Agro Bali : Agricultural Journal
Vol. 9 No. 2: 302-316, July 2026

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v9i2.2708

Organic Fertilizer Source and Rate Determine Growth, Flower Yield, and Phytochemical Quality of Butterfly Pea on Ultisol

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Article history: submitted: March 17, 2026; accepted: July 28, 2026; available online: August 10, 2026

Abstract. Butterfly pea is increasingly valued as a functional flower crop because its petals are rich in flavonoids and anthocyanins, which are used as natural colorants and antioxidants. However, production on Ultisol is frequently constrained by poor soil fertility and limited nutrient availability. This study evaluated the effects of organic fertilizer source and application rate on vegetative growth, flowering, and phytochemical quality under greenhouse conditions. The study provides an integrated factorial assessment of these response domains across contrasting organic fertilizer sources and rates on Ultisol. A 3 × 4 completely randomized factorial design with three replications compared cattle manure, poultry manure, and water hyacinth bokashi at nominal as-received rates of 5, 10, 15, and 20 t ha^{-1}. Responses included vegetative traits, flowering time, flower number, total flavonoids, and anthocyanins. Fertilizer source, application rate, and their interaction significantly affected flower number, total flavonoid content, and anthocyanin content (all $P < 0.001$). Poultry manure at 15 t ha^{-1} produced 48.11 flowers plant^{-1}, 27.00 mg mL^{-1} QE total flavonoids, and 1.65 mg 100 g^{-1} anthocyanins; flower number was 32.0% greater than with cattle manure at the same rate. Increasing poultry manure from 15 to 20 t ha^{-1} reduced flower number, total flavonoids, and anthocyanins by 20.1%, 43.5%, and 43.0%, respectively. Rate responses were non-monotonic and fertilizer-source-dependent. Poultry manure at 15 t ha^{-1} was the best-performing treatment among those evaluated for balancing flower production and phytochemical quality. These findings support source-specific organic nutrient management of butterfly pea on acidic Ultisols, although multi-location field trials and nutrient-equivalent comparisons are needed before broad recommendations can be made.

Keywords: anthocyanin; *Clitoria ternatea*; flavonoid; organic fertilizer; Ultisol

1. Introduction

Butterfly pea (*Clitoria ternatea* L.) is a multifunctional tropical legume with increasing agronomic and economic value. Its tropical adaptation and symbiotic nitrogen fixation support intercropping, crop rotation, cover cropping, and other low-input farming systems (Oguis et al., 2019). However, flower productivity and quality remain strongly influenced by soil fertility, particularly in degraded acidic soils (Jamil et al., 2018).

Beyond its agronomic role, butterfly pea has become increasingly important as a functional crop because its flowers are rich in anthocyanins and flavonoids, which are widely used as natural colorants and antioxidants. Its ternatin-based blue pigmentation is valuable to the food, cosmetic, functional-food, and plant-based health industries (Gonçalves et al., 2024; Oguis et al., 2019; Prado et al., 2019; Zeng et al., 2025). Cultivation strategies must therefore improve both flower yield and phytochemical quality, directly benefiting flower and medicinal-plant growers, organic farming practitioners, natural-colorant processors, and sustainable agriculture programs.

A major constraint to butterfly pea production in many tropical regions is the predominance of acidic soils, particularly Ultisols, which commonly have low base saturation and cation exchange capacity, high exchangeable Al, and strong phosphorus fixation (Muktamar et al., 2020; Pal et al., 2014; Regasa et al., 2025; Zhou et al., 2021). These constraints reduce root function and nutrient availability, requiring fertility management that improves nutrient supply and alleviates acidity-related limitations.

Organic fertilizers are widely regarded as a relevant approach for crop production on Ultisols. Studies on acidic soils show that organic amendments can increase soil

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v9i2.2708

carbon, improve nutrient retention, influence nutrient transformations, and alleviate some acidity-related constraints (Luo et al., 2024; Nkoh et al., 2023; Regasa et al., 2025). Their effectiveness, however, depends on fertilizer composition, C/N ratio, decomposition characteristics, application rate, and synchronization between nutrient release and crop demand. Fertilizer source and rate should therefore be evaluated jointly.

Plant responses to organic fertilizers are strongly influenced by the chemical characteristics of the applied materials. Differences in nutrient concentration, C/N ratio, and decomposition behavior alter nutrient-release patterns, so equal material rates do not necessarily provide equivalent amounts of plant-available nutrients (Geisseler et al., 2021; Lazicki et al., 2020; Smith et al., 2024). Poultry manure, cattle manure, and plant-based bokashi may therefore produce contrasting vegetative, reproductive, and phytochemical responses; on Ultisol, poultry manure may release available P more rapidly than cattle manure (Muktamar et al., 2020; Sant'Anna et al., 2024).

Nitrogen and phosphorus influence vegetative growth, reproductive development, and secondary-metabolite accumulation. Nitrogen supports shoot and meristem development, although excessive nitrate may suppress nodulation and biological N fixation in legumes (Ferguson et al., 2019; Jiang et al., 2020; Landrein et al., 2018). Phosphorus supports root growth, energy transfer, and flowering but is often poorly available in acidic soils (Baquy et al., 2022; Muktamar et al., 2020). Nutrient supply can also modify flavonoid and anthocyanin accumulation in a non-linear, context-dependent manner (Jia et al., 2025; Raza et al., 2024; Yan et al., 2024). Thus, fertilizer management should balance vegetative, reproductive, and phytochemical responses rather than maximize nutrient input alone.

Despite growing interest in butterfly pea as a natural pigment crop, previous fertilization studies have generally evaluated only selected response domains. Available studies have examined urea rate and productivity (Wardi et al., 2023), growing media and organic fertilizer effects on growth and flower yield (Maharani et al., 2024), or P and Mo supplementation and production performance (Suharto et al., 2024). None compared multiple organic fertilizer sources across a common rate gradient while simultaneously assessing vegetative growth, flowering, flower production, and phytochemical quality under Ultisol conditions. Consequently, recommendations based only on growth or yield may overlook treatment effects on the phytochemical quality that determines the crop's functional value.

