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Synergistic Effect of Phosphorus, Magnesium, and Copper on Phenolic and Flavonoid Production in Paddy Rice

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Abstract: Secondary metabolites are crucial for plant resistance to biotic and abiotic stress. Fertilization with phosphorus (P), magnesium (Mg), and copper (Cu) can enhance secondary metabolite production, supporting plant defense mechanisms. This study evaluated the effects of P, Mg, and Cu fertilization on nutrient absorption and the production of phenolic and flavonoid metabolites in Pendok rice during vegetative and generative phases. A randomized block design with seven nutrient treatments and three replications was applied, involving 21 pots in a greenhouse. P was supplied as Super Phosphate $Ca(H_2PO_4)$ (SP-36) and Phosphate Rock (PR). Mg and Cu treatments included combinations with and without their addition. Results showed that SP-36 with Mg produced the highest phenolic content during the vegetative phase, significantly higher than other treatments with 7.80 mg (GAE).g extract-1. Mg increased phenolic levels, while Mg and P had a significant effect (95% confidence level) on flavonoid and phenolic content during both growth phases. In conclusion, Mg and P fertilization effectively enhanced secondary metabolite production, improving plant resilience through nutrient optimization. These findings emphasize the role of proper nutrient management in boosting secondary metabolite quality in rice cultivation.

Keywords: Agrominerals; Biosynthesis; Enzymes; Phenylalanine ammonia-lyase

Introduction

Secondary metabolites play a vital role in plant defense mechanisms against biotic and abiotic stresses, acting as antioxidants and protecting plants from oxidative damage (Sharma et al., 2019; Yang et al., 2018). Phenolic and flavonoid compounds are particularly important for resistance formation and plant health (Kumar et al., 2023). Their production can be influenced by nutrient availability, especially phosphorus (P), magnesium (Mg), and copper (Cu).

P contributes to energy metabolism and nucleic acid synthesis, playing a key role in the biosynthesis of phenolic compounds through enzymatic reactions catalyzed by phenylalanine ammonia-lyase (PAL) (Kołton et al., 2022; Nicholls et al., 2023). Mg supports chlorophyll formation and photosynthesis, enhancing carbohydrate production as precursors for phenolic compounds (Ali et al., 2023; Tatagiba & Rodrigues, 2016; Tränkner et al., 2018). Cu acts as a co-factor in enzymatic processes, including those catalyzed by PAL, which regulates polyphenolic compound synthesis

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(Kováčik et al., 2010; Macoy et al., 2015; Tripathi et al., 2015).

Efficient nutrient management is crucial to optimizing secondary metabolite production in rice plants. Previous studies have focused on individual nutrient contributions, but limited research has examined the combined effects of P, Mg, and Cu on secondary metabolite production, particularly in specific cultivars like Pendok rice. This study explores these synergistic effects during both vegetative and generative phases, addressing gaps in existing research.

Unlike prior research, this study evaluates the combined impacts of P, Mg, and Cu fertilization on Pendok rice, a cultivar that has been understudied. It investigates nutrient interactions using agro-mineral sources (SP-36 and PR) to enhance phenolic and flavonoid levels, providing insights into sustainable nutrient management strategies for improving plant resilience and secondary metabolite production (Khare et al., 2020; Villamarin-Raad et al., 2023).

This study aims to explore the positive impact of Pendok rice plants by applying a combination of P, Mg, and Cu nutrients on the production of secondary metabolites and their uptake during both vegetative and generative phases. This research addresses a critical gap by providing insight into the multifaceted interaction of these nutrients, contributing valuable knowledge to both agronomic practices and plant biochemistry.

Method

Research Location

This study was conducted in Jember, Indonesia from February to November 2021 (8°09'50.1''S 113°42'58.6''E), with analyses performed at the soil chemistry and fertility laboratory and soil biology laboratory at Jember University. The Pendok rice variety (CDAST Collection, Jember University) was selected to be the experimental crop to evaluate the efficacy of PR sourced from the Tuban deposit. The growth medium comprised Inceptisol soil with a clay texture, having a pH of 5.73, available P concentration of 2.33 ppm, and Mg concentration of 1.47 me.100 g-1, Cu concentration of 1.06 ppm, organic carbon content of 2.30%, and total nitrogen content of 0.471%. Each experimental pot contained 10 kg of Inceptisol soil, filtered through a 50-mesh sieve, and received essential fertilizers such as urea and KCl as recommended by the Ministry of Agriculture. The nutritional treatments consisted of seven variations.

