Original research article

EFFECTS OF GUANO FERTILIZER, GOAT MANURE, AND PLANT GROWTH-PROMOTING RHIZOBACTERIA ON GROWTH, CHLOROPHYLL CONTENT, AND ANTIOXIDANT ACTIVITY OF PAKCOY (*Brassica rapa* L.)

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SUMMARY

Excessive use of inorganic fertilizers in intensive vegetable production may degrade soil quality and microbial activity, whereas organic fertilizers and beneficial rhizobacteria offer alternative inputs for more sustainable production systems. Pakcoy (*Brassica rapa* L.) is an important leafy vegetable with high market demand and nutritional value. This study aimed to evaluate the effects of guano fertilizer, goat manure, and plant growth-promoting rhizobacteria (PGPR) on growth, chlorophyll content, and antioxidant activity of pakcoy cv. Nauli-F1. The experiment was conducted in a greenhouse using a one-factor completely randomized design. The treatment factor consisted of ten levels: control without additional experimental organic fertilizer; guano fertilizer at 10, 20, and 30 g polybag^{-1}; goat manure at 15, 30, and 45 g polybag^{-1}; and PGPR at 15, 30, and 45 mL L^{-1}. Each treatment was replicated six times. Growth parameters included plant height, number of leaves, leaf area, fresh weight, and root length. Chlorophyll content was measured using UV-Vis spectrophotometry, while antioxidant activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. Growth data were analyzed using one-way analysis of variance followed by Duncan's multiple range test at the 5% level, whereas leaf-quality variables were interpreted descriptively. Organic fertilizer treatments significantly improved pakcoy growth compared with the control. The highest guano dose (30 g polybag^{-1}) produced the highest plant height and leaf area and showed the highest numerical total chlorophyll and antioxidant activity. Guano at 30 g polybag^{-1} and PGPR at 45 mL L^{-1} gave the best results for leaf number, fresh weight, and root length. The results indicate that guano fertilizer and PGPR are promising inputs for improving pakcoy growth under greenhouse conditions.

KEYWORDS: antioxidant activity, chlorophyll, DPPH, goat manure, guano, organic fertilizer, pakcoy, PGPR

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INTRODUCTION

Sustainable vegetable production requires nutrient management strategies that maintain crop productivity while reducing the negative effects of excessive inorganic fertilizer use, such as reactive nitrogen losses, greenhouse gas emissions, soil degradation, and declining soil biological quality (Xu et al., 2024; Zhang et al., 2024; Tang et al., 2025). Inorganic fertilizers are widely used because nutrients are readily available to plants. However, long-term dependence on synthetic fertilizers may contribute to soil degradation, reduced soil biological activity, and declining soil health through soil acidification, shifts in microbial community structure, reduced microbial functionality, and lower soil quality indices (Carrascosa et al., 2023; Bharathi et al., 2024; Tadesse et al., 2024; Zheng et al., 2024). Therefore, organic fertilizers and microbial biofertilizers are increasingly considered as practical alternatives or complementary inputs in vegetable production systems.

Pakcoy (*Brassica rapa* L.) is a short-duration leafy vegetable belonging to the family Brassicaceae. It is widely consumed due to its soft texture, mild taste, and nutritional value. As a leafy vegetable, pakcoy requires adequate nutrient availability, particularly nitrogen,

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phosphorus, and potassium, to support leaf development, biomass accumulation, and overall plant quality. Improving nutrient management in pakcoy cultivation is therefore important for both yield and quality improvement.

Guano fertilizer is an organic fertilizer derived from accumulated bat or bird excreta. It is generally rich in nitrogen, phosphorus, potassium, and organic matter, making it useful for improving soil fertility, nutrient availability, and vegetative growth (Dimande et al., 2023a, 2023b; Duffaut et al., 2023; Możdżer, 2024). Goat manure is another organic input commonly used in vegetable cultivation because it contains essential nutrients and contributes to soil organic matter. However, nutrient release from goat manure depends on decomposition and mineralization processes and may be slower or more gradual than that of readily degradable organic materials or liquid organic inputs (Katakula et al., 2021; Zhu et al., 2020; Zondo et al., 2025). In addition to organic fertilizers, plant growth-promoting rhizobacteria (PGPR) can enhance plant growth through nitrogen fixation, phosphate solubilization, phytohormone production, particularly indole-3-acetic acid, and improved root growth or root-associated nutrient uptake (Jiang et al., 2023; Ganesh et al., 2024; Fan et al., 2025).

