

Interactive Effects of Fertilizer Types and Shading Levels on Growth, Yield, and Piperine Content of *Piper Retrofractum* Under Tropical Agroecosystems

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Abstract

Piper retrofractum Vahl. is an economically important medicinal plant valued for its piperine content, yet its productivity and phytochemical quality remain inconsistent under tropical agroecosystem conditions due to variations in nutrient management and light availability. This study aimed to evaluate the interactive effects of fertilizer types and shading levels on growth, dry fruit yield, and piperine content of *P. retrofractum*. A factorial completely randomized design was applied with two factors: fertilizer type, consisting of NPK, biofertilizer, local microbial liquid organic fertilizer, and NPK combined with biofertilizer; and shading level, consisting of no shading, 40% shading, and 60% shading. The experiment generated 12 treatment combinations with three replications. The observed variables were productive branches, dry fruit weight per plant, and piperine content. Data were analyzed using ANOVA followed by Duncan's Multiple Range Test at the 5% level, supported by Pearson correlation and multiple regression analyses. The results showed that fertilizer type, shading level, and their interaction significantly affected all observed variables. The combination of NPK and biofertilizer under no shading produced the highest number of productive branches, dry fruit weight, and piperine content. Productive branches were strongly correlated with dry fruit weight, while dry fruit weight showed a positive relationship with piperine content. The regression model indicated that productive branches and dry fruit weight jointly explained 88% of the variation in piperine content, with dry fruit weight having the stronger contribution. This study implies that integrated nutrient management combined with adequate light exposure can be used as an effective cultivation strategy to improve both productivity and bioactive compound quality of *P. retrofractum* for medicinal, herbal, and spice industries.

Keywords: Biofertilizer; Dry Fruit Weight; NPK Fertilizer; *Piper retrofractum*; Piperine Content.

INTRODUCTION

Piper retrofractum Vahl., commonly known as Javanese long pepper, is a tropical medicinal and spice plant with substantial economic and pharmacological value, particularly in Indonesia and other Southeast Asian regions. This species belongs to the Piperaceae family and has long been used in traditional medicine, herbal formulations, food ingredients, and phytopharmaceutical products because its fruit contains various bioactive compounds, including alkaloids, flavonoids, essential oils, and especially piperine as a major marker compound. Previous studies have reported that *Piper* species and their amide alkaloids exhibit diverse biological activities, including antioxidant, anti-inflammatory, antimicrobial, anthelmintic, gastroprotective, and potential anticancer properties, which strengthen the relevance of this commodity for medicinal plant development and industrial raw materials (Carsono et al., 2022; Díaz-Guerrero et al., 2025; Salehi et al., 2019). In particular, piperine has attracted increasing scientific attention because it is associated with pungency, pharmacological activity, and bioenhancing capacity, making the

improvement of piperine content an important target in the cultivation of *P. retrofractum* and related *Piper* species (Stojanović-Radić et al., 2019; Wan-Hong et al., 2020; Ziegenhagen et al., 2021; Zou et al., 2024).

Despite its high potential, the productivity and phytochemical quality of *P. retrofractum* remain inconsistent under tropical agroecosystems. Variations in growth, fruit yield, and piperine accumulation may be influenced by genotype, environmental conditions, soil fertility, cultivation practices, and post-harvest handling. Studies on Javanese long pepper have emphasized that cultivation improvement is still needed to increase both production and active compound content, particularly because the demand for medicinal plants and herbal raw materials continues to increase in domestic and international markets (Costa et al., 2022; Gao et al., 2025; Sander et al., 2025; Shabrina et al., 2025). This problem is especially relevant in tropical production systems, where high temperature, variable rainfall, soil nutrient imbalance, and differences in light intensity may affect plant physiological processes and secondary metabolite biosynthesis. Therefore, optimizing agronomic practices is essential not only to improve plant growth and fruit yield but also to ensure the consistency of piperine content as a quality indicator of *P. retrofractum*.

Nutrient management is one of the most important agronomic factors determining plant growth, yield formation, and phytochemical accumulation. Inorganic fertilizers such as NPK can provide readily available nitrogen, phosphorus, and potassium, which are essential for vegetative growth, root development, photosynthetic efficiency, flowering, fruit filling, and enzyme regulation. However, excessive or continuous dependence on chemical fertilizers may reduce soil biological quality, disrupt microbial diversity, and contribute to long-term soil degradation, particularly when not integrated with organic or biological inputs (Guo et al., 2025; Khan et al., 2025; Tang et al., 2025; Xing et al., 2025). In contrast, biofertilizers and microbe-based organic fertilizers can improve nutrient availability through nitrogen fixation, phosphate solubilization, phytohormone production, and stimulation of beneficial rhizosphere microorganisms. Integrated nutrient management combining inorganic fertilizers with biofertilizers has therefore been widely recognized as a more sustainable approach because it can improve nutrient-use efficiency, support soil health, and enhance crop productivity while reducing the negative effects of sole chemical fertilizer application (Ibáñez et al., 2023; Kumar et al., 2022; Shahzad et al., 2025; Timofeeva et al., 2023).

In addition to nutrient availability, light intensity is a key environmental factor affecting plant growth and secondary metabolism. Light directly regulates photosynthesis, carbon assimilation, biomass partitioning, morphogenesis, flowering, and the biosynthesis of secondary metabolites such as alkaloids, phenolics, terpenoids, and flavonoids (Borbély et al., 2022; Cioć et al., 2025; Liu et al., 2023). In medicinal plants, adequate light conditions may stimulate the accumulation of bioactive compounds by increasing photosynthate supply and activating metabolic pathways related to secondary metabolite formation (KC et al., 2022; Qaderi et al., 2023; Takshak & Agrawal, 2019; S. Zhang et al., 2021). Conversely, excessive shading may reduce photosynthetic rate, limit carbohydrate availability, alter biomass allocation, and consequently decrease yield and phytochemical concentration. Although some plant species benefit from partial shading, the optimal light requirement is species-specific and depends on the interaction between plant physiology, nutrient availability, and environmental adaptation. For *P. retrofractum*, understanding the response to shading is particularly important because it is often cultivated in mixed or semi-shaded tropical agroecosystems.

