

## Growth and Yield Response of Pagoda Mustard to Nitrogen Fertilizer and Biostimulant Application on Podzolic Soil

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### ABSTRACT

Pagoda mustard is a new type of vegetable in Indonesia with high economic potential, but its cultivation is still limited. One of the challenges in its development is the use of Red-Yellow Podzolic soil, which is characterized by acidic pH, low organic matter, and limited nutrient availability, all of which can hinder plant growth. The use of biostimulants and balanced fertilization in cultivated plants can provide optimal growth and yield. The research was carried out in West Kalimantan, for two months from May to July 2024, using a Factorial Completely Randomized Design with two factors: biostimulants application (without and with biostimulants) and nitrogen fertilizer application at three levels (100, 200, and 300 kg.ha<sup>-1</sup>). The results indicate that the combined application of biostimulants and nitrogen fertilizer did not show interactions for any observed variables. However, a single treatment of biostimulants could increase the number of leaves, leaf area index, root volume, dry weight, and amino acid levels of pagoda mustard plants. Nitrogen fertilizer at 100 kg.ha<sup>-1</sup> was found to be an efficient treatment to increase the growth and yield.

**Keywords:** *Brassica narinosa*; Fertilization; Nutrient availability; Organic content; Soil acidity

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### INTRODUCTION

Pagoda mustard (*Brassica narinosa* L.) is a relatively new vegetable crop in Indonesia and has not yet been widely cultivated. However, it has high economic value and promising market prospects due to increasing consumer demand for fresh vegetables. In 2022, the production of mustard in West Kalimantan reached 4,277.5 tons, a decrease from the previous year's 5,118.4 tons (Statistics Indonesia 2023). This decline may be related to reduced soil fertility, nutrient imbalances, and suboptimal fertilization practices (Kurniawati and Very 2019). The development of pagoda mustard is particularly attractive because it has a relatively shorter harvest period than other mustard varieties, typically harvested within 40–60 days after sowing (Dwiratna et al. 2022). Additionally, pagoda mustard has a sweeter taste than other mustard types, making it more appealing to consumers who dislike the bitter taste commonly found in leafy vegetables. As a relatively new *Brassica* species in cultivation, it adds diversity to vegetable production. Thus, efforts to expand pagoda mustard production in West Kalimantan are needed.

One approach to developing pagoda mustard cultivation is the use of Red-Yellow Podzolic soils, which are widely found in West Kalimantan, covering approximately 17.28% of the province (West Kalimantan Provincial Government 2019). However, podzolic soils are often acidic, low in organic matter, and nutrient-deficient, limiting plant growth. Improving the quality of podzolic soils can be achieved through the application of biostimulants and balanced fertilization.

Biostimulants are natural or synthetic organic compounds, distinct from fertilizers, applied to plants or soils to enhance growth, productivity, and stress tolerance (Du Jardin 2015). They can positively affect plant development and can be derived from sources such as plant extracts, seaweed, microorganisms, fungi, bacteria, humic acids, amino acids, nitrogen-containing compounds, and chitosan. In this study, amino acid biostimulants derived from fish fermentation were used.

Biostimulants act as a nutrient source for microorganisms in soil, thereby increasing microbial activity and organic matter decomposition, improving nutrient exchange and soil health. They can stimulate plant growth, influence physiological processes, and enhance tolerance to environmental stress. For example, Saban et al. (2018) found that liquid biostimulants significantly increased plant height, fresh weight, and dry weight of mustard. Hattalaibessy et al. (2020) reported that biostimulant containing *Bacillus* spp applied at 3.5 mL.L<sup>-1</sup> every seven days improved plant growth and

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productivity in shallots. The effectiveness of biostimulants depends on concentration, dosage, application method, active ingredients, and plant compatibility.

Plant growth in pagoda mustard is also highly influenced by fertilizer type, particularly nitrogen fertilizer. Nitrogen is an essential macronutrient that is important for growth and development. It a crucial role in the synthesis of amino acids, proteins, and nucleic acids (Dini et al. 2021) and contributes to chlorophyll formation (Soepriyanto 2021), which is vital for photosynthesis. Nitrogen deficiency causes stunted growth and leaf chlorosis, while excess nitrogen can cause excessive vegetative growth, weak stems, and susceptibility to pests and diseases. In this study, urea fertilizer was used.