Previous studies have reported positive effects of guano fertilizer, goat manure, and PGPR on several vegetable crops. However, comparative information on their effects at different application rates on pakcoy growth and quality traits, especially chlorophyll content and antioxidant activity, remains limited. This study was designed to evaluate the effects of guano fertilizer, goat manure, and PGPR on growth, chlorophyll content, and antioxidant activity of pakcoy cv. Nauli-F1 under greenhouse conditions. The study also aimed to identify the most effective organic fertilizer treatment for improving vegetative growth and leaf quality.

MATERIAL AND METHODS

Experimental site and plant material

The experiment was conducted from May to November 2024 in the greenhouse of UNNES Ecofarm, Sekaran, Gunungpati, Semarang, Central Java, Indonesia (7.048240° S, 110.399579° E; approximately 235 m above sea level). The plant material used was pakcoy (*Brassica rapa* L.) cv. Nauli-F1.

Experimental design and treatments

The experiment used a one-factor completely randomized design. The single factor was the organic fertilizer treatment, consisting of ten treatment levels (Table 1). Each treatment was replicated six times. The treatment structure was analyzed as one factor because each treatment level represented a specific fertilizer type-dose combination. Thus, no factorial interaction was tested.

Table 1. Organic fertilizer treatments used in the experiment

Code	Treatment description
T0	Control, without additional experimental organic fertilizer
G1	Guano fertilizer, 10 g polybag^{-1}
G2	Guano fertilizer, 20 g polybag^{-1}
G3	Guano fertilizer, 30 g polybag^{-1}
K1	Goat manure, 15 g polybag^{-1}
K2	Goat manure, 30 g polybag^{-1}
K3	Goat manure, 45 g polybag^{-1}
P1	PGPR, 15 mL L^{-1}
P2	PGPR, 30 mL L^{-1}
P3	PGPR, 45 mL L^{-1}

PGPR–plant growth-promoting rhizobacteria

Crop management and data collection

The basal growing medium, prepared before the application of the experimental organic fertilizer treatments, consisted of black mineral soil, cattle compost, and rice husk mixed at a ratio of 4:4:1 (w/w/w). The black soil was characterized by a dark mineral surface layer with approximately 25% organic carbon, while the cattle compost contained 0.33% N, 0.11% P, and 0.26% K. The soil was sieved through a 16-mesh wire screen to obtain a fine and homogeneous texture before being mixed with cattle compost and rice husk. The mixed medium was fermented for seven days using 40 mL of Easyfarm F2 liquid organic fertilizer diluted in 25 L of water. After fermentation, 3 kg of the medium was placed into each black low-density polyethylene (LDPE) polybag (25 cm x 35 cm, 45 µm thickness).

Pakcoy seedlings were raised in rockwool and transplanted into polybags after 14 days in the nursery. Each polybag contained three plants, and one plant per polybag was selected randomly as the observation sample. All polybags, including the control, received the same basal inorganic fertilizer at 2.5 g polybag^{-1} five days before the experimental organic or biological fertilizer treatments were initiated. Therefore, T0 represented plants without additional experimental organic fertilizer, not plants without any nutrient input. Organic fertilizer treatments were applied according to the treatment codes starting at 14 days after transplanting and were repeated at seven-day intervals. Plants were watered to near field capacity, and daytime greenhouse temperature ranged from 28 to 31°C.

Plant growth was observed from 14 to 42 days after transplanting. Plant height was measured from the plant base to the highest leaf tip using a ruler. The number of leaves was counted based on fully developed leaves. At harvest, leaf area, fresh weight, and root length were measured. Leaf area was estimated as leaf length x leaf width x correction factor, using a pakcoy correction factor of 0.6825 as applied in previous pakcoy leaf-area measurements (Harfiani et al., 2024). Fresh weight was measured using a digital balance after the plants were harvested and cleaned from adhering soil. Root length was measured after the root system was carefully washed.

Chlorophyll content was analyzed using UV-Vis spectrophotometry following the principle of pigment absorbance measurement described by Arnon (1949). Fresh leaf samples were extracted with 96% ethanol, and absorbance was read at 645 and 663 nm. Chlorophyll a, chlorophyll b, and total chlorophyll were expressed as mg L^{-1} extract. Antioxidant activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method according to Blois (1958) and expressed as percentage inhibition.

