaxial) of the treated winged bean leaves at different concentrations of paclobutrazol, Figure 3 shows the observed illustrations of the stomata structure. From Figure 3, it could be observed that the treated leaf with the higher concentration of paclobutrazol (50 ppm, 75 ppm, and 100 ppm) had a higher number of stomata than the treated leaf with a lower concentration of paclobutrazol (25 ppm) and the control. Higher stomatal density is directly proportional to the higher number of stomata. The application of various concentrations of paclobutrazol had a significant effect on increasing the stomata density on the abaxial (lower) leaf surfaces of winged bean leaves ($\alpha<0.05$) (Table 6). The highest density of stomata was found in those plants treated with 50 ppm paclobutrazol, namely 30.111 Σ/mm^2 whereas paclobutrazol of 75 ppm or 100 ppm also increased stomata density of winged bean leaf compared to the control (0 ppm) or those plants treated with 25 ppm paclobutrazol (Table 6).

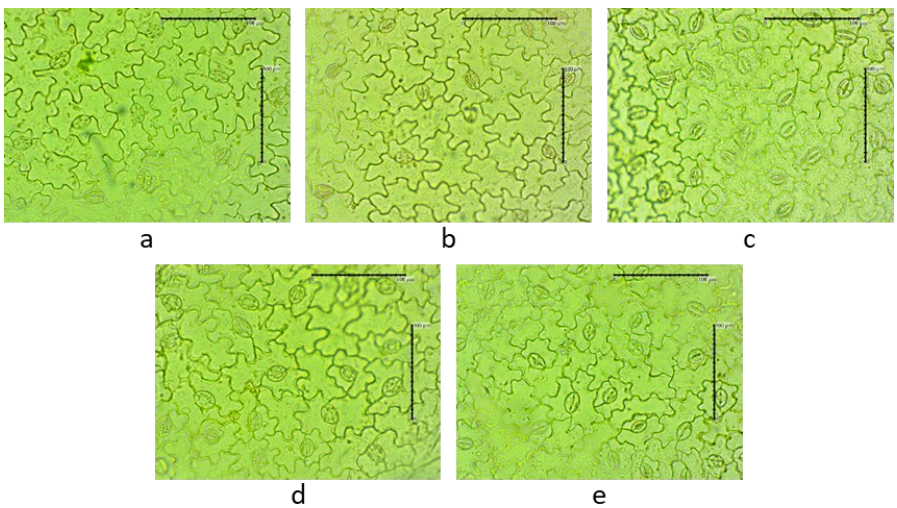

Figure 3. Anatomy of the stomata of winged bean leaves treated with

paclobutrazol at various concentration: (a) 0 ppm (b) 25 ppm (c) 50 ppm (d) 75 ppm (e) 100 ppm.

Table 6. Effect of paclobutrazol on stomatal density on the abaxial (lower) surface of winged bean leaves (*Psophocarpus tetragonolobus* (L.) DC.).

Treatment	Stomatal Density(Σ/mm²)
P0 (control)	15.526 ± 1.411[a]
P1 (25 ppm)	18.349 ± 3.293[a]
P2 (50 ppm)	30.111 ± 6.587[b]
P3 (75 ppm)	26.504 ± 2.757[b]
P4 (100 ppm)	29.954 ± 3.338[b]

Note: The same letter shows results that are not significantly different in the DMRT test with a significance of 5%.

Effect of Paclobutrazol on some Phytochemicals Levels of Winged Bean (*Psophocarpus tetragonolobus* (L.) DC.) Pods

ANOVA and DMRT test results proved that the application of paclobutrazol had a significant effect on increasing chlorophyll levels in winged bean pods ($\alpha<0.05$) (Table 7). The highest total chlorophyll was 1.144 mg/g found in plants treated with 100 ppm paclobutrazol. On the contrary, the application of various concentration of paclobutrazol had a significant effect on reducing vitamin C levels as well as protein levels in winged bean pods ($\alpha<0.05$) (Table 8).

Table 7. Effect of paclobutrazol on chlorophyll content of winged bean (*Psophocarpus tetragonolobus* (L.) DC.) leaves.

