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PREFACE

The second issue of 2025, Kultivasi Volume 24 No. 2, includes research articles and reviews from a variety of agricultural scientific fields, including plant production, soil science, plant breeding, and plant protection.

Additionally, this edition includes pieces written by authors from around the globe that offer fresh insights into agriculture. This diversity of expertise will enhance the agricultural knowledge and propel scholars' and researchers' research and publishing forward.

The Kultivasi Team works to raise the articles in order to sustain consistent success and expand internationally. We intend to keep offering the greatest support possible to other writers, editors, reviewers, and agriculture industry stakeholders. We are incredibly grateful to scholars and researchers who contribute to developing our journal, especially for this edition.

AUTHOR'S INSTRUCTIONS

Manuscript that met scientific requirements can be published. The original manuscript is sent to the editor in accordance with the writing requirements as listed below. Editors have the right to change and suggest improvements in accordance with the norms of science and scientific communication. Editors cannot accept papers that have been published in other publications.

The manuscript is typed on Microsoft Word software, on A4 size paper with a writing length ranging from 6-15 pages and followed the template. The manuscript in the Jurnal Kultivasi can be written in English with an effective and academic language style.

The full manuscript is sent to the editors accompanied by a cover letter from the author. The sent manuscript is a group of original paper, soft file of images and other suplementary materials. The editor issues the letter of manuscript acceptance to author once the paper is considered to be going to publish.

Special Requirements

Review Articles:

Articles should discuss critically and comprehensively the development of a topic that is actual public concern based on new findings supported by sufficient and up-to-date literature. Before writing an article, it is recommended that the author contact the Chairman of the Editorial Board for clarification of the selected topic.

The systematics of writing peel articles consists of: Title, author's name and correspondence address; Abstract with keywords; The Introduction contains justifications for the importance of the topic being discussed; Subject matter; Conclusion; Acknowledgment; and References.

Research Articles:

The original manuscript is compiled on the basis of the following sections:

Title

- The title must be brief and indicate the identity of the subject, the purpose of the study and contain keywords and be written in Bahasa Indonesia and English. Titles range from 6-20 words, created with capital letters except for latin names written in italics.

Author's name

- The authors must list the name without the title, profession, agency and address of the place of work and the author's email clearly in accordance with applicable ethics. If it is written by more than one author, the writing of the order of names should be adjusted according to the contribution level of each author. The writing of the name of the first author is written the last syllable first (although not the surname), while the subsequent author the initial syllable is abbreviated and the next syllable is written in full. For example: Tati Nurmala and Yudithia Maxiselly then written as Nurmala, T. and Y. Maxiselly

Abstract

- Abstract is an informative writing that is a brief description about the background, objectives, methods, results and conclusions. Abstract is written in English with a maximum of 250 words and equipped with keywords.

Introduction

- Introduction presents the background on the importance of research, underlying hypotheses, general approaches and research objectives as well as related literature reviews.

Materials and Method

- Materials and Methods contains an explanation of the time, place, technique, design, plant material and other materials of experiment as well as statistical data analysis. It should be writen in detail so that it is repeatable and reproduceable. If the method used is known in advance then the reference should be listed.

Results and Discussions

- Results and discussions are briefly outlined assisted by informative tables, graphs and photographs. The discussion is a brief and clear review of research results and refers to previous related literatures. Table or Figure Captions are written in English.

Conclusion

- Conclusion is the final decision of the conducted research and the follow-up advice for further studies.

Acknowledgment

- Acknowledgment to sponsors or parties who support the research briefly.

Reference

There are at least 20 references from the last 10 years. The references list all related libraries along with the aim of making it easier to search for readers who need it. Only list libraries that have been published either in the form of textbooks or scientific articles. Using an internationally applicable article author's name writing system. Inside the text, the reference should be written as follows:

- Two authors: Tati Nurmala and Yudithia Maxiselly *then written* Nurmala and Maxiselly (2014) or (Nurmala and Maxiselly, 2014).
- Three or more authors: Nurmala et al. (2014) or (Nurmala et al., 2014).
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  Example: Zakry FAA, Shamsuddin ZH, Rahim KA, Zakaria ZZ, Rahim AA. 2012. Inoculation of Bacillus sphaericus UPMB-10 to young oil palm and measurement of its uptake of fixed nitrogen using the 15N isotope dilution technique. Microbes Environ., 27: 257–262.

CONTENTS

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Buswar AS · Ruminta · Wicaksono FY

Rice (*Oryza sativa* L.) production, productivity, and harvested area in Karawang Regency under extreme weather from 1991-2023

Abstract. Climate change occurred in Karawang Regency due to extreme weather events, which are included in the El Nino climate anomaly phenomenon. The existence of extreme weather events in Karawang Regency has decreased rice production, which is a climate-dependent crop. Based on this problem, a study was conducted to analyze the identification of extreme weather changes and correlation analysis of rice crops in Karawang Regency to see the magnitude of extreme weather changes and the influence of extreme weather elements on rice crops. The method used is a quantitative descriptive method by analyzing extreme weather changes, correlation analysis of extreme weather elements on rice plants (production, productivity, and harvest area), and correlation graphs of extreme weather elements with rice plants. Data was obtained from BPS, BMKG, and the Karawang Regency Agriculture and Food Security Office. The results showed that Karawang Regency experienced extreme weather changes due to climate change, namely an increase in the average maximum rainfall (1.78 mm), an increase in maximum temperature (0.76 °C), a decrease in minimum temperature (-0.57 °C), a decrease in wet spell for 3 days, and an increase in dry spell for 10 days. The impact of extreme weather change, namely the wet spell element, has a real significant correlation with a moderate level to a decrease in rice production and productivity.

Keywords: Climate change · Correlation · Extreme weather · Rice · Trend analysis

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Introduction

Extreme weather events are part of the variability of the climate system, both in stable conditions and climate change, and negative impacts that arise, such as heavy rains, storms, and tropical cyclones (Vellore et al., 2020). These climate anomalies are becoming more frequent with more extreme seasonal conditions and prolonged durations that significantly impact agricultural production in many countries, including Indonesia (IPCC, 2001). Therefore, extreme weather changes can be attributed to climate change (Rehana et al., 2022). Climate indices that indicate extreme weather changes are generally related to rainfall and air temperature (Alizadeh-Choobari & Najafi, 2018). These two indicators have visible changes and can be identified regarding food cropping patterns (Ruminta, 2016).

One indication of climate change is changes in the pattern and intensity of various climate parameters, including erratic rainfall, intensity that tends to increase, and a shift in the beginning and length of the season that deviates or is extreme compared to normal conditions (Martel et al., 2021). Extreme weather informs the increasing weather and climate along with the increase of unusual natural phenomena (climate deviations) (Ummenhover & Meehl, 2017). The impact of extreme weather conditions is usually associated with increased rainfall intensity, the occurrence of hydrometeorological disasters such as flash floods and tidal floods, local storms, increased air temperatures, and drought (Kundzewicz, 2016). The impacts of extreme weather cause changes in rainfall patterns, the length of the rainy season, shifts at the beginning of the rainy season, and increased extreme weather events that seriously impact the agricultural sector, especially food crops (Mall et al., 2017).

The agricultural sector, which is highly dependent on climate, suffers losses due to extreme weather events. The agricultural sector in Indonesia is highly vulnerable to climate change, as climatic conditions affect production, yield quality, cropping patterns, and planting times (Rejekiningrum et al., 2022). One crop that is highly dependent on climate is rice (*Oryza sativa* L.). Rice strongly depends on climate elements, especially rainfall and temperature (Hussain et al., 2020). This dependence can be seen from the physiological impact of rice when rainfall decreases and temperature increases, namely the inhibition of the process of filling and ripening seeds, decreasing the rate of photosynthesis, which will affect the formation of carbohydrates, and reducing pollen viability, causing yield loss (Rahman et al., 2017). This extreme decrease in rainfall causes low productivity of rice plants, delays rice planting, decreases the area of planting and harvesting, disrupts the growth of rice plants because the average water needs of plants are reduced, and causes crop failure because rice plants are plants that are vulnerable to climate change (Hussain et al., 2020).

Extreme weather is divided into five indicators that significantly impact agriculture due to climate change: maximum rainfall, maximum temperature, minimum temperature, wet spells (consecutive rainy days), and dry spells (consecutive days without rain). Maximum rainfall can lead to flooding, waterlogging, and poor seed distribution, especially in direct-seeded rice fields. These conditions hinder germination, reduce plant populations, and increase vulnerability to disease, ultimately lowering rice yields (Darise, 2023). High maximum temperatures cause rice plants to suffer from heat stress, disrupting photosynthesis and respiration, particularly during growth stages, which can result in spikelet sterility, poor grain filling, and significant yield loss (Basak et al., 2013). Low minimum temperatures lead to cold stress during the reproductive stages (booting and heading), causing spikelet degeneration, incomplete panicle development, and impaired nitrogen uptake, all of which can drastically reduce yield (Beleten, 2019). Dry spells result in drought conditions, leading to water stress, particularly during the flowering and grain filling stages. This can cause poor seed development and, in severe cases, total crop failure (Molla et al., 2021). Wet spells, on the other hand, create waterlogged conditions, increasing the risk of root diseases and reducing oxygen availability to the roots. These effects can delay planting, hinder seedling establishment, and negatively impact crop growth and yield (Kaur et al., 2020). These five indicators are interconnected and collectively pose a significant threat to rice crops, particularly during their critical growth phases.

In addition to the decline in rice production and productivity due to extreme weather, this also impacts the planting season. Supposedly,

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three planting seasons can be carried out within one year, but currently, only two planting seasons can be carried out with planting times that are backward from the schedule (Wang et al., 2022). This extreme weather event needs to be a special concern because rice is the main food crop in Indonesia, especially in Karawang Regency, one of the most significant rice production contributing areas from West Java, which is the most significant contributor to rice production in Indonesia (Suliman & Setiawan, 2022). The decline in rice production in Karawang Regency continues to occur over the years. It can be seen that rice production in Karawang Regency has decreased, as seen in the data from 2023 to 2024, by 7.61%, from 1,131,977 tons to 1,045,879 tons (BPS, 2024). The decline in rice production is the impact of the problems in Karawang Regency, namely the problem of climate change due to extreme weather, including the El Nino event (Dewi et al., 2023).

To see the correlation of rice production, productivity, and harvest area in Karawang Regency with extreme weather events, it is necessary to analyze the data using Microsoft Excel from extreme weather elements, including maximum rainfall, minimum and maximum air temperature, consecutive rainy days (wet spell) and days without rain (dry spell), in the last 33 years (1991-2023) which are divided into 2 periods, namely the 1991-2006 period and the 2007-2023 period to determine the magnitude of extreme weather changes in Karawang Regency.

Materials and Methods

The research was conducted in Karawang Regency, and data were taken from the Karawang Regency BMKG, the Karawang Regency Food Crops and Forestry Agriculture Office, and the Karawang Regency Central Statistics Agency (BPS). The research took place from January to March 2025. The design of the method used in this research is a descriptive method with a quantitative approach to analyze the impact of extreme weather events on production, productivity, and rice harvest areas in Karawang Regency. The data used are climate data such as temperature, rainfall, and extreme weather (maximum rainfall, maximum temperature, minimum temperature, dry spell, and wet spell for 33 years, namely 1991-2023, rice crop data from 1991-2023 (production, productivity, and harvest area). The obtained data was analyzed using Microsoft Excel 2019 and Minitab 19 statistical software to obtain extreme weather results and their correlation with rice plants in Karawang Regency.

The research data were analyzed using correlation and trend analysis at a 5% significance level:

1) Pearson correlation analysis between changes in temperature and rainfall in each sub-district in Karawang Regency.

$$r = \frac{\sum_{i=1}^{n} x_i y_i - \frac{1}{n}(\sum_{i=1}^{n} x_i)(\sum_{i=1}^{n} y_i)}{\sqrt{(\sum_{i=1}^{n}(x_i)^2 - \frac{1}{n}(\sum_{i=1}^{n} x_i)^2)(\sum_{i=1}^{n}(y_1)^2 - \frac{1}{n}(\sum_{i=1}^{n} y_1)^2)}}$$

where :
r = correlation coefficient; xi = rainfall or temperature data for each sub-district in Karawang Regency; yi = production data, harvest area, and rice productivity of each sub-district in Karawang Regency.

2) Trend line equation (regression)

$$Y = b_0 + b_1 x$$
$$b_0 = \frac{(\sum_{i=1}^{n} y_i)}{\text{n}}$$
$$b_1 = \frac{\sum_{i=1}^{n}(x_i y_i)}{\sum_{i=1}^{n}(x_i)^2}$$

where: Y = trend value of rainfall or temperature or maximum rainfall or maximum temperature or minimum temperature or wet spell or dry spell in Karawang Regency; b_0 = constant value, which is the value of Y when the value of X = 0; b_1 = the value of the slope of the line, which is the additional value of Y, if X increases by one unit; X = Year period value

Results and Discussion

Analysis of Extreme Weather Changes. Karawang Regency in the 1991-2006 and 2007-2023 periods experienced extreme weather changes as shown in Table 1. The average maximum rainfall increased by 1.78 mm from the 1991-2006 period of 340.14 mm, increasing in the 2007-2023 period to 341.93 mm. The increase in maximum rainfall occurred due to an increase in Earth's temperature, which causes an increase in evaporation events and the volume of water in cloud formation, causing higher-intensity rain

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(Puspitasari et al., 2016). The maximum average temperature has increased and the minimum average temperature has decreased. The maximum average temperature from the 1991-2006 period of 35.32 °C increased by 0.76 °C in the 2007-2023 to 36.08 °C. Meanwhile, the minimum average temperature decreased by -0.57°C from 19.19 °C to 18.62 °C.

Table 1. Changes in extreme weather in Karawang Regency in the period 1991-2006 and 2007-2023

Climate Indicators	Extreme Weather Change		The Magnitude of Extreme Weather Change
	Period 1991-2006	Period 2007-2023	
Average Maximum Rainfall (mm)	340.14	341.93	1.78 mm
Maximum Average Temperature (°C)	35.32	36.08	0.76 °C
Minimum Average Temperature (°C)	19.19	18.62	-0.57 °C
Average of Wet Spell	9	6	-3
Average of Dry Spell	77	88	10

The increase in temperature, both maximum and minimum temperatures, is caused by global warming factors, with an increase in the average temperature of the Earth's surface within a specific period that occurred in Karawang Regency. In Indonesia, air temperature increased from the 1900s to the 20th century, increasing by 1.2°C (Siswanto et al., 2016). Apart from Karawang Regency, an increase in temperature has also occurred in the South Sumatra region, with an average increase in temperature of 0.5°C (Muharomah & Setiawan, 2022). The results of the study by Stocker et al. (2007) suggest that the climate will continue to warm or increase in temperature over a specific period due to the emission of gas and carbon dioxide, where the two elements will remain in the atmosphere for a hundred years even until nature can reabsorb these elements and the atmosphere returns to normal. This temperature increase occurred due to a massive increase in greenhouse gases such as CO_2 gas produced from fossil fuels and deforestation (Yoro & Daramola, 2020). This excessive concentration of greenhouse gases causes the sun's heat to be trapped in the atmosphere, which causes the temperature to rise.