Statistical analysis

Growth data were analyzed using one-way analysis of variance (ANOVA). When the treatment effect was significant, means were separated using Duncan's multiple range test (DMRT) at the 5% significance level. The

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

statistical model used was:

where Y_{ij} is the observation value, μ is the overall mean, τ_i is the effect of the i-th organic fertilizer treatment, and ε_{ij} is the experimental error. Chlorophyll and antioxidant data were obtained as descriptive laboratory values and were not subjected to DMRT; therefore, these variables are presented as numerical comparisons among treatments.

RESULTS AND DISCUSSION

Organic fertilizer treatments improved pakcoy growth compared with the control. At 42 days after transplanting, the control plants showed the lowest values for plant height, number of leaves, leaf area, fresh weight, and root length (Table 2). In contrast, the highest treatment levels of guano fertilizer and PGPR showed the strongest positive effects on vegetative growth.

Table 2. Effect of organic fertilizer treatments on pakcoy growth at 42 days after transplanting

Treatment	Plant height (cm)	Number of leaves	Leaf area (cm^2)	Fresh weight (g)	Root length (cm)
T0	21.09e	17e	68.68e	131e	10.17e
G1	23.32d	19d	82.48d	156cd	10.85d
G2	25.13b	20c	101.27bc	166c	12.61b
G3	27.27a	25a	120.57a	215a	13.44a
K1	23.09d	18d	81.32d	150cd	10.68d
K2	23.83c	20c	97.66c	190b	11.36cd
K3	24.71bc	21bc	107.19bc	193b	12.45b
P1	23.10d	18d	84.52d	161c	12.03bc
P2	24.29bc	22b	98.78c	195b	12.67b
P3	25.46b	24a	115.51b	216a	13.56a

Values followed by the same letter in the same column are not significantly different according to DMRT at p <= 0.05

The highest plant height was observed in G3, reaching 27.27 cm, followed by P3 and G2. This result indicates that guano fertilizer at 30 g $polybag^{-1}$ provided sufficient nutrients to support shoot elongation. Guano is known as a nutrient-rich organic material, particularly as a source of nitrogen and phosphorus, as shown by studies reporting high N and P contents, low C/N ratio, rapid N mineralization, and P release from bat and seabird guano (Misra et al., 2019; Browning et al., 2023; Dimande et al., 2023a, 2023b; Możdżer, 2024). Nitrogen is essential for protein synthesis and vegetative growth, while phosphorus supports root development and energy transfer. The improved nutrient availability from guano may explain the superior plant height observed in G3.

The number of leaves was highest in G3 and P3, with 25 and 24 leaves, respectively. Both treatments were statistically similar and superior to the control. Leaf number is closely associated with nitrogen availability and plant metabolic activity. The positive response in G3 may be related to nutrient supply from guano, particularly through the release of N and P during mineralization, while the response in P3 may be associated with PGPR activity in the rhizosphere, including phosphate solubilization, nitrogen fixation, indole-3-acetic acid (IAA) production, and improved root-associated nutrient uptake (Dimande et al., 2023a, 2023b; Jiang et al., 2023; Możdżer, 2024; Fan et al., 2025). PGPR can enhance nutrient uptake, stimulate root growth, and produce phytohormones that promote leaf initiation and expansion, as reflected by increases in root biomass, lateral root formation, leaf number, leaf area, chlorophyll content, and mineral nutrient accumulation in leafy vegetables and other model plants (Dasgan et al., 2023; Mei et al., 2023; Ganesh et al., 2024; Ikiz et al., 2024; Fan et al., 2025).

Leaf area also increased markedly with organic fertilizer treatments. G3 produced the highest leaf area, reaching 120.57 cm^2, followed by P3 at 115.51 cm^2. Larger leaf area increases the photosynthetic surface and may contribute to higher biomass accumulation because leaf expansion is closely associated with light interception, photosynthetic capacity, and shoot fresh or dry weight in leafy vegetables (He et al., 2022; Shohag et al., 2024). In leafy vegetables such as pakcoy, leaf area is an important agronomic trait because the harvested product is mainly composed of leaves and petioles.