Treatment	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total of Chlorophyll (mg/g)
P0 (control)	0.049 ± 0.045	0.027 ± 0.026	0.076 ± 0.072[a]
P1 (25 ppm)	0.090 ± 0.016	0.059 ± 0.004	0.149 ± 0.020[a]
P2 (50 ppm)	0.286 ± 0.010	0.141 ± 0.006	0.426 ± 0.004[b]
P3 (75 ppm)	0.363 ± 0.067	0.179 ± 0.023	0.541 ± 0.090[c]
P4 (100 ppm)	0.727 ± 0.009	0.418 ± 0.017	1.144 ± 0.018[d]

Note: The same letter shows results that are not significantly different in the DMRT test with a significance of 5%.

Table 8. Effect of paclobutrazol on vitamin C and protein levels in winged bean (*Psophocarpus tetragonolobus* (L.) DC.) pods.

Treatment	Level of Vitamin C (mg/100 g)	Level of Protein (%)
P0 (control)	91.91 ± 17.92[b]	2.70 ± 0.02[d]
P1 (25 ppm)	56.71 ± 8.96[a]	2.40 ± 0.03[c]
P2 (50 ppm)	48.89 ± 6.77[a]	2.34 ± 0.02[b]
P3 (75 ppm)	54.76 ± 3.39[a]	-
P4 (100 ppm)	54.77 ± 12.21[a]	2.00 ± 0.04[a]

Note: The same letter shows results that are not significantly different in the DMRT test with a significance of 5%, no sample was available at harvest for protein level test in those plants treated with 75 ppm paclobutrazol.

DISCUSSION

The Effect of Paclobutrazol on the Growth of Winged Bean (*Psophocarpus tetragonolobus* (L.) DC.)

The results of this study proved that the higher the concentration of paclobutrazol applied to the winged bean (*Psophocarpus tetragonolobus* (L.)

DC.), the plant height declined in those plants applied with 75 ppm or 100 ppm paclobutrazol (Table 1). This is caused by the action of paclobutrazol which inhibits the activity of enzyme that catalyzed oxidation of *ent*-kaurene to *ent*-kaurenoic acid, namely the cytochrome p450-dependent oxygenase enzyme, often called *ent*-kaurene oxidase (Soumya et al. 2017). Consequently, the KO enzyme (*ent*-kaurene oxidase) cannot catalyze the conversion of *ent*-kaurene to *ent*-kaurenol, the conversion of *ent*-kaurenol to *ent*-kaurenal and the conversion of *ent*-kaurenal to *ent*-kaurenoic acid (Olszewski et al. 2002; Soumya et al. 2017). In plants treated with paclobutrazol, the cells may continue to divide even though the level of endogenous gibberellins was reduced. However, cell elongation was hampered due to inhibition of gibberellin biosynthesis in the subapical meristem. This can reduce the rate of cell division and cell elongation. As it was reported in other species, plants treated with paclobutrazol produced stems with shorter internodes and become stunted (Meena et al. 2014).

The results of this study showed that the higher the concentration of paclobutrazol, the fewer the number of leaves produced by the winged bean (Table 1). This is probably caused by the reduction in gibberellin as well as auxin biosynthesis in those plants treated with paclobutrazol and it slow down the leaf growth. It has been reported that application of paclobutrazol may increases cytokinin levels that normally play a role in leaf morphogenesis (Fletcher et al. 2000). Cytokinins play a role in triggering the maintenance of SAM size, structure, and activity to do proliferation and growth. It contributes to the control of leaf primordia development by preventing stem cell differentiation. However, cytokinins also affecting the establishment of the auxin gradient by regulating auxin biosynthesis and transport (Wu et al. 2021). As paclobutrazol was applied at the early seedling stage by soil drenching, it seems that severe reduction in both gibberellin and auxin leads to the reduction of leaf number especially in those plants treated with paclobutrazol of 50 ppm, 75 ppm or 100 ppm. The increase in cytokinin seems manifested in the increase of chlorophyll content and delaying the leaf senescence in those plants treated with paclobutrazol.