Meanwhile, the average number of wet spells or consecutive rainy days decreased by -3 days, from 9 days in 1991-2006 to 6 days in 2007-2023. The decrease is inversely proportional to the increase in the average maximum rainfall, indicating that in Karawang Regency, the intensity of rainfall is increasing. However, the rainy time is getting shorter in both periods. The average number of dry spells or days without rain has successively increased in the two periods from the 1991-2006 period of 77 days to 88 days in the 2007-2023 period, with an increase of 10 days. The increase in dry spells indicates an increasingly dry climate in Karawang Regency.

Correlation of Extreme Weather Changes to Changes in Production, Productivity, and Harvest Area of Rice Crops in Karawang Regency. The results of the Pearson correlation of Karawang District (1991-2023) in Table 2 showed a positive and negative correlation relationship using a 5% significance level. From the table, it can be seen that the wet spell is significantly correlated with changes in the production and productivity of rice plants, with the results of negative correlation values in the relationship between the wet spell and the production and productivity of rice plants in Karawang Regency amounting to -0.49 and 0.49. The correlation value showed that the wet spell has a moderate relationship with production and productivity. The more consecutive rainy days that were not punctuated by dry days (wet spells) tended to decrease the amount of rice. Long-lasting wet spell conditions have the potential to cause waterlogging in agricultural land, reduce oxygen levels in the soil, and increase the risk of pest and plant disease attacks (Debangshi, 2021). In addition, too wet conditions can disrupt plant growth phases, especially in the early planting and flowering stages, which ultimately results in a decrease in yield (Ding et al., 2020). It can be indicated that prolonged wet conditions and low night temperatures can inhibit plant growth (Wahyuni et al., 2022). Consecutive rains lead to increased pest attacks and crop losses that can reduce rice production (Simkhada & Thapa, 2022).

Table 2. The correlation between extreme weather and the production, productivity, and harvested area of rice crops in Karawang Regency

Correlation	Production (tons)	Productivity (q/ha)	Harvest Area (ha)
Maximum Rainfall (mm)	0.16	0.12	0.27
Maximum Temperature (°C)	0.25	0.21	0.31
Minimum Temperature (°C)	-0.13	-0.04	-0.23
Wet Spell	**-0.49***	**-0.49***	-0.30
Dry Spell	0.07	0.06	0.19

Note: (*) Significant Correlation

Production changes have a medium correlation value with the wet spell. A wet spell is a consecutive period with several rainy days, usually associated with high rainfall and a more even water distribution. The negative correlation of wet spells to rice production results in a decrease in rice production due to the increase in wet spells. Too long wet spell conditions harm the growth of rice plants. Excessive rain can cause flooding, physiological stress on rice plants, and inhibit oxygen supply to rice roots, causing the death of rice roots and causing plants to die or harvest failure (Weng et al., 2017; Mahmood et al., 2019). Research in Subang showed that rice production decreased by up to 11.2% per year in several sub-districts, especially on rainfed land vulnerable to excess water due to intense wet spells (Ruminta et al., 2018).

In addition to production, productivity changes moderately correlate with wet spells. The negative correlation of wet spells to rice productivity results in a decrease in rice production due to the increase in wet spells. The wet spell can reduce the number of rice planting seasons from three times to twice a year because the land is flooded for too long, making it impossible to replant quickly (Yoon & Choi, 2020). In addition, if wet spells occur intensely, water is not absorbed optimally, which increases the risk of erosion and soil nutrient loss, so that rice productivity can decrease (Datta et al., 2017). Rice is so vulnerable to consecutive rainy days because it is tolerant to water but not to prolonged and deep inundation (Singh et al., 2017).

Between 1991 and 2023, rice cultivation in Karawang Regency faced severe challenges due to extreme wet weather conditions. This phenomenon triggered outbreaks of plant pests and diseases (PPDs), physical crop damage, and reduced planting intensity. Each rainy season or major flood event frequently led to brown planthopper infestations and bacterial leaf blight, resulting in crop failure across thousands of hectares of rice fields (Sogawa, 2014). Physical damage was also widespread, particularly during the 2013/2014 (7,700 ha) and 2023 (8,865 ha) planting seasons, where plant lodging occurred due to waterlogging (Tommi et al., 2015; Republika, 2023). These climatic disturbances, combined with infrastructure damage and land-use conversion, have significantly altered cropping patterns. The Cropping Index (CI), which previously reached CI 300 (three planting cycles per year) in irrigated areas during 1991–2005, has declined to CI 200 since the mid-2010s (Riadi, 2018). As a result, intensification strategies have shifted toward improving productivity per growing season.

Physiologically, rice plants are highly vulnerable to excessive rainfall or drought. When wet spell conditions lead to flooding in rice fields, a significant physiological impact is oxygen deficiency. This occurs because flooding induces hypoxia in the roots, reducing aerobic respiration and ATP production, which in turn disrupts root growth and function (Cai et al., 2025). Additionally, impaired gas exchange (O_2/CO_2) and chlorophyll degradation reduce photosynthesis. Turbid floodwater further reduces light penetration and photosynthetic efficiency (Gautam et al., 2015). In addition to physiological disruptions, nutrient availability is also impaired. Reduced potassium uptake results from impaired root function, and limited nitrogen availability is caused by anaerobic conditions that promote denitrification and inhibit nitrification (Tian et al., 2021).

Prolonged rainy seasons cause flooding, which disrupts the physiology and nutrition of rice plants, often leading to crop failure. Another significant impact of flooding is rice lodging, where plants fall over due to weakened stems that cannot support the weight of ripening grains (Liu et al., 2022). This condition arises during the grain-filling phase when stems begin to age. Excessive nitrogen (N) fertilization causes overly

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lush growth that weakens the stems, while increased fungal and bacterial activity further degrades plant tissues, accelerating lodging (Panja et al., 2024; Liu et al., 2024; Dong et al., 2023). This phenomenon is supported by research in Deli Serdang District, which found that in an analysis of the correlation between wet spells and rice productivity, yields tended to decrease, likely due to flooding and waterlogging (Chaniago, 2023). Wet spells also create ideal conditions for the development of rice pests and diseases, such as fungi and bacteria. These conditions increase the severity of disease outbreaks and further reduce yields, as rice plants are highly sensitive to pest and disease attacks (Asibi et al., 2019). Moreover, excessive moisture reduces the effectiveness of fertilization and pest control—nutrients are easily washed away, and pesticides become less effective (Kumar et al., 2021). As a result, crop yields decline despite stable or even increased levels of production inputs.

The observed correlation between wet spells and the decline in rice production and productivity in Karawang Regency highlights the urgent need for strategic adaptation over the next 15 years (2023–2038) to mitigate this issue. Strategic adaptation refers to deliberate adjustments, both environmental and social, in response to the adverse effects of climate change (Hessburg et al., 2021). Farmers and agricultural extension agents in Karawang can adopt several adaptive strategies, including the use of high-yield rice varieties that are tolerant to drought and flooding, such as Inpari 32, Inpari 48, and Cibatu 06 (Purbiati et al., 2024). In addition to varietal selection, appropriate cropping patterns—such as rice-rice-legume rotation—should be implemented, along with equitable irrigation water management across districts (Cox et al., 2025). Furthermore, Integrated Pest Management (IPM) should be promoted in accordance with recommendations from the Indonesian Center for Forecasting Plant Pests and Diseases (BBPOPT), including the implementation of coordinated pest control campaigns (Kusano et al., 2025).

Trends and Distribution of Extreme Weather and Their Correlation with Rice Crop Production, Productivity, and Harvested Area in Karawang Regency. Extreme weather events that correlate with rice cultivation include wet spells, which affect both rice production and productivity. As shown in Figure 1, the trend line illustrates the relationship between the number of wet spell days (consecutive rainy days) and total rice production (tons) in Karawang Regency from 1991 to 2023. An analysis of rice production reveals an average annual increase of 9,556.7 tons. Despite interannual fluctuations, this increase is largely attributed to farmers' use of high-yielding rice varieties such as Inpari 32, which is flood-tolerant, and Ciherang, which is drought-tolerant and highly productive (Aenunnisa et al., 2022). These varieties have demonstrated the ability to yield up to 9 tons per hectare, even during the dry season (Aenunnisa et al., 2022). In addition to varietal improvements, planting intensity has also increased, with farmers now practicing three rice cropping cycles per year, following recommendations from local government authorities (Mulya & Hudalah, 2024). This practice has significantly contributed to the upward trend in rice production in Karawang Regency.

Meanwhile, the number of wet spell days exhibits an inverse pattern compared to production. The annual average number of wet spell days in Karawang Regency from 1991 to 2023 has shown a declining trend of approximately 0.1319 days per year. This decline is associated with a reduction in annual average rainfall of around 47.3 mm over the 1991–2022 period, with the most significant decreases occurring during peak rainy months, such as January (approximately 39.27 mm) and March (approximately 9.7 mm) (Ruminta et al., 2024). Concurrently, the average temperature in Karawang Regency has increased by approximately 0.56 °C over the past 32 years (1991–2022), influencing evapotranspiration rates and soil moisture levels, and ultimately affecting the duration and intensity of the rainy season (Gunawan et al., 2024). The combination of reduced rainfall and rising temperatures has contributed to the declining trend in wet spell occurrences. The negative correlation between the number of wet spells and rice production is visually evident from the decline in production during years with a surge in wet spells. This phenomenon suggests that excessive consecutive rainy days can adversely affect total production—both through reduced yields per hectare and partial crop failures due to flooding, waterlogging, or disruption of critical plant growth stages (Bedane et al., 2022). Although the overall production trend shows an increase, the decreasing number of wet spells remains an external factor that may hinder the achievement of optimal rice yields.

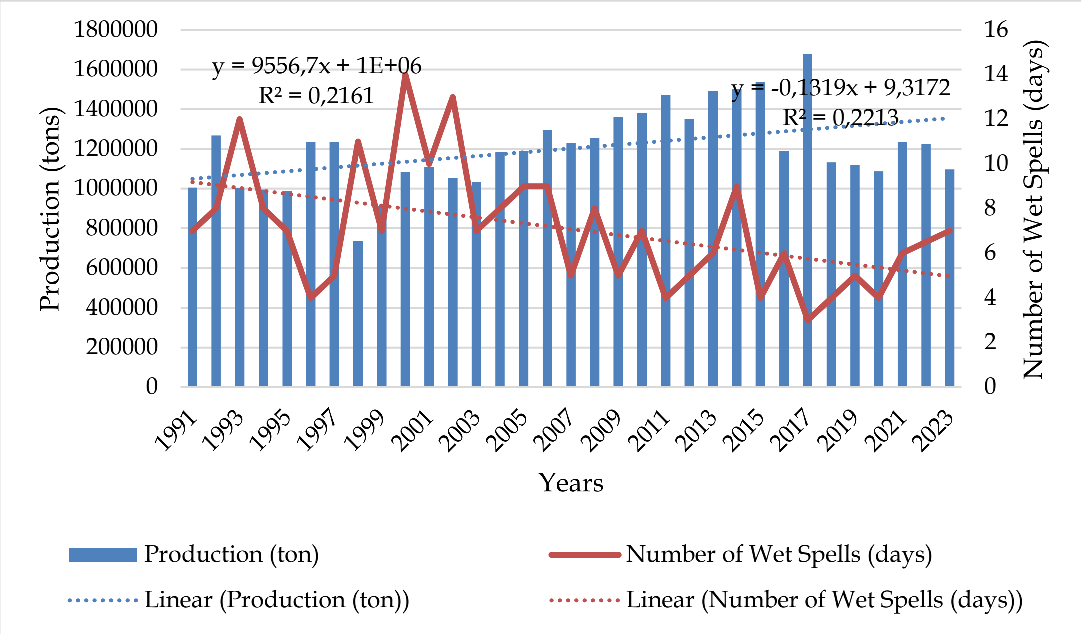

Figure 1. Distribution of wet spells and their relationship with rice production in Karawang Regency

In addition to its significant correlation with production, wet spells also show a significant correlation with rice productivity. Figure 2 illustrates the distribution of wet spells (consecutive rainy days) and rice productivity (q/ha) in Karawang Regency from 1991 to 2023. An analysis of rice productivity reveals an annual increase of 0.355 q/ha. This increase is attributed to a technical efficiency score of 0.9607, which is considered high, supported by factors such as expanded land use, the adoption of high-yielding varieties (Ciherang and Inpari 32), and the application of high-quality pesticides (Aenunnisa et al., 2022). Additionally, government intervention has played a significant role in enhancing rice productivity in Karawang Regency. This is evident through the provision of subsidized high-quality seeds, such as Inpari 32, and the distribution of modern agricultural machinery (e.g., rice threshers), which have helped farmers increase yields per hectare (Mulyani et al., 2020). The upward trend in rice productivity in Karawang Regency is therefore driven by improved technical efficiency, the use of superior rice varieties, and government support in the form of seed subsidies and agricultural infrastructure.

Meanwhile, the number of wet spells exhibits an inverse pattern with production, where the average annual wet spell duration in Karawang Regency from 1991 to 2023 shows a declining trend of -0.1319 days per year. This downward trend in wet spells is attributed to reduced rainfall and increased air temperatures, both of which are influenced by the ENSO (El Niño–Southern Oscillation) climate anomaly (Wang et al., 2017). ENSO has caused Karawang Regency to experience both El Niño and La Niña events (Frimansyah et al., 2022). The declining trend in wet spells is primarily driven by El Niño, which leads to prolonged dry seasons, less frequent rainfall, and reduced rainfall intensity—factors that shorten the duration and frequency of wet spells (Yang et al., 2021). The negative correlation between the number of wet spells and rice productivity is visually evident, with productivity declining in years when wet spells increase. This suggests that a higher number of consecutive rainy days (wet spells) tends to reduce rice productivity. Prolonged wet spells can lead to waterlogging in agricultural fields, lower soil oxygen levels, and increase the risk of pest and disease outbreaks (Sun et al., 2017). Moreover, excessively wet soil conditions can disrupt critical plant growth stages, particularly during early planting and flowering, ultimately lowering yields and interfering with the planting schedule, resulting in reduced rice productivity (Short et al., 2016).