Fresh weight followed a similar trend. The highest fresh weight was recorded in P3 and G3, with 216 and 215 g, respectively. These treatments were statistically similar and significantly higher than the control. Fresh weight is influenced by leaf expansion, water uptake, root activity, nutrient absorption, photosynthetic performance, and assimilate accumulation. The superior fresh weight in G3 may reflect improved nutrient availability from guano mineralization, particularly the release of N and P, while PGPR at 45 mL L^{-1} may have enhanced root function and nutrient absorption through rhizosphere activity, phosphate solubilization, IAA production, and improved root-associated physiological processes (Dimande et al., 2023a, 2023b; Jiang et al., 2023; Wang et al., 2023; Shohag et al., 2024).

Root length was highest in P3, followed closely by G3. Root development is important because it determines the plant's ability to absorb water and nutrients. The positive effect of PGPR on root length may be associated with the production of growth-promoting compounds, including auxins, and the ability of beneficial rhizobacteria to improve nutrient acquisition in the rhizosphere. The strong root growth in G3 may also be related to phosphorus supply from guano fertilizer, as phosphorus availability can promote root growth, root length density, and P acquisition in *Brassica* vegetables (Gao et al., 2023; Jiang et al., 2023; Mei et al., 2023; Dimande et al., 2023a, 2023b; Ganesh et al., 2024; Fan et al., 2025).

Goat manure also improved pakcoy growth compared with the control, especially at 30 and 45 g $polybag^{-1}$. However, its effect was generally lower than guano fertilizer and PGPR at the highest rates. This may be due to the slower nutrient release from goat manure, which requires microbial decomposition and mineralization before nutrients become readily available to plants (Azeez & Van Averbeke, 2010; Zhu et al., 2020; Katakula et al., 2021; Zondo et al., 2025). Nevertheless, goat manure remains useful as a source of organic matter and nutrients for soil fertility improvement.

Chlorophyll content and antioxidant activity

Organic fertilizer treatments were also evaluated for leaf quality, as indicated by chlorophyll content and antioxidant activity. Because these variables were obtained as descriptive laboratory values, no Duncan's mean separation was performed for Table 3. The highest total chlorophyll content was numerically observed in G3, followed by K3, K2, G2, and P3.

Table 3. Chlorophyll content and antioxidant activity of pakcoy leaves

Treatment	Chlorophyll a (mg L^{-1})	Chlorophyll b (mg L^{-1})	Total chlorophyll (mg L^{-1})	Antioxidant activity (%)
T0	83.77	28.30	112.07	14.07
G1	103.29	37.27	140.56	18.38
G2	109.48	38.03	147.51	27.44
G3	126.63	46.30	172.93	30.64
K1	103.84	36.33	140.17	12.45
K2	108.68	39.80	148.48	12.53
K3	115.21	39.80	155.01	25.91
P1	86.30	29.77	116.07	14.21
P2	98.02	32.58	130.60	21.45
P3	105.30	38.40	143.70	27.58

Values are descriptive laboratory measurements; no DMRT was performed for chlorophyll and antioxidant variables

The highest numerical total chlorophyll content in G3 indicates that the highest guano dose supported chlorophyll accumulation more effectively than the other tested treatments. Chlorophyll formation is strongly related to nitrogen availability because nitrogen is required for chlorophyll biosynthesis and is also a major component of photosynthetic proteins and enzymes that support photosynthetic performance (Wang et al., 2021; Chen et al., 2024; Wu et al., 2025). Improved phosphorus and potassium availability may also support energy metabolism and photosynthetic efficiency, because adequate P and K supply has been shown to increase ATP-related energy status, chlorophyll content, photosynthetic rate, electron transport, Rubisco activity, and nutrient-use efficiency in crop plants (Ma et al., 2022; Hu et al., 2023; El-Desouki et al., 2024; Liu et al., 2024).

Antioxidant activity was also numerically highest in G3, followed by P3 and G2. However, the overall antioxidant activity remained relatively low. This result suggests that organic fertilizer application can improve antioxidant capacity to some extent, but the response may depend on fertilizer type, nutrient balance, rhizosphere microbial activity, plant physiological status, and harvest stage (Zhao et al., 2007, 2009; Liao et al., 2019; Cavalheiro et al., 2020; Le et al., 2025). In leafy vegetables, antioxidant activity is influenced by phenolic compounds, vitamin C, chlorophyll -associated metabolism, carotenoids, and stress-related secondary metabolites such as flavonoids, anthocyanins, and glucosinolates, whose accumulation may vary depending on genotype, fertilization, light environment, UV exposure, and harvest stage (Liao et al., 2019; Song et al., 2020; He et al., 2022; Wessler et al., 2025).

