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## Effect of Urea Fertilizer and PNSB Photosynthetic Bacteria on the Growth and Yield of Pakcoy (*Brassica rapa* L.)

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### ABSTRACT

Pakcoy (*Brassica rapa* L.) is a high-demand leafy vegetable requiring sufficient nitrogen (N) for optimal vegetative growth. This study aimed to evaluate the effects of urea fertilizer and Purple Non-Sulfur Bacteria (PNSB) photosynthetic bacteria, as well as their interaction, on the growth and yield of pakcoy. The factorial experiment 2 x 2 was conducted from April to June 2022 using a Randomized Complete Block Design with ten replications. The first factor was dose of urea (0 and 150 kg ha<sup>-1</sup>), while the second was PNSB concentration (0 and 5 mL L<sup>-1</sup>). The data were analyzed using analysis of variance (Anova), if the results were significantly different, followed by the Least Significant Difference (LSD) test at the 5% level. Results of the Anova showed that the effect of interaction between urea and PNSB significantly different on stem height and root length. Urea application significantly different on all observed variables: stem height, leaf number (14, 21, and 28 DAT), stem diameter, root length, total leaf area, fresh weight, and dry weight per plant, while PNSB significantly increased leaf number (14, 21, and 28 DAT), stem diameter, fresh weight, and dry weight per plant. The combination of 150 kg ha<sup>-1</sup> urea and 5 mL L<sup>-1</sup> PNSB enhanced fresh weight by 73.36% compared to the control. These findings indicate that both urea and PNSB are effective in promoting pakcoy productivity, with urea showing dominant effects

## INTRODUCTION

Pakcoy (*Brassica rapa* L.) is a leafy vegetable originating from China, belonging to the Brassicaceae family. Compared to green mustard or napa cabbage, pakcoy has thicker stems and wider leaves, making it favored by consumers (Setiawan 2014). This plant can be harvested year-round and is responsive to fertilization, especially nitrogen (N), which plays an important role in chlorophyll synthesis, vegetative growth, and leaf formation (Lingga 2007).

Fertilization with urea, which contains 46% N, is a common practice among farmers to meet the N needs of plants. However, excessive use of urea can lead to N leaching, greenhouse gas emissions such as N<sub>2</sub>O, and decreased fertilization efficiency (Sari et al. 2016). Furthermore, the efficiency of urea use in short-lived vegetables like pakcoy is often low because a large portion of N is lost through volatilization and denitrification before it can be absorbed by the plant (Olivares et al. 2013). Therefore, sustainable agricultural approaches such as the use of microbe-based biofertilizers are strategic alternatives to increase fertilization efficiency while reducing the negative impact of fertilizers on the environment.

One potential microbe is Purple Non-Sulfur Bacteria (PNSB), a group of anoxygenic photosynthetic bacteria capable of performing biological nitrogen fixation through the nitrogenase enzyme (Madigan 1995; Gallon 2001). PNSB such as *Rhodopseudomonas* spp. and *Rhodobacter capsulatus* not only fix atmospheric N<sub>2</sub> but also produce phytohormones like Indole Acetic Acid (IAA) which stimulate root growth and nutrient uptake (Lee et al. 2021; Wani et al. 2016). Research by Wong et al. (2014) showed that inoculation with *Rhodopseudomonas palustris* improved pakcoy growth even under low N fertilization conditions.

However, most previous studies only evaluated PNSB separately from chemical fertilizers or in controlled systems (greenhouse/hydroponics), with little attention to the synergistic or antagonistic interactions between PNSB and urea fertilizer under tropical field conditions. Some studies report that the

presence of high concentrations of ammonium (NH<sub>4</sub><sup>+</sup>) or nitrate (NO<sub>3</sub><sup>-</sup>) can inhibit the expression of *nif* genes and the activity of the nitrogenase enzyme in N<sub>2</sub>-fixing bacteria, including PNSB (Olivares et al. 2013; Gallon 2001). This raises an important question: is PNSB application still effective when combined with the optimum urea dose commonly used by farmers?

No study has explicitly tested the interaction between the recommended urea dose (150 kg ha<sup>-1</sup>) and foliar application of PNSB (5 mL L<sup>-1</sup>) on the growth and yield of pakcoy under tropical agroecosystem conditions such as East Kalimantan, which has different soil and climate characteristics from regions where previous studies were conducted.

