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ARTICLE

Optimizing phosphorus application to enhance flowering and phytochemical profiles in black orchid (*Coelogyne pandurata* Lindl.)

Optimização da aplicação de fósforo para aumentar a floração e os perfis fitoquímicos em orquídea negra (*Coelogyne pandurata* Lindl.)

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Abstract: The black orchid (*Coelogyne pandurata*), an ornamental and medicinal plant endemic to Indonesia, exhibits seasonal flowering, limiting its commercial development. This study aimed to assess the effectiveness of varying phosphorus concentrations in inducing out-of-season flowering and enhancing secondary metabolite content and antioxidant capacity in black orchid. A randomized complete block design experiment with five phosphorus concentration (0%, 14.4%, 21.6%, 28.8%, and 36.0%) was performed. Phosphorus was applied through foliar spraying, then growth, nutrient content, and biochemical properties were analyzed. The results showed that 14.4% of phosphorus significantly promoted earlier flowering, increased the number of flowers per stem, and improved flower dimensions. Moreover, this concentration enhanced phenolic and flavonoid content, particularly in bulbs and flowers, and increased antioxidant capacity. Higher phosphorus concentrations led to nutrient imbalances and a reduction in biochemical enhancements. The study demonstrates that optimal phosphorus application can significantly improve both the ornamental and medicinal qualities of *C. pandurata*, offering insights into its commercial cultivation and pharmaceutical exploration. This research contributes to orchid horticulture by optimizing nutrient-driven flowering and phytochemical enhancement, with implications for other orchid species.
Keywords: black orchid, *Coelogyne pandurata*, flowering induction, phosphorus application, secondary metabolites.

Resumo: A orquídea negra (*Coelogyne pandurata*), uma planta ornamental e medicinal endêmica da Indonésia, exibe floração sazonal, o que limita seu desenvolvimento comercial. Este estudo teve como objetivo avaliar a eficácia de várias concentrações de fósforo na indução da floração fora de época e no aumento do conteúdo de metabólitos secundários e da capacidade antioxidante em C. pandurata. Um delineamento experimental em blocos ao acaso com cinco tratamentos de fósforo (0%, 14,4%, 21,6%, 28,8% e 36,0%) foi utilizado. O fósforo foi aplicado por pulverização foliar, e foram analisados o crescimento, o teor de nutrientes e as propriedades bioquímicas. Os resultados mostraram que 14,4% de fósforo promoveu significativamente a floração antecipada, aumentou o número de flores por haste e melhorou as dimensões das flores. Além disso, essa concentração aumentou o teor de compostos fenólicos e flavonoides, particularmente em bulbos e flores, e elevou a capacidade antioxidante. Concentrações mais altas de fósforo levaram a desequilíbrios de nutrientes e redução nos aprimoramentos bioquímicos. O estudo demonstra que a aplicação otimizada de fósforo pode melhorar significativamente tanto as qualidades ornamentais quanto medicinais de *C. pandurata*, oferecendo insights para sua exploração comercial e farmacêutica. Esta pesquisa contribui para a horticultura de orquídeas, otimizando o florescimento induzido por nutrientes e o enriquecimento fitoquímico, com implicações para outras espécies de orquídeas.
Palavras-chave: orquídea negra, *Coelogyne pandurata*, indução de floração, aplicação de fósforo, metabólitos secundários.

Introduction

The black orchid (*Coelogyne pandurata*) is a prominent endemic species of Indonesia, valued both as an ornamental and medicinal plant due to its distinctive aesthetic appeal and potential pharmaceutical benefits. This orchid, however, exhibits a seasonal flowering cycle that limits its commercial development, as it typically blooms only during specific periods in the rainy season (Heriansyah et al., 2024). The seasonal dependency of *C. pandurata* poses a significant challenge for its cultivation and commercialization. To address this matter, enhancing flowering traits through controlled nutrient applications has been proposed as a viable solution. Phosphorus, a key macronutrient, plays a vital role in flowering induction by serving as an energy source through adenosine triphosphate (ATP) formation, which supports photosynthesis and the regulation of flowering hormones like gibberellins (Biswas et al., 2021; Minasiewicz et al., 2023). While the role of phosphorus in flowering induction has been well-documented, its application in inducing out-of-season flowering and improving the biochemical profile of black orchids has not been extensively explored. Given the ornamental and pharmaceutical significance of *C. pandurata*, there is a need to investigate the dual impact of phosphorus on both flowering induction and the enhancement of secondary metabolites, such as phenolics and flavonoids, which contribute to the plant's medicinal properties (Gantait et al., 2021; Gil-Martín et al., 2022).

In the context of orchids, previous studies have highlighted the effectiveness of phosphorus in promoting flowering traits across various species but not on *C. pandurata*. For example, Suzuki et al. (2021) demonstrated that a phosphorus concentration of 33.33% significantly enhanced the flowering characteristics of Mokara orchids, including the number of flowers and spike length. Similarly, the application of NPK fertilizers with specific phosphorus concentrations has been shown to induce flowering in *Phalaenopsis amabilis* (Sunawan et al., 2020), *Cattleya* Orchid (Mubarok et al., 2024) and *Dendrobium* orchids (Shalem and Sarvanan, 2020).

While these studies provide valuable insights into the role of phosphorus in flowering induction, research specifically on *C. pandurata* remains scarce. Unlike previous studies that primarily focused on general flowering responses, this study aims to explore the biochemical mechanisms underlying phosphorus-induced flowering in *C. pandurata*, particularly its influence on secondary metabolite production. Phenolic compounds and flavonoids, which serve as key secondary metabolites, play essential roles in plant defense and contribute to the antioxidant properties of orchids, which are increasingly sought after for their health benefits (Gil-Martín et al., 2022). Although the relationship between phosphorus and flowering is well-documented, there is a limited understanding of how phosphorus application affects secondary metabolite accumulation and antioxidant capacity in black orchids. This study, therefore, seeks to bridge this research gap by examining both the physiological and

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phytochemical responses of *C. pandurata* to phosphorus treatment, providing new insights into its potential applications in both horticulture and pharmacology.