Nitrogen application promotes vegetative growth, resulting in broader, greener, and higher-quality leaves. Aminah et al. (2021) found that 150 kg.ha<sup>-1</sup> urea increased leaf length and fresh weight in pakcoy mustard. Rolanda et al. (2021) reported that 250 kg.ha<sup>-1</sup> urea produced the highest growth and yield in bitter mustard. Although both nitrogen and biostimulants have clear benefits, little research has focused on their combined effects on pagoda mustard grown in podzolic soils. Therefore, this study aimed to evaluate the growth and yield responses of pagoda mustard to biostimulant application and different nitrogen fertilizer dosages on podzolic soils.

## MATERIALS AND METHODS

The study was conducted at Rusunawa Untan, Jalan Sepakat 2, Southeast Pontianak District, Pontianak City, West Kalimantan, with coordinates 0.062° S and 109.345° E, with an altitude of ± 1 masl.. The research was conducted from May 24 to July 30, 2024. Materials included pagoda mustard seeds, podzolic soil, amino acid biostimulant, urea fertilizer, SP36, K fertilizer, chicken manure, and dolomite lime. Tools used included a machete, a hoe, buckets, watering cans, polybags, plastic sheets, rulers, digital and manual scales, labels, measuring cups, an oven, documentation tools, and stationery.

The experiment used a Factorial Completely Randomized Design (CRD) with two factors: biostimulant application (b0 = without, b1 = with biostimulant) and nitrogen (urea) fertilizer (n1 = 100 kg.ha<sup>-1</sup>, n2 = 200 kg.ha<sup>-1</sup>, n3 = 300 kg.ha<sup>-1</sup>). There were six treatment combinations with three replications, each consisting of four plants.

Soil media preparation involved mixing 7 kg of podzolic soil with 210 g of chicken manure and 3 g of dolomite per polybag, followed by two weeks of incubation.. Seeds were sown in trays containing a soil-manure mix. Seedlings with four true leaves were transplanted to polybags (one plant per polybag). Nitrogen fertilizer was applied according to treatments, along with recommended phosphorus and potassium fertilizers. Nitrogen was reapplied four weeks after planting (WAP). Biostimulants were applied weekly for four weeks until harvest. Routine maintenance included watering, replanting, weeding, and pest control. Harvesting occurred 45 days after transplanting.

Observed variables included the number of leaves, leaf area, canopy area, leaf area index, root volume, fresh weight, dry weight, chlorophyll content, protein content, and amino acid content. Data were analyzed using ANOVA, followed by the Honest Significant Difference (HSD) test at the 5% level.

## RESULTS AND DISCUSSIONS

### Number of leaves

Observations in Table 1 indicated that the application of biostimulant and nitrogen fertilizer independently increased the number of leaves in pagoda mustard, particularly at 8 WAP (weeks after planting), which represents the peak of the vegetative phase. At 4 and 6 WAP, both treatments did not show significant effects, suggesting that the physiological response of the plants to the treatments was still in the initial stage of accumulation.

Amino acids in biostimulants function as precursors for protein, enzyme, and plant hormone synthesis, thereby promoting leaf development. Hildebrandt et al. (2015) reported that amino acids support vegetative growth by enhancing photosynthetic activity and chlorophyll formation. Moreover, these compounds help optimize nitrogen absorption, thereby supporting cell division and leaf expansion. Consequently, plants treated with biostimulants exhibited better leaf growth compared to untreated plants.

The sole application of nitrogen fertilizer at 200 kg.ha<sup>-1</sup> produced the highest number of leaves, indicating that this dosage represents an optimal and efficient fertilization level for pagoda mustard in Podzolic soils. This finding is consistent with the study of Fauzi et al. (2021), who reported that nitrogen application at 200 kg.ha<sup>-1</sup> increased leaf number in Samhong King mustard. Nitrogen is a key nutrient required by plants for growth and the formation of vegetative organs such as stems, leaves, and roots (Suhastyo and Raditya 2019). It plays a vital role in chlorophyll synthesis, photosynthesis, and the acceleration of plant growth. The increase in leaf number serves as an indicator of plant growth and development, which ultimately determines crop productivity. The improvement in leaf number following nitrogen application reflects sufficient nutrient supply during the vegetative phase, thus preventing nutrient stress that could inhibit leaf formation.