The novelty of this research lies in the integrative approach that tests the single effects and interaction between inorganic urea fertilizer and PNSB as a biofertilizer in a garden-scale pakcoy cultivation system. Furthermore, this study provides empirical data on the threshold urea dose where the benefits of PNSB begin to diminish, crucial information for designing efficient and sustainable integrated fertilization strategies. Thus, these findings not only enrich the scientific literature on N<sub>2</sub>-fixing microbes but also provide practical recommendations for farmers to optimize pakcoy productivity without compromising environmental health.

The study aims to: (1) evaluate the individual effects of urea fertilizer and PNSB on the growth and yield of pakcoy, and (2) analyze the interaction between the two in improving sustainable fertilization efficiency.

## METHODS

The research was conducted from April to June 2022 in the shade net house (*para-para*), Department of Agroecotechnology, Faculty of Agriculture, Mulawarman University, Samarinda.

The materials used consisted of pakcoy seeds Variety Nauli F1, urea fertilizer, SP-36, KCl, chicken manure, PNSB culture, Furadan 3G, while the tools used were polybags sized 30 cm × 25 cm and 10 cm

× 10 cm, hand sprayer, Leaf Area Meter, analytical balance, and stationery.

The research was a 2 x 2 factorial experiment in a Randomized Complete Block Design (RCBD) with 10 replications. The first factor was urea fertilizer (P) consisting of  $p_0 = 0 \text{ kg ha}^{-1}$  and  $p_1 = 150 \text{ kg ha}^{-1}$ , while the second factor was PNSB concentration (B) consisting of  $b_0 = 0 \text{ mL L}^{-1}$  and  $b_1 = 5 \text{ mL L}^{-1}$ . There were a total of 40 experimental units.

The steps carried out in the experiment included: **Nursery.** Seeds were sown in small polybags containing a mixture of soil and rice husk (1:1) for 14 days; **Planting medium preparation.** Planting polybags were filled with 6 kg of a mixture of soil and chicken manure (1:1); **Planting.** Seedlings were transplanted (one plant per polybag). Basal fertilizers SP-36 and KCl were applied at  $100 \text{ kg ha}^{-1}$  each; **Urea fertilization.** Urea was applied in two stages, namely  $100 \text{ kg ha}^{-1}$  at planting and  $50 \text{ kg ha}^{-1}$  at 14 days after transplanting (DAT); **PNSB application.** PNSB was applied by spraying at 7 and 14 DAT at concentrations according to the treatment;

**Maintenance,** including daily watering, weeding every 7 DAT, replanting until 14 DAT, and preventive pest control; and **Harvesting:** carried out at 30 DAT by uprooting the entire plant.

Data were analyzed using analysis of variance (ANOVA) at the 5% level. If the treatment effect was significantly different, it was followed by the Least Significant Difference (LSD) test at the 5% level to compare two treatment averages.

## RESULTS AND DISCUSSION

The results of the analysis of variance showed that the interaction effect between urea and PNSB photosynthetic bacteria was not significant, except for stem height and root length. Urea fertilizer was significantly different for all variables: stem height, leaf number (14, 21, and 28 DAT), stem diameter, root length, total leaf area, fresh weight, and dry weight per plant, while the effect of PNSB was significant on leaf number (14, 21, and 28 DAT), stem diameter, fresh weight, and dry weight per plant. A recapitulation of the data and the results of the research data analysis are presented in Table 1 below.

Table 1. Recapitulation of Data and Results of Research Data Analysis

Treatment	Stem Height (cm)	Number of Leaves (sheets)			Stem Diameter (mm)	Root Length (cm)	Total Leaf Area (cm <sup>2</sup> )	Fresh Weight per Plant (g)	Dry Weight per Plant (g)
		14 DAT	21 DAT	28 DAT					
		Dose of Urea (P) (kg ha <sup>-1</sup> )							
$p_0 = 0$	7,17a	8,90a	11,25a	12,85a	6,44a	16,51a	354,42a	30,42a	2,88a
$p_1 = 150$	9,13b	12,20b	15,70b	17,85b	9,54b	20,19b	777,46b	81,34b	6,91b
ANOVA	*	*	*	*	*	*	*	*	*
LSD 5%	0,72	0,75	0,84	1,10	0,87	1,50	93,61	9,68	1,55
PNSB Concentration (B) (mL L <sup>-1</sup> )									
$b_0 = 0$	8,01	10,00a	12,80a	14,70a	7,10a	18,38	529,17	48,17a	3,61a
$b_1 = 5$	8,29	11,10b	14,15b	16,00b	8,89b	18,32	602,71	63,58b	6,18b
ANOVA	ns	*	*	*	*	ns	ns	*	*
LSD 5%	-	0,75	0,84	1,10	0,87	-	-	9,68	1,55
Interaction (P x B)									
$p_0b_0$	6,64Aa	8,30	10,40	12,00	5,80	13,98Aa	313,52	24,09	1,86
$p_0b_1$	7,70Ba	9,50	12,10	13,70	7,08	19,05Ba	395,32	36,74	3,90