In light of these considerations, this study aims to evaluate the role of phosphorus application not only in inducing out-of-season flowering but also in influencing the biochemical pathways associated with secondary metabolite production in *C. pandurata.* By examining phosphorus concentrations ranging from 0% to 36.0%, this study seeks to determine the optimal level that enhances both flowering traits and the total of phenolic and total flavonoid compounds, thereby improving the orchid's antioxidant capacity. Unlike previous studies that primarily focused on the morphological effects of phosphorus, this research integrates physiological and biochemical analyses to provide a comprehensive understanding of its dual impact.

It is hypothesized that controlled phosphorus application will not only accelerate and enhance flowering in *C. pandurata* but also stimulate the biosynthesis of key secondary metabolites, reinforcing its medicinal and antioxidant potential. This dual focus strengthens the scientific foundation for optimizing *C. pandurata* cultivation, addressing both its commercial viability in the horticultural industry and its pharmacological relevance. The findings of this study are expected to bridge the existing knowledge gap by offering new insights into the controlled use of phosphorus in improving both the ornamental and medicinal qualities of black orchids, thereby contributing to advancements in orchid horticulture and plant secondary metabolism.

Material and Methods

The research was conducted at the Orchid House of the Leuwikopo Experimental Farm, IPB University, located in Ciampea, Bogor Regency, West Java, Indonesia, at coordinates 6°33'50.3"S and 106°43'29.3"E, with an elevation of 188 meters above sea level. Additional testing was conducted at the DAGH-IPB University Testing Laboratory and the Laboratory of the Department of Biochemistry, IPB University, Indonesia. The study was carried out from March to June 2024.

The orchid plants used in the study, were in the vegetative stage, one-year post-splitting, with two leaves and two bulbs having reached their maximum size in the vegetative phase. The phosphorus foliar application was conducted during the dry season, with no rainfall occurring after the treatment was applied. A total of 45 plants were employed, divided equally across five treatments. The nutrient solution for fertilization consisted of a blend containing nitrogen (N 24.7%), potassium (25.5%), calcium (23.1%), magnesium (6.7%), all macro element percentages were obtained from the following fertilizers: Calcium Ammonium Nitrate, Potassium Nitrate, Sulphate of Potash, Monopotassium Phosphate, Magnesium Sulphate, and Ammonium Sulphate (ZA). And trace elements including FeEDTA (2.16 ppm), FeEDDHA (0.59 ppm), MnEDTA (1.11 ppm), ZnEDTA (0.14 ppm), CuEDTA (0.04 ppm), boron (0.3 ppm), molybdenum (0.028 ppm), and sodium (0.014 ppm). This formulation was selected to provide comprehensive nourishment to the plants (Bhat et al., 2024).

The experimental design adopted in this study was a non-factorial randomized complete block design (RCBD) with five treatment levels of phosphorus application: control (0%), 14.4%, 21.6%, 28.8%, and 36.0%. The material used to prepare the phosphorus compound is mono potassium phosphate (MKP) fertilizer. 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 on vegetative and generative characteristics, nutrient content, and secondary metabolite levels in the orchid organs (Heriansyah et al., 2024).

Prior to the experimental treatments, the orchids were acclimated under natural greenhouse conditions. Maximum and minimum temperatures were 32 °C and 20 °C, respectively, with an average temperature of 26 °C. The daytime PPFD ranged from 189 to 81 µmol m^{-2} s^{-1}, maximum and minimum relative humidity (RH) were 90% and 40%, respectively, photosynthetic photon flux (FFF) was 120 µmol m^{-2} s^{-1}, and the photoperiod was 12/12 h day^{-1}. The plants, each with two bulbs and four leaves, were carefully selected to ensure uniformity in age and size, reducing the variability in experimental outcomes. The plants were grown in pots filled with a uniform growing medium namely charcoal, and watering was conducted every two days to prevent water stress, but it was not performed on rainy days. Nutrient applications other than phosphorus were administered at the same dose and concentration across all treatments, while phosphorus fertilizer was applied at varying concentrations.

Phosphorus concentrations were administered via foliar spray, ensuring even distribution over the plant's leaves and bulbs. Each treatment was applied weekly, maintaining consistent environmental conditions throughout the study period. Parameters related to plant phenology were measured systematically, including the age of flower emergence, the number of flowers per stem, flower stem length, and the dimensions of floral organs such as sepals, petals, labellum, and stamens. Fresh weights of the leaves, bulbs, and flowers were recorded immediately after collection to determine treatment effects (Ashour et al., 2020).

Nutrient analysis was conducted to measure the total nitrogen (N), phosphorus (P), potassium (K), and organic carbon (C) content in the leaves, bulbs, and flowers of *C. pandurata*. The Kjeldahl method was used for nitrogen determination, involving acid digestion and titration to quantify nitrogen concentration. Phosphorus content was measured using the P-Bray 1 method and analyzed with a UV-Vis spectrophotometer (UV-1800), while potassium content was determined through the K-Bray 1 method using flame photometry. Organic carbon content was assessed by the Walkley and Black method, utilizing oxidation and titration to evaluate carbon levels (Lambers, 2022; Nurcholis et al., 2021).