### Leaf area

Leaf area is a crucial indicator for assessing a plant's photosynthetic capacity, which directly influences its productivity. Based on the results presented in Table 1, the application of 100 kg ha<sup>-1</sup> nitrogen (N) fertilizer had a significant effect on the leaf area of pagoda mustard plants. This finding indicates that a moderate application of nitrogen can effectively meet the plant's physiological needs without inducing excessive vegetative growth. Conversely, higher nitrogen doses, such as 300 kg ha<sup>-1</sup>, can lead to a growth imbalance, where the plant experiences excessive vegetative growth. This condition results in inefficient distribution of nutrients and energy for leaf formation (Hariodamar et al. 2018). Similarly, research by Handayani et al. (2020) demonstrated that using medium doses of nitrogen fertilizer significantly

increased the leaf area of mustard plants without causing the physiological stress associated with nitrogen excess. Therefore, selecting the appropriate nitrogen fertilizer dose is a critical strategy in fertilization management. It not only supports optimal leaf growth but also maintains the economic efficiency of fertilizer use.

### Canopy area

The results of this extensive canopy study, as detailed in Table 1, show that the application of a moderate dose of nitrogen combined with this biostimulant increases the efficiency of nitrogen utilization by the plants. This finding aligns with research by Sari and Roviq (2019), who found that a nitrogen fertilizer application of 100 kg ha<sup>-1</sup> led to improved vegetative growth. Specifically, it resulted in a better increase in the average number of leaves and leaf area, which directly expanded the canopy size.

The efficiency of nitrogen use significantly impacts a plant's vegetative growth, particularly its leaf area and canopy. Efficient nitrogen uptake ensures an even distribution of nutrients throughout the plant, including newly developing leaves. This promotes a well-proportioned canopy that supports optimal light absorption for photosynthesis. Efficient photosynthesis can only happen when the plant canopy has an open structure that allows light to penetrate the lower leaf layers. Conversely, high doses of nitrogen often lead to excessive vegetative growth, making the canopy too dense. A dense canopy inhibits light penetration to the lower parts of the plant, reducing photosynthetic efficiency and interfering with the development of the lower leaves, which are crucial for overall plant productivity (Yustiningsih 2019).

### Leaf area index

The Leaf Area Index (LAI) is closely related to the shape and spread of leaves within the plant canopy. The total leaf area influences the rate of transpiration, where water evaporates from the leaf surface, helping to regulate the microclimate around the plant. LAI is a key parameter used to evaluate the efficiency of photosynthesis, light use, and potential crop productivity

at the canopy scale. It is also an important component of plant growth analysis, used to determine the intensity of radiation intercepted by the leaves and to estimate biomass (Zakariyya 2016). Based on the observations shown in Table 2, a higher LAI in the amino acid biostimulant treatment indicates an increased capacity for the plant to utilize light for photosynthesis, which supports its overall growth. Amino acid biostimulants serve as precursors for the synthesis of plant proteins and hormones, such as auxins and cytokinins. These hormones play a vital role in the division and elongation of leaf cells (Du Jardin 2015). This effect leads to an expansion of the leaf surface area, which subsequently increases the efficiency of water and nutrient use. Leaves that are evenly distributed and have a large surface area can regulate the temperature and micro-humidity around the plant through stable transpiration. An increase in LAI as a result of this treatment is often positively correlated with higher yields and improved plant resistance to environmental stress.

### Root volume

Based on the observations presented in Table 2, the application of an amino acid biostimulant significantly affects root volume, with an average of 3.44 cm<sup>3</sup>, compared to the control group. Amino acid biostimulants are crucial for transporting essential ions into root cells, thereby increasing nutrient absorption efficiency and promoting stronger, healthier root growth. This finding is consistent with research by Van Oosten et al. (2017), who stated that amino acid biostimulants can accelerate root growth and enhance the plant's nutrient absorption capacity.