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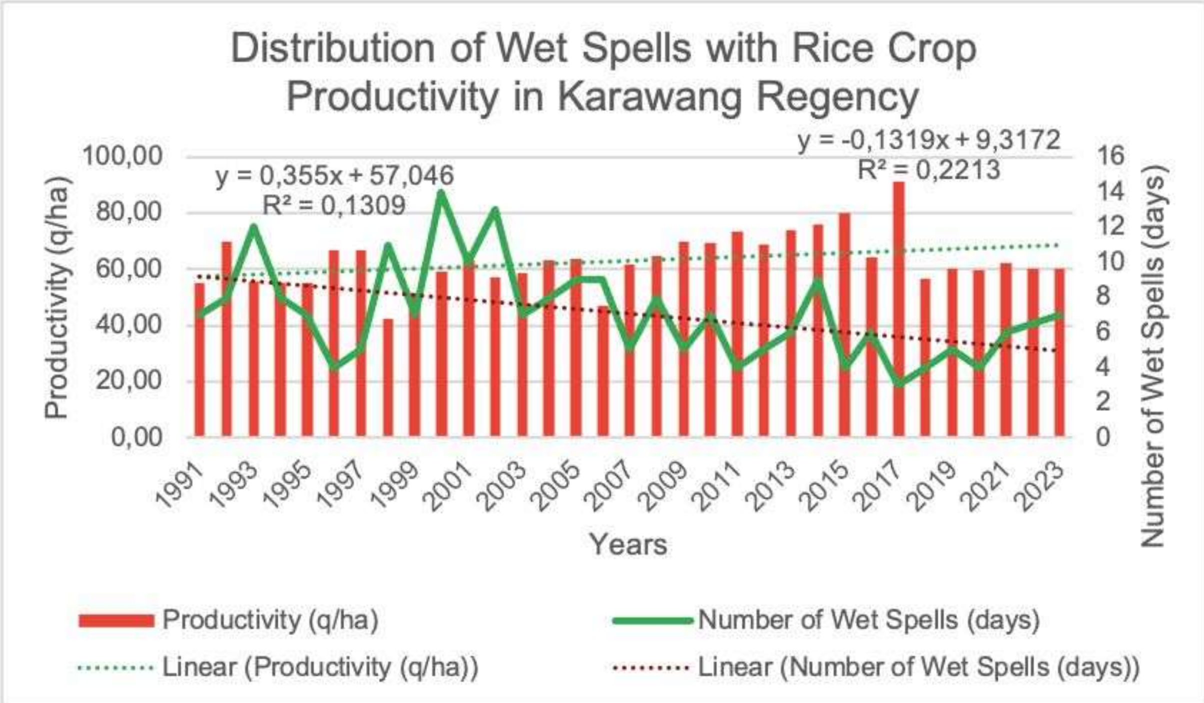

Figure 2. Distribution of wet spells and their relationship with rice plant productivity in Karawang Regency

Conclusion

Karawang Regency is experiencing climate change that impacts extreme weather events, as seen in the increase in maximum rainfall, maximum temperature, and dry spells, then a decrease in minimum temperature and wet spells in 1991-2023. Extreme weather events in Karawang Regency during the wet spell element significantly correlate with changes in rice crop production and productivity, resulting in decreased production and productivity.

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Post-drought growth recovery of tea (*Camellia sinensis*) under different techniques and doses of biofertilizer applications

Abstract. Drought stress is a major limiting factor affecting the growth, yield, and quality of tea (*Camellia sinensis*). The present study aimed to analyze the success of post-drought growth recovery of tea in response to different techniques and doses of biofertilizer applications. Field experiments were conducted from January to August 2024 in the experimental garden of the Research Institute for Tea and Cinchona, Gambung Blok A8, Bandung. This work was arranged in a split-plot design, consisting of a main plot with two biofertilizer application techniques (foliar feeding and soil drenching) and subplots with four levels of biofertilizer dosage (control (B_1), 15 L ha^{-1} (B_2), 22.5 L ha^{-1} (B_3), and 30 L ha^{-1} (B_4), with three replications. The results showed a significant interaction of biofertilizer dose and technique on shoot dry weight and plant growth rate of tea. There was an independent effect of biofertilizer dose on leaf area ratio. Applying a biofertilizer dose of 15 L ha^{-1} through soil drenching produced the best plant growth rate and shoot dry weight at the 6th harvest.

Keywords: Biofertilizer · Post-drought · Plant growth analysis · Tea (*Camellia sinensis*)

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Introduction

Tea plants are susceptible to environmental changes; non-ideal environmental conditions can cause plant stress. The impact of drought can be seen visually in tea plantations, which is the morphological response of tea plants to survive. Under drought stress, the growth of roots, stems, and leaves decreases, accompanied by a reduction in the leaf-to-stem ratio, accelerated senescence of mature leaves, and inhibited development of new foliage (Hemati et al., 2022). Environmental factors such as drought stress in tea plants are the main limiting factors that affect the growth, yield, and quality of tea plants (Qian et al., 2018). Climate change caused by global warming has led to prolonged droughts. Tea growth, yield, and quality are closely related to environmental factors such as temperature, rainfall, and soil health, rendering tea plants highly vulnerable to climate change (Omer et al., 2024). Climate change has a significant impact on plants, as photosynthesis depends on temperature, water, and nutrient availability (Dusenge et al., 2019).

Drought conditions caused decreased biomass, decreased plant height, number of roots, leaf stomatal closure, decreased photosynthetic rate, metabolic disturbances, and increased activity of enzymes such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) that play an important role in enhancing drought tolerance (He et al., 2020; Hemati et al. 2022). The crucial condition of tea plants after drought requires optimal strategies, such as fertilizers, rainwater harvesting, irrigation systems, etc. Plant management based on ecophysiology, which includes internal and external factors of tea plantations such as plants, climate, and sufficient nutrients, can improve the growth and quality of tea (Anjarsari et al., 2020). According to Kudoyarova et al. (2015), the availability of water and nutrients significantly affects plant growth and productivity. Plant shoots' development depends on the water supply and nutrients in the soil, especially nitrogen and phosphorus.

Fertilization is one of the strategies used to recover tea plants after drought; however, tea plantations generally rely on inorganic fertilizers in the cultivation process. Repeated application of chemical fertilizers will cause damage to soil properties and environmental damage (Gebrewold, 2018). According to Debele (2021), the main factor leading to decreasing soil fertility is the continuous use of inorganic fertilizers, which causes degradation of soil structure and reduces soil aggregation so that nutrients are easily lost through fixation, leaching, and increases soil acidity. Biofertilizers help the growth of tea plants post-drought, where plants are in the production phase, so they need sufficient nutrients to maintain and increase production. The biofertilizer microorganisms used in this research are categorized as nitrogen fixing (*Azospirillum* sp. and *Azotobacter* sp.) and phosphate solubilizing (*Bacillus* sp. and *Pseudomonas* sp.). Microorganisms in biofertilizers can provide nutrients and promote plant growth under stressful conditions by solubilizing phosphate, fixing nitrogen, and providing other macro or micronutrients (Singh et al., 2022).

The previous study by Reetz et al. (2015) reported that efficient and effective fertilization can be obtained by paying attention to the type of fertilizer, fertilizer dosage, fertilizer application time and technique. According to Niu et al. (2020), the application of fertilizer through the leaves allows plants to absorb nutrients directly through the leaves (stomata), while fertilization through the soil is absorbed by the plant's roots. Application of biofertilizers in excessive doses cause competition between microbes for nutrients, and the performance of biofertilizers is less effective if the application of excessive or insufficient doses (Sinulingga et al., 2015). Rhizosphere microorganisms get energy sources from root exudates, while phyllosphere microorganisms get energy sources from carbohydrates released by leaves (Ali et al., 2024; Bashir et al., 2022).

After drought, plants experience physiological disorders due to a lack of water, nutrients, and oxidative damage. Applying biofertilizers at optimal doses combined with proper fertilization techniques can support tea plant growth by fixing nitrogen, dissolving phosphate, increasing nutrients, and phytohormones (gibberellin, cytokinin, auxin). The effectiveness of fertilizer dosage is determined not only by the amount applied but also by the technique of application, which affects the efficiency of nutrient uptake by the plant. Lower doses may be able to provide optimal results under proper fertilization techniques. The decline in tea productivity due to drought requires appropriate recovery strategies. The

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application of biofertilizers with proper fertilizer application techniques has the potential to enhance nutrient uptake efficiency and accelerate plant recovery. This study aims to evaluate biofertilizers' effectiveness and determine the optimal fertilizer application technique and dosage to support tea plants' growth and leaf yield after drought stress.

Materials and Methods

Field experiments were conducted from January to August 2024 in the experimental field of the Research Center for Tea and Chincona, Gambung block A8, Bandung district. The experimental field is 1,350 meters above sea level (MASL) with Andisol soil order. The average rainfall is 2,960 mm $year^{-1}$. The materials used in this research include: 1) 27-year-old tea plant clone GMB-7, 2) liquid biofertilizer containing nitrogen-fixing microorganisms *Azospirillum* sp. and *Azotobacter* sp. (1.24 x 10^{11} CFU/ml), and phosphate-solubilizing microorganisms *Bacillus* sp. and *Pseudomonas* sp (1.36 x 10^{11} CFU/ml), 3) Inorganic fertilizers (Urea, TSP, KCl, and Kieserit). The tools used in the research are hand sprayer for biofertilizer application, measuring cup, bamboo, plastic clips, measuring type Krisbow 7.5 m, hoe, tea harvesting net, permanent marker, analytical scales, and scanner Canonscan Lide 200.

The experimental design used a split-plot design, consisting of a main plot with two levels of biofertilizer application techniques and a subplot with four levels of biofertilizer dosage, with three replications. The main plot consisted of foliar feeding (A_1) and soil drenching (A_2). In contrast, the subplots consisted of different dosage treatments: without bio-fertilizer (B_1), 15 L ha^{-1} (B_2), 22.5 L ha^{-1} (B_3), and 30 L ha^{-1} (B_4), which were based on the recommended application doses for tea plants and represent decreasing levels to evaluate the optimal dosage for enhancing post-drought recovery. Liquid biofertilizer was dissolved in 500 ml of water according to the calibration of tools in the field, applied according to the treatment, namely without biofertilizer (500 ml of water), 15 L ha^{-1} (13.5 ml/plant + 486.5 ml of water), 22.5 L ha^{-1} (20.25 ml/plant + 479.75 water), and 30 L ha^{-1} (27 ml/plant + 473 ml of water). The biofertilizer was applied a day after the pre-plucking, with an application interval of 25 days, 6 times. The biofertilizer application technique was carried out according to the treatment, namely, sprayed on the leaves evenly using a hand sprayer and drenched into the soil using a measuring cup. Each experimental unit measured 2 x 5 m with 10 plants, totaling 24 units. A row of tea plants was used as a border between plots.

The plucking is done using plucking scissors. The type of medium plucking is shoots consisting of pecco shoots with two leaves (p+2), three young leaves (p+3m), or dormant shoots with two or three young leaves (b+2m, b+3m). The observation parameters included leaf area, shoot dry weight, leaf area ratio, and plant growth rate. Leaf area and shoot dry weight were obtained from 50 g of fresh shoots taken from each plucking. Plucking was conducted every 25 days over six sampling periods (Sepriana et al., 2023). After sampling, leaf area was measured using an image scanner and ImageJ software.

Leaf area ratio and plant growth rate were calculated using the formula according to (Pandey et al., 2017). The data from the analysis and observations were processed and statistically analyzed using the SmartstatXL program. The data were organized into an analysis of variance (ANOVA) table and analyzed using the F-test (Fisher) at a 95% confidence level. If the analysis of variance indicated significant differences, the results were further tested using Duncan's Multiple Range Test at the 5% significance level.

1. Crop Growth Rate (CGR): The increase in plant dry matter production per unit area per unit time was measured at 25-day intervals over six sample periods.

$$\text{CGR} = \frac{W_2 - W_1}{GA(t_2 - t_1)} \ \text{g m}^{-2}\ \text{day}^{-1}$$

   Where:
   W = shoot dry weight
   t = time
   GA = Ground Area
2. Leaf Area Ratio (LAR): the ratio of leaf area to dry weight, which indicates the efficiency of leaf surface in producing dry matter, was measured at 25-day intervals over six sampling periods.

$$\text{LAR} = A/W \quad \text{cm}^2\ \text{g}^{-1}$$

   Where:
   A = leaf area
   W = shoot dry weight

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Results and Discussion

Leaf Area. Leaves are the main organs of plants where photosynthesis occurs. According to Huang et al. (2019), plant leaves are where light energy is processed into chemical energy and carbohydrates (glucose) through photosynthesis. According to Tondjo et al. (2015), plant leaves play a role in the formation of biomass through the process of photosynthesis. In tea production, the apical shoots along with the first two to three leaves are regularly harvested continuously, so the tea leaf area is an important component in studying tea plant physiology (Jayasinghe et al., 2015). Figure 1 shows the leaf area of tea plants for 6 times of pluckings. Different fertilizer application techniques did not significantly affect the leaf area of tea plants in a short period. Post-drought tea plants have a plucking layer thickness that is less than optimum (5-10 cm), implying that plant lacks assimilate to expand the leaves. The ideal plucking layer thickness is around 15-20 cm (Anjarsari et al., 2021). Leaf expansion requires an adequate supply of carbohydrates from source organs, mainly from mature leaves (Costa et al., 2007). The photosynthetic capacity of young tea leaves, young buds, and shoots depends on the layer of maintenance foliage to supply the assimilates (Hajiboland, 2017).

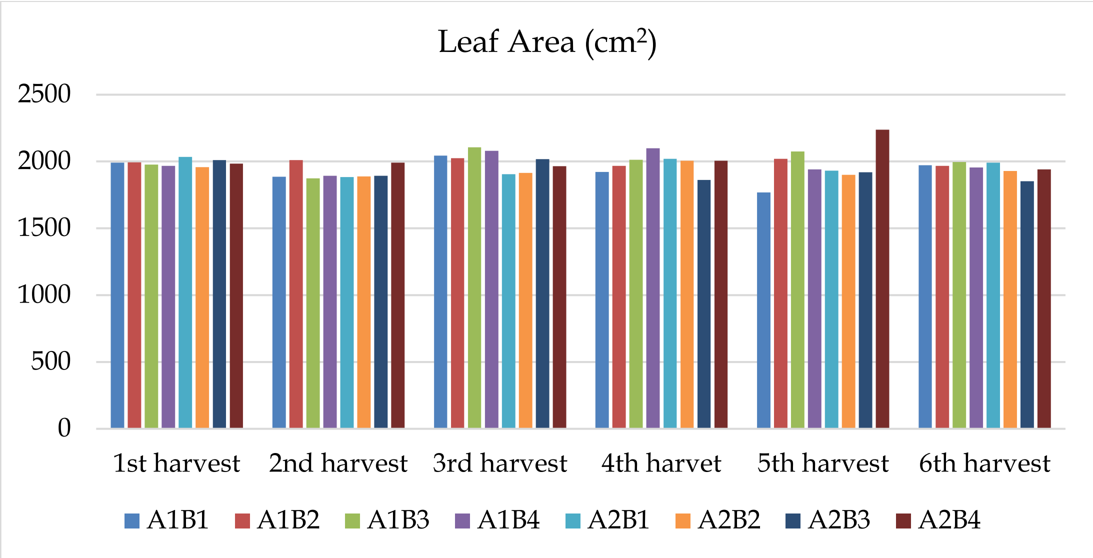

Figure 1. Leaf area (cm^2) of tea plants for 6 times of harvests in response to different biofertilizer application techniques and doses

Note: foliar feeding + without bio-fertilizer (A1B1), 15 L ha^{-1} via foliar feeding (A1B2), 22.5 L ha^{-1} via foliar feeding (A1B3), 30 L ha^{-1} via foliar feeding (A1B4), soil drenching + without bio-fertilizer (A2B1), 15 L ha^{-1} via soil drenching (A2B2), 22.5 L ha^{-1} via soil drenching (A2B3), 30 L ha^{-1} via soil drenching (A2B4).

Table 1. Interaction effect of biofertilizer application techniques and doses on shoot dry weight (g) at 2nd harvest.

Treatment	Biofertilizer Doses (L ha^{-1})			
	B_1 : 0 L	B_2 : 15 L ha^{-1}	B_3 : 22.5 L ha^{-1}	B_4 : 30 L ha^{-1}
A_1 : Foliar feeding	12.67 a B	13.77 a A	12.97 a AB	13.10 a AB
A_2 : Soil drenching	12.81 a AB	12.31 b B	12.93 a AB	13.35 a A

Note: The mean values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 0.05 significance level. Lowercase letters (a,b) read vertically, comparing the two techniques at the same dose (within a column). Uppercase letters (A, B) read horizontally, comparing four doses for the same technique (within a row).