The interaction between fertilization and light availability is theoretically important because nutrient uptake and utilization are closely linked to photosynthetic capacity. Plants grown under sufficient light may use available nutrients more efficiently to support vegetative growth, reproductive development, and metabolite biosynthesis (Y. Li et al., 2020; Lu et al., 2021; Selwal et al., 2023; Wu et al., 2025). Conversely, under shaded conditions, the positive effect of fertilization may be constrained by limited carbon assimilation and lower metabolic activity. Previous studies on medicinal and horticultural crops have indicated that integrated nutrient

management and environmental regulation can influence both yield and secondary metabolite accumulation, but the magnitude and direction of these effects depend on plant species, cultivation system, and the specific metabolite being targeted (Ali et al., 2024; Lv et al., 2024; Venkatasai et al., 2025; Zhao et al., 2023). Therefore, evaluating fertilizer type and shading level simultaneously is necessary to identify cultivation strategies that can improve both the quantity and quality of medicinal plant products.

Although previous research has discussed the pharmacological potential, phytochemical composition, and general cultivation aspects of *P. retrofractum*, empirical studies examining the interactive effects of fertilizer type and shading level on growth, dry fruit yield, and piperine content remain limited. Most available studies have tended to focus either on the medicinal properties of *P. retrofractum*, the general role of piperine, or single-factor agronomic practices, while fewer studies have quantitatively linked vegetative traits, yield components, and piperine accumulation under different nutrient and light regimes. This creates an important research gap because medicinal plant cultivation should not only aim to increase biomass or yield but also to improve the concentration of bioactive compounds that determine product quality and economic value. Moreover, the use of correlation and regression analyses to clarify the relationship among productive branches, dry fruit weight, and piperine content is still rarely emphasized in *P. retrofractum* cultivation studies.

Based on this gap, the present study aimed to evaluate the interactive effects of fertilizer types and shading levels on the growth, dry fruit yield, and piperine content of *Piper retrofractum* under tropical agroecosystem conditions. Specifically, this study examined the effects of NPK fertilizer, biofertilizer, local microbial liquid organic fertilizer, and the combination of NPK and biofertilizer under different shading levels on productive branches, dry fruit weight per plant, and piperine percentage. In addition, this study analyzed the relationships among these variables using Pearson correlation and multiple regression analyses. The findings are expected to provide an agronomic basis for developing a sustainable cultivation strategy that can simultaneously enhance productivity and bioactive compound quality in *P. retrofractum*.

METHODS

The study was conducted in 2025 at the experimental field of Universitas Islam Batik Surakarta, Central Java, Indonesia, under tropical agroecosystem conditions. The experimental site is located at an altitude of approximately 105 m above sea level and is characterized by regosol soil, which is commonly found in tropical agricultural areas and requires appropriate nutrient management to support optimal crop growth. The study used one year old *Piper retrofractum* Vahl plants as the experimental material. The research was designed to evaluate the effects of fertilizer type, shading level, and their interaction on plant growth, dry fruit yield, and piperine content (Ahmed et al., 2023; Gelaye, 2025; Gokkus, 2025). A factorial completely randomized design was applied with two experimental factors. The first factor was fertilizer type, consisting of four levels: NPK fertilizer, biofertilizer, local microbial liquid organic fertilizer, and a combination of NPK fertilizer and biofertilizer. The second factor was shading level, consisting of three levels: no shading, moderate shading at 40%, and heavy shading at 60%. These factors generated 12 treatment combinations, and each treatment was replicated three times, resulting in 36 experimental units.

The observed variables included the number of productive branches, dry fruit weight per plant, and piperine content in dried fruit. Productive branches were counted by recording the number of branches capable of supporting reproductive growth, while dry fruit weight was measured after harvested fruits had been dried to a stable weight and expressed in grams per plant. Piperine content was measured as a percentage of dried fruit material to determine the quality of the harvested product as a medicinal raw material. These variables were selected because they represent vegetative performance, yield formation, and phytochemical quality, respectively (Izadi et al., 2022; Jahan et al., 2023; Shah et al., 2020; Wan et al., 2022). The

integration of these three parameters allowed the study to assess whether agronomic treatments could simultaneously improve productivity and bioactive compound accumulation in *P. retrofractum* under tropical conditions.

The collected data were analyzed using analysis of variance at a 5% significance level to determine the main effects of fertilizer type and shading level as well as their interaction effect (L. Li et al., 2022; Wadood et al., 2023; H. Zhang et al., 2021). When the ANOVA results indicated significant differences among treatments, Duncan's Multiple Range Test at the 5% level was applied to compare treatment means. To strengthen the interpretation of treatment effects, Pearson correlation analysis was used to examine the relationships among productive branch number, dry fruit weight, and piperine content. Furthermore, multiple regression analysis was performed to determine the contribution of productive branches and dry fruit weight to piperine content. The regression model used in this study was expressed as $Y = a + b_1X_1 + b_2X_2$, where Y represents piperine content, X_1 represents the number of productive branches, X_2 represents dry fruit weight per plant, a represents the regression constant, and b_1 and b_2 represent the regression coefficients of each independent variable. The coefficient of determination was used to assess the proportion of variation in piperine content explained by the independent variables. The interpretation of the statistical results was based on the significance value, correlation coefficient, regression coefficient, and coefficient of determination, thereby enabling a more comprehensive understanding of the relationship between growth, yield, and piperine accumulation in *P. retrofractum*.

RESULT AND DISCUSSION

Productive Branches of Piper retrofractum

The number of productive branches is an important growth parameter because it reflects the plant's capacity to form reproductive organs. In *Piper retrofractum*, productive branches serve as the main sites for flower and fruit development; therefore, a higher number of productive branches may indicate greater yield potential. The results showed that fertilizer type, shading level, and their interaction influenced the number of productive branches per plant.