Furthermore, amino acid biostimulants applied to the soil influence the microbial activity in the rhizosphere (the area around the roots). These microorganisms utilize amino acids as an energy source, which helps to improve the soil structure, making it more conducive for plant root growth. This is supported by Rouphael and Colla (2020), who reported that biostimulants increase rhizosphere microbial activity and improve soil conditions, thereby supporting optimal root growth.

**Table 1.** Number of leaves, leaf area, and canopy area of pagoda mustard plants in podzolic soil due to the application of biostimulants and nitrogen fertilizers

| Treatments                    | Number of Leaves |              |                 | Leaf Area (cm²)  | Canopy Area (cm²) |
|-------------------------------|------------------|--------------|-----------------|------------------|-------------------|
|                               | 4 WAP            | 6 WAP        | 8 WAP           |                  |                   |
| Biostimulant                  |                  |              |                 |                  |                   |
| b0 (Without)                  | 14,72 ± 0,40     | 26,31 ± 1,03 | 59.44 ± 1.48 b  | 432,91           | 432,91 ± 18,8     |
| b1 (With)                     | 15,61 ± 0,52     | 28,17 ± 1,17 | 68.14 ± 2.68 a  | 426,61           | 426,61 ± 12,8     |
| Sig. (p)                      | 0,09             | 0,25         | 0,01            | 0,07             | 0,57              |
| Nitrogen Fertilizer           |                  |              |                 |                  |                   |
| N1 (100 kg.ha <sup>-1</sup> ) | 15,58 ± 0,50     | 28,21 ± 1,24 | 56.80 ± 1.72 b  | 474.61 ± 1.09 a  | 474.61 ± 18.9 a   |
| N2 (200 kg.ha <sup>-1</sup> ) | 14,67 ± 0,66     | 27,00 ± 1,47 | 68.04 ± 3.01 a  | 438.99 ± 1.22 ab | 438.99 ± 19.4 a   |
| N3 (300 kg.ha <sup>-1</sup> ) | 15,25 ± 0,53     | 26,50 ± 1,38 | 66,54 ± 2,93 ab | 375.69 ± 1.11 b  | 375.69 ± 15.4 b   |
| Sig. (p)                      | 0,95             | 0,67         | 0,00            | 0,03             | 0,01              |

**Table 2.** Leaf area index, root volume, fresh weight, and dry weight of pagoda mustard plants in podzolic soil due to the application of biostimulants and nitrogen fertilizers

| Treatments                         | Leaf Area Index | Root Volume (cm <sup>3</sup> ) | Fresh Weight (g) | Dry Weight (g) |
|------------------------------------|-----------------|--------------------------------|------------------|----------------|
| <b>Biostimulant</b>                |                 |                                |                  |                |
| <b>b0 (Without)</b>                | 2.94 ± 0.06 b   | 2.67 ± 0.17 b                  | 121.50 ± 1.93    | 7.95 ± 0.32 b  |
| <b>b1 (With)</b>                   | 3.40 ± 0.01 a   | 3.44 ± 0.18 a                  | 136.51 ± 4.48    | 9.92 ± 0.50 a  |
| <b>Sig. (p)</b>                    | 0.04            | 0.01                           | 0.07             | 0.01           |
| <b>Nitrogen Fertilizer</b>         |                 |                                |                  |                |
| <b>N1 (100 kg.ha<sup>-1</sup>)</b> | 3.19 ± 0.23     | 3.00 ± 0.26                    | 136.26 ± 4.18    | 9.29 ± 0.67    |
| <b>N2 (200 kg.ha<sup>-1</sup>)</b> | 3.11 ± 0.29     | 3.00 ± 0.26                    | 128.36 ± 5.95    | 8.99 ± 0.68    |
| <b>N3 (300 kg.ha<sup>-1</sup>)</b> | 3.21 ± 0.18     | 3.17 ± 0.31                    | 122.41 ± 4.52    | 8.53 ± 0.67    |
| <b>Sig. (p)</b>                    | 0.92            | 0.85                           | 0.36             | 0.64           |

Research by [Tahapary et al. \(2020\)](#) on lettuce plants showed that applying amino acid biostimulants, either through the leaves or soil, significantly increased the fresh weight, dry weight, and root length. A more developed root system allows the plant to absorb more water and nutrients from the soil, which has a direct positive impact on the plant's overall growth, as reflected in its dry weight. The dry weight of a plant, which is the total amount of organic compounds, depends on the rate of photosynthesis and nutrient absorption by the roots. Healthy roots have a high capacity to absorb essential nutrients from the soil. These nutrients are then used in various physiological processes, all of which contribute to an increase in the plant's dry weight.