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Shoot dry weight. There was an interaction effect between biofertilizer application technique and dose on shoot dry weight at the 2nd, 5th, and 6th plucking. At the first plucking, the application of biofertilizer with various fertilization techniques did not show any significant effect on shoot dry weight. Meanwhile, during the 3rd and 4th pluckings, the decrease in rainfall likely influenced the plant's response to fertilizer application, thereby affecting the resulting shoot dry weight. In May and June, the rainfall decreased to 128.3 mm/month and 220.4 mm/month, respectively.

Table 1 shows the interaction effect between biofertilizer application technique and dose at the 2nd harvest. The treatment sprayed on the leaves at a dose of 15 L ha^{-1} was able to produce the highest dry weight, while application through the soil requires a higher dose of 30 L. This is probably because nutrients are absorbed more quickly through the leaves. Dry weight shows that plants get enough nutrients, because bacteria in biofertilizers help nitrogen fixation and phosphate solubilization. This indicates that plants get enough nutrients. According to Anjarsari et al. (2021), the dry weight of tea shoots reflects the net accumulation of CO_2 in a unit of time based on the plucking cycle, where the increase in dry matter becomes the main parameter in quantitative analysis of plant growth. According to Niu et al. (2020), fertilizer application through the leaves accelerates nutrient absorption, and nutrients enter through the stomata. Fertilizer application through the leaves is absorbed quickly through the pores and leaf surface, so nutrients are absorbed faster than through the roots (Gupta et al., 2023).

Table 2 shows an interaction effect of biofertilizer application doses and techniques on shoot dry weight at the 5th harvest. The treatment of soil drenching at a dose of 30 L ha^{-1} gives the best results, while in foliar application, the dose levels showed relatively similar values. When applied to the soil, microbes need time to adapt and colonize, so the application of fertilizer to the soil requires a longer time to be able to increase the dry weight of shoots. According to Demir et al. (2023), when biofertilizers are applied to the soil, microorganisms will colonize the rhizosphere and help increase the efficiency of plant nutrient absorption. According to Fageria et al. (2009) plants respond to fertilizers applied through the soil for a longer time than foliar fertilization. Nutrients applied through the soil have a long-term effect on plant growth, while foliar application is only temporary. Rainfall aids nutrient dissolving in the soil and can also affect the performance of foliar-applied biofertilizers. The 5th plucking was done in July with 320 mm/month of rainfall. Rainfall has a negative effect on foliar application. Strong winds and rain can directly affect bacterial colonization on leaf surfaces (Bashir et al., 2022).

Table 3 shows an interaction effect of biofertilizer application doses and techniques on the dry weight of shoots at the 6th harvest. The biofertilizer treatment sprayed onto the leaves at a dose of 22.5 L ha^{-1} gives the best results, while application through soil showed relatively similar values at the different dose levels. 6th plucking was conducted in August, where the experimental field rainfall was below optimal at 79.1 mm/month, so the biofertilizer application technique would affect the effectiveness of nutrient absorption. According to Supriadi & Rokhmah (2014), optimal rainfall for tea plant growth ranges from 223-417 mm/month. According to Mandic et al. (2015), foliar application of nutrients can reduce the impact of abiotic stress caused by low rainfall.

Table 2. Interaction effect of biofertilizer application techniques and doses on shoot dry weight (g) at 5th harvest.

Treatment	Biofertilizer Doses (L ha^{-1})			
	B_1 : 0 L	B_2 : 15 L ha^{-1}	B_3 : 22.5 L ha^{-1}	B_4 : 30 L ha^{-1}
A_1 : Foliar feeding	15.11 a	16.14 a	16.53 a	15.66 b
	A	A	A	A
A_2 : Soil drenching	15.12 a	14.07 b	15.07 a	17.54 a
	B	B	B	A

Note: The mean values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 0.05 significance level. Lowercase letters (a,b) read vertically, compare two techniques at the same dose (within a column). Uppercase letters (A, B) read horizontally, compare four doses for the same technique (within a row).

Table 3. Interaction effect of biofertilizer application techniques and doses on shoot dry weight (g) at 6th harvest.

Treatment	Biofertilizer Doses (L ha^{-1})			
	B_1 : 0 L	B_2 : 15 L ha^{-1}	B_3 : 22.5 L ha^{-1}	B_4 : 30 L ha^{-1}
A_1 : Foliar feeding	14.78 a	16.42 a	17.80 a	17.29 a
	C	B	A	AB
A_2 : Soil drenching	15.19 a	16.20 a	15.14 b	16.19 a
	A	A	A	A

Note: The mean values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 0.05 significance level. Lowercase letters (a,b) read vertically, comparing between two techniques at the same dose (within a column). Uppercase letters (A, B) read horizontally, comparing four doses for the same technique (within a row).

Table 4. Independent effect of biofertilizer application techniques and doses on leaf area ratio (cm^2 g^{-1}) at 6th Harvest

Treatment	Leaf Area Ratio (cm^2 g^{-1})
Biofertilizer application technique	
A_1 : Foliar feeding	119.69 a
A_2 : Soil drenching	123.53 a
Biofertilizer application dose (L ha^{-1})	
B_1 : 0 L	132.36 a
B_2 : 15 L ha^{-1}	120.34 b
B_3 : 22.5 L ha^{-1}	117.14 b
B_4 : 30 L ha^{-1}	116.61 b

Notes: The mean values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 0.05 significance level.

Many foliar microorganisms are capable of producing extracellular polysaccharides (EPS) that enable cell aggregation and contribute to protection against drought and osmotic stress (Bashir et al., 2022). According to Kakisina et al. (2023), optimal nutrient availability—specifically nitrogen, phosphorus, and potassium—in biological organic fertilizers enhances photosynthetic efficiency, leading to greater photosynthate allocation for building plant tissues and organs, which ultimately increases biomass accumulation and plant dry weight.

Leaf Area Ratio. Leaf area ratio represents the efficiency of the leaf surface in producing dry matter. Based on Table 8, there is an independent effect of the dose of biofertilizer on the leaf area ratio (LAR). The treatment without biofertilizer produced the highest LAR. The higher the dose of biofertilizer given, the smaller the LAR value obtained. According to Anjarsari et al. (2021), the LAR value is influenced by the area and dry weight of the leaves, which depend on their wet weight, while the wet weight of the plant is related to the transportation of photosynthetic products to the parts that utilize them, such as leaves and stems.

The relatively small leaf area can cause a decrease in LAR compared to leaf dry weight, which can indicate thicker leaves and higher dry matter. Higher leaf weight means that the application of biofertilizer is influenced by the availability of nutrients provided through the leaves and soil. As leaf dry weight increases, the increase in leaf area will decrease. Large leaves can improve the efficiency of light absorption on the leaf surface, but require biomass to increase leaf area (Huang et al. 2019; Sun et al., 2017). Spongy tissue contributes a relatively large amount to leaf thickness and plays a role in storing metabolites and nutrients related to tea quality, such as polyphenols, caffeine, and glucose (Sun et al., 2023).

The microorganisms in the biofertilizer used consist of nitrogen-fixing microorganisms (*Azotobacter* sp. and *Azospirillum* sp.) and phosphate-solubilizing bacteria (*Bacillus* sp. and *Pseudomonas* sp.). These microorganisms help convert substances into a form available to plants, thus indirectly helping to increase the growth of tea plants. Biofertilizers fix nitrogen, dissolve phosphate, secrete growth-promoting substances, reduce the use of chemical fertilizers,

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and improve overall environmental quality (Kumar et al. 2017). According to Figiel et al. (2025), Nitrogenase enzymes can fix and reduce free Nitrogen into a form that plants can absorb, but nitrogenase enzymes are susceptible to oxygen, so nitrogen-fixing bacteria in biofertilizers help bind oxygen and keep the enzyme active. According to Anand et al. (2016), phosphate-solubilizing bacteria promote the dissolution of insoluble phosphate compounds through the secretion of organic acids and the enzymes phosphatase and phytase.

Plant Growth Rate. At the 1st and 2nd plant growth rates, there was no interaction or independent effect of biofertilizer application doses and techniques. This is because the plants were still recovering due to drought. The application of biofertilizers can improve plant growth and development by producing growth hormones such as indole acetic acid (IAA) and cytokinin (Chaudhary et al., 2022).

Table 5 shows the interaction effect of biofertilizer application and fertilization technique at the 4th plant growth rate (PGR). Soil drenching of 30 L ha^{-1} biofertilizer gave the best results, while in foliar application, the dose levels showed relatively similar values. When applied to the soil, microorganisms in biofertilizers can colonize, nitrogen-fixing bacteria (*Azospirillum* sp. and *Azotobacter* sp.) help fix nitrogen, which affects the vegetative growth of plants, and phosphate-solubilizing bacteria help solubilize phosphate, which affects plant metabolism (Mahanty et al., 2017).

Table 6 shows an interaction effect of biofertilizer application doses and techniques on the 5th PGR. At the 6th plucking, applying biofertilizer through the soil at a dose of 15 L ha^{-1} gave the best results, while foliar application requires a dose of 30 L ha^{-1}. This was thought to be due to the presence of previously applied microbes, which still remain in the soil, so the addition of a low dose in the following application was enough to affect the increasing PGR. According to Demir et al. (2023b), when biofertilizers are applied to the soil, the microorganisms colonize the rhizosphere and help improve the absorption efficiency of plant nutrients. According to Ajmal et al. (2018), biofertilizers provide plant nutrients and accelerate microbial activity to provide enough and balanced nutrients for soil and plants.

Table 5. Interaction effect of biofertilizer application techniques and doses on 4th plant growth rate (g m^{-2} day^{-1})

Treatment	Biofertilizer Doses (L ha^{-1})			
	B_1 : 0 L	B_2 : 15 L ha^{-1}	B_3 : 22.5 L ha^{-1}	B_4 : 30 L ha^{-1}
A_1 : Foliar feeding	0.703 a	0.709 a	0.709 a	0.703 b
	A	A	A	A
A_2 : Soil drenching	0.704 a	0.705 a	0.706 a	0.714 a
	B	B	B	A

Note: The mean values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 0.05 significance level. Lowercase letters (a,b) read vertically, comparing the two techniques at the same dose (within a column). Uppercase letters (A, B) read horizontally, compare four doses for the same technique (within a row).

Table 6. Interaction effect of biofertilizer application techniques and doses on the 5th plant growth rate (g m^{-2} day^{-1})

Treatment	Biofertilizer Doses (L ha^{-1})			
	B_1 : 0 L	B_2 : 15 L ha^{-1}	B_3 : 22.5 L ha^{-1}	B_4 : 30 L ha^{-1}
A_1 : Foliar feeding	0.705 a	0.708 a	0.711 a	0.713 a
	B	AB	AB	A
A_2 : Soil drenching	0.707 a	0.715 a	0.707 a	0.702 b
	B	A	B	B

Note: The mean values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 0.05 significance level. Lowercase letters (a,b) read vertically, comparing the two techniques at the same dose (within a column). Uppercase letters (A, B) read horizontally, comparing four doses for the same technique (within a row).

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Conclusion

There is a significant interaction effect of biofertilizer application techniques and doses on the shoot dry weight and the growth rate of tea plants. There was an independent effect of biofertilizer dose on leaf area ratio. Soil application at 15 L ha^{-1} and 30 L ha^{-1} resulted in the highest 4th and 5th plant growth rates, and the highest shoot dry weight at the 2nd and 5th harvests. Meanwhile, foliar application at 15 L ha^{-1} and 22.5 L ha^{-1} produced the best shoot dry weight at the 2nd and 6th harvests.

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Effect of preharvest paclobutrazol and nitrogen fertilizers on the sprouting performance of 'median' potato seed G_0 tuber

Abstract. Various intensifications of potato cultivation, such as the provision of paclobutrazol and nitrogen (N) fertilizer, are thought to impact the quality of the seeds produced. This study aims to evaluate the effects of different N fertilizer doses and paclobutrazol concentrations applied in the preharvest period on the sprouting performance of G_0 potato tuber seeds after storage. Nine treatment combinations, each repeated three times, were tested, using 50%, 100%, and 150% of the recommended N dose and paclobutrazol concentrations of 50, 100, and 150 part per million (ppm), applied at 30 and 45 days after planting, respectively. The interaction effect between N fertilizer and paclobutrazol concentration was not significantly affected on all observed variables. Preharvest application of 100% N fertilizer produced the largest seedlings, indicated by the highest shoot length at 56 and 74 days after storage. Preharvest application of 150 ppm paclobutrazol produced the highest shoot length, shoot emergence rate, and seedling dry weight than other treatments. The present study implied the importance of preharvest N and paclobutrazol for improving the sprouting performance of G_0 potato seed tuber.

Keywords: Intensification · Potato cultivation · Seed · Shoot emergence rate · Shoot length

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Introduction

Potato (*Solanum tuberosum* L.) is a horticultural commodity known to have tubers with high carbohydrate content, so it is used as an alternative carbohydrate source (Kanter and Elkin 2019). In Indonesia, potatoes have been widely utilized. As the population increases, the demand for potatoes is expected to grow every year. For instance, there will be 70%in food production by 2050 due to the demand of the 9.7 billion world population (FAO et al., 2018). This situation is directly impacting the increasing need for potato seeds. According to data from the Direktorat Jenderal Hortikultura (2022), the need for potato seeds in Indonesia in 2021 reached 143,740 tons. Still, domestic production was only able to meet 8.6% or 12,361 tons, consisting of 7,045 tons of local seeds and 5,316 tons of imported seeds. In addition to the quantity aspect, the quality of potato seeds also needs to be assessed, considering that seed quality plays an important role in efforts to increase potato harvest yields.

Low potato seed quality is often caused by repeated use of seeds from the same crop (Mburu et al., 2023), which potentially leads to virus accumulation over time. In addition, low seed production is also related to the limited area available for potato cultivation, which is generally still focused on the highlands and is threatened by high land conversion. In 2023, the potato harvest area will only reach around 63 thousand hectares, or around 1.63% of the total 3.8 million hectares of agricultural land in Indonesia (BPS, 2024). Potato cultivation, which has been concentrated in tropical highlands, needs to be gradually shifted to medium plains to reduce the environmental impact—such as increased flood risk—caused by land degradation and overuse in highland regions. However, this transition poses challenges due to differences in the ecological carrying capacity of the new growing environments.

This limitation is related to environmental factors that are suitable for potato cultivation, which are also limited. Potatoes were first cultivated more than 7,000 years ago in the Andes, South America, which has a cool temperature (Momčilović, 2019). In tropical countries like Indonesia, ideal environmental conditions for potato plants are only found in highlands with an altitude of around 1,500 meters above sea level (Ademe et al., 2024). Potato development stages, such as germination, leaf initiation, and leaf area development, are greatly influenced by temperature (Adekanmbi et al., 2023). The range of temperature between 17–25°C produced normal growth and yield of potato (Enoch et al., 2017). Temperatures above 30°C can slow down tuber initiation and development, and cause physical damage to tubers (Aien et al., 2017).