Secondary metabolite analysis involved the quantification of total phenolic and flavonoid content, along with antioxidant capacity. Phenolic content was assessed using the Folin-Ciocalteu method with a nano spectrophotometer (SPECTRO$^{star\ Nano}$ BMG LABTECH). The absorbance was measured at 765 nm, and the results were expressed as mg of gallic acid equivalent (GAE) per gram of dry weight. Flavonoid content was determined by the colorimetric method using aluminium chloride as a reagent, and absorbance was recorded at 415 nm, expressed as mg of quercetin equivalent (QE) per gram of dry weight (Haryoto et al., 2024). Antioxidant capacity was measured using the DPPH (2,2'-diphenyl-1-picrylhydrazyl) radical scavenging assay. Samples of black orchid extracts were prepared, and the reduction of DPPH absorbance at 517 nm was used to calculate the antioxidant activity in µmol Trolox equivalent per gram of dry weight (Nurcholis et al., 2024).

The collected data were analysed using analysis of variance (ANOVA) to determine the significance of the differences between treatments. Tukey's Honestly Significant Difference (HSD) test was employed for post-hoc comparisons at a 5% significance level to identify which phosphorus concentrations significantly influenced the growth and biochemical characteristics of *C. pandurata*. Statistical analyses were conducted using R Studio and SPSS (Version 26.0).

Results and Discussion

Observations on the phenological characteristics of *C. pandurata* revealed that phosphorus application significantly influenced various vegetative and generative traits. Treatment with 14.4% phosphorus concentration promoted increased growth in the vegetative phase, specifically evident in the longer leaf length, greater leaf width, and larger bulb size (Table 1). In the generative phase, the same concentration resulted in the earliest flower emergence, with an average emergence time of 47.33 days. Higher phosphorus concentrations delayed flowering, with the longest emergence time recorded at 74.00 days for plants treated with 36.0% phosphorus. The number of flowers per stem was highest in plants treated with 14.4% phosphorus, producing an average of 5.33 flowers per stem. In contrast, the number of flowers decreased at higher phosphorus concentrations. Significant differences were also observed in the dimensions of floral organs. Sepal length was longest in the 14.4% treatment group, while petal and labellum lengths similarly peaked at this concentration (Fig. 1). These findings indicate that 14.4% phosphorus application optimally supports both vegetative growth and flower development in *C. pandurata*.

Table 1. Observational results of phenology in black orchid with phosphorus spraying.

Observed Variables	Phosphorus Concentration (%)				
	Control	14.4	21.6	28.8	36.0
Bulb Length (cm)	4.20 ± 0.05 c	7.10 ± 0.05 a	6.70 ± 0.14 a	6.10 ± 0.05 b	5.77 ± 0.03 b
Bulb Width (cm)	2.83 ± 0.08 d	4.17 ± 0.03 a	3.60 ± 0.05 b	3.17 ± 0.03 c	2.93 ± 0.03cd
Bulb Wet Weight (g)	22.53 ± 0.26 e	28.27 ± 0.08 a	27.20 ± 0.05 b	26.40 ± 0.20 c	24.17 ± 0.08 d
Leaf Length (cm)	22.53 ± 0.31 d	28.63 ± 0.23 a	26.23 ± 0.06 b	24.60 ± 0.20 c	23.23 ± 0.12 d
Leaf Width (cm)	3.13 ± 0.06 c	5.57 ± 0.08 a	4.53 ± 0.08 b	4.40 ± 0.57 b	3.43 ± 0.33 c
Leaf Wet Weight (g)	15.97 ± 0.14 e	21.80 ± 0.05 a	20.03 ± 0.12 b	18.23 ± 0.08 c	17.17 ± 0.03 d
Flower Emergence Age (days)	-	47.33 ± 1.20 a	58.33 ± 0.88 b	65.00 ± 1.00 c	74.00 ± 0.57 d
Flower Number per Stem	-	5.33 ± 0.33	3.67 ± 0.33	4.00 ± 0.57	3.67 ± 0.33
Flower Stem Length (cm)	-	39.00 ± 0.57 a	35.67 ± 0.66 ab	33.67 ± 1.20 b	33.00 ± 0.57 b
Sepal Length (cm)	-	5.33 ± 0.33 a	4.33 ± 0.33 a	4.33 ± 0.57 a	3.00 ± 0.33 b
Petal Length (cm)	-	4.67 ± 0.88 a	4.10 ± 0.05 b	3.93 ± 0.03 bc	3.77 ± 0.03 c
Labellum Length (cm)	-	3.03 ± 0.03 a	2.70 ± 0.05 b	2.43 ± 0.06 c	2.20 ± 0.05 c
Stamen Length (cm)	-	1.57 ± 0.03 a	1.40 ± 0.05 ab	1.33 ± 0.06 b	1.23 ± 0.03 b
Flower Wet Weight (g)	-	10.17 ± 0.03 a	9.47 ± 0.12 b	8.33 ± 0.03 c	8.10 ± 0.05 c

Note: Numbers with different letters in the same row indicate significant differences according to the Tukey test at 5% level.

Fig 1. Plant performance at 74 days after treatment with Phosphorus Spraying: Control, 14.4%, 21.6%, 28.8%, and 36.0% Phosphorus

The results demonstrated a significant influence of phosphorus concentrations on both the vegetative and generative phases. Application of 14.4% phosphorus notably enhanced vegetative traits, including leaf length, leaf width, and bulb size (including diameter and length) compared to other concentrations (Table 1). These improvements suggest that 14.4% phosphorus optimally supports vegetative growth, likely due to its role in ATP synthesis and energy metabolism, which facilitates cellular expansion and overall plant development (Péret et al., 2014)

In addition to promoting vegetative growth, phosphorus at 14.4% also induced the earliest flowering, significantly reducing the time to flower emergence (Table 1). This finding supports the hypothesis that moderate phosphorus levels serve as key regulators of flowering by interacting with plant hormonal pathways, particularly gibberellins, which are known to influence floral initiation (Mohammed et al., 2018). These results align with previous studies on *Mokara* and *Dendrobium* orchids, where phosphorus application similarly enhanced flowering traits (Shalem and Sarvanan, 2020; Mubarok et al., 2024).