### Fresh weight

Based on the observation results, the biostimulant and nitrogen fertilizer treatments did not show a significant effect on the fresh weight of the plants, as indicated in [Table 2](#). Although biostimulants can increase nutrient absorption efficiency and vegetative growth, their effects are highly dependent on soil conditions and plant species. A study by [Gofar et al. \(2022\)](#) on red chili plants showed that while biostimulant application improved soil quality and nutrient content, its effect on the plant's fresh weight was not statistically significant. This finding suggests that the combined application of nitrogen fertilizer and biostimulants does not always produce a synergistic effect. It highlights the complexity of the interaction between nutrients and the plant's response to these treatments. The physiological response of a plant to agronomic inputs is often not linear. It is influenced by various factors, such as the plant's growth stage, weather, and other biotic and abiotic factors. Therefore, even if there is an increase in nutrient absorption, it does not necessarily translate into a significant increase in fresh weight.

### Dry weight

Dry weight is a direct reflection of accumulated biomass and crop productivity. The increase in a plant's dry weight is an indicator of growth and is a direct result of the photosynthesis process. The biostimulant

treatment, as shown in [Table 2](#), resulted in a higher average dry weight (9.92 g) compared to the control group (without biostimulants).

A greater dry weight indicates that the photosynthesis process is more efficient and that tissue cell productivity and development are higher and faster, leading to better overall plant growth. This aligns with the observation that a larger leaf area and a greater number of leaf blades can result in the highest dry weight ([Sarif 2017](#)). Research by [Kocira et al. \(2020\)](#) also supports these findings, stating that the application of seaweed with amino acids increases the growth and productivity of *Phaseolus vulgaris* plants. Amino acid biostimulants can improve a plant's physiological balance and increase enzymatic activity, which supports biomass formation. Amino acids aid in metabolic processes, such as the synthesis of chlorophyll and proteins, which are essential for forming organic matter. An increase in the growth of vegetative organs—such as an increase in the number of leaves, plant height, and root elongation—and the efficiency of assimilate distribution to different parts of the plant, will directly impact the increase in dry weight ([Anjarwati et al. 2017](#)).

### Leaf chlorophyll levels

Nitrogen is a primary constituent of chlorophyll. Chlorophyll levels are often used as an indicator of a plant's nitrogen content ([Pratama and Sukmaningsih 2019](#)). Chlorophyll a and chlorophyll b are the two main types of chlorophyll, absorbing light most strongly in the red spectrum (600–700 nm) and least in the green spectrum (500–600 nm). The presence of chlorophyll gives leaves their characteristic green color; the higher the chlorophyll content, the deeper the green color ([Mandal and Dutta 2020](#)). The results presented in [Table 3](#) show that the highest level of chlorophyll b was achieved with a nitrogen fertilizer dose of 200 kg ha<sup>-1</sup>, producing 25.73 mg l<sup>-1</sup>. This is consistent with research [Damanik et al. \(2019\)](#), which demonstrated that higher doses of nitrogen fertilizer significantly increase the chlorophyll content in plants compared to lower doses.

**Table 3.** Leaf chlorophyll levels, protein levels, and amino acid levels of pagoda mustard plants in podzolic soil due to the application of biostimulants and nitrogen fertilizers