Table 1. Combination of Fertilizer Treatments in Rice Fields

Combination treatment code	Description
P1C0M0	SP36 (control)
P1C1M0	SP-36 + Cu
P1C0M1	SP-36 + Mg
P1C1M1	SP-36 + Cu + Mg
P2C1M0	PR + Cu
P2C0M1	PR + Mg
P2C1M1	PR + Cu + Mg

Experimental Design

The study adopted a randomized block design with seven nutritional treatments, each replicated three times, totaling 21 experimental pots. Greenhouse experiments were conducted through the application of P, Cu, and Mg nutrients. The P factor comprised SP-36 and PR fertilization, while the Cu factor contained treatments without and with Cu fertilization. Similarly, the Mg factor included treatments without and with the addition of Mg fertilization. PR and SP-36 treatments were applied at a rate of 100 kg SP-36 per hectare. Cu, a micro-element, was administered at a dosage of 20 kg per hectare, while Mg was provided at 40 kg per hectare. The control treatment in this study was established based on the recommendations from the Rice Research Center providing baseline fertilization guidelines for optimal rice cultivation. It is important to note that the control in this experiment was not based on unfertilized soil; therefore, the control was not without treatment but rather followed standard recommended fertilization practices, based on the results from previous reviews, which are detailed in Table 2.

Table 2. Characteristics of Fertilizers for Research

Nutrient	Fertilizer/Agromineral	Formula	Concentration
Phosphor (P)	Superphosphate (SP-36)	P_2O_5	36% P_2O_5; S 5%
	Phosphate Rock	$Ca_{10}(PO_4)6(OH)_2$	22,1% P_2O_5; 66.0% CaO
Copper (Cu)	Micro Cu EDTA	$C_{10}H_{12}N_2O_8CuNa_2$	15 % Cu
Magnesium (Mg)	Macro Mg	$MgSO_4$	16% MgO; 13% S

Phosphate rock (PR) was derived from Tuban deposits and comprised 22.1% P_2O_5, 66.0% CaO, 6.19% Fe_2O_3, 2.2% Al_2O_3, 1.65% MnO, and 0.24% CuO. The results showed that PR was calcium apatite phosphate, with the formula $Ca_5(PO_4)3(OH)$ or $Ca_{10}(PO_4)6(OH)_2$. Scanning electron microscope (SEM) imagery, as shown in Figure 1, showed its high reactivity, with a P_2O_5 concentration dissolved in 2% citric acid reaching 26.96%. According to the Diamond classification system

(1979), PR from the Tuban deposit fell into the high standard category, exceeding 9.40%. When the reactivity of natural phosphate released potentially plant- available P, its agronomic effectiveness in the field relied on various factors (Center for Soil Research, 2009).

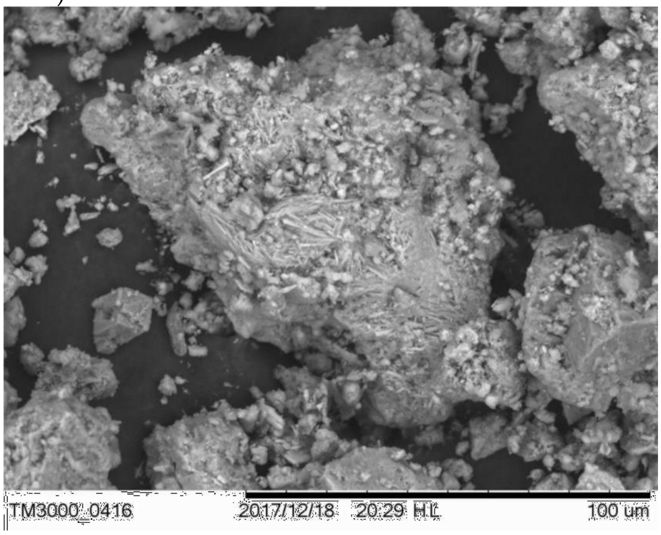

Figure 1. SEM images of calcium phosphate minerals (phosphate rock) Tuban Deposite

Research Management

Cultivation and plant care

The rice plant preparation procedure covered the activities such as seedlings and transplanting. Seeds were initially sown in trays filled with standard growing medium (devoid of additional nutrients) for approximately 14 days to allow rice to sprout with one leaf and attain a robust and erect stance. Seedlings showing optimal growth, typically around five plants, were transplanted into experimental pots. However, close monitoring of the growing medium's condition and management of pest and disease outbreaks were prioritized throughout the growth phase. Soil and plant specimens were subsequently collected for laboratory analysis during the final growth stage.