In this study the leaf number of those plants treated with either 25 ppm, 50 ppm, 75 ppm or 100 ppm paclobutrazol was not significantly different statistically even though the value tends to decrease as the concentration of paclobutrazol applied to the plants increased. This is because the number of leaves is influenced by genetic and environmental factors (Tumewu et al. 2012).

The results of this study indicated that the higher the concentration of paclobutrazol applied, the smaller leaf area of winged bean observed (Table 2). Application of paclobutrazol reduces gibberellin levels, thus reducing the intensity of proliferation and expansion of leaf plate meristem cells (Du et al. 2018). The plate meristem is one of the leaf meristem tissues, which is located at the base of leaf blade primordia, long-lived, consisting of parallel layers of anticlinal dividing cells. It has been implied that paclobutrazol plays a major role in inhibiting leaf morphogenesis, including growth of leaf size until the mature phase (Yeshitela et al. 2004; Du et al. 2018).

Effect of Paclobutrazol Application on the Development of Winged Bean (*Psophocarpus tetragonolobus* (L.) DC.)

The results of this study show that the paclobutrazol treatment of 25 ppm, 75 ppm or 100 ppm leads to the initiation of flowering in winged bean that was not significantly different compared to control, the fastest

flowering time was found in plants treated with paclobutrazol of 50 ppm (Table 3). This is probably caused by paclobutrazol which already limits vegetative growth due to inhibition of gibberellin biosynthesis, but there is an accumulation of photosynthate in the stroma of the chlorophyll. The photosynthate will be used to support vegetative growth initially, but as vegetative growth of those plants treated with 50 ppm paclobutrazol was inhibited then photosynthate is allocated to induce shoots to enter generative growth (Wardani et al. 2020). In the winged bean treated with 50 ppm paclobutrazol, flowering was earlier because plant height can be reduced by about 42.8% compared to the control. The photoassimilate is sufficient for vegetative growth and then it was seems to be allocated for the formation of flowers.

However, in winged bean applied with higher concentration of paclobutrazol (75 ppm or 100 ppm) flowering time was slightly delayed compared to control. This is probably caused by limited levels of gibberellins that found in those plants. Besides that, flowering initiation is influenced by genetic and enviromental factors that regulate flowering patterns. When the winged bean has not yet reached the mature phase, delayed flowering initiation may occured because of paclobutrazol treatment (Harpitaningrum et al. 2014). In addition, each plant possibly has a different levels of sensitivity to paclobutrazol treatment (Ardigusa & Sukma 2015). Plant height in those winged bean treated with 75 ppm and 100 ppm paclobutrazol was significantly reduced by about 96.2% and 96.6%, respectively. It indicated that the plants were severely stunted. In addition, the number of leaves was also reduced in those plants. Consequently, the amount of photosynthate produced was drastically reduced and it is still be used to support vegetative growth rather than generative growth. The application of paclobutrazol in this study resulted in the number of pods per plant of winged bean appearing less (Table 4). This is probably due to the paclobutrazol that also make assimilate transport ineffective because the phloem cells may become smaller or denser. Application of paclobutrazol shortens the distance between internodes or stem internodes. The leaves emerge from the nodes of the stems, resulting in densely growing leaves that narrow the leaf's light-receiving area. There is a possibility of reduced photosynthetic activity so that the supply of carbohydrates for flower and fruit formation is reduced. The number of fruit that is successfully formed depends on the amount of available photosynthetic products, namely carbohydrates. Carbohydrates have a role as a food supply which is stored in winged bean pods. Competition for photosynthetic assimilation also occurs in flowers and fruit in the same plant. The imbalance between supply and demand makes flowers more at risk of falling on stunted plants treated with paclobutrazol as it was reported in *Cucumis sativus* (Harpitaningrum et al. 2014). This also results in less pod production in dwarf winged bean plants.