| Treatments                    | Leaf Chlorophyll Levels (mg.L <sup>-1</sup> ) |                 |                   | Protein Levels (%) | Amino Acid Levels(%) |
|-------------------------------|-----------------------------------------------|-----------------|-------------------|--------------------|----------------------|
|                               | Chlorophyll A                                 | Chlorophyll B   | Chlorophile Total |                    |                      |
| Biostimulant                  |                                               |                 |                   |                    |                      |
| b0 (Without)                  | 13.25 ± 1.47                                  | 22.99 ± 2.01    | 36.19 ± 2.19      | 1.69 ± 0.12        | 2.17 ± 0.29 b        |
| b1 (With)                     | 15.43 ± 2.43                                  | 20.10 ± 1.82    | 35.59 ± 3.20      | 1.82 ± 0.17        | 3.41 ± 0.25 a        |
| Sig. (p)                      | 0.55                                          | 0.17            | 0.83              | 0.57               | 0.01                 |
| Nitrogen Fertilizer           |                                               |                 |                   |                    |                      |
| N1 (100 kg.ha <sup>-1</sup> ) | 13.88 ± 2.24                                  | 16.63 ± 2.59 b  | 30.46 ± 2.89      | 1.74 ± 0.27        | 3.00 ± 0.44          |
| N2 (200 kg.ha <sup>-1</sup> ) | 15.08 ± 2.79                                  | 25.73 ± 1.55 a  | 40.42 ± 2.87      | 1.81 ± 0.17        | 2.86 ± 0.40          |
| N3 (300 kg.ha <sup>-1</sup> ) | 14.06 ± 2.68                                  | 22.28 ± 1.11 ab | 36.78 ± 2.99      | 1.71 ± 0.05        | 2.50 ± 0.47          |
| Sig. (p)                      | 0.94                                          | 0.01            | 0.06              | 0.93               | 0.62                 |

The addition of nitrogen also triggers the upregulation of genes associated with flavonoid production, primarily through the activity of the enzyme chalcone synthase, which plays a crucial role in the biosynthesis of anthocyanins (Wan et al. 2015). Nitrogen fertilizers directly affect plant chlorophyll levels by supplying the essential nitrogen elements needed for chlorophyll synthesis. An adequate nitrogen application will increase chlorophyll levels, which is visible in the more intense green color of the leaves, indicating sufficient chlorophyll for efficient photosynthesis. Conversely, a lack of nitrogen will cause a decrease in chlorophyll levels, resulting in leaf yellowing (chlorosis). However, excessive nitrogen application can lead to poor or weak growth of young shoots, soft and easily collapsible stems, and increased susceptibility to diseases and pests.

### Protein levels

The observation results showed that the treatments with biostimulants and nitrogen fertilizers had no significant effect on the protein levels of pagoda mustard, as seen in Table 3. While amino acid biostimulants are known to improve nutrient absorption efficiency, increase stress tolerance, and support vegetative growth, their direct effect on protein synthesis in plant tissues can vary. This variability depends on factors such as plant species, environmental conditions, and the specific dose applied. On the other hand, nitrogen application in the form of fertilizers plays a direct role in building organic nitrogen components, including amino acids and proteins. Nitrogen is known to accelerate plant growth and increase the levels of protein and fats in plants (Pramitasari et al. 2016). Interestingly, different doses of nitrogen did not result in significantly different protein levels. This can occur because plants utilize nitrogen only up to a certain optimal level of need (Ulfah 2015). Once this optimal threshold is exceeded, nitrogen tends to be allocated more toward vegetative growth, such as in leaves and stems, rather than to an increase in protein content within plant tissues. The effect of amino acid biostimulants and nitrogen fertilizers on plant protein content is influenced by the interaction between the

applied doses, the plant's growth phase, nutrient balance, and environmental factors.

### Amino acid levels

The biostimulant treatment resulted in a higher average plant amino acid level (3.41%) compared to the control group (without biostimulants), as shown in Table 3. This is because amino acid biostimulants contain various amino acids that plants can directly absorb through their leaves and roots. Research by Bari (2023) revealed that applying biostimulants based on 40 amino acids and hormones to corn (*Zea mays* L.) can improve crop growth and yield quality. This study indicates that biostimulant treatment increases the nutritional content, including amino acids, in corn plants. By giving supplement with exogenous amino acids, plants can directly utilize them for protein synthesis and other metabolic processes.

### CONCLUSIONS AND SUGGESTIONS

The application of amino acid biostimulants enhanced growth parameters such as number of leaves, leaf area index, root volume, dry weight, and amino acid content of pagoda mustard. Nitrogen fertilizer at 100 kg.ha<sup>-1</sup> was the most efficient dosage for improving growth and yield in Red-Yellow Podzolic soil. Although no significant interaction was observed between biostimulants and nitrogen fertilizer, the independent application of biostimulants showed positive effects on plant performance.

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