Table 1. Effect of fertilizer types and shading levels on the number of productive branches per plant

Fertilizer Type	Shading Percentage			Mean
	F1	F2	F3	
P1	18.92c	17.22d	14.32g	16.82e
P2	20.43b	18.72c	16.17e	18.44c
P3	19.08c	17.93d	15.54f	17.52d
P4	23.27a	20.84b	17.24d	20/45b
Mean	20.43b	18.68c	15.82f	

Note: Values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 5% level.

As shown in Table 1, the highest number of productive branches was obtained from the combination of NPK and biofertilizer under no shading (P4F1), with 23.27 branches per plant. Conversely, the lowest number of productive branches was recorded in the NPK treatment under 60% shading (P1F3), with 14.32 branches per plant. The mean values indicate that the combined application of NPK and biofertilizer produced the highest average number of productive branches, followed by biofertilizer, local microbial liquid organic fertilizer, and NPK alone. These findings suggest that integrated fertilization can promote better vegetative development than single fertilizer application.

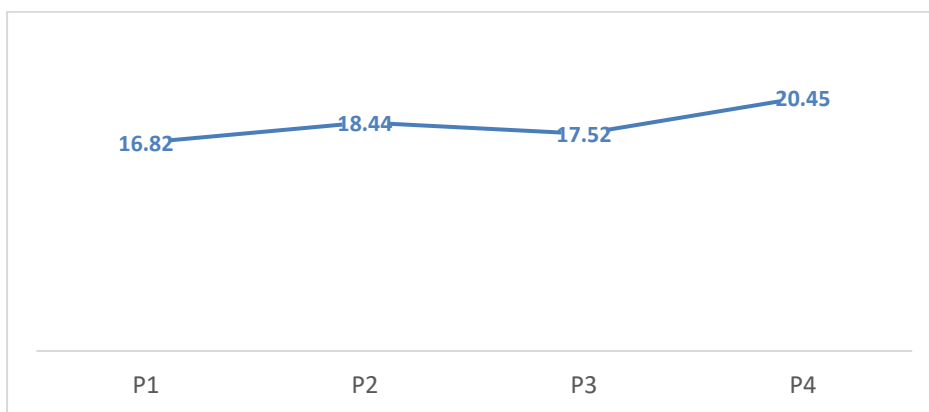


Figure 1. Effect of fertilizer types on the number of productive branches per plant

Figure 1 should present the mean number of productive branches under each fertilizer treatment. This figure visually confirms that the P4 treatment, namely the combination of NPK and biofertilizer, produced the highest average number of productive branches. The trend indicates that the integration of inorganic fertilizer and biofertilizer improved vegetative growth more effectively than NPK, biofertilizer, or local microbial liquid organic fertilizer applied separately.

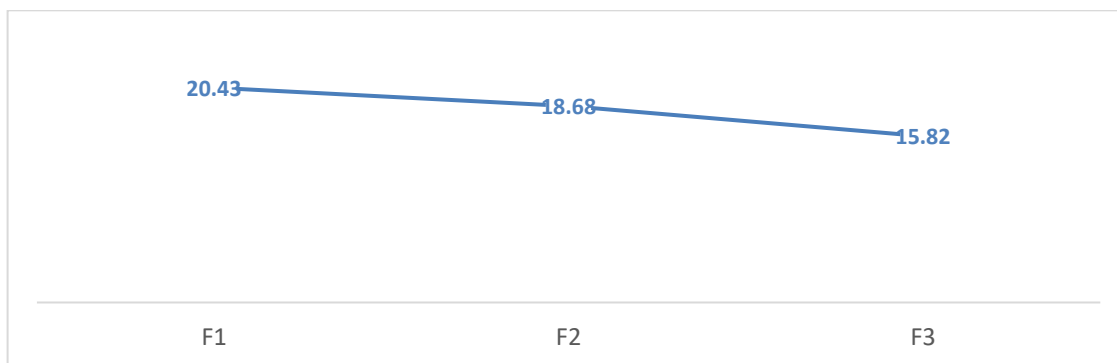


Figure 2. Effect of shading levels on the number of productive branches per plant

Figure 2 should show the effect of shading levels on productive branch formation. The highest mean number of productive branches was recorded under no shading, followed by 40% shading and 60% shading. This pattern indicates that increasing shading intensity reduced productive branch development. The decline under 60% shading suggests that limited light availability may restrict photosynthesis and reduce the formation of new productive branches.

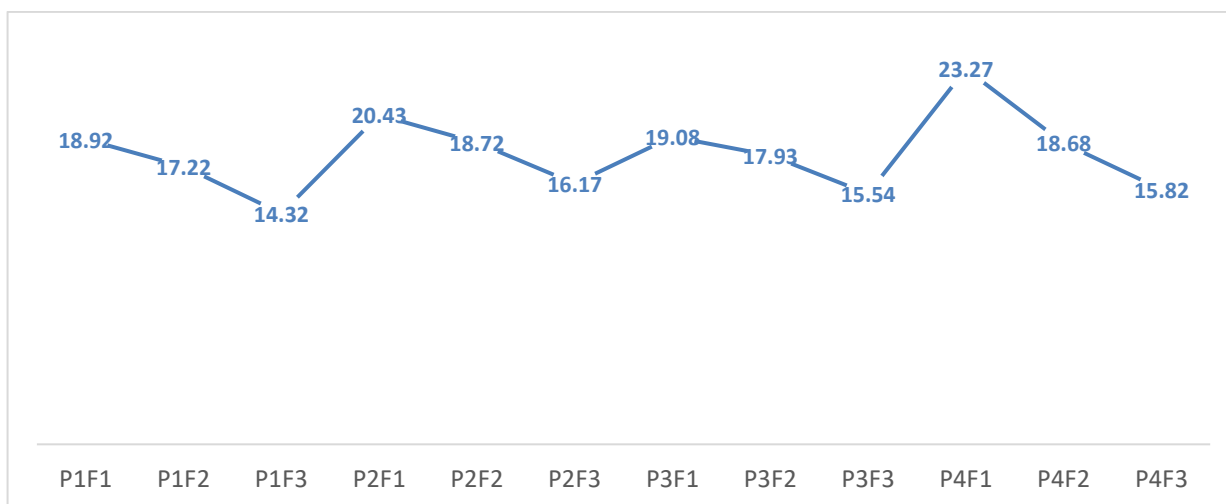


Figure 3. Interaction effect of fertilizer types and shading levels on productive branches per plant