The results of the study showed that the higher the concentration of paclobutrazol, the shorter the length of the winged bean fruit (Table 5). The decrease in winged fruit length was not significantly different at various concentration of paclobutrazol, namely 0 ppm, 25 ppm, 50 ppm and 75 ppm. It can be caused by several factors, namely genetic factors, environmental habitat, and treatment at harvest. Genetic factors may be very dominant in influencing fruit length growth compared to environmental factors and growth inhibitory substances which help to increase assimilate translocation to sink organs, such as those found in cucumber (*Cucumis sativus* L.) venus cultivars that were treated with paclobutrazol (Harpitaningrum et al. 2014). Treatment of 100 ppm

paclobutrazol reduced fruit length because of paclobutrazol concentration that were too high could reduce the rate of cell division, weight gain, and cell size. As a result, fruit growth becomes stunted so they don't grow bigger. Similar finding has been reported in water melon treated with paclobutrazol (Jasmine et al. 2014).

The results also showed that the higher the concentration of paclobutrazol, the fresh weight per fruit of winged bean was lighter (Table 5). This may be due to the influence of paclobutrazol which already acting since growth of seedlings. Paclobutrazol inhibits gibberellins biosynthesis, especially in the *ent*-kaurene oxidation phase. Paclobutrazol can also increase ABA biosynthesis due to the accumulation of precursors in the terpenoid pathway which triggers its biosynthesis (Soumya et al. 2017). The increased concentration of ABA causes stomatal guard cells to lose ions, followed by loss of turgor and water which ends with stomata closing (Hopkins & Hüner 2009). According to Chaves et al. (2009) in Wardani et al. (2022), closing stomata causes photosynthesis to be hampered due to decreased stomatal conductance. As a result, the amount of assimilates (carbohydrates) that will be translocated to sinks decreases, so that the fresh weight of sink organ (fruit) produced is less.

Effect of Paclobutrazol on Stomatal Density of Winged Bean (*Psophocarpus tetragonolobus* (L.) DC.)

The stomata of winged bean leaves which are located on the abaxial side of the leaves have a parasitic type (Figure 3). Parasitic stomata are stomata that have a single neighboring cell with a parallel arrangement of the connecting walls to the guard cells (Hostettmann 2014). The results of this study show that the higher the concentration of paclobutrazol, the greater the number of stomatal density of winged bean leaves (Table 6). The application of paclobutrazol at 50 ppm is best for increasing stomatal density. However, the application of paclobutrazol at 75 ppm and 100 ppm produced stomatal density that was not significantly different from the 50 ppm paclobutrazol treatment. This may be caused by the application of paclobutrazol that can increase cytokinin biosynthesis. Paclobutrazol is one of the compounds from the triazole group with the performance of inhibiting the cytochrome P-450 dependent monooxygenase enzyme which plays a role in gibberellin biosynthesis. This resulted in the inhibition of the catalytic reaction in a stepwise oxidation reaction involving the conversion of *ent*-kaurene to *ent*-kaurenoic acid. This inhibition results that one of the precursor for isoprenoid pathway, namely isopentenyl diphosphate, is not used for gibberellin biosynthesis. Consequently, isopentenyl diphosphate is diverted for cytokinin biosynthesis (isopentyl adenosine) so cytokinin levels increase (Fletcher et al. 2000). Cytokinin is one of the phytohormones that has several roles in regulating cell division and differentiation by influencing the control of the cell division cycle (Taiz & Zeiger 2010). It has been reported that paclobutrazol causes the stimulation of division and differentiation of leaf epidermal cells to become stomata (Willmer & Fricker 1996; Hostettmann 2014).

Stomatal density is the number of stomata per unit area of the leaf surface. Stomatal density affects the amount of CO_2 that enters or is fixed into the leaf for photosynthesis and regulates transpiration, in which the water vapor will diffuse out of the leaf. Both of the processes affect plant productivity. CO_2 acts as the reactant on the Calvin cycle or dark reaction of photosynthesis. The Calvin cycle begins with the fixation of CO_2 with the acceptor that called RuBP (ribulose-1,5-bisphosphate) and pro-

duces phosphorylated carbon intermediates (glyceraldehyde-3-phosphate) which will become photo-assimilate. This photo-assimilate will be converted to sucrose in the mesophyll vacuole or starch in the chloroplast stroma. Sucrose and starch will be translocated to non-photosynthetic organs and tissues. Excessive transpiration causes productivity decline. This is due to the function of water as a temperature regulator in plants cells, a suitable medium for absorbing and distributing soluble compounds, and a reactant or product in the biochemical reactions (Hopkins & Hüner 2009). The results of the study proved that enhancement of stomata density may support plant growth and development, especially at the initiation of flowering.

