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Enhancing total phenolic, flavonoid, and antioxidant capacity in black orchid (*Coelogyne pandurata* Lindl.) with paclobutrazol application

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Abstract. The Black Orchid (*Coelogyne pandurata* Lindl.) is a species with promising secondary metabolite content, including total phenolics, total flavonoids, and antioxidant capacity, making it highly valuable for horticultural applications. This research investigated the influence of Paclobutrazol application on the total phenolic content, total flavonoid content, and antioxidant capacity of Black Orchid. The study was conducted using a randomized complete block design (RCBD) with Paclobutrazol treatments at concentrations of 0, 50, 100, and 150 ppm. Each experimental unit was conducted with three replicates. The data were analyzed using ANOVA, followed by Tukey's HSD test at a 5% significance level to compare treatment means. Total phenolic, flavonoid, and antioxidant capacities were analysed using a Nano-spectrophotometer. Results indicated that treatment with 150 ppm Paclobutrazol significantly increased the flavonoid concentration in the leaves, showing a significant difference compared to the control. However, no significant effect was observed on the total phenolic content or antioxidant activity in either the leaves or bulbs.

1 Introduction

The black orchid (*Coelogyne pandurata* Lindl.) is an orchid with potential as a medicinal orchid [1], This endemic orchid from Indonesia has aesthetic potential and has the opportunity to be developed into medicinal raw materials as a source of secondary metabolite compounds. The most dominant secondary metabolites in *C. pandurata* orchids are phenolic compounds, flavonoids, and antioxidants.

Phenolic compounds can be utilized as antioxidant, anti-inflammatory, anticancer, and antibacterial agents. Plants naturally use these compounds as a defense against the stress of various external factors. One compound that belongs to the group of phenolic compounds is flavonoid compounds [2].

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Flavonoid is a secondary metabolite in plants that have a basic structure in the form of a C6-C3-C6 skeleton. This compound consists of two aromatic rings (A and B) connected by a triple carbon chain (ring C). Flavonoids are produced through the phenylpropanoid biosynthesis pathway, which starts from phenylalanine acid. These flavonoid compounds can be antioxidant agents [3].

Antioxidant capacity is the ability of plants to fight oxidative stress caused by free radicals and reactive oxygen species (ROS). Plants produce both enzymatic and non-enzymatic antioxidant compounds to protect cells from oxidative damage that can affect growth and productivity. In the pharmaceutical industry, antioxidants can be used to prevent degenerative diseases such as cancer, diabetes, and cardiovascular disease. In the food sector, natural antioxidants are used as preservatives to increase product shelf life, and plant antioxidants are used in skin care products to protect against free radical damage [4].

The content of phenolic compounds, flavonoids, and antioxidants in plants has been studied by [5] which examined the increase in phenolic and flavonoid content by spraying paclobutrazol 150 mg L^{-1} on rice plants. In *Salvia officinalis* plants spraying paclobutrazol, 17 µM increased the content of plant's biochemical content [6]. However, no information specifically examines the increase in the content of phenolic compounds, and flavonoids. and antioxidants in *C. pandurata* orchid plants by spraying paclobutrazol. The purpose of this study was to increase the content of phenolic compounds, flavonoids, and antioxidants in *C. pandurata* orchid plants by spraying paclobutrazol.

2 Material and method

2.1 Research time and place

This study was conducted between January and July 2024 at the Leuwikopo Orchid House, located within the experimental garden of IPB University in Ciampea, Bogor Regency, West Java, Indonesia (2404905 "S 3005646 "E), at an altitude of 188 meters (masl).

2.2 Planting material preparation

The orchids used in this study were *C. pandurata*, which have entered the early vegetative phase, aged 6 months after transplanting, with morphological conditions consisting of 2 bulbs, and 4 leaves.

2.3 Research design

This study employed a non-factorial randomized complete block design (RCBD) with five levels of Paclobutrazol treatment, including a control (0 ppm), 50 ppm, 100 ppm, and 150 ppm. Each treatment was replicated nine times, resulting in 45 samples. Phosphorus was applied through foliar spraying using the designated concentrations, allowing for an assessment of the impact on the growth and biochemical properties of *C. pandurata*. Data collection focused organs on vegetative and generative characteristics and secondary metabolite levels.