Figure 3 should illustrate the interaction between fertilizer types and shading levels. The highest treatment combination was P4F1, while the lowest was P1F3. This interaction pattern indicates that the beneficial effect of fertilizer application was stronger under sufficient light conditions. Although fertilization improved productive branch development, its effectiveness decreased under higher shading levels, particularly at 60% shading.

Overall, the results show that productive branch formation was optimized when plants received both balanced nutrient input and full light exposure. The combination of NPK and biofertilizer under no shading provided the most favorable condition for vegetative growth in *P. retrofractum*.

Dry Fruit Weight per Plant

Dry fruit weight is a major yield indicator in *Piper retrofractum* because the harvested product is commonly used in dried form as a medicinal and spice raw material. The results showed that fertilizer type, shading level, and their interaction affected dry fruit weight per plant.

Table 2. Effect of fertilizer types and shading levels on dry fruit weight per plant

Fertilizer Type	Shading Percentage			Mean
	F1	F2	F3	
P1	182.4c	165.8e	142g	163.4e
P2	195.7b	178.9d	150.4f	175d
P3	190.6c	172.3d	148.7f	170d
P4	214.8a	196.5b	168.9e	193.4b
Mean	195.88b	178.38d	152.5f	

Note: Values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 5% level.

Table 2 shows that the highest dry fruit weight was recorded in the P4F1 treatment, reaching 214.8 g per plant. The lowest dry fruit weight was found in P1F3, with 142.0 g per plant. Based on the fertilizer mean values, the combination of NPK and biofertilizer produced the highest average dry fruit weight. This indicates that the integration of inorganic and biological nutrient sources supported better yield formation than single fertilizer treatments.

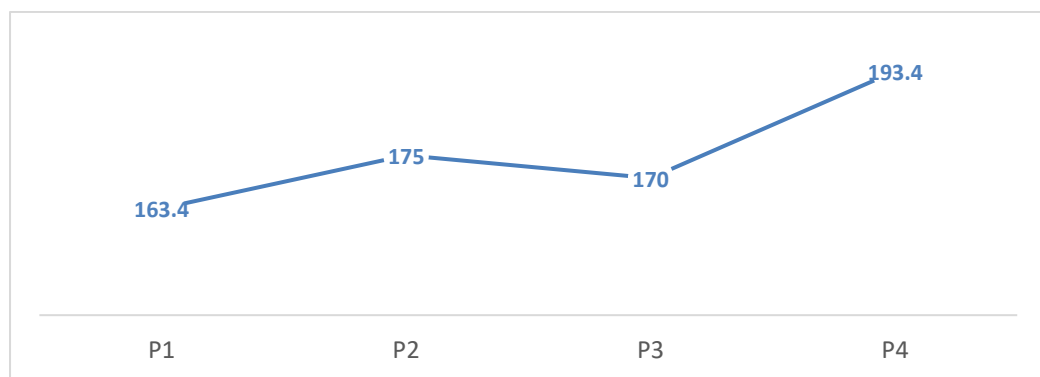


Figure 4. Effect of fertilizer types on dry fruit weight per plant

Figure 4 should present the average dry fruit weight obtained from each fertilizer treatment. The figure should show that P4 produced the highest mean dry fruit weight, followed by P2, P3, and P1. This trend indicates that the combination of NPK and biofertilizer was more effective in increasing fruit biomass than individual fertilizer application.

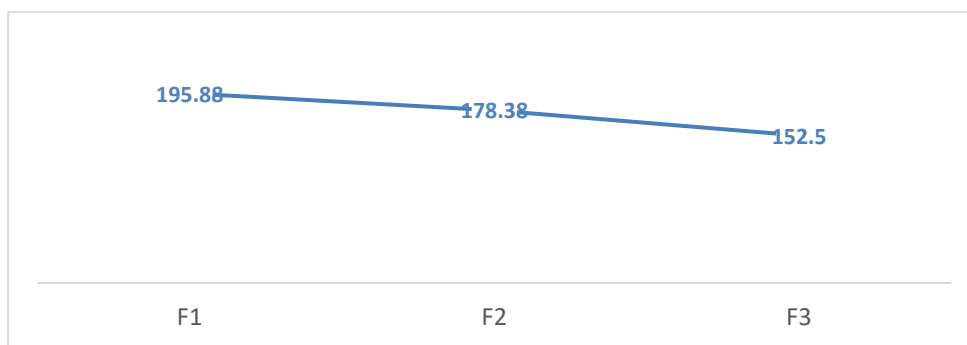


Figure 5. Effect of shading levels on dry fruit weight per plant

Figure 5 should present the effect of shading levels on dry fruit weight. The highest mean dry fruit weight was obtained under no shading, while the lowest was recorded under 60% shading. This result indicates that dry fruit production decreased as shading intensity increased. The reduction in dry fruit weight under shaded conditions may be associated with lower photosynthetic activity and reduced assimilate supply for fruit development.