Effect of Paclobutrazol on Phytochemical Levels of Winged Bean (*Psophocarpus tetragonolobus* (L.) DC.) Pods

The results of this study show that the higher the concentration of paclobutrazol, the higher the chlorophyll content of winged bean leaves (Table 7). Winged bean leaves that were applied with paclobutrazol became darker in color. This is due to the performance of paclobutrazol which inhibits gibberellin biosynthesis by inhibiting the cytochrome p450-dependent oxygenase enzyme. The geranylgeranyl pyrophosphate precursor accumulates from the terpenoid pathway so that it can be used for the synthesis of phytol, which is a precursor compound for the chlorophyll component (Chaney 2005; Soumya et al. 2017).

In addition, the results in Table 8 show that the higher the concentration of paclobutrazol, the lower the vitamin C content in winged bean young pods. This may be caused by the application of paclobutrazol which shortens the internodal distance. The effect of paclobutrazol on vitamin C is probably derived from the amount of photosynthate produced. Photosynthesis plays a role in producing soluble carbohydrates. Soluble carbohydrates are needed as precursors of vitamin C biosynthesis such as D-glucose-6-phosphate (Paciolla et al. 2019). Extreme dwarfing of plants results in a drastically reduced amount of photosynthate produced. As a result, the vitamin C content of young winged bean pods is reduced due to lack of precursors for biosynthesis.

The results also show that the higher the concentration of paclobutrazol, the lower the protein content in winged bean young pods (Table 8). The application of paclobutrazol on winged bean causes an increase in leaf chlorophyll content. The increase in chlorophyll accumulation supposedly causes the rate of photosynthesis to increase thereby affecting assimilate translocation (Sambeka et al. 2012). Nitrogen is one of the elements that make up the chlorophyll and protein components (Hopkins & Hüner 2009). The availability of N in plant organs is obtained from absorption of N from the soil or remobilisation of N from source organs to sink organs (Tekalign & Hammes 2005). Photo-assimilate competition for nitrogen supply occurs between chlorophyll synthesis for leaves and protein synthesis for pods. The supply of photo-assimilates and nitrogen may be more focused on increasing leaf chlorophyll synthesis in those plants treated with paclobutrazol. The imbalance between supply and demand causes protein levels in winged bean pods to decrease.

CONCLUSIONS

Paclobutrazol has a significant effect on the growth of winged beans, which reduces the plant height, leaf number, leaf area. Paclobutrazol affects on the yield of winged bean pods significantly, which reduces number of pods per plant, pod length and fresh weight per fruit. Paclobutrazol has a significant effect on reducing the levels of winged bean pod phytochemicals, namely levels of vitamin C and protein levels.

Paclobutrazol application gave the best results in increasing the stomatal density, enhancing chlorophyll content and accelerating flower initiation. The flowering time of winged beans can be accelerated by the application of paclobutrazol at 50 ppm. The chlorophyll content of winged bean leaves tend to increase by paclobutrazol application. The stomatal density of the abaxial surface of winged bean leaves was highest with paclobutrazol application of 50 ppm.

AUTHORS CONTRIBUTION

J.S.W. collected and analysed the data, as well as wrote and revised the manuscript. K.D. designed and supervised the research processes, critically proofread the manuscript writing and participated in revising the manuscript.

ACKNOWLEDGMENTS

The authors would like to express our gratitude to the Plant Physiology Laboratory and Sawit Sari Research Station Staff, Faculty of Biology, Universitas Gadjah Mada, for providing the facilities, assistance, and equipment that were required for this research. We also appreciated the assistance in determining the protein content of the young winged bean pods from the Department of Food and Agriculture Products Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada.

CONFLICT OF INTEREST

There is no conflict of interest regarding research and research funding.

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