Modification offered to support potato production in medium plains is the use of plant growth regulators/retardants such as paclobutrazol (Desta and Amare 20021; Mubarok et al., 2022) and prohexadion-Ca (Hernawati et al., 2022a). Paclobutrazol has been reported to increase potato yields (Hamdani et al., 2018), because the inhibition of gibberellin biosynthesis due to paclobutrazol directs growth more towards tuber enlargement than just vegetative growth that is too dominant, so that the dominance of vegetative growth can be suppressed, and photosynthate is translocated for tuber formation (Hamdani et al., 2024). In addition to retardants, nitrogen, as an important macronutrient for potato plant growth, also needs to be studied. Nitrogen can stimulate vegetative plant growth, and nitrogen deficiency can slow growth. Nitrogen increases vegetative growth, which results in higher assimilation to supply tuber formation and increases protein and starch content in tubers. Previous studies have proven the role of nitrogen fertilization on growth, leaf area, stomatal conductance, photosynthesis rate, number of stolons, number of tubers per plant, and potato tuber weight (Hernawati et al., 2022b). G_0 potato tubers modified with pre-harvest application of paclobutrazol and N fertilizer need to be studied further; hence, limited studies have been reported concerning their sprouting ability as seed tubers. Therefore, this study aims to analyze the sprouting performance of G0 potato tubers as the impact of preharvest administration of nitrogen fertilizer and paclobutrazol.

Materials and Methods

This study used 'Median' G_0 potato seed tubers, which were produced by PT. Horti Agro Makro, Cisurupan, Garut. The cultivation was also carried out in the same place from July to October 2023, following the maintenance instructions in the previous study (Hernawati et al. 2022b), with differences in nitrogen (N) and paclobutrazol factors. The plant was arranged in a randomized complete block design to accommodate two factors, namely N and paclobutrazol. The N fertilizer levels were 50%, 100%, and 150% of the recommended N fertilizer dose of 400 kg ZA/ha, applied separately at planting and 30 days after planting (DAP). According to the technical guidelines for potato cultivation, the seed tuber requirement per hectare is 1,200 kg, with an average tuber weight of 30 g. This corresponds to a planting density of approximately 40,000 plants per hectare (BPTP Jawa Barat, 2015). For instance, plants were treated with 5, 10, and 15 g ZA/plant for doses of 50%, 100%, and 150%, respectively. The second factor is the concentration of paclobutrazol, which consists of three levels, namely 50 ppm, 100 ppm, and 150 ppm, with an application time of 45 DAP. G_0 potato seed tubers with differences in N and paclobutrazol were harvested at the age of 90 DAP, and stored in storage boxes.

This experiment tested 9 treatment combinations, each repeated 3 times, so there were 27 storage plastic boxes. There were 10 tubers in each storage box. This storage test was conducted in the experimental room at Ciparanje Garden, Jatinangor, Sumedang, from November to December 2023. Potato tubers were stored for 60 days, after which they were cultivated in a nursery for 2 weeks. Tuber weight loss was measured by an analytical scale at 60 days after storage (DAS). Tuber weight loss (%) was calculated by subtracting the post-storage tuber weight (g) from their initial weight (g), dividing the result by the initial weight, and then multiplying by 100 to express the loss as a percentage. Shoot emergence time was defined as the breaking of dormancy, indicated when at least 50% of seed tubers produced sprouts with a minimum shoot length of 2 mm (Nuraini et al., 2019). Sprouting percentage was determined at the end of storage by calculating the proportion of tubers that have produced shoots (above a defined minimum length, e.g., 2 mm) relative to the total number of tubers observed. Shoot length (mm) was measured twice, at 56 and 74 DAS, on all emergent shoots. Shoot dry weight (g) was destructively tested on all emerged sprouts at 74 DAS, using the oven method (80 ^{0} C, 48 hours). The experimental data were tabulated in Microsoft Excel and processed statistically using the analysis of variance approach and Duncan's test using Smartstat XL V.3.6.5.3 add-in for Microsoft Excel.

Results and Discussion

Tuber weight loss . The present study revealed an insignificant interaction between the dose of N fertilizer and the concentration of paclobutrazol on the tuber weight loss. The effect of the treatment of the dose of N fertilizer and the concentration of paclobutrazol on the tuber weight loss is presented in Table 1. This incident of reduced sweet potato weight is related to the loss of photosynthates for the purposes of forming and enlarging the emergent shoots. The longer the storage period, the higher the loss of potato tuber weight (Degebasa, 2020).

Table 1. Percentage of tuber weight loss in response to dfifferent preharvest nitrogen and paclobutrazol applications

Treatment	Tuber Weight Loss (%) at 60 DAS
Preharvest Nitrogen (N)	
N_1 : 50%	4.04 a
N_2 : 100%	4.76 a
N_3 : 150%	4.48 a
Preharvest Paclobutrazol (P)	
P_1 : 50 ppm	4.38 a
P_2 : 100 ppm	4.06 a
P_3 : 150 ppm	4.04 a

Means followed by the same letter within the same column and factor are not significantly different, based on Duncan's test at 0.05 significance. DAS – days after storage.

Sprouting percentage. There was an insignificant interaction effect between the dose of N fertilizer and the concentration of paclobutrazol on the sprouting percentage of potato tuber (Table 2). Similarly, the single factor of preharvest N also showed an insignificant effect on sprouting performance. It is likely that the preharvest N fertilizer range of 50–150% remains adequate to support healthy potato growth and yield, as indicated by relatively uniform tuber size. Tuber size, in turn, reflects the carbohydrate reserves available for sprout development. Potato tubers are actually vegetative reproductive organs, not true seeds, and therefore are equipped with meristem tissue

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buds, which are temporarily dormant. Once dormancy is broken, the tuber's eye buds become physiologically active, triggering cell division and elongation in the apical meristem. This growth process is supported by the tuber's nutrient reserves, which are primarily stored in the form of starch. This starch is enzymatically broken down into soluble sugars by amylase. These nutrient reserves serve as the source of growth during the early stages of shoot development.

In contrast, preharvest paclobutrazol had a significant effect on sprouting performance, with the 150 ppm treatment (P_3) showing significantly better results than the 50 ppm treatment (P_1). However, both P1 and P3 were not significantly different from the 100 ppm treatment (P_2). Sprouting performance is related to the accumulation of starch contained in potato tubers; the more paclobutrazol is applied to potato plants, the more starch is contained in the seeds of the potato. The starch is then hydrolyzed into glucose as a food reserve by hydrolytic enzymes such as α-amylase, whose activity can be triggered by the gibberellin hormone (GA_3). Furthermore, glucose is broken down through the Krebs cycle to produce energy in the form of ATP. This energy plays a role in potato metabolism so that it can stimulate seed germination.

Table 2. Shoot growing rate of potato seed tuber in response to different preharvest nitrogen and paclobutrazol applications

Treatment	Shoot Growing Rate (%)
Preharvest Nitrogen (N)	
N_1 : 50%	64.44 a
N_2 : 100%	67.67 a
N_3 : 150%	68.29 a
Preharvest Paclobutrazol (P)	
P_1 : 50 ppm	57.72 a
P_2 : 100 ppm	68.81 ab
P_3 : 150 ppm	76.31 b

Means followed by the same letter within the same column and factor are not significantly different, based on Duncan's test at 0.05 significance.

Shoot emergence time. Neither the interaction between preharvest N and paclobutrazol, nor their individual effects, significantly affected the time of shoot emergence (Table 3). Potato seeds that were stored for 60 days had already undergone an emergent shoot growth at 50-54 DAS. The timing of shoot emergence is related to the dormancy breaking of potato seeds (Nuraini et al., 2019). Dormancy breaking of seed tubers is related to tuber size, as tuber size is a proxy for photosynthate sufficiency to support subsequent sprout growth (Park et al., 2021). Because this study produced relatively similar seed tuber sizes, dormancy breaking did not differ significantly. We suspect that shoot emergence in potato tubers is more predominantly influenced by endogenous dormancy-regulating mechanisms and post-harvest environmental conditions than pre-harvest interventions.

Table 3. Shoot emergence time of potato seed tuber in response to different preharvest nitrogen and paclobutrazol applications

Treatment	Shoot Emergence Time (DAS)
Preharvest Nitrogen (N)	
N_1 : 50%	53.56 a
N_2 : 100%	52.75 a
N_3 : 150%	50.81 a
Preharvest Paclobutrazol (P)	
P_1 : 50 ppm	54.88 a
P_2 : 100 ppm	52.33 a
P_3 : 150, ppm	50.88 a

Means followed by the same letter within the same column and factor are not significantly different, based on Duncan's test at 0.05 significance**.** DAS – days after storage.

Emergent shoot length. Shoot length measurements were carried out on tubers stored periodically at 56 DAS and 2 weeks later in the nursery stage (74 DAS). Similar to previous measured variables, there was an insignificant effect of the interaction of both preharvest factors on shoot length; however, the single factor effect of N and paclobutrazol led to significant emergent shoot length variation (Table 4). The dose of 100% N fertilizer produced a higher shoot length and was significantly different compared to other treatments at 56 and 74 DAS. 100% N fertilizer dose meets the potato plants' nitrogen requirements at optimal levels. It was supported by a previous study that concluded the urgency of N to support potato plant growth (Mubarok et al., 2024). N fertilizer contains ammonium ion compounds with a more stable nitrogen supply and low evaporation, so that it is easily absorbed by plants (Khalil, 2014).

At 70 DAS, a concentration of 150 ppm paclobutrazol produced a higher shoot length compared to a concentration of 50 ppm. The administration of paclobutrazol inhibits vegetative growth, so that assimilation is focused on tuber growth (Azima et al., 2017). With higher concentrations, the accumulation of photosynthate in tubers is greater, encouraging shoot growth. This accumulation can be converted into energy for shoot formation and increase food reserves in tubers. Tuber with larger food reserves produces stronger and more shoots than small tubers.

Table 4. Emergent shoot length from potato seed tuber in response to different preharvest nitrogen and paclobutrazol applications

Treatment	Shoot Length (mm)	
	56 DAS	74 DAS
Preharvest Nitrogen (N)		
N_1 : 50%	2.34 a	2.42 a
N_2 : 100%	2.37 b	3.27 b
N_3 : 150%	2.19 a	2.99 b
Preharvest Paclobutrazol (P)		
P_1 : 50 ppm	2.17 a	2.07 a
P_2 : 100 ppm	2.29 a	3.04 ab
P_3 : 150 ppm	2.24 a	3.32 b

Means followed by the same letter within the same column and factor are not significantly different, based on Duncan's test at 0.05 significance. DAS – days after storage.

Emergent shoot biomass. There was an insignificant interaction effect between preharvest N and paclobutrazol on emergent shoot biomass. However, a single factor of paclobutrazol resulted in a significant impact. Paclobutrazol 150 ppm (P_3) significantly increased shoot dry weight by 0.517 g compared to treatments of 50 ppm (P_1) and 100 ppm (P_2). The provision of a higher concentration of paclobutrazol caused the resulting seeds to have more food reserves, which is in line with the results of the shoot length at the end of the 70-day storage period, with the highest results in the treatment of paclobutrazol concentration 150 ppm (P_3). This is also supported by the germination power of seeds in the 150 ppm treatment (P_3), producing the highest results to support seed growth. Plant dry weight is the result of photosynthate assimilation, which is distributed to the roots and all parts of the plant.

Table 5. Emergent shoot biomass from potato seed tuber in response to different preharvest nitrogen and paclobutrazol applications

Treatment	Emergent Shoot Biomass at 74 DAS (g)
Preharvest Nitrogen (n)	
n_1 : 50%	0.263 a
n_2 : 100%	0.321 a
n_3 : 150%	0.271 a
Preharvest Paclobutrazol (p)	
p_1 : 50 ppm	0.243 a
p_2 : 100 ppm	0.424 b
p_3 : 150 ppm	0.517 c

Means followed by the same letter within the same column and factor are not significantly different, based on Duncan's test at 0.05 significance.

Conclusion

There was an insignificant interaction effect between the effect of preharvest N fertilizer and paclobutrazol application on all observed parameters. However, the dose of N fertilizer affected the shoot length, implying the urgency of preharvest N in improving sprouting performance. For instance, N 100% (10 g/plant) significantly produced the highest shoot length, indicating a seedling growth booster after storage. Additionally, 150 ppm paclobutrazol is recommended to significantly increase shoot length, seedling growth rate, and shoot biomass of G_0 potato seed tubers.

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Growth and yield of curly red chili (*Capsicum annum* L.) in response to mulching and chicken manure application in the erosion-prone area of Loto, Ternate island

Abstract. Information on soil resources in volcanic landscapes presents a high potential for agricultural land development, but its development is always faced with the potential for continuous land degradation. This study aims to reduce the rate of land degradation through the application of vegetative conservation in erosion-prone Loto agrotourism area, Ternate Island. The research location is focused on erosion-prone areas. The research consisted of a soil resource inventory and a vegetative conservation application. The vegetative conservation application method used a Randomized Block Design (RBD) pattern, composed of 2 factors. The first factor is mulching, namely: no mulching (M0) and mulching (M1), while the second factor is chicken manure-based organic fertilizer, specifically at 10 tons ha^{-1}(P1), and 20 tons ha^{-1}(P2). Observation parameters include soil parameters as well as plant growth and yield parameters. The results showed that the combination of mulching and chicken manure had an insignificant effect on plant height, number of flowers, number of fruits, or number of branches. However, the application of mulching was found to increase the fruit weight of curly chili plants. This finding implied that erosion-prone areas require improved land management, such as mulching, to optimize soil resources and reduce erosion risk.

Keywords: Curly red chilies · Geomorphic process · Land management · Volcanic landscapes

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Introduction

Land resources are an important indicator to support the sustainability of agricultural sector development. In general, the function of soil is to serve as a medium for growing plants, providing nutrients, and regulating the water cycle. One of the developments in the agricultural sector is on volcanic landscapes. This landscape is widespread in Indonesia (Ashari & Purwantara, 2022; Hartati et al., 2023; Aji et al., 2024). Volcanic landscapes have high potential. This factor is supported by the soil's ability to provide complete nutrient availability derived from volcanic ash material.

Conversely, the topography of the region is distinguished by undulating and steep terrain, accompanied by a multitude of natural hazards, including erosion, landslides, and drought. These factors contribute to a heightened susceptibility to accelerated land degradation and the occurrence of land degradation events. Land degradation is indicated by a decrease in the ability of land to support the ecosystem conditions in it (Aji et al., 2020) and has an impact on reducing the level of soil fertility (Hartati et al., 2023). Erosion is one of the geomorphic processes that play a role in determining soil quality and crop productivity. Land mismanagement has been identified as a primary catalyst in the acceleration of geomorphic processes. Consequently, the development of an effective approach to mitigating the adverse effects of geomorphic processes on land functions is imperative. The application of vegetative conservation is regarded as a potential solution to decelerate geomorphic processes. Vegetative conservation activities include fertilization, the planting of ground cover plants, and the creation of terraces (Fronning et al., 2008), which are a subset of these activities. This approach has been previously explored by researchers, who have implemented diverse mechanical conservation measures on sloping terrain (Rofita et al., 2022). Conversely, the implementation of vegetative conservation measures within volcanic landscapes has been extensively documented, though their application in specific regions remains limited. Agritourism is defined as the integration of agricultural activities with tourism activities (Kurniasanti, 2019; Nasution et al., 2024). This concept is believed to improve economic, social, and cultural conditions (Kurniasanti, 2019) and can be used as an alternative in other aspects, such as educational and recreational facilities (Nurani et al., 2020). However, the implementation of vegetative conservation techniques in such contexts frequently overlooks the intricate interplay between cultivation practices and geomorphic processes. To address this knowledge gap, an approach centered on the study of geomorphic processes is imperative. This approach will facilitate a more profound understanding of the intricate interplay between vegetation, topography, and soil dynamics. Consequently, conservation strategies can be designed with greater specificity and efficacy, aligning with the unique characteristics of each local environment. A case study of an agrotourism area is relevant for testing the geomorphic process approach in a specific context and for providing practical recommendations for area managers in designing sustainable conservation strategies.