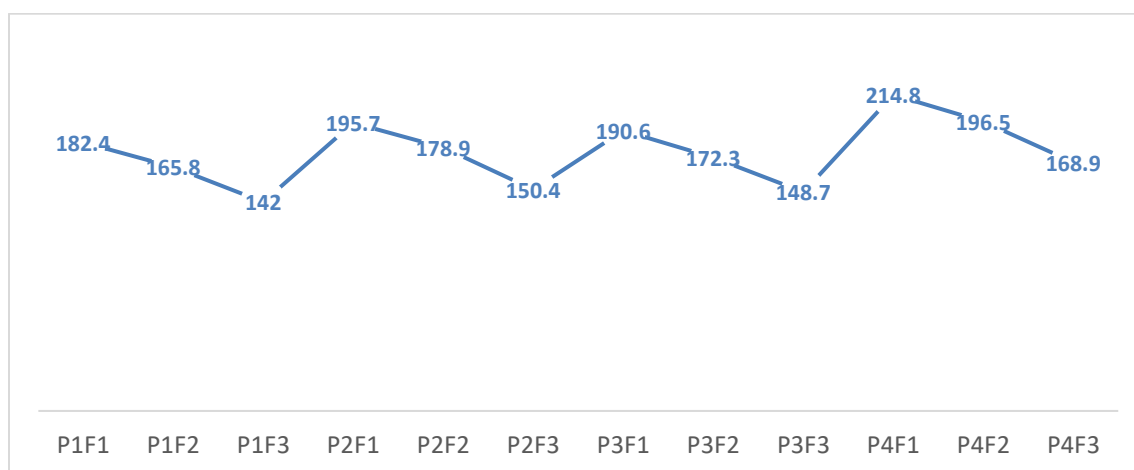


Figure 6. Interaction effect of fertilizer types and shading levels on dry fruit weight per plant

Figure 6 should display the interaction between fertilizer types and shading levels on dry fruit weight. The P4F1 combination produced the highest dry fruit weight, whereas P1F3 produced the lowest value. This interaction indicates that the positive effect of fertilization was maximized under no shading. In contrast, under 60% shading, dry fruit weight decreased across all fertilizer treatments, suggesting that light limitation reduced the plant's ability to convert nutrient availability into fruit biomass.

The dry fruit weight results demonstrate that yield formation in *P. retrofractum* is strongly influenced by the combined availability of nutrients and light. Adequate fertilization improves nutrient uptake and plant physiological activity, while sufficient light supports photosynthesis and assimilate accumulation. Therefore, the combination of NPK and biofertilizer under no shading was the most effective treatment for increasing dry fruit yield.

Piperine Content of Dried Fruits

Piperine content is a key quality parameter in *Piper retrofractum* because piperine is the major bioactive compound associated with pharmacological value, pungency, and industrial utilization. The results showed that fertilizer type, shading level, and their interaction affected piperine content in dried fruits.

Table 3. Effect of fertilizer types and shading levels on piperine content of dried fruits

Fertilizer Type	Shading Percentage			Mean
	F1	F2	F3	
P1	3.82cd	3.61de	3.28g	3.57de

P2	4.05b	3.74cd	3.36f	3.55e
P3	3.96bc	3.69d	3.32f	3.66d
P4	4.28a	3.98bc	3.54e	3.93bc
Mean	4.03a	3.76cd	3.38f	

Note: Values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 5% level.

As presented in Table 3, the highest piperine content was recorded in the P4F1 treatment, with 4.28%. The lowest piperine content was obtained from P1F3, with 3.28%. The mean values show that the combination of NPK and biofertilizer resulted in the highest average piperine content. This finding suggests that integrated nutrient management can support not only yield improvement but also the accumulation of bioactive compounds.

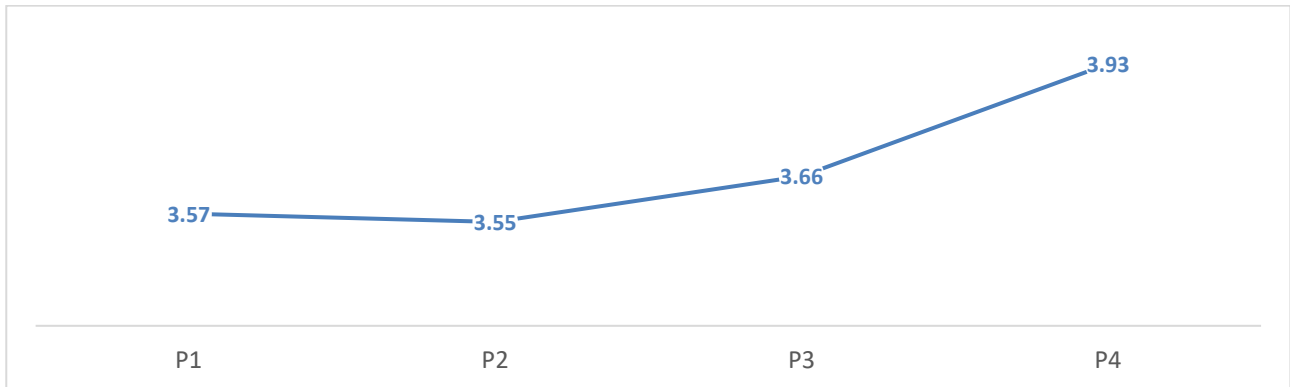


Figure 7. Effect of fertilizer types on piperine content of dried fruits

Figure 7 should show the mean piperine content under each fertilizer treatment. The figure should indicate that P4 produced the highest piperine content compared with the other fertilizer treatments. This pattern suggests that balanced nutrient availability and microbial activity may stimulate secondary metabolite biosynthesis in *P. retrofractum*.