Accordingly, this study addressed the following research question: Which organic fertilizer source–rate combination constitutes the best-performing treatment for balancing vegetative growth, flower production, and phytochemical quality of butterfly pea grown on Ultisol? We hypothesized that poultry manure applied at an intermediate rate would be the best-performing treatment among the tested combinations. The objective was to determine the individual and interactive effects of cattle manure, poultry manure, and water hyacinth bokashi applied at 5, 10, 15, and 20 t ha^{-1} on vegetative growth, flowering time, flower number, total flavonoids, and anthocyanins under greenhouse conditions using a 3 × 4 factorial completely randomized design. To our knowledge, this is among the first integrated factorial evaluations of vegetative, reproductive, and phytochemical responses of Ultisol-grown butterfly pea across contrasting organic fertilizer sources and a common rate gradient. By testing whether the treatment maximizing vegetative growth also maximizes flower production and phytochemical quality, the study provides a more precise basis for nutrient management by growers, organic farming practitioners, natural-colorant and functional-food industries, and sustainable agriculture programs.

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v9i2.2708

2. Materials and Methods

The study was conducted from June to September 2024 in a greenhouse at the Department of Agronomy, Faculty of Agriculture, University of Lambung Mangkurat, Banjarbaru, Indonesia (3°26′44.55″S, 114°50′44.95″E). Butterfly pea seeds were collected from a single mother plant, and one seedling was maintained per polybag. This reduced genetic heterogeneity but limited inference to that maternal source.

Plants were grown in 35 × 35 cm polybags containing 10 kg of air-dried, sieved, and homogenized Ultisol collected from the 0–20 cm layer. Before treatment application, each polybag received 10 g dolomite polybag^{-1} (approximately 2 t ha^{-1}) to ameliorate acidity and reduce potential Al toxicity. No inorganic fertilizer was applied.

Before amendment, the soil contained 68.73% sand, 29.16% silt, and 2.11% clay; pH 4.53; organic C 0.90%; total N 0.11%; Bray-I available P 2.96 mg kg^{-1}; exchangeable K 0.13 cmol(+) kg^{-1}; cation exchange capacity 9.22 cmol(+) kg^{-1}; base saturation 25.81%; and exchangeable Al 1.25 cmol(+) kg^{-1}. Soil pH was measured in a 1:5 soil-to-water suspension, organic C by Walkley–Black, total N by Kjeldahl, available P by Bray-I, exchangeable bases and cation exchange capacity with 1 N NH_4OAc at pH 7, and exchangeable Al by KCl extraction and titration.

The first factor comprised cattle manure (CA), poultry manure (PO), and water hyacinth bokashi (WHB); their chemical properties are presented in Table 1. Fertilizers were compared at equal nominal material rates, not nutrient-equivalent rates. A 3 × 4 completely randomized factorial design combined the three fertilizer sources with four rates (5, 10, 15, and 20 t ha^{-1}), producing 12 treatment combinations. Rates were calculated on an as-received basis because fertilizer moisture was not measured; thus, equal nominal rates did not necessarily provide equal dry-matter or nutrient inputs. Each treatment combination had three replications, each comprising three plant subsamples. Subsample values were averaged, so analyses used 36 independent experimental-unit means from 108 plants. Fertilizers were incorporated two weeks before planting. Irrigation was applied twice daily for the first 10 days and once daily thereafter; weeds and pests were managed manually and uniformly. Vegetative traits (leaf number, stem length, stem diameter, and branch number) were measured at 4, 5, and 6 weeks after planting (WAP). Reproductive variables were days to first flowering and flower number per plant.

Fertilizer total N, P, and K were measured after H_2SO_4 wet digestion, organic C by Walkley–Black, and pH in a 1:5 (w/v) suspension. Moisture content, electrical conductivity, cation exchange capacity, and micronutrients were not determined. Flowers were harvested at a uniform developmental stage (Oguis et al., 2019; Zeng et al., 2025). Total flavonoids were measured by the $AlCl_3$ colorimetric method using quercetin as the standard (Wahyanto & Agustini, 2024), and anthocyanins were measured by the pH differential method at 520 nm with 700 nm correction (Chusak et al., 2018; Santana & Ribeiro, 2025). Results were expressed as mg mL^{-1} quercetin equivalents and mg 100 g^{-1} sample, respectively.

Replicate means were analyzed by factorial ANOVA using:

$$Y_{ijk} = \mu + S_i + R_j + (S \times R)_{ij} + \varepsilon_{ijk} \ldots (1)$$

where Y_{ijk} is the experimental-unit mean, μ is the overall mean, S_i is the effect of the fertilizer source, R_j is the effect of the application rate, $(S \times R)_{ij}$ is the fertilizer source × application rate interaction, and ε_{ijk} is the random error associated with the replicate k. Measurements at 4, 5, and 6 WAP were analyzed separately. Residual normality and variance homogeneity were assessed using Shapiro–Wilk and Brown–Forsythe tests, supported by Q–Q and residual-versus-fitted plots; no observations were excluded. Shapiro–Wilk and Brown–Forsythe *P*-values ranged from 0.055 to 0.599 and from 0.619 to 0.999, respectively. Significant interactions

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v9i2.2708

were interpreted using Tukey HSD simple-effect comparisons based on the pooled residual mean square; otherwise, marginal means were compared. Significance was set at $P < 0.05$, and analyses used pandas, SciPy, and statsmodels in Python.

The greenhouse conditions, 10-kg polybags, one Ultisol source, one season, and one maternal seed source strengthened internal comparability but limited field-scale and genetic inference.

3. Results and Discussion

3.1 Chemical characteristics of organic fertilizers

Chemical analysis showed clear differences among the three organic fertilizers in terms of nutrient composition and C/N ratio (Table 1). Poultry manure had the highest organic C (14.53%), total N (2.41%), total P (3.92%), and total K (0.57%) concentrations, together with the lowest C/N ratio (6.03). Its total N and total P concentrations were approximately 2.5 and 7.7 times those of cattle manure, respectively. Water hyacinth bokashi had the highest C/N ratio (10.73), whereas cattle manure had the lowest total P and total K concentrations. Because fertilizers were applied at equal nominal as-received rates and their moisture contents were not measured, the treatments were not necessarily equivalent in dry-matter or nutrient inputs.

Table 1. Selected chemical properties of the organic fertilizers used in the study.