Analysis of plant and soil nutrients

The levels of nitrogen, P, Cu, and Mg in plant shoots and roots were assessed using the wet digestion method with H_2SO_4 + H_2O_2 extraction. Total nitrogen content was determined by introducing 5% NaOCl and measuring it with a spectrophotometer set at a wavelength of 636 nm. The levels of P were assessed at a wavelength of 693 nm after adding a color reagent. Similarly, the levels of Mg and Cu nutrients were determined using an Atomic Absorption Spectrophotometer (AAS) (Kim, 2005). Nutrient uptake was calculated by multiplying the nutrient content by the dry weight of the plant. The analysis of soil included assessing soil pH and determining nitrogen, P, Cu, and Mg nutrient levels.

Analysis of secondary metabolites production

The analysis of secondary metabolites primarily focused on phenolic and flavonoid compounds. However, phenolic was analyzed using the Folin Ciocalteu method, as described by Conde et al. (1997). This method covered placing 0.1 mL of the extract solution into a test tube with the addition of 0.1 mL of 50% Folin Ciocalteu reagent. The resulting mixture was measured using UV-Vis spectrophotometry at a wavelength of 750 nm. The analysis of flavonoid compounds was conducted using the method outlined by Meda et al. (2005), where 2 ml of the sample was combined with 2 ml of 2% aluminum chloride dissolved in ethanol. The resulting mixture was measured using UV-Vis spectrophotometry at a wavelength of 415 nm.

Data analysis

The collected data were subjected to analysis of variance (ANOVA) followed by additional testing using the Tukey test or the Duncan Multiple Range Test (DMRT) at a confidence level of 95%. Furthermore, a correlation test was conducted to assess the relationship between variables.

Result and Discussion

Phosphorous

This study showed that the application of SP-36, Cu, and Mg fertilizers significantly increased the availability of soil P to be 20.25 ppm compared to the use of PR, Cu, and Mg fertilizers with 12.53 ppm (Figure 2). SP-36 along with the addition of Cu and Mg, has shown efficacy in augmenting the availability of soil P. The results show the significance of tailored fertilization strategies in optimizing uptake and availability for rice plants. Furthermore, the study showed that the availability of soil P from PR fertilization did not have a significant difference compared to the application of SP-36 fertilizer. In summary, the availability of soil P differs between PR and SP-36 fertilizers due to the higher solubility of SP-36 (Rivaie, 2015).

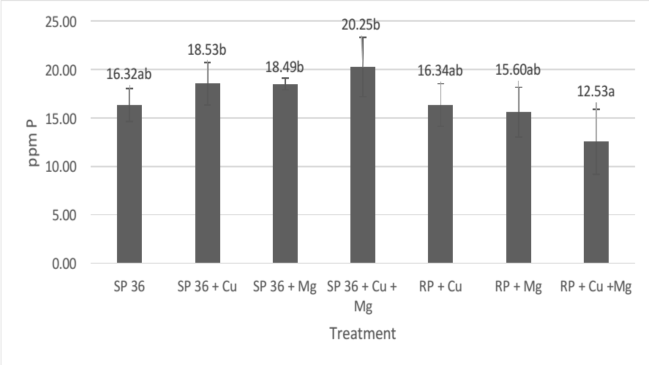

Figure 2. Concentration of phosphorus available in the soil

Potassium

The results showed that the joint application of SP-36 and Cu fertilization positively influenced the availability of soil K with 146.7 ppm K, leading to elevated K levels compared to other treatments (Figure 3). The application of SP-36 fertilizer, rich in P and Cu, stimulated mechanisms that facilitated optimal K release and availability in the soil. This method represented an effective strategy for improving the K nutrient status in rice plant growth environment. Furthermore, the study showed that the application of an equal dose of K fertilizer resulted in similar values of available nutrient in rice plants, suggesting the consistent impact of a fixed fertilizer dosage on K availability surrounding plant roots.