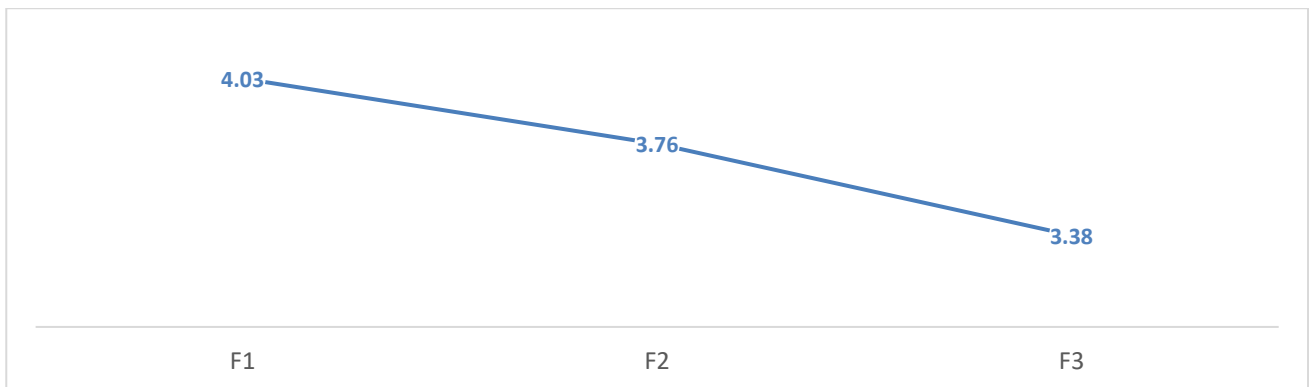


Figure 8. Effect of shading levels on piperine content of dried fruits

Figure 8 should present the effect of shading levels on piperine content. The highest piperine content was observed under no shading, while the lowest was found under 60% shading. This result indicates that piperine accumulation decreased with increasing shading intensity. Sufficient light may enhance photosynthesis and provide carbon precursors required for secondary metabolite biosynthesis, including alkaloid formation.

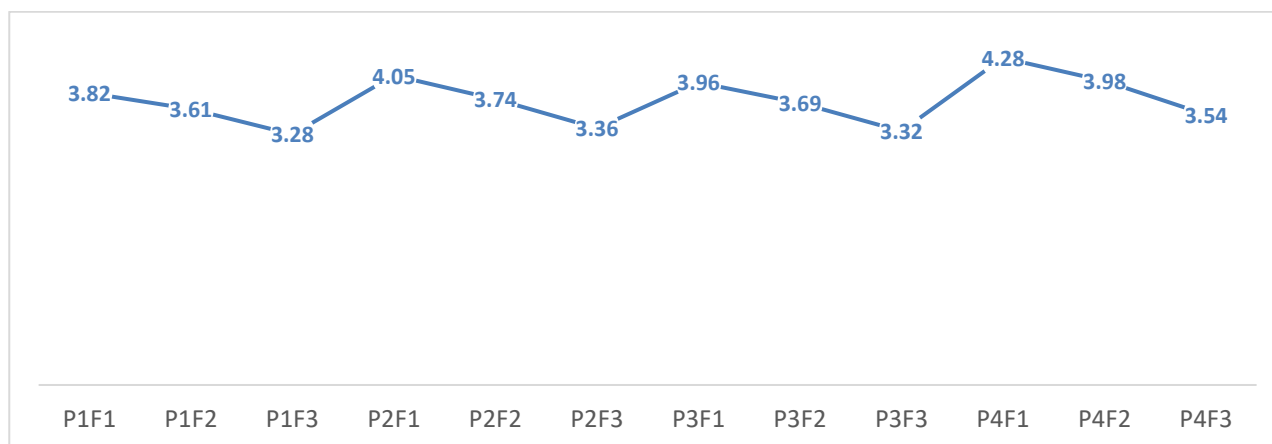


Figure 9. Interaction effect of fertilizer types and shading levels on piperine content of dried fruits

Figure 9 should illustrate the interaction between fertilizer type and shading level on piperine content. The P4F1 treatment produced the highest piperine content, while P1F3 produced the lowest. This interaction indicates that the highest piperine accumulation occurred when plants received both balanced fertilization and sufficient light exposure. Under higher shading levels, piperine content declined even when fertilizer was applied, suggesting that light availability is a limiting factor for secondary metabolite biosynthesis.

Overall, the piperine content results indicate that the quality of *P. retrofractum* dried fruits can be improved through appropriate nutrient and light management. The combination of NPK and biofertilizer under no shading was the most effective treatment for enhancing piperine accumulation.

Correlation among Productive Branches, Dry Fruit Weight, and Piperine Content

Correlation analysis was conducted to determine the relationship among productive branches, dry fruit weight, and piperine content. This analysis is important because it explains whether vegetative growth and yield components are associated with the quality of dried fruits.

Table 4. Correlation among productive branches, dry fruit weight, and piperine content

Variable	Productive Branches	Dry Fruit Weight (gram)	Piperine Content (%)
Productive branches	1.00	0.88**	0.61*
Dry fruit weight	0.88**	1.00	0.69**
Piperine content	0.61*	0.69**	1.00

Note: r = Pearson correlation coefficient; * significant at the 5% level; ** significant at the 1% level.

Table 4 shows that productive branches had a very strong positive correlation with dry fruit weight ($r = 0.88$). This indicates that plants with more productive branches tended to produce higher dry fruit weight. Productive branches also showed a moderate positive correlation with piperine content ($r = 0.61$), suggesting that better vegetative growth may support higher secondary metabolite accumulation. In addition, dry fruit weight had a strong positive correlation with piperine content ($r = 0.69$), indicating that higher fruit yield was associated with higher piperine content.

These results suggest that yield and quality traits in *P. retrofractum* are positively associated. The strong correlation between productive branches and dry fruit weight confirms that productive branch formation is a key determinant of yield potential. Meanwhile, the positive relationship between dry fruit weight and piperine content indicates that increased fruit production did not reduce phytochemical quality. Instead, favorable growth conditions may simultaneously enhance fruit biomass and piperine accumulation.

Regression Analysis of Piperine Content

Multiple regression analysis was conducted to determine the contribution of productive branches and dry fruit weight to piperine content. The regression model used in this study was:

$$Y = 1.84 + 0.021X_1 + 0.034X_2$$

where Y represents piperine content, X_1 represents the number of productive branches, and X_2

represents dry fruit weight per plant.