Fertilizer type	pH	C-org (%)	N-total (%)	C/N	P-total (%)	K-total (%)
Cattle manure	7.31	9.79	0.98	10.03	0.51	0.19
Poultry manure	7.88	14.53	2.41	6.03	3.92	0.57
Water hyacinth bokashi	7.33	11.84	1.10	10.73	1.90	0.44

Note: Values are laboratory measurements of the analyzed fertilizer materials. Moisture content, electrical conductivity, cation exchange capacity, and micronutrient concentrations were not determined. Application rates were calculated on an as-received weight basis.

The contrasting chemical characteristics of the organic fertilizers provide a plausible basis for understanding the differential responses of butterfly pea grown on Ultisol. The lower C/N ratio and greater measured N and P concentrations of poultry manure are consistent with potentially more rapid nutrient release, whereas the wider C/N ratio of water hyacinth bokashi may support a more gradual release pattern. The C/N ratio of organic amendments can influence microbial biomass development and N mineralization, and evidence from Ultisol incubation indicates that poultry manure may release available P more rapidly than cattle manure (Muktamar et al., 2020; Smith et al., 2024). However, temporal soil nutrients, plant nutrient uptake, and fertilizer moisture contents were not measured. Fertilizer composition therefore provides explanatory context but does not demonstrate nutrient-release dynamics or actual nutrient delivery to the plants.

This interpretation is particularly relevant to the experimental Ultisol, which before treatment had a pH of 4.53, organic C of 0.90%, total N of 0.11%, Bray-I available P of 2.96 mg kg^{-1}, exchangeable K of 0.13 cmol(+) kg^{-1}, a cation exchange capacity of 9.22 cmol(+) kg^{-1}, base saturation of 25.81%, and exchangeable Al of 1.25 cmol(+) kg^{-1}. These measured properties indicate strong acidity, low nutrient-retention capacity, and very low available P. Although dolomite was applied uniformly, post-application soil chemical properties were not measured; therefore, the potential improvement in acidity and P availability remains contextual rather than a demonstrated treatment pathway (Regasa et al., 2025).

Poultry and cattle manures should not be treated as equivalent organic inputs because

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v9i2.2708

their effects may extend beyond total nutrient concentration to changes in soil chemistry and microbial-community structure (Sant'Anna et al., 2024). In acidic soil, organic fertilizer can also increase N mineralization and nitrification, potentially increasing both plant-available N and nitrate loss risk (Luo et al., 2024). However, mineralization, leaching, microbial activity, and nutrient balances were not measured. Because treatments were applied at equal as-received material rates, source effects cannot be fully separated from possible differences in moisture, dry-matter input, and N, P, and K loading.

3.2 Vegetative growth responses

Residual diagnostics supported the assumptions of factorial ANOVA across all response variables (Shapiro–Wilk *P*-values = 0.055–0.599; Brown–Forsythe *P*-values = 0.619–0.999), and no observations were excluded. Relatively large studentized residuals represented valid observations and did not materially affect the balanced analysis.

For leaf number, the fertilizer source × rate interaction was not significant at 4 WAP ($P = 0.094$) or 5 WAP ($P = 0.156$), but was significant at 6 WAP, $F(6, 24) = 8.05$, $P < 0.001$ (Table 2). At 4 WAP, fertilizer source ($P = 0.024$) and rate ($P = 0.008$) had significant main effects, whereas only rate was significant at 5 WAP (P = 0.004). At 6 WAP, poultry manure produced 25.67 and 26.89 leaves plant^{-1} at 10 and 20 t ha^{-1}, respectively, compared with 15.56 leaves plant^{-1} under water hyacinth bokashi at 15 t ha^{-1} (Figure 1A).

Table 2. Leaf number of butterfly pea at 4, 5, and 6 WAP as affected by organic fertilizer source, application rate, and their interaction.

Source	Rate (t ha^{-1})	4 WAP	5 WAP	6 WAP
CA	5	5.11 ± 0.29	10.11 ± 0.48	17.56 ± 0.62 ^{bB}
CA	10	5.89 ± 0.48	12.56 ± 0.87	21.22 ± 0.78 ^{aC}
CA	15	4.33 ± 0.33	11.00 ± 0.51	20.11 ± 0.48 ^{aA}
CA	20	5.44 ± 0.29	12.11 ± 0.59	22.33 ± 0.38 ^{aB}
PO	5	5.67 ± 0.19	11.00 ± 0.77	20.89 ± 0.89 ^{bA}
PO	10	5.67 ± 0.33	13.33 ± 1.17	25.67 ± 0.51 ^{aA}
PO	15	5.11 ± 0.22	10.56 ± 1.09	19.78 ± 0.62 ^{bA}
PO	20	6.78 ± 0.29	14.56 ± 1.39	26.89 ± 0.59 ^{aA}
WHB	5	5.44 ± 0.29	11.56 ± 0.99	18.44 ± 0.59 ^{cB}
WHB	10	5.56 ± 0.62	13.00 ± 1.17	23.33 ± 0.51 ^{aB}
WHB	15	4.89 ± 0.29	9.44 ± 0.48	15.56 ± 0.62 ^{dB}
WHB	20	4.89 ± 0.11	10.33 ± 0.38	20.89 ± 0.22 ^{bB}
ANOVA *P*: source		0.024	0.131	<0.001
ANOVA *P*: rate		0.008	0.004	<0.001
ANOVA *P*: source × rate		0.094	0.156	<0.001
CV (%)		10.84	13.20	4.86

Note: Values are means ± SE of three experimental units. CA = cattle manure; PO = poultry manure; WHB = water hyacinth bokashi. Rates are expressed on an as-received material-weight basis. For significant source × rate interactions, lowercase letters compare rates within a fertilizer source and uppercase letters compare sources within a rate according to Tukey HSD at $P < 0.05$. Letters are omitted for nonsignificant interactions; significant main effects are described in the text. ANOVA *P*-values are from the two-way factorial model.

Stem length showed a significant fertilizer source × rate interaction at 4, 5, and 6 WAP (all $P < 0.001$; Table 3), indicating that rate rankings differed among fertilizer sources. At 6 WAP, poultry manure produced stem lengths of

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v9i2.2708

163.67 and 170.00 cm at 10 and 20 t ha^{-1}, respectively. Cattle manure reached its highest mean at 15 t ha^{-1} (145.83 cm), whereas water hyacinth bokashi decreased to 115.78 cm at 15 t ha^{-1} and increased to 148.28 cm at 20 t ha^{-1}. These crossing response profiles are illustrated in Figure 1B.