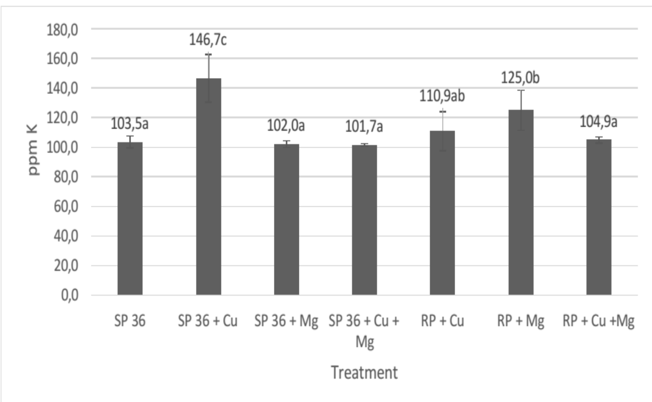

Figure 3. The concentration of potassium available in the soil in several treatments

Nitrogen

Figure 4 presented the total soil nitrogen content in the fertilizer treatments applied to rice plant, showing varying values for each treatment. The SP-36 + Cu + Mg fertilizer treatments had the highest total soil nitrogen value with 0.78%, significantly distinct from the PR + Cu with and PR + Mg treatments with 0.72%, respectively, which had the lowest value at 0.72%. In contrast, the PR + Cu + Mg combination.

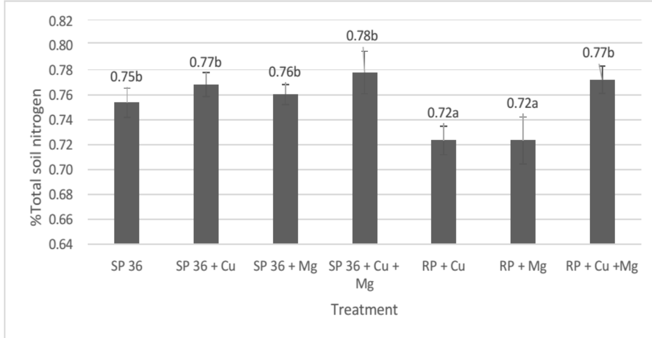

Figure 4. Soil nitrogen concentration in several treatments for rice plants

Copper

This study presented that Cu fertilization substantially increased the available Cu content in the soil compared to rice plant without such fertilization (Figure 5). The results showed the important role of Cu in fostering plant growth and suggested its administration through fertilization, could effectively address deficiencies of nutrient in the soil.

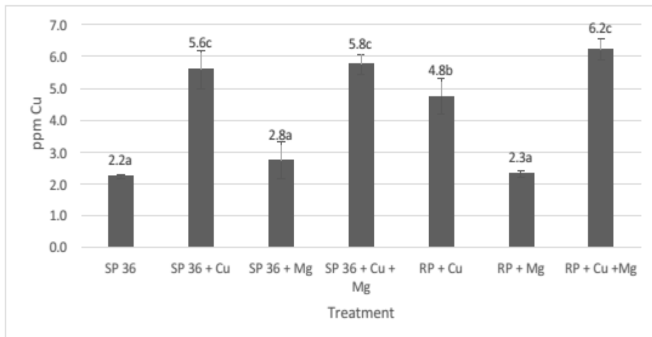

Figure 5. The concentration of Cu available in the soil in several treatments

Magnesium

The study provided valuable insights into the effect of Mg fertilizer treatment on soil Mg availability. The results showed that applying SP-36 in combination with Mg fertilizer to rice plant led to the highest Mg availability, with a concentration of 171.6 ppm (Figure 6). The concentration surpassed both the control treatment at 161.2 ppm and other treatments lacking Mg fertilization. The results showed that the application of Mg could effectively increase soil Mg availability, paving the way for the development of more efficient fertilization strategies.