Table 5. Contribution of productive branches and dry fruit weight to piperine content

Variable	Regression Coefficient	Effect
Productive branches	0.021	Positive
Dry fruit weight	0.034	Positive

Table 5 shows that both productive branches and dry fruit weight had positive effects on piperine content. The regression coefficient of productive branches was 0.021, indicating that each additional productive branch increased piperine content by 0.021%. Meanwhile, the regression coefficient of dry fruit weight was 0.034, indicating that each 1 g increase in dry fruit weight increased piperine content by 0.034%.

The regression model was highly significant, with an F-value of 32.71 and $p < 0.01$. The coefficient of determination was $R^2 = 0.88$, indicating that 88% of the variation in piperine content could be explained by productive branches and dry fruit weight. The remaining 12% may be influenced by other factors, such as microclimate, soil nutrient availability, genetic variation, light-use efficiency, and other physiological processes. The higher regression coefficient of dry fruit weight indicates that fruit biomass had a stronger contribution to piperine content than productive branches.

Taken together, the regression results support the correlation findings by showing that growth and yield components are important predictors of piperine accumulation. These findings indicate that improving productive branch formation and dry fruit yield may contribute to the enhancement of piperine content in *P. retrofractum*.

Discussion

The discussion section demonstrates that fertilizer type, shading level, and their interaction significantly affected the growth, dry fruit yield, and piperine accumulation of *Piper retrofractum* under tropical agroecosystem conditions. The combination of NPK fertilizer and biofertilizer under full-light conditions consistently produced the highest number of productive branches, dry fruit weight, and piperine content, indicating that integrated nutrient management and adequate light availability function synergistically in optimizing both plant productivity and phytochemical quality. These findings support the concept of sustainable agronomic intensification in medicinal plant cultivation, where crop performance is enhanced through the integration of inorganic and biological nutrient inputs rather than reliance on a single fertilization source. Similar findings were reported by Zhang et al. (2021), who demonstrated that biofertilizers improve crop productivity through microbial community modulation and enhanced nutrient-use efficiency. Likewise, Kumar et al. (2022) explained that biofertilizers contribute to environmental sustainability while improving nutrient cycling and plant growth. The present findings therefore reinforce the view that combining NPK fertilizer with biological inputs provides a more balanced nutrient environment that supports vegetative growth and physiological activity in medicinal plants.

The higher number of productive branches obtained from the integrated fertilizer treatment confirms that nutrient availability strongly influences vegetative architecture and reproductive potential in *P. retrofractum*. Productive branches represent the primary sites of flower and fruit formation; consequently, their increase directly contributes to yield enhancement. This result is consistent with the findings of Rizvi et al. (2022), who reported that plant growth-promoting rhizobacteria stimulate vegetative growth and phytochemical accumulation in medicinal crops through phytohormone production and nutrient mobilization. Similarly, Timofeeva et al. (2023) emphasized that beneficial soil bacteria improve nutrient uptake through nitrogen fixation and phosphate solubilization, thereby supporting plant growth and metabolic processes. From a national perspective, Diana Nurus Sholehah & Millatul Ulya (2023) also highlighted that the cultivation performance of Javanese long pepper is strongly associated with soil fertility and appropriate environmental management. These studies collectively support the present result that balanced nutrient availability promotes productive

branch formation and improves reproductive performance in *P. retrofractum*.

The dry fruit weight findings further indicate that fruit biomass accumulation was highly dependent on the interaction between fertilization and light exposure. The highest dry fruit weight was observed under the combination of NPK and biofertilizer without shading, whereas severe shading significantly reduced fruit production across all fertilizer treatments. This finding aligns with Li et al. (2020), who explained that environmental conditions and nutrient availability jointly regulate biomass accumulation and secondary metabolite production in medicinal plants. Lu et al. (2021) also demonstrated that light deficiency inhibits plant growth by reducing photosynthetic efficiency and metabolic activity. In horticultural crops, Ali et al. (2024) reported that integrated nutrient management strategies improve biomass production only when environmental conditions support active photosynthesis and assimilate partitioning. Therefore, the present study confirms that nutrient availability alone is insufficient to maximize fruit production when light intensity becomes a limiting factor.

The effect of shading on plant performance was particularly evident in this study because increasing shading intensity consistently reduced productive branches, dry fruit weight, and piperine content. This result indicates that *P. retrofractum* under the studied tropical agroecosystem conditions responded more positively to full-light exposure than to moderate or heavy shading. The finding is consistent with Borbély et al. (2022), who explained that light intensity directly regulates photosynthetic efficiency, redox balance, and metabolic activity in plants. Likewise, Wu et al. (2025) reported that light availability dynamically controls plant growth and sugar metabolism, which subsequently affects biomass production and physiological performance. From an Indonesian context, Diana Nurus Sholehah & Millatul Ulya (2023) described *P. retrofractum* as a species that can tolerate semi-shaded conditions; however, the present study demonstrates that under integrated fertilization systems and tropical field conditions, full-light exposure generated superior agronomic responses. This discrepancy may be attributed to differences in genotype, environmental conditions, cultivation systems, and shading intensity applied across studies.

The increase in piperine content under no shading and integrated fertilization confirms the critical role of light and nutrient availability in secondary metabolite biosynthesis. Piperine is an alkaloid compound whose biosynthesis depends on sufficient carbon assimilation and nitrogen metabolism. Liu et al. (2023) explained that light regulates the biosynthesis of alkaloids, phenolics, and terpenoids by activating metabolic pathways associated with secondary metabolite formation. Similarly, Zhang et al. (2021) demonstrated that adequate light intensity enhances the accumulation of secondary metabolites in medicinal plants by increasing photosynthate availability and enzymatic activity. Qaderi et al. (2023) further emphasized that environmental factors such as light intensity significantly influence secondary metabolite production through physiological and biochemical regulation. These findings strongly support the present result that reduced shading promoted higher piperine accumulation because sufficient light likely increased photosynthetic activity and the availability of carbon precursors required for alkaloid biosynthesis.