2.4 Determination of total phenolic content, total flavonoid content, and antioxidant capacity

Plant samples were isolated from leaves, bulbs, and flowers, followed by sample extraction with the microwave method. Phenolic analysis using the *Folin-Ciocalteu* method with nanospectro (SPECTROstar Nano BMG LABTECH). The absorbance will be measured using a wavelength of 765 nm. The results will be presented as milligrams of gallic acid equivalent (GAE) per gram of dry weight. Flavonoid content was measured using a colorimetric method with aluminum chloride as the reagent, and absorbance was recorded at 415 nm, with the results expressed as milligrams of quercetin equivalent (QE) per gram of dry weight. The antioxidant capacity was evaluated using the DPPH (2,2'-diphenyl-1-picrylhydrazyl) radical scavenging assay. Extract samples of black orchid were prepared, and the reduction in DPPH absorbance at 517 nm was used to determine antioxidant activity, expressed in µmol Trolox equivalent per gram of dry weight.

2.5 Data analysis

The collected data were subjected to analysis of variance (ANOVA) to assess the significance of differences among treatments. Post-hoc comparisons were performed using Tukey's Honestly Significant Difference (HSD) test at a 5% significance level to identify which paclobutrazol concentrations significantly affected the growth and biochemical properties of *C. pandurata*. Statistical analyses were conducted using R Studio and SPSS (Version 26.0).

3 Result and discussion

3.1 Total phenolic content

Observations on the plants showed that foliar application of paclobutrazol up to 150 ppm increased the total phenolic content in the leaves. However, in the bulbs, paclobutrazol application increased only up to 100 ppm, which remained lower than the control. The total phenolic content in leaves and bulbs of black orchid at different paclobutrazol treatments are presented in Fig 1.

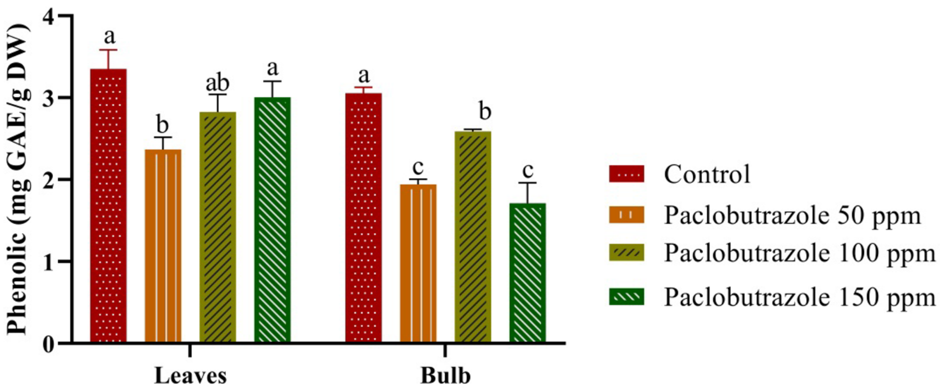


Fig. 1. Total Phenolic Content in Leaves and Bulbs of Black Orchid (*Coelogyne pandurata* Lindl.) at Different Paclobutrazol Treatments.

Phenolic compounds are essential for plant defense and antioxidant activity. Paclobutrazol treatment significantly influenced phenolic content in *C. pandurata* (Fig 1). The control group had the highest levels, while increasing Paclobutrazol concentrations led to a decline, with the lowest phenolic accumulation at 150 ppm, especially in bulbs. This suggests that Paclobutrazol affects phenolic metabolism, potentially altering plant adaptation and biochemical properties.

The increase in total phenolic content in plant leaves with paclobutrazol application appears to have not reached its optimal level, indicating the potential for further enhancement with higher concentrations. Paclobutrazol (PBZ) can increase phenolic compound levels in plants by influencing stress response and secondary metabolite pathways. PBZ inhibits gibberellin biosynthesis, leading to altered hormonal balances, particularly an increase in abscisic acid (ABA) and a decrease in auxin. This hormonal shift enhances the plant's antioxidant defenses and phenolic biosynthesis as a response to stress. Phenolics play critical roles in plant protection and stress mitigation, making PBZ a valuable tool in improving plant resilience and secondary metabolite production [1].