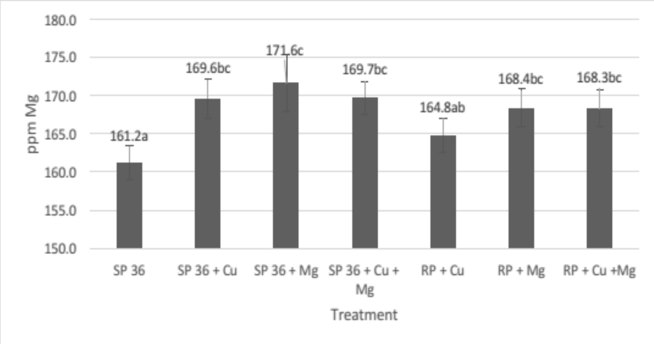

Figure 6. The concentration of Mg available in soil in several treatments

The concentration of P in rice plant tissues ranged from 0.26 to 0.37 ppm across treatments. Specifically, treatments including the addition of SP-36 and a combination of PR + Cu + Mg showed significantly higher results compared to others. Regarding the levels of K in rice plant tissues, the concentration ranged from 1.60 to 1.81 ppm, with the control treatment showing the highest concentration. Conversely, the SP-36 + Cu + Mg treatment showed the lowest K concentration. It was also observed that nitrogen values in rice plant tissues ranged from 0.71 to 1.19%, with SP-36 + Cu + Mg treatments resulting in the highest value.

Uptake and Nutrients in Plant Tissues

Table 3. Nutrient Concentration of Plant Tissue for Several Parameters

Treatment	Nutrient concentration on plant tissue (%)				
	P	K	N	Cu	Mg
SP36 (control)	0.27a	1.81c	0.85ab	0.49ab	1.63bc
SP-36 + Cu	0.36b	1.70abc	1.04b	0.54b	1.50a
SP-36 + Mg	0.37b	1.76bc	0.71a	0.43a	1.73c
SP-36 + CU + Mg	0.37b	1.60a	1.19bc	0.50ab	1.69bc
PR + Cu	0.26a	1.62ab	0.72a	0.73c	1.58ab
PR + Mg	0.33ab	1.71abc	1.03b	0.49ab	1.68bc
PR + Cu + Mg	0.34b	1.75bc	0.97b	0.72c	1.74c

Note: the numbers followed by the same notation are not significantly different in Duncan's multiple distance test at 95% level

Table 3 presented the Cu concentration in rice plant tissues across various treatments, ranging from 0.43 to 0.73 ppm. Specifically, treatments without the addition of Cu fertilization had lower concentrations. In terms of Mg concentration in rice plant tissues, values ranged from 1.50 to 1.74 ppm, showing minimal differences between treatments. The highest value was observed in PR + Cu + Mg treatments, while treatments without Mg fertilization tended to have lower values.

The application of PR alongside Cu and Mg resulted in nutrient element concentrations of P and Mg similar to those achieved with SP-36 treatment combined with Cu and Mg. It was only the K absorption concentration that showed insignificant differences among treatments. Specifically, all treatments had similar trends across parameters. The combination of PR with Cu and Mg also resulted in the highest nutrient uptake in Cu. However, variations in plant uptake parameters for P, nitrogen, and Mg were observed, with variable concentrations obtained when applying SP-36 with Cu and Mg (Table 4).

Table 4. Plant Uptake Values for Several Treatment Parameters

Treatment	Plant uptake ($mg.pot^{-1}$)				
	P	K	N	Cu	Mg
SP36 (control)	30.76a	204.14a	96.18ab	54.86ab	184.09ab
SP-36 + Cu	42.61bc	199.40a	121.93b	62.93b	175.45a
SP-36 + Mg	40.58bc	183.89a	77.53a	47.54a	190.29abc
SP-36 + CU + Mg	45.22c	196.87a	146.37c	62.10b	208.72c
PR + Cu	30.22a	185.97a	82.11a	83.34c	180.96a
PR + Mg	38.31b	196.65a	118.07b	56.43ab	193.46abc
PR + Cu + Mg	40.47bc	206.21a	114.25b	84.76c	205.27bc

The joint application of Cu and Mg with PR resulted in a higher concentration of all nutrients in the shoots. The combination of all these fertilizers also led to greater nutrient uptake than PR combined with Cu or Mg. Observably, adequate levels of P, Mg, and Cu positively impacted the efficiency of nutrient uptake.

Phenolic and Flavonoid

Phenolic secondary metabolites content during the vegetative phase showed considerable variation. The highest content was observed in SP-36 combined with Mg treatments, significantly differing from others. Adding Mg to the treatment positively affected the increase in phenolic secondary metabolites. Similarly, the SP-36 treatments combined with Mg showed the highest phenolic content during the vegetative phase, with a value of 4.12 mg (GAE).g extract^{-1} (Table 5).