Ternate Island, with its hilly topography and many erosion-prone areas, has challenges in managing agricultural activities. The Loto agrotourism area is administratively located in Loto Village, West Ternate District. This village is used as an agrotourism-based agricultural development area that focuses on horticultural crops. This area has an area of 0.04 km^2. This area was initiated by the City Agriculture Office in collaboration with the Ternate City Tourism Office (Tourism Office, 2023). In addition, this sub-district has an area of 33.8 km^2 (BPS, 2021). Located on the volcanic slopes of Mount Gamalama, this area is strategically suited for tourism and vegetable cultivation, offering potential to boost productivity while serving as an educational site (Tourism Office, 2023).

Mulching is one of the important land management technologies to increase plant productivity that plays a role in protecting soil temperature fluctuations and moisture levels (Chakraborty et al., 2023; Kader et al., 2017; Guo et al., 2024), thus creating a conducive environment (Chakraborty et al., 2023) for plant growth. Mulching has also had a positive impact on crop yields. Similar research has also been conducted, but with a combination of mulch with biochar at regular intervals for three years, which increased the yield by 23.4% (Zhang et al., 2024). The use of mulch is believed to be an effective approach to reduce the impact of erosion (Iqbal et al., 2020). Mulch also helps protect the soil surface from direct rain. Furthermore, the application of manure is a type of vegetative conservation

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action. Manure plays a role in soil aggregation systems, improving root systems, and plant productivity (Rofita et al., 2022). Chemically, organic fertilizers help in providing essential nutrients, increase soil biodiversity, and play a role in the formation of complex compounds (Pasang et al., 2019). Several similar studies have been conducted by previous researchers that manure has a positive effect on plant productivity (Liu, 2016; Syahputra, 2016; Emir, 2017; Risal & Mukhlishah, 2019; Risal & Halim, 2020).

Curly red chili plant (*Capsicum annum L.*) is one of the agricultural commodities that has been developed and neatly arranged in the Loto agrotourism area, but the production produced is still not optimal, especially when looking at the results of each slope, which still show different results. Curly red chili pepper (*Capsicum annum L.*) is also one of the important horticultural commodities that are widely cultivated because of its high economic value. Curly red chili has nutritional content such as protein, fat, carbohydrates, calcium, vitamins A and C (Rindani, 2015). The content of curly red chili has 90.9% water content, 31 calories, 1 g protein, 0.3 fat, 7.3 g carbohydrates, 29 mg calcium, 24 mg phosphorus, 47 mg vitamin A, and 18 mg Vitamin C (Sutrisni, 2016). This plant is also able to be grown in various topographic conditions, both in the highlands and in the lowlands.

This research aims to reduce land degradation through the application of vegetative conservation in erosion-prone areas. This research not only contributes to better management of land resources but also supports sustainable development goals, particularly in maintaining terrestrial ecosystems and promoting sustainable agricultural practices.

Materials and Methods

The research was carried out in the Loto agrotourism area, Loto Village, West Ternate District (Figure 1). The implementation time starts from March – October 2024. Laboratory analysis was carried out at the Soil Science Laboratory, Faculty of Agriculture, Universitas Khairun, and the Soil Laboratory, East Java Agency for Agricultural Assembly and Modernization. Furthermore, the materials used in this study include curly red chili seeds and chicken manure. Meanwhile, the tools used include hoes, meters, shovels, sample rings, plastic mulch, treatment nameplates, and writing stationery.

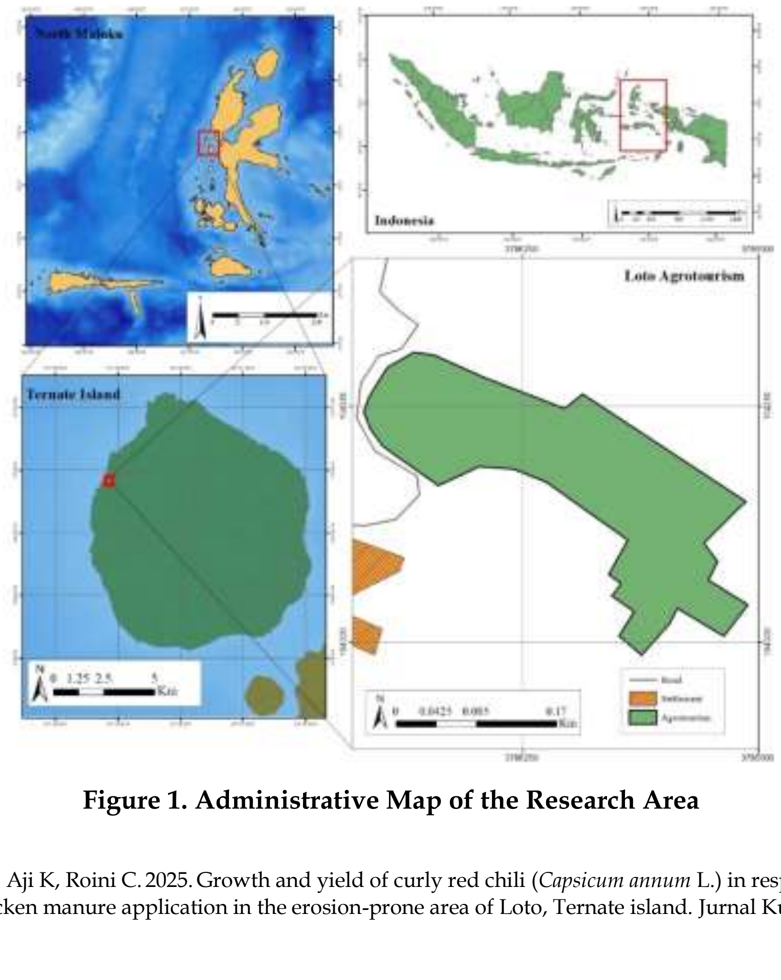

Figure 1. Administrative Map of the Research Area

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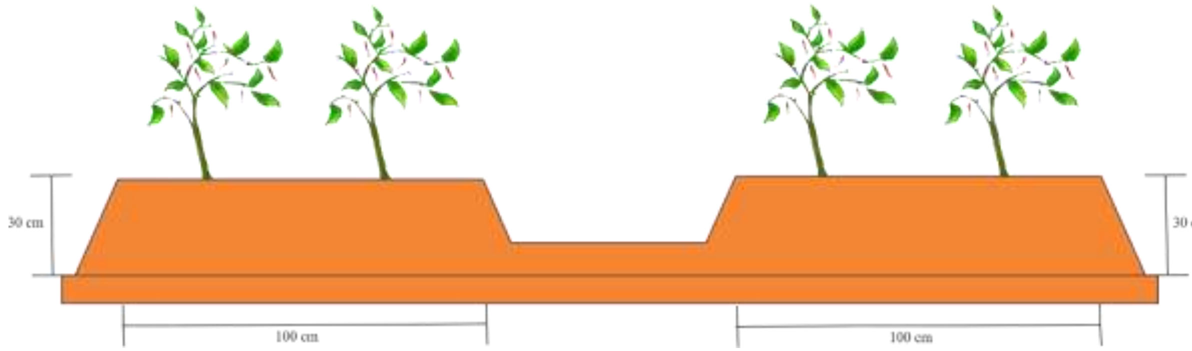

Figure 2. Chili Plant Bed

The study focused on erosion-prone locations with steep slopes (25 – 45%). The study was carried out in two stages: first, soil sampling for preliminary analysis; second, vegetative conservation through red chili cultivation. The soil sampling method employing the soil boring technique is based on the USDA Field Book Procedures for Describing and Sampling Soils (Schoeneberger et al., 2012), and soil properties are analyzed using the standard of the Agricultural Instrument Standardization Agency of the Ministry of Agriculture. Soil physicochemical analysis includes analysis of bulk density (ring method), particle density (pycnometer method), soil porosity, soil texture (hydrometer method) with based on the principle of sedimentation or deposition of soil grains in water. The texture classification criteria based on USDA classification including, clay (Φ: <0.002 mm), silt (Φ: 0.05 mm - 0.002 mm) and sand (Φ: 2 mm - 0.05 mm), soil pH (1:5) (electrometric method; pH meter), soil organic carbon (Walkley & Black method), soil total nitrogen (Kjeldahl Titrimetric method), soil P_2O_5 content (Olsen method; Spectrophotometer), soil K_2O content (AAS method), exchangeable cations (K, Na, Ca, Mg,), and Soil CEC (Percolation method NH_4OAc 1 M, pH 7 + NaCl 10%; Titrimetric). The second phase of research involved manufacturing beds of 1 x 3 m with a height of 30 cm each (Figure 2). This step employs the Randomized Block Design (RBD), which includes two elements, namely mulching and chicken manure-based organic fertilizer (Table 1).

Each treatment combination was repeated five times, yielding a total of 20 experimental units (2x2x5). Plant growth and yield metrics are monitored, such as plant height, number of flowers, number of fruits, number of branches, and fruit weight.

Table 1. Combination of Treatments

Code	Combination of Treatments
M0P1	No mulching + 10 tons ha^{-1} chicken manure
M0P2	No mulching + 20 tons ha^{-1} chicken manure
M1P1	Mulching+ 10 tons ha^{-1} chicken manure
M1P2	Mulching + 20 tons ha^{-1} chicken manure

The analysis of soil observation data was carried out in a composite manner in each treatment, while the data from plant observation was analyzed by multi-fingerprint analysis (ANOVA). The treatment with a real effect will be further tested using the Duncan multiple range test (DMRT) at the 5% significance level by using Minitab software ver.18.

Results and Discussion

Soil Analysis Results. The soil order in the research region is classified as Entisols, with the subgroup Typic Udorthents. This type of soil is distinguished by little soil profile development and a distinctive okric horizon above the soil surface (Fiantis, 2016). The physicochemical analysis results are utilized to determine the carrying capacity for vegetative conservation applications. The initial soil analysis of the location where vegetative conservation is applied shows that sand fractions dominate, with 90% in the upper layer and 85% in the lower layer, followed by clay and dust fractions, resulting in a clay-sand texture (Table 2). With such a texture, this soil has a low ability to store soil moisture, as well as water and nutrient storage capacity, due to the vast pore space, which requires a large amount of water to meet field capacity.

Table 2. Initial soil analysis before treatment

No	Properties of Soil	Depth	
		0 - 30 cm	30 - 60 cm
Physical Properties of Soil:			
1.	Texture (%):		
	Sand	90	85
	Silt	3	8
	Clay	7	7
	Texture Classes	Loamy sand	Loamy sand
2.	Bulk Density ($g.cm^{-3}$)	1.89	1.77
3.	Particle Density ($g.cm^{-3}$)	2.56	2.62
4.	Porosity	26.25	32.53
Chemical Properties of Soil:			
1.	pH H_20	7.4	7
	pH KCl	6.3	5.8
2.	Organic Carbon (%)	1.5 (l)	1.16 (l)
3.	Total Nitrogen (%)	0.02 (vl)	0.13 (l)
4.	C/N Ratio	75 (h)	8.92 l)
5.	Organic Matter (%)	2.60	2.01
6.	P_2O_5 available (ppm)	21 (m)	12 (vl)
7.	P_2O_5 Potensial (mg.100 g^{-1})	73	57
8.	K_2O (mg.100 g^{-1})	57 (h)	12 (l)
9.	Cations can be exchanged:		
	K (cmol (+) kg^{-1})	0.26 (l)	0.4 (l)
	Ca (cmol (+) kg^{-1})	3.99 (l)	2.93 (l)
	Mg (cmol (+) kg^{-1})	0.82 (l)	0.04 (vl)
	Na (cmol (+) kg^{-1})	0.3 (l)	0.22 (l)
10.	CEC (cmol (+) kg^{-1})	5.26 (l)	4.63 (vl)
11.	Base Saturation	102.09 (vh)	71.92 (h)

Source: the Soil Laboratory, East Java Agency for Agricultural Assembly and Modernization (2024) and Soil Science Laboratory of the Faculty of Agriculture, Universitas Khairun (2024)
Description: (1) vl: Very Low; (2) l: Low; (3) m: moderate (4) T: High; (4) st: Very High

The BD value in the upper layer was 1.89 gr cm^{-3}, while the lower layer was 1.77 gr cm^{-3}, indicating that the soil at the research site is quite dense. The weight of the volume can describe the density of the soil; the higher the weight, the higher the proportion of solids or the more compacted. In general, coarse-textured soils (Ø 2.00 mm) have a higher volume weight than fine-textured soils (Ø 0.002 mm). The assumption is that plant roots will have an easier time penetrating soil with a high bulk density because there is enough pore space for air and groundwater. On the other hand, in soils with a low soil volume weight, plant roots will be difficult to develop since the density level is likewise low, causing the roots to climb to the surface in search of nutrients (Aji et al., 2020) and water to promote production.

Soil chemical analysis at the research site typically yields very low to low values. The total nitrogen content of 0.02% qualifies as very low. Nitrogen is lost into the atmosphere as a result of its volatile nature and is oxidized to generate ammonium. Furthermore, the washing process in the soil body may contribute to nitrogen loss (Xiao et al., 2019; Chen et al., 2021; Batubara et al., 2024). Furthermore, the low P concentration is available because the P factor is bound by Al and Fe metals, making it unavailable to plants. The potassium component in the form of K_2O has a rather high yield, 57 mg per 100 g. This is due to the inorganic nature of the K concentration in the soil, which allows it to move freely throughout the soil body and easily deliver nutrients to plants. In addition, volcanic landscapes contribute potassium content through orthoclase [(K, Na)$AlSi_3O_8$], Microlins [(Na, K)$AlSi)_4$], Muscovite [K$(AlSi)_3$ $O_{10}(OH)_2$], and Biotite [K$(Mg.Fe^{2+})_3AlSi_3$ $O_{10}(OH)_2$]. Furthermore, the benefit of family planning and CEC is inversely related. This is because the soil pedogenesis process is influenced by soil texture factors, which are still dominated by sand fractions, resulting in a low exchange capacity of cations in the soil. After all, the soil is easily washed out, causing alkaline cations to be lost through the surface through erosion or washed through eluviation in the soil body.

Chili Plants Growth and Yield. The analysis of variance revealed that while the treatment had no significant influence on plant height, number of flowers, number of fruits, or number of branches, it did have a significant effect on fruit weight. The average results of plant height in each treatment produce values that differ; this condition can be seen in the graph of plant height rise at each observation (Figure 3).

The M0P2 treatment (no mulching + 20 tons ha^{-1} chicken manure) had the lowest plant height, while the M1P2 treatment (mulching + 20 tons ha^{-1} chicken manure) had the greatest (Figure 3). The addition of chicken manure to soil can improve its high water absorption, in certain cases it may facilitate the easy mineral accessibility to plants (Widowati, 2004; Suryana, 2008). Furthermore, mature organic fertilization can also benefit the soil by increasing colloidal humus, which facilitates the release of nutrients (K, Ca, and Mg) (Brar et al., 2015; Neina, 2019). On the other hand, using the same fertilizer but also applying mulch might increase the height of chili plants.