The positive influence of integrated fertilization on piperine content also supports previous studies emphasizing the role of nutrient availability in phytochemical accumulation. Nitrogen is closely associated with amino acid synthesis, which serves as a precursor for nitrogen containing secondary metabolites such as alkaloids. Phosphorus contributes to energy transfer and ATP formation, while potassium regulates enzyme activation and physiological stability. Li et al. (2020), Yu et al. (2024) and Zhao et al. (2023) explained that secondary metabolite accumulation in medicinal plants is controlled by multilayered interactions between nutrient status and environmental factors. Likewise, Lv et al. (2024) reported that microorganisms associated with biofertilizers can stimulate secondary metabolite production through biochemical signaling and improved nutrient availability. The present findings therefore indicate that the combined use of NPK fertilizer and biofertilizer not only improved plant growth but also enhanced piperine biosynthesis in *P. retrofractum*.

The correlation analysis revealed that productive branches were strongly associated with dry fruit weight, while dry fruit weight was positively correlated with piperine content. These findings indicate that vegetative growth, yield formation, and phytochemical quality were positively integrated rather than antagonistic. In medicinal plant cultivation, increases in biomass are sometimes accompanied by reduced secondary metabolite concentration due to resource allocation trade offs. However, the present study demonstrates that optimal agronomic management can simultaneously improve yield and phytochemical quality. Similar findings were reported by Selwal et al. (2023), who stated that favorable environmental and nutritional conditions can stimulate both plant growth and secondary metabolite accumulation without necessarily creating physiological trade-offs. Venkatasai et al. (2025) also emphasized that environmental optimization strategies in medicinal plant cultivation may improve both biomass production and phytochemical concentration simultaneously.

The regression analysis further strengthened this interpretation by demonstrating that productive branches and dry fruit weight jointly explained 88% of the variation in piperine content. This high coefficient of determination indicates that vegetative and yield components are important predictors of phytochemical accumulation in *P. retrofractum*. The stronger regression coefficient of dry fruit weight suggests that fruit biomass contributed more substantially to piperine accumulation because piperine is concentrated in fruit tissues. This finding is important because it provides a practical agronomic indicator for predicting phytochemical quality in medicinal plant cultivation systems. Similar relationships between biomass accumulation and secondary metabolite production have been observed in medicinal plant studies reported by KC et al. (2022) and Venkatasai et al. (2025), both of whom highlighted the close interaction between physiological growth performance and metabolite biosynthesis.

The novelty of this study lies in its comprehensive integration of agronomic, physiological, and phytochemical analyses within a single experimental framework. Previous studies on *Piper retrofractum* have generally focused on pharmacological activity, phytochemical characterization, or individual cultivation factors. In contrast, the present study simultaneously evaluated fertilizer type, shading level, vegetative growth, dry fruit yield, piperine content, correlation patterns, and regression-based predictive relationships. This integrative approach provides a more holistic understanding of how nutrient management and light availability jointly regulate productivity and bioactive compound accumulation in *P. retrofractum* under tropical agroecosystem conditions. Moreover, the incorporation of correlation and regression analyses contributes additional scientific value because it quantitatively explains the interrelationship among growth, yield, and phytochemical quality variables, which has rarely been emphasized in previous *Piper* cultivation studies.

The practical implications of this study are substantial for medicinal plant cultivation and sustainable agricultural development. The findings suggest that the combined application of NPK fertilizer and biofertilizer under full-light conditions can be recommended as an effective cultivation strategy for improving both the quantity and quality of *P. retrofractum* production. For farmers, this strategy may increase dry fruit yield and economic profitability. For herbal and phytopharmaceutical industries, higher piperine content can improve raw material quality and support product standardization. From a sustainability perspective, the integration of biofertilizers with inorganic fertilizers may reduce excessive dependence on chemical inputs while maintaining productivity and soil health. Scientifically, the study also provides empirical evidence that productive branches and dry fruit weight can serve as practical agronomic indicators for estimating piperine accumulation in medicinal plant production systems.

Despite these contributions, this study has several limitations. First, the experiment was conducted at a single location and under one tropical agroecosystem condition, which may limit the generalizability of the findings across different environments, soil types, and climatic conditions. Second, only three shading levels and four fertilizer treatments were evaluated; therefore, the optimum fertilizer dosage and more precise light-intensity thresholds were not fully identified. Third, the study focused primarily on productive branches, dry fruit weight, and

piperine content, while other important physiological and biochemical variables, such as chlorophyll concentration, photosynthetic rate, nutrient uptake efficiency, soil microbial diversity, and metabolomic profiling, were not measured. Fourth, genetic variation and seasonal effects were not included in the experimental design, even though these factors may significantly influence piperine biosynthesis and plant adaptation. Future studies should therefore conduct multi-location and multi-season trials, incorporate different *P. retrofractum* genotypes, evaluate soil and plant physiological responses, and apply advanced metabolomic analyses to obtain a more comprehensive understanding of the mechanisms underlying piperine accumulation and agronomic performance in medicinal plant cultivation systems.

CONCLUSION

This study concludes that fertilizer type, shading level, and their interaction significantly influenced the growth, dry fruit yield, and piperine content of *Piper retrofractum* under tropical agroecosystem conditions. The combination of NPK and biofertilizer under no shading consistently produced the highest number of productive branches, dry fruit weight, and piperine content, indicating that balanced nutrient supply and sufficient light availability are essential for optimizing both productivity and phytochemical quality. The correlation analysis showed that productive branches were strongly associated with dry fruit weight, while dry fruit weight was positively related to piperine content, suggesting that improved vegetative and yield performance can support bioactive compound accumulation. The regression model further confirmed that productive branches and dry fruit weight jointly explained a high proportion of variation in piperine content, with dry fruit weight contributing more strongly. These findings demonstrate that integrated nutrient management combined with adequate light exposure can serve as an effective cultivation strategy for improving the yield and quality of *P. retrofractum*. Therefore, the combined use of NPK and biofertilizer under full-light conditions is recommended as a practical agronomic approach to support sustainable production of high-quality Javanese long pepper for herbal, pharmaceutical, and spice industries.

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