However, higher phosphorus concentrations (28.8% and 36.0%) resulted in diminished flowering traits, including delayed flower emergence and a reduction in flower count per stem, indicating a possible saturation effect or nutrient imbalance in *C. pandurata.* Excessive phosphorus may interfere with nutrient transport and disrupt hormone signalling pathways, leading to suppressed flowering performance (Cai et al., 2024). Furthermore, the decline in floral organ dimensions, such as reductions in sepal and petal lengths, suggests that an overabundance of phosphorus may induce physiological stress, disrupt metabolic homeostasis, and contribute to osmotic imbalance (Muhammad et al., 2024).

These findings highlight the importance of controlled phosphorus application, particularly at 14.4%, as an optimal strategy for balancing both vegetative and reproductive growth in *C. pandurata.* This study provides scientific insights into the precise role of phosphorus in flowering regulation, reinforcing its potential for application in orchid cultivation to enhance both horticultural and commercial value. The analysis of nutrient content in the leaves, bulbs, and flowers revealed differential responses to varying phosphorus concentrations (Table 2 and Fig. 2). Exogenous application of 14.4% phosphorus spray significantly increased nitrogen, phosphorus, and potassium content in flower organs but decreased organic carbon content and the C/N ratio.

Table 2. Nutrient content in organs under different phosphorus concentrations

Organ	Phosphorus Concentration (%)	N	P	K	C-ORGANIC	C/N Ratio
Leaves	Control	1.69 ± 0.03	0.11 ± 0.00	0.93 ± 0.18	51.39 ± 0.42 a	30.37 ± 0.36
	14.4%	1.81 ± 0.30	0.14 ± 0.01	1.18 ± 0.15	47.53 ± 0.95 ab	27.55 ± 3.66
	21.6%	2.00 ± 0.06	0.15 ± 0.00	1.15 ± 0.10	48.54 ± 0.72 ab	24.37 ± 0.88
	28.8%	1.89 ± 0.15	0.15 ± 0.01	1.07 ± 0.22	41.81 ± 2.85 b	22.17 ± 1.10
	36.0%	1.72 ± 0.11	0.13 ± 0.00	0.78 ± 0.09	46.43 ± 2.27 ab	27.02 ± 0.57
Bulb	Control	0.65 ± 0.00	0.14 ± 0.04	1.11 ± 0.18	42.99 ± 0.83	66.15 ± 1.22
	14.4%	0.62 ± 0.14	0.15 ± 0.02	0.77 ± 0.19	40.17 ± 0.15	71.20 ± 13.7
	21.6%	0.66 ± 0.14	0.17 ± 0.01	1.19 ± 0.19	43.88 ± 0.80	73.11 ± 15.6
	28.8%	0.62 ± 0.06	0.17 ± 0.01	0.82 ± 0.09	40.00 ± 1.16	65.77 ± 8.16
	36.0%	0.65 ± 0.27	0.17 ± 0.00	0.77 ± 0.05	41.97 ± 1.64	109.87 ± 57.7
Flower	Control	0.00 ± 0.00 b	0.00 ± 0.00 b	0.00 ± 0.00 b	0.00 ± 0.00 b	0.00 ± 0.00 b
	14.4%	1.58 ± 0.04 a	0.18 ± 0.00 a	2.16 ± 0.47 a	0.00 ± 1.20 a	0.00 ± 1.22 a
	21.0%	1.31 ± 0.18 a	0.12 ± 0.03 a	2.02 ± 0.19 a	38.35 ± 1.21 a	30.14 ± 3.11 a
	28.8%	1.52 ± 0.10 a	0.18 ± 0.01 a	1.36 ± 0.06 a	41.04 ± 2.22 a	27.50 ± 3.18 a
	36.0%	1.45 ± 0.032 a	0.17 ± 0.00 a	1.75 ± 0.12 a	42.05 ± 0.17 a	28.89 ± 0.61 a

Note: Numbers with different letters in the same columns indicate significant differences according to the Tukey test at 5% level.

The nutrient analysis revealed distinct physiological responses to phosphorus treatments across different plant organs. Nitrogen levels in leaves were highest at 14.4% phosphorus, suggesting that this concentration optimally facilitates nitrogen uptake and assimilation. This effect is likely attributed to the synergistic role of phosphorus in enhancing nitrogen transporter activity and assimilation pathways, which are crucial for protein synthesis and overall metabolic efficiency (Lambers, 2022).

Interestingly, phosphorus levels remained relatively stable across all treatments, indicating that phosphorus absorption in *C. pandurata* may have reached a saturation threshold beyond 14.4%. This observation aligns with the findings of Grzebisz et al. (2024), who reported that excessive phosphorus availability can exceed the plant's uptake capacity, potentially leading to nutrient imbalances and reduced efficiency in phosphorus utilization.

A strong positive correlation between nitrogen, phosphorus, and organic carbon in leaves suggests that these nutrients are intricately linked to primary metabolic functions, particularly photosynthesis and carbon fixation. Phosphorus, as a key component of ATP, plays a pivotal role in these processes by supplying the necessary energy for carbon metabolism and biochemical reactions (Vukmirović et al., 2024).