p <sub>1</sub> b <sub>0</sub>	9,37Ab	11,70	15,20	17,40	8,40	22,79Bb	744,82	72,25	5,36
p <sub>1</sub> b <sub>1</sub>	8,88Ab	12,70	16,20	18,30	10,69	17,59Aa	810,09	90,42	8,46
ANOVA	*	ns	ns	ns	ns	*	ns	ns	ns
LSD 5%	1,01	-	-	-	-	2,13	-	-	-

Note: Mean values followed by the same lowercase letter in the same column and the same uppercase letter in the same column indicate not significant difference based on the LSD test at 5% (\* = significantly different; ns = not significantly different)

### Interaction between Urea and PNSB

The results showed that the interaction effect between urea fertilizer and PNSB photosynthetic bacteria was significant only on two growth variables: stem height and root length (Table 1). The combination of 150 kg ha<sup>-1</sup> urea without PNSB (p<sub>1</sub>b<sub>0</sub>) produced the highest values for both variables (stem height = 9.37 cm; root length = 22.79 cm), while the addition of PNSB at the same urea dose (p<sub>1</sub>b<sub>1</sub>) actually decreased growth performance (stem height = 8.88 cm; root length = 17.59 cm). This finding indicates functional antagonism between the inorganic nitrogen source (urea) and the activity of biological nitrogen-fixing microbes like PNSB.

The decrease in PNSB effectiveness under high inorganic nitrogen conditions can be explained by the regulatory mechanism of the nitrogenase enzyme. This enzyme, responsible for atmospheric N<sub>2</sub> fixation by diazotrophic bacteria like *Rhodopseudomonas* spp., is highly sensitive to the concentration of environmental ammonia (NH<sub>3</sub>/NH<sub>4</sub><sup>+</sup>). When the availability of inorganic nitrogen (such as NH<sub>4</sub><sup>+</sup> from the hydrolysis of urea by soil urease) is abundant, the expression of *nif* genes encoding nitrogenase is transcriptionally repressed through the Ntr (Nitrogen regulatory system) (Huergo & Chandra 2018). Consequently, the biological nitrogen fixation activity by PNSB becomes inefficient or even stops, so the contribution of PNSB to plant growth becomes minimal.

This phenomenon is supported by the findings of Olivares et al. (2013) who stated that under conditions of excess nitrogen, N<sub>2</sub>-fixing microbes tend to shift their metabolic energy from nitrogen fixation to cellular growth processes, because N<sub>2</sub> fixation is a highly energetic process (requiring 16 ATP per N<sub>2</sub> molecule). With an easily available N

source (such as from urea), the bacteria no longer need to "waste energy" fixing N<sub>2</sub>.

Furthermore, the addition of PNSB to a nitrogen-rich medium can trigger indirect competition between plants and microbes in nutrient uptake. Although PNSB are generally mutualistic, under certain conditions, especially when organic carbon availability is limited, PNSB can compete with plant roots for nutrients such as phosphorus and potassium (Lee et al. 2021). This may explain why root length in the p<sub>1</sub>b<sub>1</sub> treatment was shorter than in p<sub>1</sub>b<sub>0</sub>, as roots may experience inhibition in expansion due to less optimal energy allocation or changes in hormone balance due to microbial stress.

This finding is also in line with research by Adi et al. (2022) who reported that the interaction between urea and biochar on pakcoy plants only showed a synergistic response at a moderate urea dose (100 kg ha<sup>-1</sup>), while at a high dose (150 kg ha<sup>-1</sup>), the additional effect of biochar became non-significant. This reinforces the hypothesis that the efficiency of external inputs (both chemical and biological) highly depends on the nutrient balance in the soil-plant system, and that the optimal dose must be determined holistically, not just based on a single response to one input.

Furthermore, the results of Wong et al. (2014) showed that PNSB (*Rhodopseudomonas palustris*) was most effective in enhancing pakcoy growth precisely under low nitrogen fertilization conditions. Under those conditions, PNSB not only provided nitrogen through biological fixation but also improved water and nutrient use efficiency through the production of phytohormones such as IAA and cytokinins. However, because in this study urea was applied at a dose that already met the plant's N requirement (150 kg ha<sup>-1</sup>), the role of PNSB as an N

provider became redundant, so its main benefits, such as increased fresh weight and stem diameter, were not observed in structural variables like height and root length.