Table 3. Stem length (cm) of butterfly pea at 4, 5, and 6 WAP as affected by organic fertilizer source, application rate, and their interaction.

Source	Rate (t ha^{-1})	4 WAP	5 WAP	6 WAP
CA	5	51.67 ± 0.51 ^{cC}	86.56 ± 0.64 ^{cB}	131.78 ± 1.24 ^{cB}
CA	10	59.61 ± 1.02 ^{bB}	96.00 ± 1.29 ^{bC}	138.67 ± 1.35 ^{bC}
CA	15	65.94 ± 0.82 ^{aA}	95.44 ± 1.26 ^{bB}	145.83 ± 2.43 ^{aA}
CA	20	64.56 ± 0.91 ^{aB}	106.94 ± 0.75 ^{aB}	138.22 ± 1.93 ^{bC}
PO	5	57.78 ± 0.62 ^{cA}	99.28 ± 0.65 ^{cA}	139.83 ± 1.69 ^{cA}
PO	10	66.39 ± 0.39 ^{bA}	115.94 ± 1.66 ^{bA}	163.67 ± 1.29 ^{bA}
PO	15	60.28 ± 0.72 ^{cB}	99.94 ± 0.91 ^{cA}	145.83 ± 1.09 ^{cA}
PO	20	76.11 ± 1.06 ^{aA}	127.06 ± 0.78 ^{aA}	170.00 ± 1.58 ^{aA}
WHB	5	54.56 ± 0.71 ^{bB}	98.44 ± 0.59 ^{bA}	127.78 ± 1.49 ^{bB}
WHB	10	64.44 ± 0.87 ^{aA}	103.78 ± 1.54 ^{aB}	154.06 ± 1.88 ^{aB}
WHB	15	38.33 ± 0.69 ^{dC}	72.17 ± 1.44 ^{dC}	115.78 ± 1.31 ^{cB}
WHB	20	48.67 ± 0.25 ^{cC}	93.50 ± 1.17 ^{cC}	148.28 ± 1.06 ^{aB}
ANOVA *P*: source		<0.001	<0.001	<0.001
ANOVA *P*: rate		<0.001	<0.001	<0.001
ANOVA *P*: source × rate		<0.001	<0.001	<0.001
CV (%)		2.21	1.95	1.90

Note: Statistical notation follows Table 2.

Table 4. Stem diameter (mm) of butterfly pea at 4, 5, and 6 WAP as affected by organic fertilizer source, application rate, and their interaction.

Source	Rate (t ha^{-1})	4 WAP	5 WAP	6 WAP
CA	5	2.78 ± 0.11	4.44 ± 0.11	5.78 ± 0.11
CA	10	3.33 ± 0.19	4.33 ± 0.19	5.56 ± 0.11
CA	15	2.67 ± 0.19	3.67 ± 0.19	5.56 ± 0.22
CA	20	2.56 ± 0.11	3.78 ± 0.40	5.44 ± 0.40
PO	5	2.44 ± 0.29	4.67 ± 0.19	6.33 ± 0.19
PO	10	3.67 ± 0.19	4.22 ± 0.29	6.33 ± 0.19
PO	15	2.11 ± 0.11	3.61 ± 0.06	5.89 ± 0.11
PO	20	3.00 ± 0.19	4.67 ± 0.19	7.22 ± 0.29
WHB	5	3.11 ± 0.20	4.11 ± 0.22	5.67 ± 0.19
WHB	10	3.00 ± 0.51	4.00 ± 0.19	5.89 ± 0.29
WHB	15	2.56 ± 0.22	4.00 ± 0.19	5.22 ± 0.29
WHB	20	2.44 ± 0.22	3.89 ± 0.29	5.89 ± 0.29
ANOVA *P*: source		0.946	0.178	<0.001
ANOVA *P*: rate		<0.001	0.016	0.033
ANOVA *P*: source × rate		0.064	0.128	0.112
CV (%)		14.59	9.57	7.11

Note: Statistical notation follows Table 2.

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v9i2.2708

The fertilizer source × rate interaction was not significant for stem diameter at 4, 5, or 6 WAP (P = 0.064, 0.128, and 0.112, respectively; Table 4). Application rate had a significant main effect at all observation times, whereas fertilizer source was significant only at 6 WAP (P < 0.001). At 6 WAP, poultry manure averaged 6.44 mm, compared with 5.58 mm under cattle manure and 5.67 mm under water hyacinth bokashi. Although poultry manure at 20 t ha^{-1} had the largest cell mean (7.22 mm), the nonsignificant interaction precludes interpreting it as a source-specific rate response (Figure 1C).

For branch number, the fertilizer source × rate interaction was not significant at 4 WAP (P = 0.213), but was significant at 5 WAP (P = 0.003) and 6 WAP (P = 0.007; Table 5). At 4 WAP, fertilizer source (P = 0.004) and rate (P < 0.001) had significant main effects. At 6 WAP, poultry manure at 10 t ha^{-1} produced 6.00 branches plant^{-1}, more than twice the 2.56 branches plant^{-1} produced by water hyacinth bokashi at 15 t ha^{-1} (Figure 1D).

Table 5. Branch number of butterfly pea at 4, 5, and 6 WAP as affected by organic fertilizer source, application rate, and their interaction.