Conversely, the negative correlation between phosphorus and potassium in bulbs highlights a potential nutrient antagonism, where excessive phosphorus may competitively inhibit potassium uptake. This interference is likely mediated through disruptions in potassium transport proteins or ion channels, as reported in previous studies on nutrient interactions in plants (Costa et al., 2024). Given the essential role of potassium in enzymatic activation, water balance, and carbohydrate translocation, maintaining a balanced phosphorus-to-potassium ratio is critical for sustaining optimal growth and physiological stability in *C. pandurata.* These findings emphasize the importance of controlled phosphorus application to maintain nutrient homeostasis and prevent potential antagonistic interactions that could compromise both vegetative and reproductive development. Understanding these nutrient dynamics provides a foundation for optimizing fertilization strategies, ensuring sustainable growth, and enhancing the metabolic efficiency of *C. pandurata.*

Phosphorus application significantly influenced the phenolic and flavonoid content in *C. pandurata* organs (Table 3). The phenolic content in leaves was not significant, while in bulbs, phenolic levels peaked at 14.4%. In flowers, the optimal phenolic content was recorded at 14.4% phosphorus concentration, with a decline in phenolic levels at higher concentrations. Similarly, the flavonoid content in leaves peaked at 14.4%, but decreased beyond this concentration. In bulbs, however, flavonoid levels continued to increase with higher phosphorus applications, reaching a maximum at 36.0%. In flowers, the highest flavonoid content was recorded at 28.8%, after which no further increase was observed. Antioxidant capacity showed a varying trend across plant organs, with the highest capacity recorded in leaves at 28.8%, followed by a decline at higher and lower concentrations. In bulbs, antioxidant activity decreased with increasing phosphorus levels, while in flowers, the highest capacity was noted at 28.8%, followed by a reduction in activity at higher concentrations.

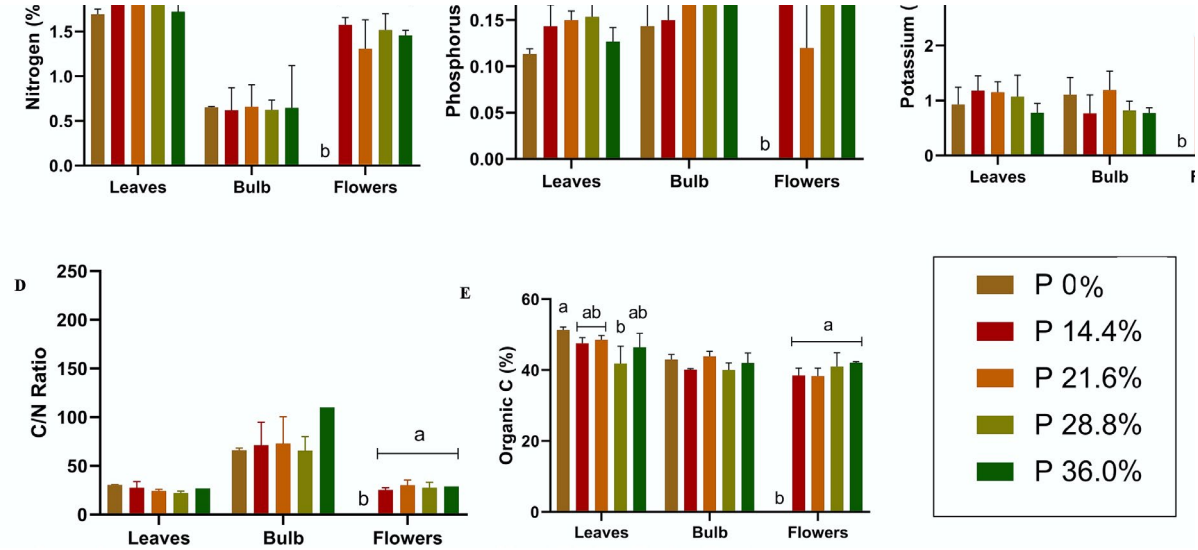

Fig 2. Changes in Nutrient Levels with Exogenous Phosphorus Spraying: (A) Nitrogen (N), (B) Phosphorus (P), (C) Potassium (K), (D) Organic Carbon, and (E) C/N Ratio in leaves, Bulb, and Flower Organs with Phosphorus Application at Concentrations of 0% P (Dark Brown) 14.4% P (Red), 21.6% P (Light Brown), 28.8% P(Light Green), and 36.0% P (Dark Green).

Table 3. Total phenolic and total flavonoid content, and antioxidant capacity in organs under different phosphorus concentrations.

Organ	Phosphorus Concentration (%)	Total Phenolic (mg GAE/g DW)	Total Flavonoid (mg QE/g DW)	Antioxidant Capacity (mg TE/g DW)
Leaves	Control	2.10 ± 0.10	3.74 ± 0.12 ab	9.29 ± 0.02 ab
	14.4%	2.30 ± 0.17	3.86 ± 0.07 ab	9.09 ± 0.09 ab
	21.6%	2.11 ± 0.12	3.47 ± 0.14 b	9.31 ± 0.09 a
	28.8%	2.17 ± 0.06	4.30 ± 0.19 a	8.84 ± 0.16 ab
	36.0%	1.86 ± 0.06	3.80 ± 0.05 ab	8.75 ± 0.14 b
Bulb	Control	1.66 ± 0.10 ab	0.60 ± 0.02 b	9.52 ± 0.11 a
	14.4%	1.80 ± 0.01 a	0.60 ± 0.03 b	8.87 ± 0.16 ab
	21.6%	1.60 ± 0.13 ab	0.60 ± 0.02 b	8.16 ± 0.18 c
	28.8%	1.23 ± 0.09 c	0.76 ± 0.99 ab	7.82 ± 0.11 c
	36.0%	1.36 ± 0.07 ab	1.00 ± 0.04 a	8.46 ± 0.13 bc
Flower	Control	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c
	14.4%	2.80 ± 0.35 a	0.98 ± 0.09 a	9.92 ± 0.03 a
	21.6%	2.57 ± 0.08 a	1.07 ± 0.00 a	10.14 ± 0.03 a
	28.8%	1.07 ± 0.12 b	0.37 ± 0.03 b	7.50 ± 0.11 b
	36.0%	0.89 ± 0.05 b	0.27 ± 0.02 b	7.32 ± 0.11 b

Note: Numbers with different letters in the same columns indicate significant differences according to the Tukey test at 5% level