3.2 Total flavonoid content

The observations and analysis of total flavonoid content in plant leaves revealed that paclobutrazol application significantly enhanced the total flavonoid content in the leaves, with the highest effect observed at a concentration of 150 ppm. However, no significant effect was noted on the total flavonoid content in the bulbs. The total flavonoid content in leaves and bulbs of black orchid different paclobutrazol treatments are presented in Fig 2.

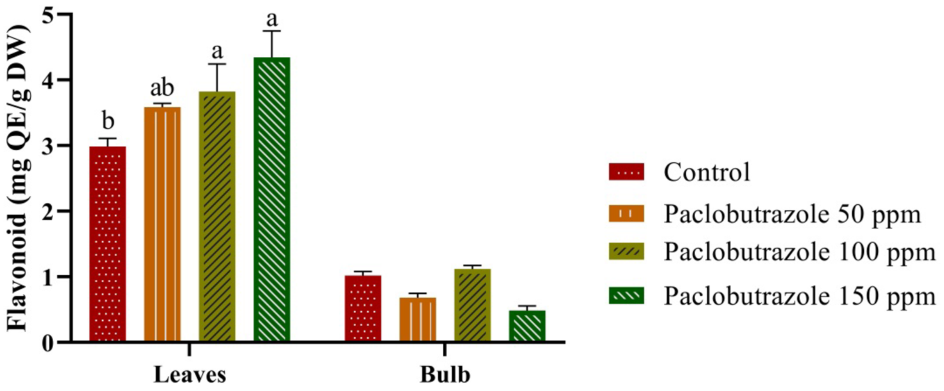


Fig. 2. Total Flavonoid Content in Leaves and Bulbs of Black Orchid (*Coelogyne pandurata* Lindl.) at Different Paclobutrazol Treatments.

Flavonoids are key secondary metabolites involved in plant defense and antioxidant activity. Paclobutrazol treatment significantly influenced flavonoid accumulation in *C. pandurata*. In leaves, flavonoid content increased with higher Paclobutrazol concentrations, with the highest levels recorded at 150 ppm (Fig 2). However, in bulbs, flavonoid levels remained relatively low across all treatments. These findings suggest that Paclobutrazol enhances flavonoid biosynthesis mainly in leaves, potentially influencing the plant's physiological and biochemical responses.

The physiological mechanism behind the increase in total flavonoid content in plant leaves due to paclobutrazol application is primarily linked to its role in altering hormonal balance and metabolic pathways. Paclobutrazol, a triazole-based plant growth regulator, inhibits gibberellin biosynthesis, reducing vegetative growth and reallocating resources toward secondary metabolite production. This metabolic change promotes the activity of essential enzymes such as phenylalanine ammonia-lyase (PAL) which catalyzes the initial steps in the phenylpropanoid pathway, a precursor route for flavonoid biosynthesis. Studies have shown that increased PAL activity directly correlates with higher flavonoid accumulation, as observed in various species under similar treatments [7].

3.3 Antioxidant capacity

Antioxidants play a vital role in protecting plants from oxidative stress by neutralizing free radicals. Paclobutrazol treatment on antioxidant activity in *Coelogyne pandurata*. The control group exhibited the highest antioxidant levels in both leaves and bulbs (Fig 3). In contrast, Paclobutrazol application led to a significant reduction, particularly at 50 ppm, with slight variations at higher concentrations. These results suggest that Paclobutrazol may influence antioxidant metabolism, potentially altering the plant's physiological responses to stress.

The observations of antioxidant capacity in the leaf and bulb organs of plants treated with paclobutrazol showed no significant increase compared to the control plants.
The absence of increased antioxidant capacity in plant leaves and bulbs following paclobutrazol application can be attributed to the physiological mechanisms it influences. Paclobutrazol primarily inhibits gibberellin biosynthesis, which reallocates energy toward secondary metabolite production, such as phenolics and flavonoids. However, this does not necessarily result in a proportional increase in antioxidant capacity [8].

Antioxidant activity is influenced by the concentration of phenolics and flavonoids and the presence and activity of enzymatic antioxidants such as superoxide dismutase, catalase, and peroxidase. Studies suggest that paclobutrazol may not significantly enhance the activity of these enzymatic antioxidants, leading to limited changes in overall antioxidant capacity despite increased flavonoid or phenolic content [9].