Source	Rate (t ha^{-1})	4 WAP	5 WAP	6 WAP
CA	5	1.00 ± 0.00	1.67 ± 0.19 ^{bA}	3.00 ± 0.19 ^{bB}
CA	10	1.44 ± 0.11	3.00 ± 0.19 ^{aAB}	4.67 ± 0.19 ^{aB}
CA	15	1.11 ± 0.11	1.89 ± 0.11 ^{bA}	4.33 ± 0.33 ^{aA}
CA	20	1.33 ± 0.19	2.33 ± 0.19 ^{abB}	4.33 ± 0.19 ^{aAB}
PO	5	1.00 ± 0.00	2.11 ± 0.29 ^{bA}	4.44 ± 0.40 ^{bA}
PO	10	1.56 ± 0.11	3.11 ± 0.29 ^{aA}	6.00 ± 0.19 ^{aA}
PO	15	0.89 ± 0.11	1.44 ± 0.22 ^{bA}	4.28 ± 0.31 ^{bA}
PO	20	1.44 ± 0.11	3.22 ± 0.11 ^{aA}	5.11 ± 0.22 ^{abA}
WHB	5	1.00 ± 0.19	1.67 ± 0.19 ^{abA}	3.33 ± 0.33 ^{bcB}
WHB	10	1.11 ± 0.11	2.33 ± 0.19 ^{aB}	5.00 ± 0.19 ^{aB}
WHB	15	0.78 ± 0.11	1.44 ± 0.11 ^{bA}	2.56 ± 0.22 ^{cB}
WHB	20	0.89 ± 0.11	1.44 ± 0.11 ^{bC}	4.11 ± 0.22 ^{abB}
ANOVA *P*: source		0.004	<0.001	<0.001
ANOVA *P*: rate		<0.001	<0.001	<0.001
ANOVA *P*: source × rate		0.213	0.003	0.007
CV (%)		18.40	15.80	10.56

Note: Statistical notation follows Table 2.

The factorial ANOVA revealed both additive main effects and fertilizer source-dependent responses to application rate. Significant fertilizer source × rate interactions were observed for leaf number at 6 WAP, stem length at all observation times, and branch number at 5 and 6 WAP, whereas stem diameter showed no significant interaction. Increasing fertilizer rate therefore did not produce uniformly proportional growth responses; for interacting traits, profiles crossed or changed rank, showing that the direction and magnitude of the rate response depended on fertilizer source.

The measured fertilizer composition provides a plausible context for these vegetative responses, but it does not establish causation. Poultry manure had higher measured total N and total P concentrations than the other sources, and N availability can influence shoot-meristem activity through cytokinin-related processes (Landrein et al., 2018). In legumes, increasing external nitrate may also restrict nodulation and biological N fixation (Ferguson et al., 2019; Jiang et al., 2020). Because plant N status, chlorophyll, hormones, nodulation, and N fixation were not measured, these mechanisms remain hypotheses.

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v9i2.2708

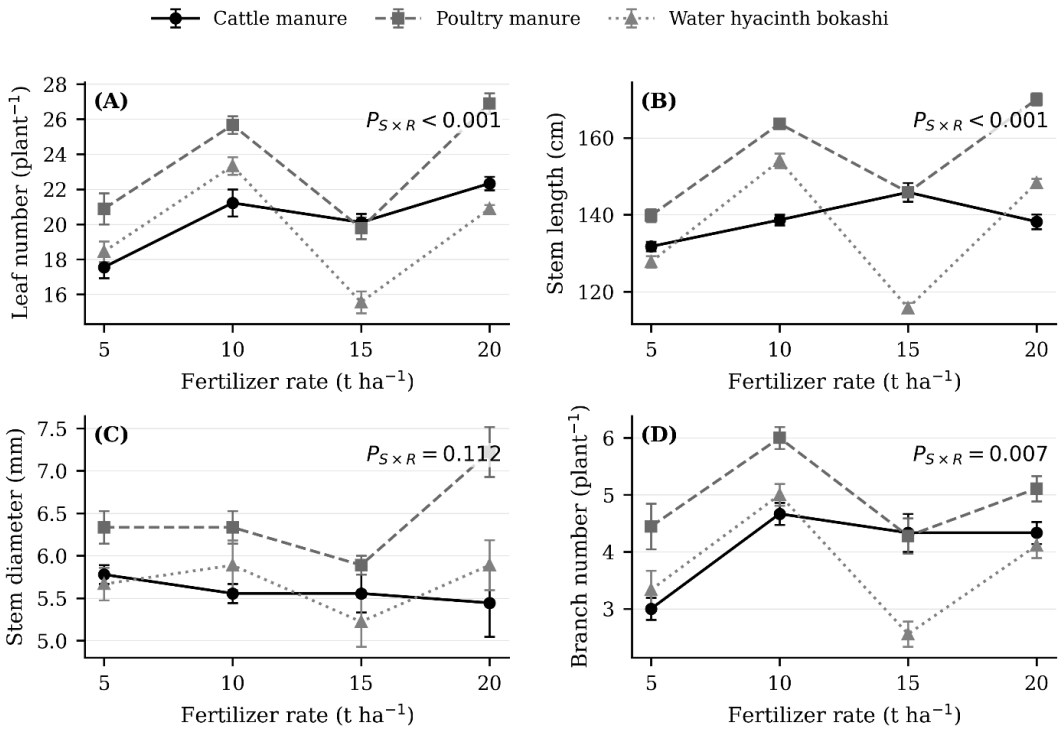

Figure 1. Fertilizer source × application rate interaction profiles for vegetative traits of butterfly pea at 6 WAP: leaf number (A), stem length (B), stem diameter (C), and branch number (D). Points represent the means ± SE of three experimental units; the interaction *P*-value from the factorial ANOVA is shown in each panel.

The divergence among vegetative traits is itself informative. Poultry manure at 20 t ha^{-1} produced the greatest leaf number and stem length and the largest cell mean for stem diameter at 6 WAP, whereas branch number under poultry manure peaked at 10 t ha^{-1}. Thus, greater axial and foliar growth did not consistently increase branching, demonstrating that vegetative traits differed in their response to fertilizer rate. The underlying physiological processes cannot be determined from morphological measurements alone.

This non-linearity is important because it indicates that the agronomic value of an organic fertilizer cannot be inferred from application rate alone. Differences among fertilizer sources may partly reflect contrasting C/N ratio and nutrient-release timing. Organic-material C and N contents and their ratio can regulate microbial biomass development and N mineralization, while coarse soil texture can influence moisture retention, decomposition, and the physical protection of organic substrates (Geisseler et al., 2024; Smith et al., 2024). However, temporal mineralization and nutrient leaching were not measured; the results demonstrate source-dependent rate responses, not the mechanisms producing them.

A recent field study on *C. ternatea* similarly reported a non-monotonic response to NPK, with moderate rates providing a more stable balance between vegetative performance and flavonoid accumulation. However, several flowering and yield traits were not significantly affected in that study (Fatika et al., 2026). This combination of agreement and conflict indicates that fertilizer responses depend on fertilizer form, soil properties, genotype, climate, and experimental environment.