During the vegetative period, rice plant had significant variation in flavonoid content. The SP-36 treatments combined with Mg showed the highest content, reaching 3.45 mg QE.g extract^{-1}, significantly different from others. Conversely, the control treatment without adding Cu or Mg, showed the lowest value during the vegetative phase. During the generative phase, PR + Cu + Mg treatments had the highest value of flavonoid secondary metabolites, measuring 2.15 mg QE.g extract^{-1}. Despite having the highest value during the vegetative phase, the SP-36 + Cu treatments still showed the lowest value during the generative phase.

Flavonoid a subset of phenolic secondary metabolites played a crucial role in plant physiology (Ratnasari et al., 2023). The highest average content was observed in the Cu and Mg fertilizer, measuring 2.003 mg QE.g extract^{-1}, significantly differing from treatments solely receiving Cu or Mg fertilizer. Simultaneously, the application of Cu and Mg fertilizers resulted in higher production in rice plant compared to the application of Cu or Mg fertilizers individually. The concentration of flavonoid ranged from 2.17 to 3.44 mg QE.g extract-1 during the vegetative period and from 0.94 to 2.15 mg QE.g extract^{-1} during the generative period.

Table 5. Values of Phenolic and Flavonoid Secondary Metabolites in Each Treatment

Treatment	Phenolic mg (GAE).g extract^{-1}		Flavonoid mg QE.g extract^{-1}	
	Vegetative	Generative	Vegetative	Generative
SP36 (control)	6.36ab	3.77a	1.56a	1.24a
SP-36 + Cu	6.87b	3.88ab	2.64b	1.29a
SP-36 + Mg	7.80c	4.12b	3.45c	1.03a
SP-36 + Cu + Mg	6.96b	3.88ab	2.68b	2.09bc
PR + Cu	5.83a	3.72a	2.53b	1.61abc
PR + Mg	6.15ab	3.79a	2.62b	1.39ab
PR + Cu + Mg	6.69b	3.79a	2.62b	2.15c

Discussion

Fertilizer treatments containing P (both inorganic and agro mineral), Mg, Cu, and their combinations have significantly enhanced various agronomic traits such as plant height, tiller number, and total plant growth (Cho et al., 2021; Mulyati et al., 2022; Surya et al., 2024). The most favorable responses regarding these characteristics were observed in treatments combining P, Mg, and Cu fertilizers, followed by treatments using a combination of different fertilizer types. However, it was evident that fertilizer application affected agronomic traits, plant growth (Aksarah et al., 2024; Jannah et al., 2024), and the concentrations of phenolic and flavonoid secondary metabolites in plants (Sutrisno & Yusnawan, 2018).

Fertilization treatments in this study using SP-36 showed a significantly higher average P value than PR fertilization, for instance 20.25 ppm P on SP-36, Mg and Cu treatment. Both treatments enhanced soil P availability, but SP-36 outperformed PR. According to Khan et al. (2023), increasing P availability could stimulate enzyme activity (Gho et al., 2020) and augment the synthesis pathway of secondary metabolites, resulting in increased accumulation of these bioactive compounds, serving as a fundamental component of the ATP molecule, which became the primary energy source in plant cells (Poirier et al., 2022; Yoskader et al., 2023). Therefore, SP-36 treatments combined with Mg had the highest value of phenolic compounds. P and Mg play an important role in numerous plant functions, including nucleic acid metabolism, enzyme activation or deactivation, redox reactions, nitrogen fixation, and carbohydrate metabolism (Mou et al., 2020).

The application of Phosphor fertilizer using SP-36 resulted in greater P uptake by rice plant compared to the use of PR. However, PR led to higher uptake than the control, which relied solely on SP-36 fertilizer without additional P supplementation. The highest Phosphor concentration of plant tissue was observed in treatments combining SP-36 with Mg and SP-36 with Cu and Mg in the same level, namely 0.37%. A positive relationship was found between P content in rice plant tissues and soil phosphor content. Specifically, the treatments having the highest P content in soil and plant tissues was SP-36 combined with Cu and Mg with 20.25 ppm P and 0.37%, respectively. The enhanced P availability within such treatments facilitated the absorption of P by plant, thereby aiding in the formation of phenolic and flavonoid secondary metabolites (Lambers, 2022).