The study's results demonstrated that phosphorus application significantly affected the levels of phenolic and flavonoid compounds in *C. pandurata*, with the highest phenolic content observed at 14.4% phosphorus concentration in bulbs and flowers (Figure 2). This finding aligns with previous research indicating that moderate phosphorus levels enhance phenolic synthesis through increased ATP availability and the activation of metabolic pathways involved in secondary metabolite production (Gil-Martín et al., 2022; Gantait et al., 2021). The decline in phenolic content at higher phosphorus concentrations suggests that excessive phosphorus may disrupt the balance between anabolic and catabolic pathways, limiting the energy available for secondary metabolism (Wu et al., 2024)

Flavonoid content followed a similar trend, with the highest levels recorded at 14.4% and 28.8% phosphorus concentrations in leaves and flowers, respectively. This trend indicates that phosphorus plays a dual role in promoting both phenolic and flavonoid biosynthesis by facilitating glycolysis and ATP production, which are essential for the formation of flavonoid precursors (Zhao et al., 2023). The findings support the hypothesis that moderate phosphorus application enhances the secondary metabolite profile of *C. pandurata*, thereby increasing its potential medicinal value.

The observed increase in antioxidant capacity, particularly in flowers and leaves treated with 14.4% phosphorus, underscores the role of phenolic and flavonoid compounds as key antioxidants in *C. pandurata* (Fig. 2).

Antioxidants are crucial in mitigating oxidative stress and enhancing plant resilience to environmental fluctuations (Gil-Martín et al., 2022). The correlation between phenolic content and antioxidant activity suggests that the increase in phenolic compounds directly contributes to the enhanced antioxidant capacity, as indicated by the positive correlation coefficients in Fig. 3. These findings highlight the significance of optimizing phosphorus application to achieve a dual improvement in both aesthetic and medicinal qualities of *C. pandurata*.

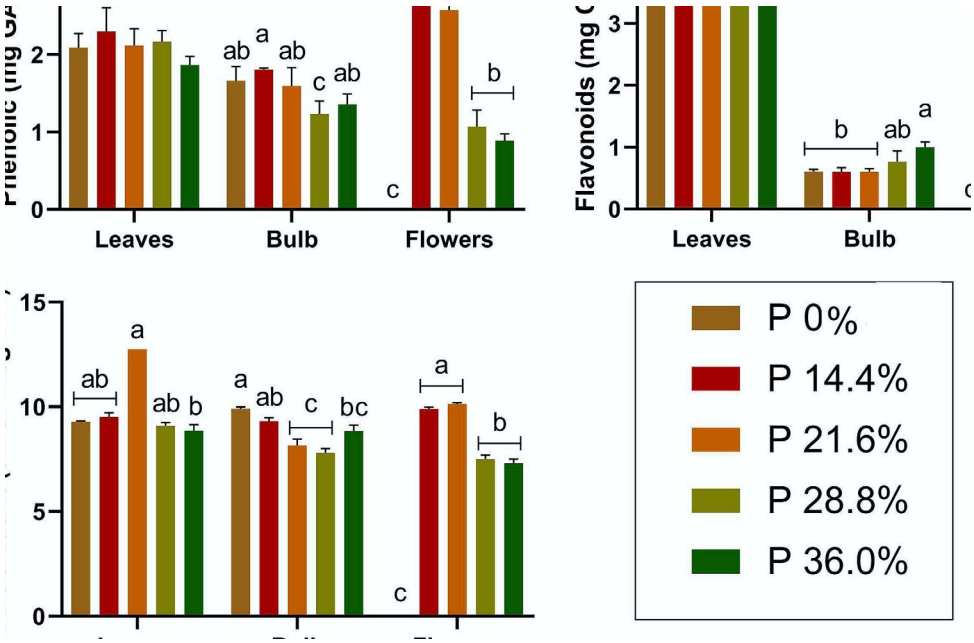

Fig 3. (A) Total Phenolic, (B) flavonoid, and (C) antioxidant in leaves, bulb, and flower organs with phosphorus application at concentrations of 0% P (Dark Brown) 14.4% P (Red), 21.6% P (Light Brown), 28.8% P (Light Green), and 36.0% P (Dark Green).

The results indicate a significant correlation between phosphorus application and key plant characteristics. Nitrogen positively correlated with phosphorus, potassium, and organic carbon, indicating that nitrogen levels supported nutrient absorption and secondary metabolite synthesis. Phosphorus concentration was also positively correlated with phenolic content and antioxidant activity, particularly in the leaves and flowers, suggesting that phosphorus played a key role in enhancing these biochemical properties. In contrast, a negative correlation was observed between flavonoid content and potassium levels, highlighting that higher potassium concentrations may inhibit flavonoid biosynthesis. Figure 3 illustrates these correlations across the measured variables, indicating that phenolic compounds exhibited a strong positive correlation with antioxidant capacity in all plant organs.

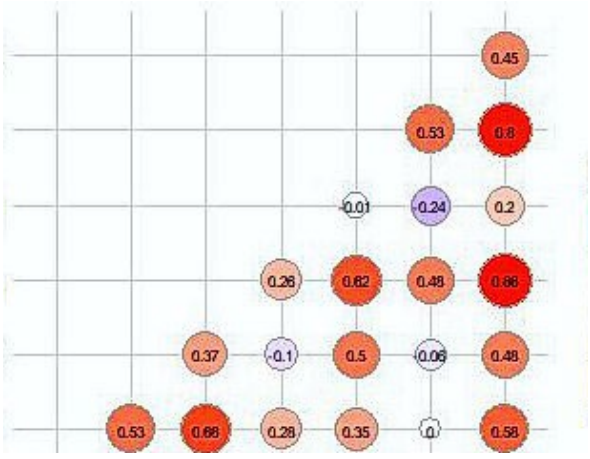

Fig 4. Pearson's correlation in the leaf, bulb, and flower organs with phosphorus spraying at 14.4%, 21.6%, 28.8%, and 36.0% concentrations. TPC (Total Phenolic Content), TFC (Total Flavonoid Content), C_Org (C-Organic), C_N (C/N Ratio)