The combined interpretation of growth and quality traits indicates that the highest guano dose (30 g polybag^{-1}) was the most consistent treatment. It produced the highest plant height and leaf area and the highest numerical total chlorophyll and antioxidant activity, while also producing high fresh weight and root length. PGPR at 45 mL L^{-1} was particularly effective in increasing leaf number, fresh weight, and root length. Based on their positive effects on plant growth, nutrient availability, rhizosphere microbial activity, and biomass production, guano fertilizer and PGPR may be recommended as promising inputs for pakcoy cultivation under greenhouse conditions, although guano-specific validation in pakcoy should be further confirmed in subsequent trials in controlled and field conditions (Wang et al., 2022; Dimande et al., 2023a, 2023b; Jiang et al., 2023; Ikiz et al., 2024; Fan et al., 2025).

CONCLUSIONS

The application of organic fertilizer significantly improved the growth of pakcoy cv. Nauli-F1 compared with the control. Among the tested treatments, the higher guano dose (30 g polybag-1) was the most consistent treatment for increasing plant height and leaf area and showed the highest numerical values for total chlorophyll content and antioxidant activity. The higher guano dose and the highest PGPR concentration (45 mL L-1) produced the best responses for fresh weight and root length. Goat manure improved growth compared with the control, although its response was generally lower than that of guano fertilizer and PGPR at the highest rates. These findings suggest that guano fertilizer and PGPR can be used as alternative inputs to support pakcoy production in more sustainable vegetable cultivation systems.

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SAŽETAK

EFEKTI GUANO ĐUBRIVA, KOZJEG STAJNJAKA I PGP-RIZOBAKTERIJA NA RAST, SADRŽAJ HLOROFILA I ANTIOKSIDATIVNU AKTIVNOST KINESKOG KUPUSA (*Brassica rapa* L.)

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Prekomerna upotreba mineralnih đubriva u intenzivnoj proizvodnji povrća može smanjiti aktivnost mikroorganizama i degradirati kvalitet zemljišta, dok primena organskih đubriva i korisnih rizobakterija predstavlja alternativno rešenje koje rezultira održivijim sistemima proizvodnje. Kineski kupus (*Brassica rapa* L.) je lisnato povrće velike hranljive vrednosti i za njim postoji značajna tržišna potražnja. Cilj rada je bila procena efekata guano đubriva, kozjeg stajnjaka i rizobakterija koje podstiču rast biljaka (plant growth-promoting rhizobacteria, PGPR) na rast, sadržaj hlorofila i antioksidativnu aktivnost hibrida kineskog kupusa Nauli-F1. Ogled je postavljen u plasteniku, a biljke su gajene u polietilenskim kesama. Primenjeni su tretmani: kontrola bez dodavanja organskog đubriva, guano đubrivo u količini od 10, 20 i 30 g kesa^{-1}, kozji stajnjak u količini od 15, 30 i 45 g kesa^{-1} i PGPR u količini od 15, 30 i 45 mL L^{-1}. Ogled je postavljen po potpuno slučajnom rasporedu, u šest ponavljanja. Ispitani su parametri rasta biljaka: visina, broj listova, površina lista, težina sveže biljke i dužina korena. Sadržaj hlorofila je izmeren UV-vis spektrofotometrijski, dok je antioksidativna aktivnost određena DPPH (2,2-difenil-1-pikrilhidrazil) metodom. Podaci o parametrima rasta biljaka su obrađeni analizom varijanse i sredine su upoređene Dankanovim testom višestrukog raspona na nivou od 5%, dok su parametri kvaliteta lista interpretirani deskriptivno. Tretmani organskim đubrivom značajno su poboljšali rast kineskog kupusa u poređenju sa kontrolom. Pri najvećoj dozi guana (30 g kesa-1) su zabeleženi najbolji rezultati u pogledu: visine biljke, površine lista, kao i ukupne hlorofilne i antioksidativne aktivnosti. Broj listova, težina sveže biljke i dužina korena su bili najveći pri upotrebi najveće doza guana i najviše koncentracije PGPR-a. Rezultati pokazuju da guano đubrivo i PGPR poboljšavaju rast i kvalitet kineskog kupusa gajenog u plasteniku.
KLJUČNE REČI: antioksidativna aktivnost, DPPH metod, guano, hlorofil, kineski kupus, kozji stajnjak, organsko đubrivo, PGP -rizobakterije

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