High content of potassium supported plant growth by optimizing processes such as photosynthesis, nutrient translocation, and osmotic pressure regulation (Johnson et al., 2022; Padafani, 2022). The optimization stimulated the biosynthesis pathways of phenolic and flavonoid compounds in response to environmental cues. Potassium also served as a co-factor for various enzymes (Cui & Tcherkez, 2021). The availability of potassium regulated osmotic pressure in plant cells and controlled stomata opening and closing (Sardans & Peñuelas, 2021). This affected transpiration and photosynthesis rates.

Although potassium treatment was not included in the study's fertilization regime, Microorganisms create K compounds through metabolic processes, which utilize free potassium ions in organic materials (Murnita et al., 2024). Understanding the nutrient level was necessary due to its usage in multiple aspects of plant metabolism, including enzyme activity associated with bioactive compound synthesis. The results showed soil potassium concentration variations, with SP-36 combined with Cu 146.7 ppm K and PR combined with Mg 125.0 ppm K. No direct relationship was observed between potassium concentrations in plant tissues and soil. The nutrient level could be reduced due to plant absorption or soil binding, resulting in disparities between soil and tissue potassium concentrations (Rawat et al., 2016). This phenomenon was evident in the substantial uptake in the control treatment (204.14 mg.pot-1), leading to elevated concentration in plant tissues (1.81%). However, the soil potassium concentration was higher in the SP-36 treatments combined with Cu with 146.7 ppm K.

PAL was considered a crucial enzyme in the biosynthesis pathway, requiring activation by critical enzymes. In this activation process, the availability of nitrogen in plants was essential, as PAL formation was contingent upon the nutrient's availability (Hyun et al., 2011). PAL catalyzed the conversion of phenylalanine into trans-cinnamic acid, marking the initial step in phenolic compound formation (Son et al., 2021). Flavonoid biosynthesis pathway, trans- cinnamic acid was transformed into flavonoid compounds through enzymatic reactions such as chalcone synthase (CHS), chalcone isomerase (CHI), and flavanone 3-hydroxylase (F3H) (Zhuang et al., 2023).

Although nitrogen was not included as a treatment in the study, understanding the nutrient's level was necessary, as it could enhance the synthesis of the precursor compound phenylalanine. Previous reviews showed variations in total soil nitrogen values, with the highest values observed in the SP-36 treatments combined with Cu and Mg 0.78%. The treatments had the highest accumulation of the nutrient, resulting in elevated nitrogen levels in plant tissues, surpassing other treatments (Taqiyyah et al., 2023). This uptake was attributed to the substantial nitrogen content in the soil, with plant absorbing high levels, reaching 146.37 $mg.pot^{-1}$.

Cu fertilization could positively influence the production of phenolic and flavonoid secondary metabolites in plant. Despite being a micronutrient required in small amounts, the nutrient played an important role in enzyme activity within biosynthetic pathways of bioactive compounds. This showed Cu fertilization not only enhanced total plant productivity but also facilitated the accumulation of bioactive compounds vital for plant health and resilience against environmental stressors. In this study, Cu fertilization, combined with SP- 36 and PR, was conducted. The results showed a significant increase in phenolic and flavonoid production with Cu supplementation compared to treatments without Cu. Cu concentration introduced through fertilization significantly enhanced the activity of PAL enzyme, serving as an essential co-factor for PAL and modulating its activity to stimulate polyphenolic compound production (Chi Yu et al., 2005; Printz et al., 2016).

The application of Cu fertilization resulted in an increase in Cu concentration within the soil, leading to a significant uptake. Therefore, the nutrient content within rice plant tissues showed a substantial rise. An increased Cu level played a crucial role in enhancing the production of secondary metabolites, including phenolic and flavonoid (Kováčik et al., 2010). A high concentration of the nutrient triggered the activation of enzymes used in the biosynthesis pathways of bioactive compounds (Proklamasiningsih et al., 2019), thereby facilitating the heightened accumulation of the compounds. This accumulation could positively influence plant defense responses and the characteristics of secondary metabolites in rice plants (Isah, 2019).