The findings of this study have significant implications for the commercial cultivation and pharmaceutical utilization of *C. pandurata*. The results confirm the hypothesis that controlled phosphorus application at an optimal concentration of 14.4% can induce out-of-season flowering while simultaneously enhancing phenolic and flavonoid content. This dual benefit not only improves the ornamental appeal of *C. pandurata* but also increases its antioxidant capacity, potentially expanding its use in the production of health-related products. The alignment of these findings with previous studies on orchids supports the broader applicability of the research to other orchid species (Ashour et al., 2020; Sunawan et al., 2020).

The study's findings also underscore the importance of nutrient balance in orchid cultivation. The negative correlations observed between excessive phosphorus and potassium uptake, as well as the diminishing returns on phenolic and flavonoid content at higher phosphorus concentrations, highlight the risks of over-fertilization. These results suggest that growers should carefully manage nutrient applications to avoid nutrient imbalances that could adversely affect plant health and secondary metabolite production (Costa et al., 2024). By optimizing phosphorus levels, it is possible to achieve a balance between growth, flowering induction, and biochemical enhancement, making *C. pandurata* a valuable candidate for commercial and medicinal applications.

Conclusions

Phosphorus application significantly influenced flowering, growth, and secondary metabolite accumulation. Plants without phosphorus treatment did not flower, while 14.4% phosphorus induced out-of-season flowering and improved key floral and growth parameters. Higher phosphorus levels (28.8%) increased flavonoid content in leaves and bulbs, while 14.4% and 21.6% enhanced flavonoids in flowers. Antioxidant capacity improved with 21.6% phosphorus in leaves and 14.4% in bulbs. These results highlight phosphorus's role in flowering regulation and metabolite production, with potential applications in agriculture and plant-based industries. Further research should explore the underlying mechanisms and optimal phosphorus concentrations.

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Author Contribution

PH: Conceptualization, Data Curation, Formal Analysis, Writing – Original Draft, Visualization, Project Administration. **SAA:** Conceptualization, Investigation, Methodology, Supervision, Writing - Review & Editing. **DS:** Resources, Software, Supervision, Validation, Writing - Review & Editing. **WN:** Resources, Software, Supervision, Validation, Writing - Review & Editing.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

Data will be made available upon request to the authors.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that the use of AI and AI-assisted technologies was not applied in the writing process.

REFERENCES

ASHOUR, H.A.; ESMAIL, S.E.; KOTB. M.S. Combined effects of NPK fertilizer with foliar application of benzyladenine or gibberellic acid on *Dracaena marginata* 'Bicolor' grown in different potting media. **Ornamental Horticulture**, v.27, n.1, p.88-102, 2020. https://doi.org/10.1590/2447-536X.v26i4.2141

BHAT, M.A.; MISHRA, A.K.; SHAH, S.N.; BHAT, M.A., JAN, S.; RAHMAN, S.; BAEK, K.-H.; JAN, A.T. Soil and mineral nutrients in plant health: a prospective study of iron and phosphorus in the growth and development of plants. **Current Issues in Molecular Biology**, v.46, n.6, p.5194-5222, 2024. https://doi.org/10.3390/cimb46060312

BISWAS S.S.; SINGH, D.R.; DE, L.C.; KALAIVANAN, N.S.; PAL, R.; JANAKIRAM, T. A comprehensive scenario of orchid nutrition–a review. **Journal of Plant Nutrition**, v.44, n.6, p.905-17, 2021. https://doi.org/10.1080/01904167.2021.1871758

CAI, K.; ZHU, S.; JIANG, Z.; XU, K.; SUN, X.; LI, X. Biological macromolecules mediated by environmental signals affect flowering regulation in plants: A comprehensive review. **Plant Physiology and Biochemistry**, 108931, 2024. https://doi.org/10.1016/j.plaphy.2024.108931

COSTA, M.G.; MANTOVANI, C.; DE MELLO PRADO, R. Optimizing nutrient solution for vegetative growth of *Dendrobium* Tubtim Siam and *Phalaenopsis* Taisuco Swan through plant tissue nutrient balance estimation. **BMC Plant Biology**, v.24, n.1, p.280, 2024. https://doi.org/10.1186/s12870-024-04931-x

GANTAIT, S.; DAS, A.; MITRA, M.; CHEN, J.T. Secondary metabolites in orchids: Biosynthesis, medicinal uses, and biotechnology. **South African Journal of Botany**, v.1, n.139, p.338-51, 2021. https://doi.org/10.1016/j.sajb.2021.03.015

GIL-MARTÍN, E.; FORBES-HERNÁNDEZ, T.; ROMERO, A.; CIANCIOSI, D.; GIAMPIERI, F.; BATTINO, M. Influence of the extraction method on the recovery of bioactive phenolic compounds from food industry by-products. **Food Chemistry**, v.378, p.131918, 2022. https://doi.org/10.1016/j.foodchem.2021.131918

GRZEBISZ, W.; NIEWIADOMSKA, A.; POTARZYCKI, J.; ANDRZEJEWSKA, A. Phosphorus hotspots in crop plants production on the farm—mitigating critical factors. **Agronomy**, v.14, n.1, p.200, 2024. https://doi.org/10.3390/agronomy14010200

HARYOTO, H.; HIDAYATI, S.; KURNIATIN, P.A.; AISYAH, S.I.; NURCHOLIS, W. Optimal extraction solvent for *Purslane (Portulaca grandiflora Hook.)* flowers: A study on phenolic and flavonoid contents and antioxidant activities using microwave-assisted extraction. **Chemical Engineering Transactions**, v.111, p.715-720, 2024. https://doi.org/10.3303/CET24111120