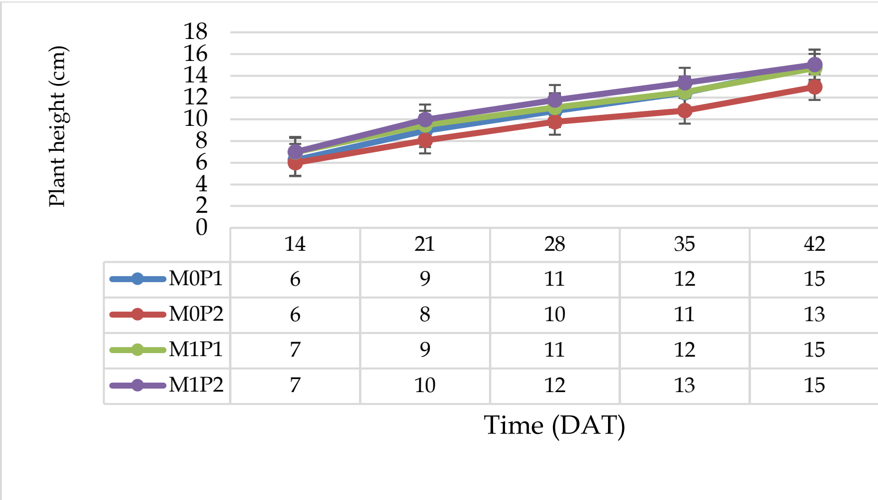

Figure 3. The increase in chili plant height from 14 to 42 days after transplanting is insignificantly affected by mulching and chicken manure treatment
Note: no mulching + 10 tons ha^{-1} chicken manure (M0P1), no mulching + 20 tons ha^{-1} chicken manure (M0P2), mulching+ 10 tons ha^{-1} chicken manure (M1P1), mulching + 20 tons ha^{-1} chicken manure (M1P2)

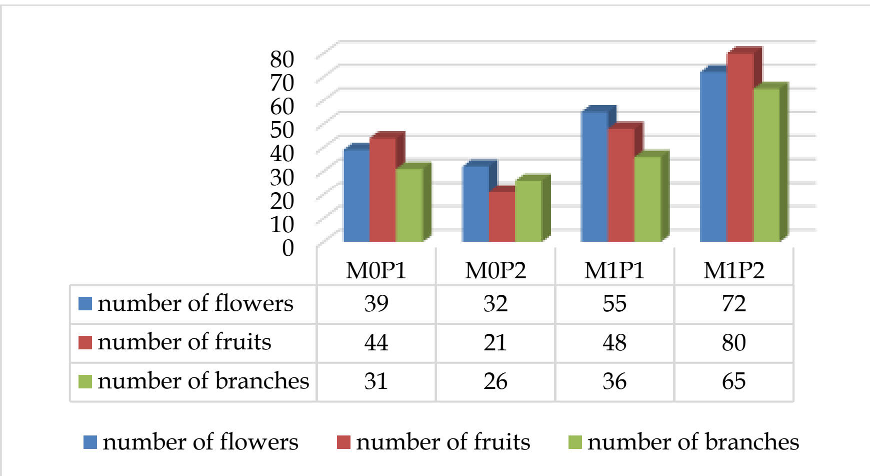

Figure 4. The number of flowers, fruits, and branches of chili plant is insignificantly affected by mulching and chicken manure treatment
Note: no mulching + 10 tons ha^{-1} chicken manure (M0P1), no mulching + 20 tons ha^{-1} chicken manure (M0P2), mulching+ 10 tons ha^{-1} chicken manure (M1P1), mulching + 20 tons ha^{-1} chicken manure (M1P2)

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Similar to the data on plant height in the flower count, the mulching treatment also increased the number of fruits and branches (Figure 4). The average differential test findings for mulching treatment on the weight of chili plants also revealed a significant difference compared to no mulching (Figure 5). This scenario demonstrates that mulching can boost chili plant growth and productivity. Plastic mulch can boost microbial activity, which in turn can boost plant growth and yield by raising the carbon dioxide content in the planting area. Some research results also show that the use of black and silver plastic mulch increases the yield of several vegetable crops such as red chili peppers (Harsono, 1997; Syamiah, 1997; Fahrurrozi et al., 2006; Soetiarso, 2006), tomatoes (Decoteau et al., 1988; Decoteau et al., 1989), and paprika (Decoteau et al., 1990).

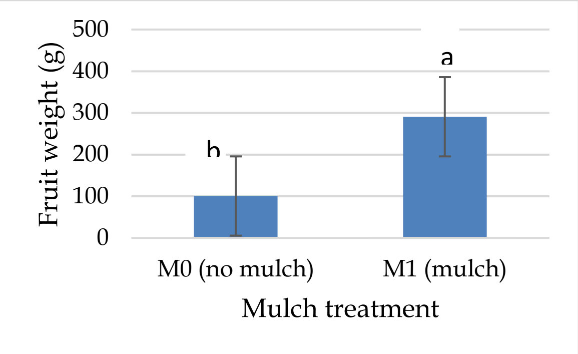

Figure 5. The fruit weight of chili plant is significantly affected by mulching

The effects of environmental factors on productivity and growth are correlated and interconnected. One of them is the soil type. The presence of an early-stage soil type in the study area is due to the dominance of Entisols. This soil type is often found in erosion-prone regions (Soil Survey Staff, 2022). Open agricultural practices in erosional zones in the research site further promote severe erosion. Unsuitable spatial and temporal land conversion is believed to accelerate this process (Rahmadi & Wibowo, 2023). If not addressed, such conditions can lead to ongoing soil degradation (Sartohadi et al., 2012; Nasution et al., 2024). Erosion commonly strips away the topsoil layer (Sulaeman & Westhoff, 2020), which is the most fertile part of the soil because it contains high levels of organic matter, minerals, and essential nutrients for plant growth.

Conclusion

Mulching and chicken manure application, as vegetative conservation treatment, are thought to be important and potential strategies to increase the productivity of curly red chili in the erosion-prone or nutrient-poor land. However, the combination of mulching and chicken manure application had no significant influence on plant height, number of flowers, number of fruits, or number of branches. On the other hand, mulching has been found to significantly increase the fruit weight, implying the urgency of this practice in erosion-prone cultivation land.

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High temperature in potato: plant responses and adaptive cultivation strategies to increase production

Abstract. Climate change, with global temperatures rising by 1.09°C from 1850–1900 to 2011–2020, threatens potato production, a critical staple crop, by exceeding the optimal temperature range of 15–20°C. This review synthesizes over 45 peer-reviewed studies published between 2015 and 2025 from Google Scholar and ScienceDirect to evaluate the physiological, morphological, and tuber quality responses of potatoes to high temperatures and to identify adaptive cultivation strategies for sustainable production. High temperatures reduce photosynthetic efficiency through chlorophyll degradation and stomatal closure, increase respiration, and divert photosynthates to vegetative growth, leading to 18–32% yield losses globally by the 2050s. Heat-tolerant varieties, such as Atlantic (11.47 tons/ha), Merbabu-17 (11.04 tons/ha), and Granola (3.61 tons/ha), maintain productivity in medium-altitude lands. Plant growth regulators (PGRs), including BAP, melatonin, and paclobutrazol, enhance tuber yield by regulating hormonal balance and antioxidant activity. Drip irrigation and mulching (e.g., straw, wheat, plastic films) improve water use efficiency and buffer soil temperature. These integrated strategies of heat-tolerant varieties, PGRs, irrigation, and mulching offer practical solutions to mitigate heat stress and ensure sustainable potato production under changing climate conditions.

Keywords: High temperature · Heat-tolerant varieties · Plant growth regulator · Irrigation · Mulch

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Introduction

Potatoes (*Solanum tuberosum* L.) are horticultural crops with the tuber as their economic organ. Based on data Statistics Indonesia (2023), potato production increased by 6.2% from 1.28 million tons in 2020 to 1.36 million tons in 2021), yet it fails to meet the annual demand of 6.16 million tons, driven by rising consumption of processed products like French fries and chips (Yulinarti et al., 2021; Asmara et al., 2022). Potato consumption in Indonesia grew by 2.79% from 2016 to 2020 (Agricultural Data and Information System Center, 2021). Optimal potato growth requires highland environments (>1,000 masl), 9–10 hours of daily irradiation, 1,500 mm/year rainfall, and temperatures of 20–25°C during vegetative growth and 15–20°C during tuber formation (Setiadi, 2009; Diwa et al., 2015; Rykaczewska, 2016; Struik, 2007).

Rising global temperatures due to climate change are altering the morphological and physiological responses of potato plants, significantly affecting productivity (Hancock et al., 2014; Jagadish et al., 2021). The Intergovernmental Panel on Climate Change (IPCC) noted that the 2011-2020 global average temperature was 1.09 ºC higher than the period 1850-1900. Exposure to high temperatures disrupts plant metabolism processes that divert growth mechanisms into plant protection to survive in abiotic stress conditions (Cramer et al., 2011). In potato plants, signals that trigger tuber formation are inhibited, respiration increases, and photosynthate accumulation is diverted to shoot growth due to high temperature (Hamdani et al., 2021; Muthoni & Kabira, 2015; Ningsih et al., 2021). As a result, there is a reduction in yield due to extreme environmental conditions. It can also lower starch content (Mubarok et al., 2022), similar to reduced oil content in high-temperature rapeseed (Qaseem et al., 2019). Potato yield losses due to high temperature are predicted to reach 18-32% by the 2050s (Hijmans, 2003).

This review examines the global impact of high temperatures on potato crop, with a particular focus on focusing on physiological, morphological, and tuber quality responses, and also explores adaptive cultivation practices such as the use of selected heat-tolerant cultivars, plant growth regulators, drip irrigation, and mulching to support sustainable potato cultivation under climate change. Literature selection criteria included scientific journals published in the 2015–2025 timeframe obtained through ScienceDirect and Google Scholar.

Morpho-physiological Response of Potato Plants to High Temperature

Photosynthesis is an important process in plants to produce energy. Temperatures that exceed the optimum range of potato plants cause changes in plant growth and development. Stomata are the place of CO_2 exchange, the release of water vapor during transpiration, and the place where the photosynthesis process takes place (Lawson & Matthews, 2020). High temperatures (>25°C) disrupt potato physiology and morphology, affecting photosynthesis, respiration, and tuber development (Hancock et al., 2014). Most studies indicate that Rubisco activity declines above 30°C, particularly in cultivars like Desiree and Russet Burbank, due to degradation of Rubisco activase enzymes (Wang et al., 2018; Perdomo et al., 2017). Stomatal closure, triggered by increased abscisic acid (ABA) levels, reduces CO_2 uptake and photosynthetic rates (Thakur et al., 2019; Urban et al., 2017). Chlorophyll degradation further limits light absorption, with studies on cultivars like Atlantic showing up to 20% reductions in chlorophyll content at 35°C (Hamdani et al., 2020; Singh et al., 2020). High temperatures also cause leaf metabolic dysfunction by damaging thylakoid membranes, disrupting electron transport, and inactivating oxygen-producing enzymes, leading to oxidative stress (Djanaguiraman et al., 2018). High temperature increases water transpiration through stomata, disrupting plant water status and reducing growth potential (Matthews et al., 2018; Urban et al., 2017).

Morphologically, high temperatures increase gibberellin levels, promoting canopy growth (stems, branches, leaves) at the expense of tuberization (Ohtaka et al., 2020). This leads to taller plants, increased leaf area, and shading, which reduces photosynthetic efficiency (Nunes et al., 2019). Studies on Granola and Kennebec cultivars report a 15–25% reduction in tuber number and weight at temperatures above 25°C due to reduced photosynthate translocation to stolons (Ramadhani et al., 2024; Zhang et al., 2021) Soil temperature is critical during tuberization (15–20°C optimal), as higher temperatures (e.g., 25–

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30°C in Inceptisols, common in tropical potato fields) reduce soil water availability and porosity, particularly in clay-rich soils with low organic matter, inhibiting tuber formation (Hamdani et al., 2019; Khan et al., 2015). For example, 35% of digested carbon is lost to respiration at high temperatures, reducing assimilate availability for tubers (Muthoni & Kabira, 2015). High temperature-induced stress not only leads to yellowing leaves due to chlorophyll degradation (Hancock et al., 2014) but also reduces stomatal conductance and chlorophyll content, as reported in potato, rice, and wheat (Djanaguiraman et al., 2018; Hamdani et al., 2020; Vinitha et al., 2020; Singh et al., 2020; Yuan et al., 2021). Gibberellin, a hormone responsible for cell division and elongation, especially in shoot apices and stolons, also increases with temperature (Latifa & Indriyatmoko, 2022; Nuraini et al., 2016). During the vegetative phase, gibberellin promotes canopy development (stem elongation, branching, and leaf area expansion), thus increasing the relative growth rate (Sardoei et al., 2024). It also supports stolon elongation, enhancing potential tuber formation sites (Meng et al., 2024).

In the source-sink relationship, leaves act as sources producing sucrose via photosynthesis, while stolons act as sinks for storage (Golovko & Tabalenkova, 2019). However, increased canopy growth caused by high gibberellin levels and elevated temperatures can disturb the source-sink balance (Meng et al., 2024; Ohtaka et al., 2020; Rademacher, 2015). During the vegetative phase, the hormone gibberellin plays an important role in promoting canopy through stem elongation and increasing the number of branches, leaves, and leaf area, but its excess disrupts carbon allocation to tubers, leading to fewer fewer and smaller tubers (Sardoei et al., 2024; Durand et al., 2018; Ludewig & Sonnewald, 2016). These findings are supported by several studies reporting reductions in both the number and weight of tubers under high temperature conditions (Ramadhani et al., 2024; Zhang et al., 2024; Mubarok et al., 2022; Singh et al., 2020). This reduction is attributed to the fact that stolons are meristem tissues, and elevated endogenous levels under heat stress promote continuous stolon elongation, which limits assimilate allocation for tuber enlargement (Hamdani et al., 2016; Mariana & Hamdani, 2016; Nuraini et al., 2016). Based on Nuraini et al. (2016), the reduction in tuber mass due to uninhibited gibberellin activity is approximately 21% compared to p3 (30 mL L^{-1} paclobutrazol).

Potato Quality Response to High Temperature

High temperatures have a direct effect on potato tuber quality by altering photosynthate allocation and disrupting metabolic processes, which leads to changes in physical and chemical composition (Jagadish et al., 2021). Elevated temperatures increase reducing sugar levels (e.g., glucose, fructose) in tubers, leading to browning during processing (e.g., potato chips) due to the Maillard reaction (Kusandriani, 2016; Ahmed et al., 2018). For example, cultivars like Eramosa show increased reducing sugars at 30°C, reducing marketability (Zhang et al., 2021). High temperatures also elevate solanine levels a toxic glycoalkaloid in tuber exposed to direct sunlight or soil temperatures above 25°C, causing bitterness and health risks like nausea and diarrhea, and potentially exceeding the 200 mg/kg safety threshold adopted by several countries, although the FAO/WHO Joint Expert Committee (JECFA) has reported natural levels ranging from 20–100 mg/kg and concluded that no specific safe intake level could be established due to limited toxicological data (Karaca & Erbaş, 2024; Schrenk et al., 2020). Additionally, heat stress induces irregular tuber shapes, skin cracking, and pre-harvest sprouting, reducing commercial value (Momčilović, 2019; Muhie, 2022; PengBo et al., 2019). Several studies have shown that planting potatoes in high temperature environments results in small tuber weights (Dewi et al., 2024; Hamdani et al., 2019, 2024; Hernawati et al., 2022; Mariana & Hamdani, 2016). The growth rate of tubers decreases due to high temperature because of the reduction of starch content in tuber (Mubarok et al., 2022; Ramadhani et al., 2024). According to Muhie (2022), heat stress can induce sprouting before harvest, which reduces tuber quality.