The introduction of Mg through fertilization altered its level within the soil, subsequently influencing Mg accumulation in plant tissues. This effect resulted in intricate mechanisms, affecting the synthesis of phenolic and flavonoid secondary metabolites (Ahlawat et al., 2023). This study showed that the application of Mg in fertilization resulted in higher concentration, particularly evident in the SP-36 combined with Mg treatments (171.6 ppm Mg). The nutrient's uptake significantly contributed to chlorophyll structure, essential for photosynthesis (Farhat et al., 2016). Increased availability of Mg enhanced plant photosynthetic capacity, elevating carbohydrate production (Ba et al., 2020). Such carbohydrates served as precursors in phenolic compounds' biosynthesis pathway since Mg upheld cell membrane integrity (Tian et al., 2021). By preserving cell membrane stability, the nutrient influenced the transport and accumulation of phenolic compounds within plant cells. Additionally, Mg participated in nutrient translocation and ATP structure formation, providing a foundation for efficient nutrition and ample energy to support phenolic compound production (Guo et al., 2016).

Augmenting Mg levels in the soil, particularly in the SP-36 treatments combined with Mg, resulted in its significant absorption by rice plants. Therefore, there was a marked increase in the nutrient content, specifically in the SP-36 combined with Mg (1.73%) and the PR treatments combined with Mg and Cu (1.74%). The elevated Mg level in plant tissues within the SP-36 treatments combined with Mg positively influenced secondary metabolite production, including phenolic and flavonoid. Comparative analysis with other treatments showed that the concentration of secondary metabolites was higher in the SP-36 treatments combined with Mg especially phenolic in vegetative and generative with 7.80 and 4.12 mg (GAE).g $extract^{-1}$, respectively. The results offered deeper insights into the role of Mg in enhancing plant responsiveness to the synthesis of specific secondary metabolites within the context of SP-36 fertilization in rice plant. The primary factors influencing total soil nitrogen included denitrifying bacteria and nitrate.

Phenolic vegetative = 7,476 + 9,901 shoot phosphor – 2,474 shoot magnesium (1)

Flavonoid vegetative = 0,147 + 7,380 shoot phosphor (2)

Phenolic generative = 0,367 + 0,681 shoot magnesium – 0,973 shoot magnesium + 2,054 shoot phosphor (3)

Flavonoid generative = -2,326 + 0,180 Copper + 1,882 shoot magnesium (4)

Although stepwise regression analysis conducted on flavonoid and phenolic secondary metabolites during both the vegetative and generative phases showed diverse results, there was a consistent pattern. It was observed that the parameters having the most substantial influence on these compounds were the Mg and the P nutrients. The two variables showed significant influence at a 95% tolerance level (α=0.05) on the values of phenolic and flavonoid secondary metabolites in rice plants across both vegetative and generative phases.

The study highlights the significant role of nutrient fertilization in enhancing agronomic traits and secondary metabolite production in rice plants. Fertilizer treatments combining phosphorus (SP-36 and PR), magnesium, and copper demonstrated superior performance in promoting plant growth, nutrient uptake, and the synthesis of phenolic and flavonoid secondary metabolites. SP-36 combined with Mg emerged as the most effective treatment, enhancing soil and plant P availability and boosting phenolic production. Magnesium contributed to photosynthetic efficiency and energy generation, serving as a precursor in phenolic biosynthesis, while Cu facilitated the activity of key enzymes like PAL, essential for polyphenolic synthesis. Despite variations in soil and tissue nutrient concentrations, the results emphasized the pivotal influence of P and Mg on secondary metabolite production.

Conclusion

SP-36 was proven to be more effective than PR in increasing the availability and absorption of P by plants. P and Mg nutrients had the greatest influence on the production of secondary metabolites in both the vegetative and generative phases, with values of 7.80 and 4.12 mg (GAE).g extract-1, respectively. These results emphasize the importance of proper nutrient management to improve plant maintenance mechanisms and productivity sustainably.

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Author Contributions
Conceptualization, T.C.S., L.M., and A.W.; methodology, T.C. S., L.M., and A.W.; validation, T.C.S., L.M., and A.W.; formal analysis, D. F. and V. K. R.; investigation, l. E. S.; resources, T. C. S. and L. E. S..; data curation, S. E.; writing—original draft preparation, T. C. S. and S. E..; writing—review and editing, T. C. S. and S. E.: visualization, L. E. S. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest
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