HERIANSYAH, P.; AZIZ, S.A.; SUKMA, D.; NURCHOLIS, W. Antioxidant capacity of *Coelogyne pandurata* extracts at different phenological phases. **Revista Brasileira de Engenharia Agrícola e Ambiental**, v.29, n.2, e279352, 2024. https://doi.org/10.1590/1807-1929/agriambi.v29n2p1-9

LAMBERS, H. Phosphorus acquisition and utilization in plants. **Annual Review of Plant Biology**, v.73, n.1, p.17-42, 2022. https://doi.org/10.1146/annurev-arplant-102720-125738

MINASIEWICZ, J.; ZWOLICKI, A.; FIGURA, T.; NOVOTNÁ, A.; BOCAYUVA, M.A.; JERSÁKOVÁ, J.; SELOSSE, M.A. Stoichiometry of carbon, nitrogen and phosphorus is closely linked to trophic modes in orchids. **BMC Plant Biology**, v.23, p.422, 2023. https://doi.org/10.1186/s12870-023-04436-z

MOHAMMED, A.N.; MOHAMMED, A.L.; MAHDI, M.H. Effect of phosphorus and gibberellic acid on growth and yield of grape (*Vitis vinifera* L.). **Research on Crops**, v.19, n.4, po.643-648, 2018. https://doi.org/10.31830/2348-7542.2018.0001.41

MUBAROK, S.; ALISSYA, A.; DRIKARSA, D.; FARIDA, F.; NURAINI, A.; JAYA, M.H.I.S.; ABDULLAKASIM, S. Combination effects of NPK fertilizer and benzyl amino purine (BAP) in accelerating *Cattleya* Orchid vegetative growth. **Ornamental Horticulture**, v.30, p.1-5, 2024. https://doi.org/10.1590/2447-536X.v30.e242787

MUHAMMAD, N.; LUO, T.; GUI, H.; DONG, Q.; WANG, Q.; PANG, N.; ZHANG, X.; WANG, X.; MA, X.; SONG, M. Effects of phosphorus-mediated alleviation of salt stress on cotton genotypes: Biochemical responses and growth adaptations. **Agronomy**, v.14, n.8, p.1707, 2024. https://doi.org/10.3390/agronomy14081707

NURCHOLIS, W.; RAHMADANSAH, R.; ASTUTI, P.; PRIOSOERYANTO, B.P.; ARIANTI, R.; KRISTÓF, E. Comparative analysis of volatile compounds and biochemical activity of *Curcuma xanthorrhiza* Roxb. essential oil extracted from distinct shaded plants. **Plants**, v.13, n.19, p.2682, 2024. https://doi.org/10.3390/plants13192682

NURCHOLIS, W.; SYA'BANI PUTRI, D.N.; HUSNAWATI, H.; AISYAH, S.I.; PRIOSOERYANTO, B.P. Total flavonoid content and antioxidant activity of ethanol and ethyl acetate extracts from accessions of *Amomum compactum* fruits. **Annals of Agricultural Sciences**, v.66, n.1, p.58-62, 2021. https://doi.org/10.1016/j.aoas.2021.04.001

PÉRET, B.; DESNOS, T.; JOST, R.; KANNO, S.; BERKOWITZ, O.; NUSSAUME, L. Root Architecture responses: in search of phosphate. **Plant Physiology**, v.166, n.4, p.1713-1723, 2014. https://doi.org/10.1104/pp.114.244541

SHALEM, T.; SARVANAN, S. Effect of different of macro nutrients on growth and spike yield of *Dendrobium* orchid var. sonia red. **International Journal of Chemical Studies**, v.8, n.5, p.2410-2412, 2020. https://doi.org/10.22271/chemi.2020.v8.i5ag.10680

SUNAWAN, HANDOKO, R.N.S.; RAHAYU, I.R.; AFANDHI, A. GA_3 and NPK Fertilization Applications Affect *Phalaenopsis amabilis* L. orchid for Plant Growth. **J-Pal**, v.11, n.1, p.1-6, 2020. https://doi.org/10.21776/ub.jpal.2012.010.01.01

SUZUKI R.M.; TAMAKI, V.; NIEVOLA, C.C.; COSTA J.P.; GUARDIA, M.C.; CACHENCO, M.V.; KANASHIRO, S.; BAPTISTA, W.; SHIDOMI, Y.; SANTOS JUNIOR N.A. Prior fertilization enables higher survival of relocated terricolous orchids?. **Rodriguesia**, v.30, n.72, e02452019, 2021. https://doi.org/10.1590/2175-7860202172033

VUKMIROVIĆ, A.; ŠKVORC, Ž.; BOGDAN, S.; KRSTONOŠIĆ, D.; BOGDAN, I.K.; KARAŽIJA, T.; SEVER, K. Photosynthetic response to phosphorus fertilization in drought-stressed common beech and sessile oak from different provenances. **Plants**, v.13, n.16, p.2270, 2024. https://doi.org/10.3390/plants13162270

WU, Q.; LIU, Y.; JIN, C.; ZHAO, Y.; GAO, M.; GUO, L. Removal mechanisms and metabolic responses of *Chlorella pyrenoidosa* to dissolved organic phosphorus. **Bioresource Technology**, v.406, p.130999, 2024. https://doi.org/10.1016/j.biortech.2024.130999

ZHAO, Q.; ZENG, D.; LUO, Z.; CHEN, A.; XU, G.; LI, Y. Flavonoids mediate the modulation of phosphate uptake and phosphate-starvation signaling in tobacco. **Journal of Plant Growth Regulation**, v.42, n.11, p.7229-7239, 2023, https://doi.org/10.1007/s00344-023-11011-1