Furthermore, the environmental conditions and plant species significantly affect how paclobutrazol impacts antioxidant profiles. In some cases, it may enhance the production of specific secondary metabolites without substantially altering the total antioxidant activity, likely due to metabolic trade-offs and the plant's prioritization of specific physiological needs [10]. The antioxidant capacity content in leaves and bulbs of black orchid at different paclobutrazol treatments are presented in Fig 3.

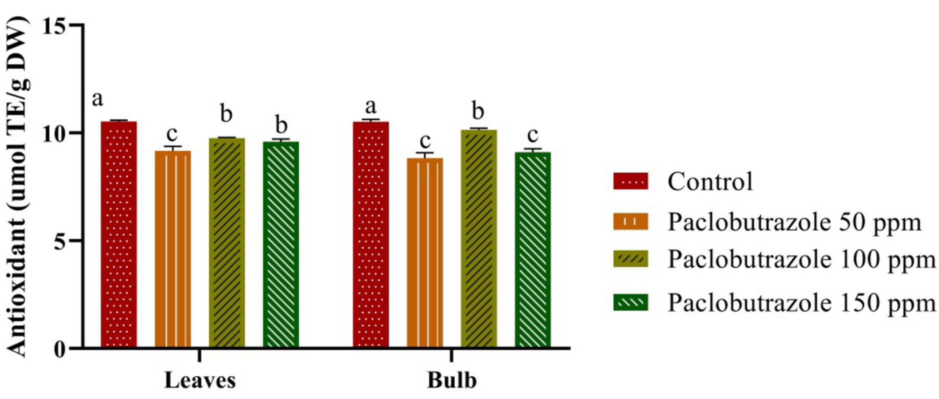


Fig. 3. Antioxidant capacity Content in Leaves and Bulbs of Black Orchid (*Coelogyne pandurata* Lindl.) at Different Paclobutrazol Treatments.

3.4 Correlations between phenolic, flavonoid, and antioxidant

The correlation analysis results between the total phenolic content, flavonoids, and antioxidants in plant bulbs revealed a positive correlation among the observed components, including a significant positive correlation between TPC and TFC with R^2 0.7691. The correlation between TPC and Antioxidants is positively correlated with R^2 0.8380 and the correlation between TFC and antioxidants is positively correlated with R^2 0.7213. Correlations between bulb phenolic, flavonoid content, and antioxidant at different paclobutrazol treatments are presented in Fig 4.

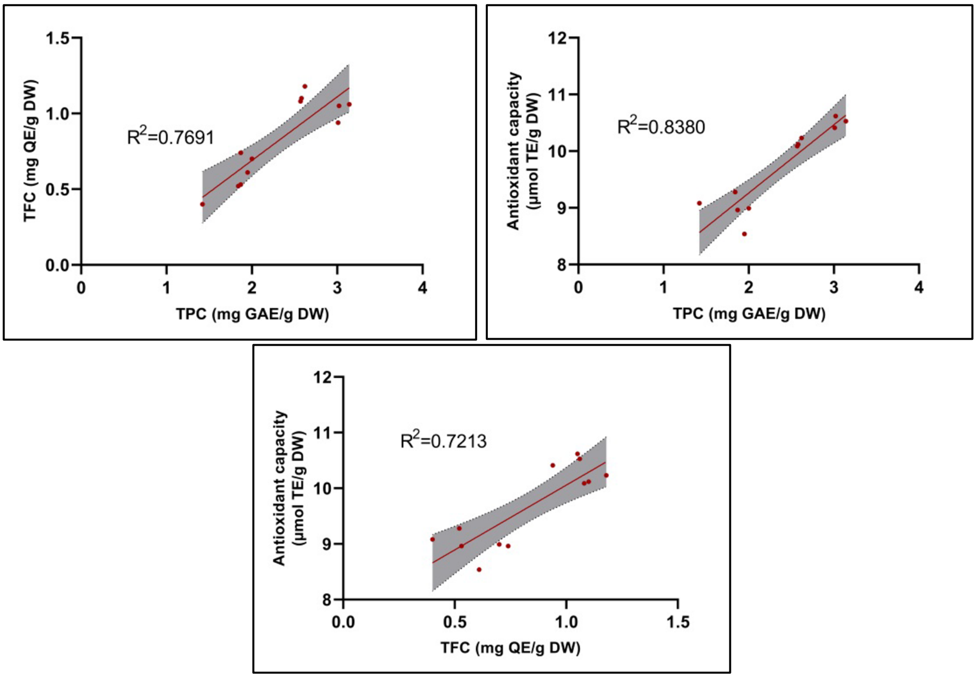
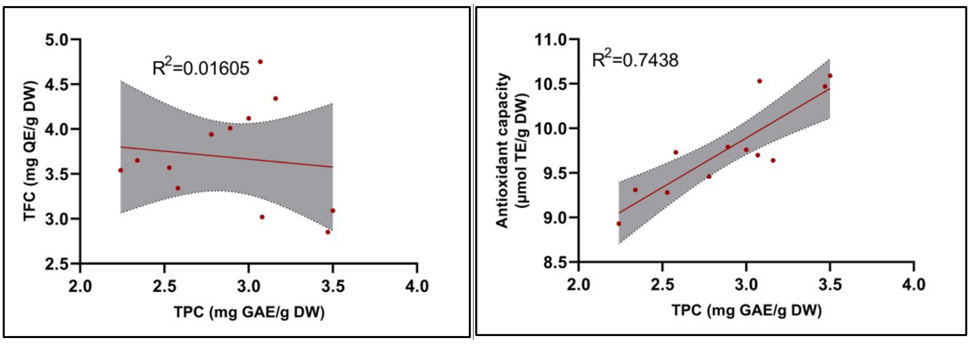