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v9i2.2708

3.3 Flowering response and phytochemical accumulation

Flowering time was governed by a significant fertilizer source × rate interaction, $F(6, 24) = 8.16$, $P < 0.001$ (Table 6). The earliest means occurred with poultry manure at 20 t ha^{-1} (50.22 DAP) and water hyacinth bokashi at 10 t ha^{-1} (50.33 DAP), whereas the latest flowering occurred with water hyacinth bokashi at 5 t ha^{-1} (61.44 DAP). The crossing profiles indicate that the effect of application rate on flowering time depended on fertilizer source (Figure 2A).

Within cattle manure, 20 t ha^{-1} produced earlier flowering than 10 t ha^{-1}; within poultry manure, 20 t ha^{-1} flowered earlier than 5 t ha^{-1}; and within water hyacinth bokashi, 10 t ha^{-1} flowered earlier than the other three rates according to Tukey HSD. These source-specific differences confirm that no single rate produced the earliest flowering across all fertilizer sources.

Table 6. Flowering time, flower number, total flavonoid content, and anthocyanin content of butterfly pea as affected by organic fertilizer source, application rate, and their interaction.

Source	Rate (t ha^{-1})	Flowering time (DAP)	Flower number (plant^{-1})	Total flavonoids (mg mL^{-1} QE)	Anthocyanins (mg 100 g^{-1})
CA	5	55.56 ± 0.29 ^{abB}	41.78 ± 1.28 ^{aA}	23.25 ± 0.18 ^{bB}	1.32 ± 0.02 ^{bB}
CA	10	60.33 ± 0.33 ^{aA}	35.56 ± 1.06 ^{bA}	20.80 ± 0.28 ^{cB}	1.10 ± 0.02 ^{dB}
CA	15	55.11 ± 0.22 ^{abB}	36.44 ± 1.46 ^{bB}	24.88 ± 0.19 ^{aB}	1.64 ± 0.01 ^{aA}
CA	20	52.50 ± 0.48 ^{bB}	37.06 ± 1.20 ^{bA}	19.50 ± 0.46 ^{dA}	1.20 ± 0.01 ^{cA}
PO	5	58.11 ± 0.91 ^{aAB}	44.44 ± 0.68 ^{aA}	24.88 ± 0.17 ^{bA}	1.60 ± 0.01 ^{aA}
PO	10	52.33 ± 1.07 ^{bcB}	36.22 ± 1.06 ^{bA}	25.75 ± 0.25 ^{bA}	1.63 ± 0.01 ^{aA}
PO	15	56.33 ± 2.83 ^{abAB}	48.11 ± 0.59 ^{aA}	27.00 ± 0.18 ^{aA}	1.65 ± 0.04 ^{aA}
PO	20	50.22 ± 1.37 ^{cB}	38.44 ± 0.56 ^{bA}	15.25 ± 0.13 ^{cB}	0.94 ± 0.02 ^{bC}
WHB	5	61.44 ± 2.74 ^{aA}	33.00 ± 1.45 ^{bB}	15.12 ± 0.12 ^{cC}	0.91 ± 0.03 ^{bC}
WHB	10	50.33 ± 0.84 ^{bB}	28.44 ± 1.13 ^{cB}	19.38 ± 0.19 ^{abC}	1.04 ± 0.02 ^{aB}
WHB	15	60.33 ± 1.71 ^{aA}	35.22 ± 0.56 ^{abB}	18.88 ± 0.45 ^{bC}	0.93 ± 0.01 ^{bB}
WHB	20	58.11 ± 0.95 ^{aA}	38.67 ± 1.35 ^{aA}	19.88 ± 0.12 ^{aA}	1.05 ± 0.02 ^{aB}
ANOVA *P*: source		0.012	<0.001	<0.001	<0.001
ANOVA *P*: rate		<0.001	<0.001	<0.001	<0.001
ANOVA *P*: source × rate		<0.001	<0.001	<0.001	<0.001
CV (%)		4.42	4.97	2.07	2.69

Note: Statistical notation follows Table 2.

Flower number showed a significant fertilizer source × rate interaction, $F(6, 24) = 12.01$, $P < 0.001$ (Table 6). At 15 t ha^{-1}, poultry manure produced 48.11 flowers plant^{-1}, which was 32.0% higher than cattle manure and 36.6% higher than water hyacinth bokashi. Within poultry manure, 15 t ha^{-1} produced 25.1% more flowers than 20 t ha^{-1}. However, fertilizer sources did not differ significantly at 20 t ha^{-1}, confirming that the source effect depended on application rate (Figure 2B).

The best-performing treatment for flower production differed among fertilizer sources: cattle manure at 5 t ha^{-1} (41.78 flowers plant^{-1}), poultry manure at 15 t ha^{-1} (48.11 flowers plant^{-1}), and water hyacinth bokashi at 20 t ha^{-1} (38.67 flowers plant^{-1}). Thus, application-rate recommendations must be specific to the fertilizer source.

The differences in flowering time and flower number indicate that fertilizer management affected reproductive growth as well as vegetative growth. Treatments producing the strongest vegetative growth did not necessarily produce the most flowers; therefore, plant size was not a reliable proxy for reproductive performance.

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v9i2.2708

Nitrogen and phosphorus are both relevant to reproductive development, but their contributions cannot be separated in the present material-rate comparison. Poultry manure had higher measured total N and total P concentrations than the other fertilizers, and available P was initially very low in the experimental soil. Evidence from Ultisol incubation indicates that poultry manure can release available P more rapidly than cattle manure (Muktamar et al., 2020). The stronger flower response may therefore be associated with fertilizer composition, although nutrient release and plant uptake were not measured directly.

The highest reproductive performance did not occur at the highest fertilizer rate. Within poultry manure, increasing the rate from 15 to 20 t ha^{-1} reduced flower number by 20.1%. This response is consistent with context-dependent allocation between vegetative growth and reproduction but does not demonstrate a causal growth–reproduction trade-off (Dutta et al., 2024). Poultry manure at 15 t ha^{-1} should therefore be described as the best-performing treatment among those evaluated, not as a universal agronomic optimum.

Nutrient management in butterfly pea should therefore consider reproductive output rather than plant size or vigor alone. Using 15 rather than 20 t ha^{-1} of poultry manure reduced as-received material input by 25% while producing 25.1% more flowers. This may reduce material acquisition and handling requirements, but production costs, nutrient-use efficiency, nutrient inputs, and nutrient losses were not measured. Any economic or environmental advantage must therefore be verified through field-scale cost and nutrient-balance analyses.