Thus, strategies for integrating chemical and biofertilizers must consider the appropriate dose and timing of application. The use of PNSB is more recommended for sustainable agricultural systems with reduced doses of inorganic fertilizer (30-50%), not as an addition to conventional high-fertilizer systems. This approach not only increases fertilization efficiency but also reduces the risk of environmental pollution due to excess nitrogen.

#### **Effect of Urea Fertilizer on the Growth and Yield of Pakcoy**

The effect of urea fertilizer was significant on all observed growth and yield variables, including number of leaves, stem height, stem diameter, root length, total leaf area, fresh weight, and dry weight per plant (Table 1). These results indicate that nitrogen (N), the main element in urea, plays a central role in promoting the vegetative growth of pakcoy plants. Nitrogen is an essential component in the synthesis of amino acids, proteins, enzymes, and chlorophyll, all of which directly contribute to tissue expansion, cell division, and photosynthetic efficiency (Marschner 2012). The increase in the number of leaves and leaf area in the 150 kg ha<sup>-1</sup> urea treatment reflects the plant's physiological response to sufficient N availability, which allows the formation of more photosynthetic units (leaves) and increases carbon assimilation capacity.

This finding is consistent with research by Sari et al. (2016) which reported that N fertilization on pakcoy increased the photosynthesis rate by up to 35%, directly impacting biomass accumulation. Additionally, Lakitan (2012) explained that N increases cell turgor pressure through the accumulation of osmotic compounds like amino acids and sugars, thus promoting cell expansion and increased fresh weight. The research results showing a 62.60% increase in fresh weight in the urea treatment compared to the control support this hypothesis (Table 1).

Furthermore, a positive response to urea was also seen in root growth. Nitrogen not only stimulates shoot growth but also stimulates root system development through the regulation of hormones such as cytokinins and auxins (Kiba & Krapp 2016). Although roots are not the main N storage organ, increased root length expands the zone of nutrient and water uptake, which indirectly supports the growth of the upper plant parts. This is consistent with the results of Oviyanti et al. (2016) on mustard plants (*Brassica juncea* L.), which showed that N application increased root length by up to 40% compared to no fertilization.

However, it is important to note that the efficiency of N use from urea is highly influenced by environmental conditions, especially soil pH, moisture, and soil microbial activity. In tropical environments like Samarinda, ammonia volatilization from urea can occur quite rapidly if not immediately incorporated into the soil (Setiyono et al. 2017). In this study, the gradual application of urea (100 kg ha<sup>-1</sup> at planting and 50 kg ha<sup>-1</sup> at 14 DAT) and the use of a mixed medium of soil and chicken manure (rich in organic matter) most likely minimized N loss, thereby increasing fertilization efficiency.

This finding is also in line with the principle of balanced fertilization, where optimal N availability must be balanced with other nutrients such as phosphorus (P) and potassium (K). In this study, basal fertilizers SP-36 and KCl were applied evenly to all treatments, so the plant response to urea could be validly isolated. This strengthens the conclusion that the increased growth of pakcoy indeed originated from the additional N supply, not from unexpected interactions with other nutrients.

Agronomically, the dose of 150 kg ha<sup>-1</sup> urea proved effective for pakcoy in an intensive polybag cultivation system. However, from a sustainability perspective, the long-term use of urea without an integrative approach (e.g., with biofertilizers or organic matter) risks causing soil quality degradation and environmental pollution through nitrate (NO<sub>3</sub><sup>-</sup>) runoff (Zhang et al. 2020). Therefore, although urea

showed a dominant growth response, sustainable fertilization strategies should combine urea with biological agents like PNSB, even though in this study their synergy was not significant, possibly due to the urea dose already meeting the plant's needs, thus inhibiting the N<sub>2</sub> fixation activity of PNSB by the presence of free ammonia (Olivares et al. 2013).

Thus, the use of urea remains the primary choice for increasing pakcoy productivity in the short term, but it needs to be balanced with environmentally friendly agricultural practices to maintain the sustainability of vegetable production systems in the future.