Fig. 4. Correlations between bulb phenolic, flavonoid content, and antioxidant at Different Paclobutrazol Treatments.

Both phenolics and flavonoids are products of the phenylpropanoid pathway, which begins with the enzyme phenylalanine ammonia-lyase (PAL). PAL converts phenylalanine into cinnamic acid, a precursor for phenolic compounds, including flavonoids. This shared pathway establishes a direct relationship between their synthesis [11].



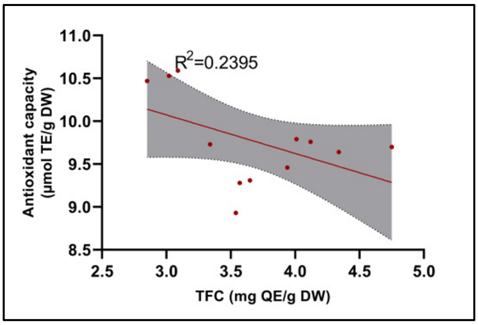


Fig. 5. Correlations between phenolic, flavonoid content, and antioxidants in the leaves at different paclobutrazol treatments.

Phenolic compounds and flavonoids act as antioxidants by scavenging reactive oxygen species (ROS) and protecting cellular structures from oxidative stress. Their hydroxyl groups donate electrons to neutralize free radicals, reducing oxidative damage. Flavonoids, a subclass of phenolics, often exhibit stronger antioxidant activity due to their ability to chelate metal ions and stabilize ROS. Studies have demonstrated that the concentration of phenolics and flavonoids directly correlates with the total antioxidant capacity of plant tissues. Plants with higher phenolic and flavonoid content tend to show stronger antioxidant activity due to their combined effects in neutralizing oxidative stress [12].

Correlations in plant leaves showed a negative correlation between TPC and TFC with R^2 0.016605. The correlation between TPC and antioxidants showed a positive correlation with R^2 0.7438, and a negative correlation between TFC and antioxidants, with R^2 0.2395 (Fig 5). Physiologically, phenolic compounds in leaves do not have a mutually supportive relationship in function, because in leaves there are many phenolic compounds other than flavonoid compounds that have a higher content when compared to flavonoid compounds. This shows that the dominating phenolic compounds in the leaves are not flavonoid compounds, but other compounds in the phenolic compound class [13].

Phenolic compounds have antioxidant activity in leaves because these compounds regulate various signals received both exogenously and endogenously related to plant responses [14]. The biosynthesis of phenolic compounds will support the formation of antioxidant activity in plant leaves. Meanwhile, flavonoid compounds in leaves do not have antioxidant activity, this is because other phenolic compounds have antioxidant activity in plant leaves [15].

4 Conclusion

Results indicated that treatment with 150 ppm Paclobutrazol significantly increased the flavonoid concentration in the leaves. No notable effect was observed on the total phenolic content or antioxidant activity in leaves and bulbs.

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