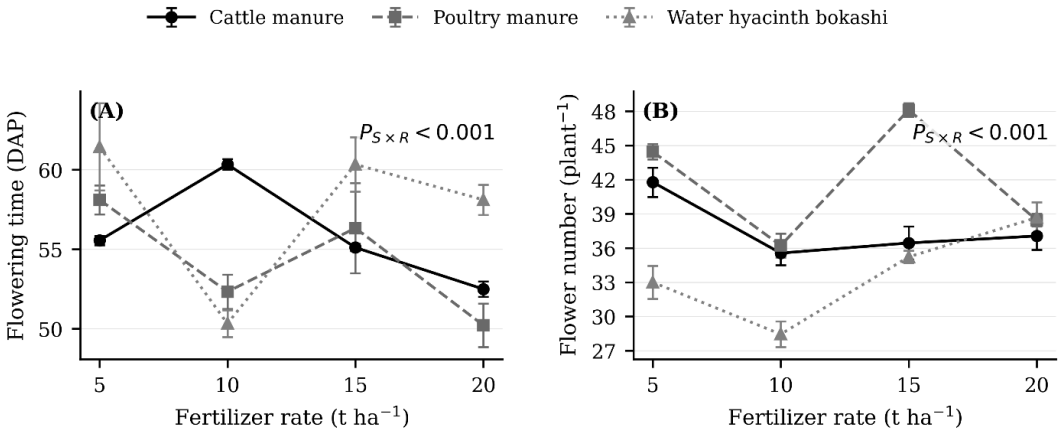

Figure 2. Fertilizer source × application rate interaction profiles for flowering time (A) and flower number (B) of butterfly pea. Points represent the means ± SE of three experimental units. The interaction *P*-value from the factorial ANOVA is shown in each panel.

Total flavonoid content showed a strong fertilizer source × rate interaction, $F(6, 24) = 189.38$, $P < 0.001$ (Table 6). Poultry manure at 15 t ha^{-1} produced the highest value (27.00 mg mL^{-1} QE), followed by cattle manure at 15 t ha^{-1} (24.88 mg mL^{-1} QE), whereas water hyacinth bokashi reached the highest value at 20 t ha^{-1} (19.88 mg mL^{-1} QE). Thus, the rate maximizing flavonoid content differed among fertilizer sources (Figure 3A).

The decline at the highest poultry-manure rate was substantial. Increasing the nominal as-received rate from 15 to 20 t ha^{-1} reduced total flavonoids from 27.00 to 15.25 mg mL^{-1} QE, a reduction of 43.5%.

Anthocyanin content likewise showed a significant fertilizer source × rate interaction, $F(6, 24) = 159.71$, $P < 0.001$ (Table 6). Poultry manure at 5, 10, and 15 t ha^{-1} produced statistically similar values of 1.60,

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v9i2.2708

1.63, and 1.65 mg 100 g^{-1}, respectively, but anthocyanin content declined to 0.94 mg 100 g^{-1} at 20 t ha^{-1}, representing a 43.0% reduction relative to 15 t ha^{-1}. At 15 t ha^{-1}, cattle manure (1.64 mg 100 g^{-1}) and poultry manure did not differ significantly, whereas water hyacinth bokashi produced 0.93 mg 100 g^{-1} (Figure 3B).

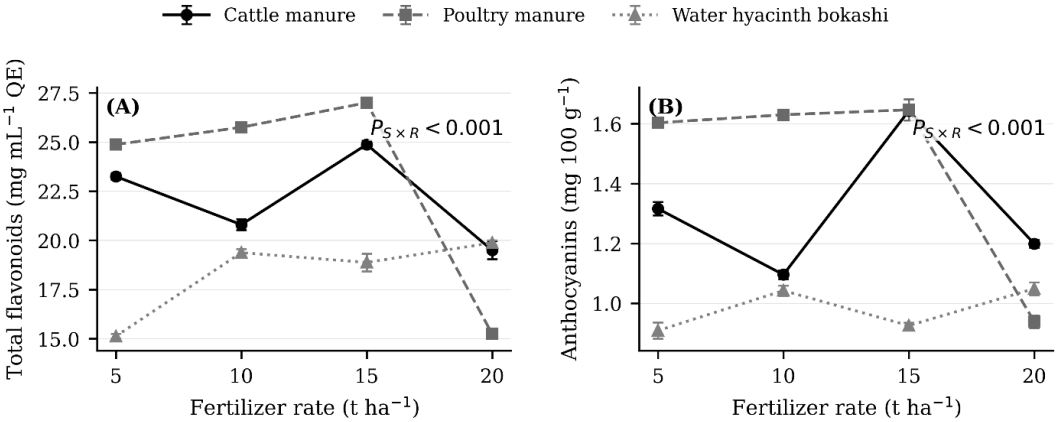

Figure 3. Fertilizer source × application rate interaction profiles for total flavonoids (A) and anthocyanins (B) in butterfly pea flowers. Points represent the means ± SE of three experimental units. The interaction *P*-value from the factorial ANOVA is shown in each panel.

3.4 Integrated interpretation, theoretical contribution, and practical implications

Taken together, significant interactions for flowering time, flower number, total flavonoids, and anthocyanins show that fertilizer-rate effects cannot be generalized across organic fertilizer sources. Poultry manure at 20 t ha^{-1} maximized vegetative traits, whereas poultry manure at 15 t ha^{-1} produced the strongest combined reproductive and phytochemical response. Increasing the as-received rate from 15 to 20 t ha^{-1} raised material input by 33.3% but reduced flower number, total flavonoids, and anthocyanins by 20.1%, 43.5%, and 43.0%, respectively. Thus, poultry manure at 15 t ha^{-1} was the best-performing treatment among those evaluated, not a universal agronomic optimum.

A recent field study likewise reported non-linear fertilization responses, with moderate NPK rates better balancing vegetative performance and flavonoid accumulation than the highest rate (Fatika et al., 2026). However, several flowering and yield traits were unaffected, partly contrasting with the strong interactions observed here. Differences in fertilizer form, soil, genotype, climate, and environment may explain this divergence.