### **Effect of PNSB Photosynthetic Bacteria on the Growth and Yield of Pakcoy**

The results showed that the application of PNSB photosynthetic bacteria at a concentration of 5 mL L<sup>-1</sup> significantly increased the number of leaves (14-28 DAT), stem diameter, fresh weight, and dry weight of pakcoy plants (Table 1). This increase is consistent with the dual role of PNSB as agents of biological nitrogen fixation and producers of growth phytohormones.

Photosynthetic bacteria PNSB, especially the genera *Rhodopseudomonas* and *Rhodobacter*, are known to be able to fix atmospheric nitrogen (N<sub>2</sub>) into ammonium (NH<sub>4</sub><sup>+</sup>) form through the activity of the nitrogenase enzyme under anoxic or microaerobic conditions (Madigan 1995; Gallon 2001). This process allows plants to obtain an additional nitrogen source without complete dependence on chemical fertilizers. In this study, the increase in the number of leaves and stem diameter in the PNSB treatment indicates that the nitrogen from fixation had been absorbed and utilized efficiently by the plant for the synthesis of proteins, chlorophyll, and cell structures.

Furthermore, PNSB are known to produce Indole Acetic Acid (IAA), one of the main auxins in plants, through tryptophan metabolism (Lee et al. 2021). Indole Acetic Acid plays an important role in stimulating cell division and elongation, especially in root and stem meristem tissues. The production of IAA by PNSB not only increases lateral root growth

but also enhances the capacity for water and nutrient uptake, which ultimately supports biomass accumulation (Wani et al. 2016). This explains the increase in fresh weight (24.24%) and dry weight (71.2%) of plants inoculated with PNSB compared to the control (Table 1).

This finding is in line with research by Wong et al. (2014) which reported that inoculation with *Rhodopseudomonas palustris* on pakcoy (*Brassica rapa* L.) increased vegetative growth by up to 30% even under low nitrogen fertilization conditions. Similarly, the results of Merugu et al. (2012) showed that *Rhodobacter capsulatu*\* increased plant height, number of tillers, and total nitrogen content in rice, confirming the potential of PNSB as a universal biofertilizer for food crops.

However, in this study, the effect of PNSB was not significant on stem height and root length. This phenomenon can be explained through two physiological mechanisms. First, the translocation of fixed nitrogen appears to be directed more towards photosynthetic organs (leaves) than stem elongation tissues, so the growth response is more dominant in the number of leaves and leaf area. Second, soil chemical analysis showed that phosphorus (P = 61.31 ppm) and potassium (K = 510.33 ppm) content were in the optimal to very high range. Phosphorus is a key element in the development of the root system, while potassium plays a role in the regulation of turgor pressure and stem cell elongation (Hardjowigeno 2007). The abundance of available P and K likely caused the growth response of roots and stems to have reached a saturation point, so the additional contribution from PNSB was no longer statistically significant.

Furthermore, the effectiveness of PNSB is also influenced by the micro-environmental interactions around the roots and leaf surfaces. Although PNSB are facultative and able to live under both aerobic and anaerobic conditions, nitrogen fixation activity is most optimal under dim light and high humidity conditions (Lee et al. 2021). In a polybag cultivation system in an open field like in this study, fluctuations in daily light intensity and temperature may have limited the metabolic

efficiency of PNSB, so the growth response was not as strong as reported in controlled systems (greenhouse or hydroponics).

It should also be noted that although PNSB provided significant benefits, its effect was still less dominant compared to urea fertilizer. This indicates that in the short term, chemical fertilizers remain the most efficient nitrogen source for driving rapid growth of short-lived vegetables like pakcoy. However, from a sustainable agriculture perspective, PNSB offers a strategic solution to reduce dependence on synthetic fertilizers, minimize environmental pollution due to nitrogen runoff, and improve nutrient use efficiency through plant-microbe systems (Elbadry & Elbanna 1999; Olivares et al. 2013).

Thus, the use of PNSB as a component in an integrated fertilization system, not a total replacement for chemical fertilizers, is a more realistic and sustainable approach. Further research is recommended to evaluate the optimum dose of PNSB in combination with a gradual reduction of urea dose to achieve a balance between productivity and environmental sustainability.

## CONCLUSION

The interaction effect between urea and PNSB was significant on stem height and root length. Urea fertilizer at 150 kg ha<sup>-1</sup> significantly increased all growth and yield variables of pakcoy plants. PNSB photosynthetic bacteria at 5 mL L<sup>-1</sup> increased the number of leaves, stem diameter, and plant weight. Their combination increased fresh weight by 73.36% compared to the control. However, at high urea doses, PNSB did not provide significant additional benefits, indicating the need for dose adjustment to achieve optimal synergy.

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