Phytochemical quality depended on fertilizer source × rate. Nutrient regimes can alter phenylpropanoid metabolism, and nutrient responses may be compound-specific and non-linear (Jia et al., 2025; Raza et al., 2024; Yan et al., 2024). The decline under poultry manure at 20 t ha^{-1} may reflect altered carbon allocation, metabolite dilution, nutrient imbalance, or pathway regulation, although none was measured directly.

A simple growth–defense trade-off is insufficient because nutrient effects vary with genotype, organ, species, and environment (Dutta et al., 2024). Applied N increased anthocyanin in one black-rice genotype but not another (Thapa et al., 2024), whereas greater nutrient availability enhanced both growth and chemical defense in a tropical vine (Morrison et al., 2024). The decline under poultry manure at 20 t ha^{-1} should therefore not be generalized beyond the genotype, tissue, soil, and greenhouse conditions evaluated here.

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v9i2.2708

Although treatment differences in flavonoid and anthocyanin concentrations were clear, tissue nutrients, biomass allocation, enzyme activity, and phenylpropanoid-related gene expression were not measured.

Soil biological mediation also remains plausible because manures can differentially alter soil chemistry and bacterial communities, while organic fertilizer can modify N transformations in acidic soils (Sant'Anna et al., 2024; Luo et al., 2024). These mechanisms require direct measurement.

The findings contribute to nutrient-management theory in three ways. First, fertilizer source and nominal rate jointly determined plant response. Second, vegetative, reproductive, and phytochemical maxima occurred under different treatments, showing that agronomic and product-quality optima may diverge. Third, nutrient management for functional crops is a multi-objective problem integrating plant growth, reproductive output, and harvested-product quality.

The greenhouse reduced variation in water supply and soil heterogeneity but restricted leaching, root exploration, environmental fluctuations, and field-scale microbial interactions. Because soil texture, rainfall, and fertilizer transformation affect nutrient retention and loss, performance may differ in the field (Geisseler et al., 2024; Luo et al., 2024). Multi-location and multi-season trials are required before poultry manure at 15 t ha^{-1} can be broadly recommended.

For growers and processors, poultry manure at 15 t ha^{-1} was the best-performing treatment among those evaluated for balancing flower quantity and pigment-related quality under the tested conditions. Relative to 20 t ha^{-1}, it requires less material while producing higher flavonoid and anthocyanin concentrations. Economic and environmental benefits remain unverified and require partial-budget, nutrient-balance, runoff, and leaching assessments.

4. Limitations and Future Directions

The results indicate that nutrient management in butterfly pea should balance vegetative growth, flower production, and phytochemical quality rather than maximize vegetative vigor alone. The significant source × rate interactions show that recommendations should be fertilizer-source specific.

The principal limitations were greenhouse and polybag conditions, one homogenized Ultisol source, one season, and one maternal seed source, which constrained field-scale and genetic generalization. Equal nominal as-received rates were used without measuring fertilizer moisture; therefore, dry-matter and nutrient inputs were not necessarily equivalent. Unmeasured nutrient dynamics, microbial processes, nutrient losses, and costs further constrained mechanistic, environmental, and economic interpretation.

Within these constraints, poultry manure at 15 t ha^{-1} was the best-performing treatment among those evaluated for flower productivity and phytochemical quality under greenhouse conditions. Because only four discrete rates were evaluated, 15 t ha^{-1} should not be interpreted as a universal agronomic optimum.

Future research should validate these responses through multi-location and multi-season field trials across contrasting Ultisols using multiple genotypes. Long-term trials should assess cumulative effects on soil properties, crop performance, and phytochemical stability.

Future experiments should compare fertilizer sources at nutrient-equivalent N, P, and K inputs while retaining equal-material-rate treatments. Fertilizer moisture should be measured, narrower rate intervals around 15 t ha^{-1} should be tested, and partial-budget analyses, nutrient-balance assessments, and leaching and runoff measurements should be included.

Mechanistic studies should measure soil acidity and nutrient dynamics, plant tissue

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v9i2.2708

nutrients, microbial biomass, enzyme activity, mineralization, rhizosphere microbiomes, biomass and carbohydrate allocation, and phenylpropanoid-related enzymes or gene expression. These measurements would test the proposed explanations for phytochemical responses.

5. Conclusion

Fertilizer source, rate, and interaction affected vegetative growth, productivity, and phytochemical quality of butterfly pea grown on Ultisol. Source × rate interactions were significant for flower number, total flavonoids, and anthocyanins (all $P < 0.001$). Poultry manure at 15 t ha^{-1} produced 48.11 flowers plant^{-1}, 27.00 mg mL^{-1} QE total flavonoids, and 1.65 mg 100 g^{-1} anthocyanins. Increasing the rate to 20 t ha^{-1} reduced these responses by 20.1%, 43.5%, and 43.0%, respectively. Although the performance of poultry manure at 15 t ha^{-1} may relate to its lower C/N ratio and greater nutrient concentrations, nutrient-release dynamics and plant nutrient status were not measured. Maximum vegetative growth therefore did not coincide with maximum flower production or phytochemical concentration.

These findings demonstrate that rate responses depend on fertilizer source and that vegetative, reproductive, and phytochemical maxima may occur under different treatments. Nutrient management should balance flower productivity and phytochemical quality rather than vegetative vigor alone. Field validation and nutrient-equivalent comparisons are required before broad recommendations can be made.

Under greenhouse conditions, poultry manure at 15 t ha^{-1} was the best-performing treatment among those evaluated and used 25% less material than the 20 t ha^{-1} treatment. This treatment provides a provisional option for growers and the functional-food and natural-colorant industries, but profitability and environmental benefits require field verification.

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

The authors declare that no Gen AI was used in the creation of this manuscript.

Authorship Contribution Statement

MLQS and HS conceptualized and designed the study. MLQS and AHS conducted the experiment and performed the laboratory analyses. HS, GR, Z, and ID supervised the research activities and coordinated the experimental work. MLQS performed the statistical analyses. MLQS and HS drafted the manuscript. All authors reviewed, revised, and approved the final manuscript.

Declaration of Competing 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.

Acknowledgements

The authors acknowledge financial support from the Institute for Research and Community Services (LPPM), University of Lambung Mangkurat, through the Lecturer Mandatory Research Program (PDWM), Decree No. 1090.176/UN8.2/PG/2024.

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