According to Mori et al. (2015), high soil temperatures cause discoloration in sweet potatoes to brown due to decay. Based on research by Momčilović (2019) that heat stress produces irregular tuber shape by disrupting dilation and elongation in the tuber enlargement phase, which constricts the tuber base. Heat reduces the soil water supply, causing low starch content due to breakdown into sucrose, which leads to increased sugar content, revealing translucent tubers and jelly-like texture changes that reduce tuber quality and tuber resistance from bacterial and fungal attack (Momčilović, 2019; Muthoni & Shimelis, 2020).

In addition, high temperatures cause the potato skin to crack. This is due to the skin cell layer developing during tuberization due to the continuous expansion of the potato (Molteberg, 2017). High temperature reduces the resistance of potato plants infected with Potato virus Y, which can lead to a decrease in yield quantity and quality (Choi et al., 2017; Makarova et al., 2018). Thus, there is a need for adaptive potato cultivation practices to deal with temperature changes to increase and maintain sustainable production.

Adaptive Cultivation Practices: Election of Superior Varieties

Heat-tolerant potato varieties are critical for maintaining productivity under high temperatures. Varieties like Atlantic, Merbabu-17, and Granola exhibit tolerance due to enhanced stomatal efficiency, reduced chlorophyll degradation, and stable tuberization at temperatures above 25°C (Djuariah et al., 2016; Firdausy et al., 2024). For instance, Atlantic maintains yields of 11.47 tons/ha in medium-altitude lands by sustaining photosynthetic rates, while Granola's tolerance stems from efficient photosynthate translocation (Prabaningrum et al., 2015). Thus, planting with superior varieties contributes to the sustainability of production under climate change conditions. High temperature tolerant potato varieties are presented in Table 1.

Adaptive Cultivation Practices: Plant Growth Regulator under Heat Stress

Plant Growth Regulators (PGRs) are natural or synthetic compounds that influence physiological processes at low concentrations (Emilda, 2020). High temperatures elevate ABA, gibberellin, and ethylene levels, which disrupt normal tuber development by promoting excessive vegetative growth and inhibiting assimilate partitioning to the tuber, inducing stomatal closure, and reducing photosynthesis (Thakur et al., 2019). Stomatal closure restricts CO_2 uptake, limiting photosynthetic activity (Lawson & Matthews, 2020). In contrast, PGRs like BAP (cytokinin) promote stomatal opening by stimulating guard cell turgor and improving photosynthetic efficiency (Pal et al., 2016). Retardants like paclobutrazol, chlorocholine chloride, and prohexadione-Ca inhibit gibberellin synthesis, redirecting assimilates from canopy growth to tuberization (Hamdani et al., 2016). Additionally, high temperatures elevate methylglyoxal levels, a toxic compound that induces ROS in plants and reduces chlorophyll, thereby inhibiting photosynthesis and plant growth (El-Yazied et al., 2022; Takagi et al., 2016). In response, exogenous melatonin application enhances antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (G-POX), and ascorbate peroxidase (APX), preserving cell membranes, promoting chlorophyll synthesis, supporting photosynthetic activity, and delaying senescence (El-Yazied et al., 2022; Jahan et al., 2021; Youssef et al., 2021; Arnao & Hernández-Ruiz, 2018). Melatonin improves potato yield under heat stress by inhibiting ABA transport from root to shoot and maintaining soil water availability, stimulating root growth, and supporting photosynthetic efficiency by increasing chlorophyll content, which can delay plant senescence (Arnao & Hernández-Ruiz, 2018; Ibrahim et al., 2020; El-Yazied et al., 2022). Several studies have shown that melatonin application can increase photo-synthetic efficiency with increased chlorophyll content in tomato (Jahan et al., 2021), wheat (Buttar et al., 2020), and kiwi (Liang et al., 2018).

Numerous studies confirm the effectiveness of PGRs in improving potato growth and yield under heat stress. For instance, BAP (40 ppm) applied at 40 and 60 DAP significantly increased tuber weight, number, chlorophyll content, and harvest index (Abouelsaad & Brengi, 2022). Melatonin (23.23 ppm and 50 ppm) applied from 42 to 70 DAP enhanced leaf water status, reduced ABA, improved antioxidant enzyme activity, and increased yield (El-Yazied et al., 2022; Amelia et al., 2023). Paclobutrazol (100 ppm) applied at 30–40 DAP improved harvest quantity and quality by up to 108%, enhancing stolon formation and chlorophyll levels (Hamdani et al., 2024). Similarly, chlorocholine chloride (2000 ppm) and prohexadione-Ca (50–100 ppm) suppressed excessive shoot growth and solanine synthesis, while improving tuber yield and quality (Tak et al., 2024; Ramadhani et al., 2024). These empirical results highlight the significant role of PGRs in mitigating heat stress in potato cultivation. Table 2 presents a summary of the major PGRs and their physiological effects under elevated temperature conditions.

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Table 1. High temperature tolerant potato varieties

Varieties	Location	Effect	Tolerance Mechanism	Reference
Granola	Medium land	Yield 3.61 tons/ha; tuber dry weight 62.45 g; growth rate 0.87 g/plant/day	Efficient photosynthate translocation	(Prabaningrum et al., 2015; Rogi et al., 2016)
Atlantic	Medium land	Yield up to 11.47 tons/ha	High stomatal efficiency	(Djuariah et al., 2016)
Merbabu-17	Medium land	Yield reached 11.04 tons/ha	Reduced chlorophyll degradation	(Firdausy et al., 2024)

Table 2. Effect of different types of PGRSs on potato plants

Hormone	Concentration	Time Application	Effect	Reference
BAP	40 ppm	40 and 60 DAP	Increase leaf chlorophyll, tuber weight and number of tubers, harvest index, mineral, protein, and ascorbic acid content of potato tubers	(Abouelsaad & Brengi, 2022)
Melatonin	23.23 ppm*	42, 49, 56, 63, and 70 DAP	Reduce ABA levels and increase leaf chlorophyll, leaf water status, antioxidant enzyme activity, and yield	(El-Yazied et al., 2022)
	50 ppm		Produce the highest weight and harvest index	(Amelia et al., 2023)
Paclobutrazol	100 ppm	30 DAP	Increase harvest quality and quantity by up to 108% in high temperature	(Hamdani et al., 2016)
		30 and 40 DAP	Suppress plant height and biomass, increase stomatal conductance, chlorophyll, number of stolon, percentage of stolon, forming tubers, number and weight of tuber per plant	(Hamdani et al., 2024; Ramadhani et al., 2024)
		40, 50, and 60 DAP	Increase tuber growth rate, number and weight of tubers per plant	(Mubarok et al., 2024)
Chlorocholine-chloride	2000 ppm	25 and 45 DAP	Suppress plant height and secondary metabolite solanine and increase leaf chlorophyll content, tuber weight, yield, starch content	(Tak et al., 2024)
Prohexadione-Ca	50 ppm	40 DAP	Increase chlorophyll content, stomatal conductance, number, and weight of tubers	(Hernawati et al., 2022)
	100 ppm	-	Increase chlorophyll content index, number and weight of tubers per plant	(Ramadhani et al., 2024)

Note: *: Convertion mM to ppm; DAP: Days After Planting

Growth retardants are specialized PGRs that inhibit gibberellin biosynthesis, thereby reducing excessive vegetative growth (Chen et al., 2020; Hamdani et al., 2016). By limiting gibberellin levels, these retardants redirect assimilates from canopy development to tuber formation, enhancing yield (Desta & Amare, 2021; Ramadhani et al., 2024; Tak et al., 2024). The mechanism of paclobutrazol inhibition in gibberellin synthesis is to inhibit the activity of ent-kaurene oxidase, which plays a role in catalyzing ent-kaurene into ent-kaurenoic acid (Kumar et al., 2023; Tesfahun, 2018). Chlorocholine chloride inhibits the early biosynthesis of gibberellins by blocking the conversion of GGPP (geranylgeranyl pyrophosphate) to ent-kaurene (Khella, 2018). Prohexadione-Ca interferes with the late-stage conversion of GA20 to GA1 by altering the related oxidase enzyme (Pal & Johal, 2019). Beyond gibberellin suppression, retardants enhance heat stress tolerance by stimulating antioxidant enzyme activity (Azmi et al., 2022; Kamran et al., 2020; Liu et al., 2022; Skłodowska et al., 2021). In addition, retardant application can improve water use efficiency, making it suitable for dry climate areas (Bhattarai, 2017; Mabvongwe et al., 2016; Teixeira et al., 2021).

Adaptive Cultivation Practices: Irrigation and Mulch Application

High temperature reduces soil water availability during the tuberization phase, decreasing the number and weight due to potato shallow root systems (Li et al., 2016; Aliche et al., 2018; Joshi et al., 2016). The use of irrigation and mulch can be used as a practice for potato cultivation under heat stress conditions (Table 3). Application of irrigation can increase the efficiency of water use in the soil, so that plant growth and development are not hampered (Biswas et al., 2015). Among irrigation methods, drip irrigation appears most effective under heat stress, as it directly targets the root zone and improves tuber weight by 15–20%, and supports quality traits like vitamin C content and yield, unlike furrow irrigation, which despite saving water, performs poorly in heavy soils (clay-rich inceptisols) and sprinkler irrigation less suitable in water-limited regions due its high water demand (Dianawati et al., 2019; Wang et al., 2020; Yang et al., 2019).

Table 3. Effect of different types of irrigation and mulch on potato plants

Type	Treatment	Effect	Reference
Drip irrigation	Water volume 300 ml/polybag and frequency 5 times	Increase primary and secondary stolon growth, number, weight, and harvest index	(Dianawati et al., 2019)
	-	Increases soil moisture content, suppresses canopy growth, and increases yields	(Zhou et al., 2018)
Alternate furrow irrigation)	Frequency of application 4 times	Increases harvest index, saves water use by 35% and increases water productivity by 50% suitable for use in dry environments	(Kumer et al., 2019)
Sprinkler irrigation	10 mm water volume	increases yield, reduces soil N_2O emissions, and saves water	(Yang et al., 2019)
Straw mulch	-	Reduces maximum soil temperature, increases yields by 30–40%, and produces safe food without herbicides	(Adamchuk et al., 2016)
Wheat mulch	-	Reduced soil temperature by 1–2 °C and increased soil moisture by 42%, water use efficiency, and crop yields	(Goel et al., 2020)
Wheat + straw mulch	Dose of 4.5 tons/ha	Increased tuber yield by 19.5%, ready to sell tubers (> 40 mm) by 21.2%, water use efficiency, soil water availability, number of tuber, and retained soil moisture	(Král et al., 2019)
Silver black mulch	-	Increased leaf area index, plant growth rate, harvest index, and tuber weight of G1 and G3 seedlings	(Jella et al., 2017)
	-	Increases number of tuber per plot, tuber weight per sample, and tuber weight per plot	(Ismadi et al., 2021)
Plastic film mulch	-	Increased yield and nitrogen use efficiency	(Wang et al., 2019)
White/black plastic mulch	-	Retained leaf nutrients and yield increased by 38.7%.	(Ruíz-machuca et al., 2015)

Mulch is a soil surface cover made from either organic material, such as decomposed plant parts that contribute nutrients to the soil, or inorganic material, such as plastic (Yetnawati & Hasnelly, 2021; Irianti et al., 2017). In terms of mulch, organic mulches (e.g., straw, wheat) are more beneficial in hot climates due to their cooling and water retention capacity, whereas inorganic mulches (e.g., silver-black plastic) enhance light reflection but are less effective in water retention (Goel et al., 2020; Ruíz-Machuca et al., 2015; Nurbaiti et al., 2017). By lowering soil temperature, mulch creates favorable conditions for stolon initiation, thereby increasing potato yield (Banerjee et al., 2016; Chen et al., 2019; Dash et al., 2018). Both types of mulch suppress weed growth, enhance water use efficiency by lowering evaporation, improving soil structure, and reducing competition for nutrients, water, and growing space (Irianti et al., 2017; Li et al., 2018; Wang et al., 2019). Lack of water in the soil causes reduced sugar in the potato due to increased levels of ROS that damage cells (Muttucumaru et al., 2015; Nasir & Toth, 2022; Dvořák et al., 2015). Therefore, combining drip irrigation with organic mulch appears to be the most effective strategy to sustain potato growth and yield under heat stress by improving water efficiency and maintaining soil quality.

Future Implications

Future research should investigate the synergistic effects of multiple PGRs, particularly the combined application of melatonin, BAP, and gibberellin inhibitors under varying heat stress intensities. Field-based validation is also crucial to determine optimal dosages, application timing, and cost-effectiveness. Moreover, molecular studies exploring gene expression linked to ROS scavenging and ABA transport in response to PGRs can deepen the understanding of their regulatory mechanisms. In addition, integrating heat-tolerant varieties with PGRs and precision irrigation in field trials remains underexplored and should be prioritized to assess their combined effectiveness.

Conclusion

High temperatures (>25°C) reduce potato yield and quality by impairing photosynthesis, increasing respiration, and altering tuber chemistry, with global yield losses projected at 18–32% by the 2050s. This review, synthesizing 45 studies, highlights heat-tolerant varieties, PGRs, irrigation, and mulching as effective strategies to mitigate these impacts, particularly in tropical regions. Limitations include the lack of field-scale studies combining multiple practices and insufficient economic data on adoption feasibility. This review contributes a comprehensive framework for sustainable potato cultivation under heat stress, guiding future research and policy.

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Effect of potassium fertilizer on the nutrition and physical quality of Job's tears (*Coix lacryma-jobi* L.) seeds

Abstract. Job's tears is a cereal crop that has beneficial nutrients. Potassium is known to affect the growth and yield of cereal crops, but there has been limited study on whether potassium affects the nutrition and physical quality of Job's tears seeds. This study aimed to analyze the effect of K fertilizer on the nutritional content and physical quality of Job's tears seeds. This research was conducted from January to May 2024 at Ciparanje Experimental Field, Faculty of Agriculture, Universitas Padjadjaran, Sumedang, Indonesia. This experiment used the Randomized Block Design (RBD) method with six treatments and four replications. Various doses of K fertilizer (KCl) were tested, i.e., 0, 62.5, 125, 250, 375, and 500 kg/ha KCl. The measured nutritional content was the extraction rates of carbohydrate, protein, lipid, calcium, and potassium, while the physical quality was represented by the weight of husked grain, size of seeds, and seed hardness. Results showed that potassium increased the extraction rates of carbohydrate, protein, lipid, calcium, and potassium. Potassium also increased the weight of husked grain and size of seeds. A dosage of 500 kg/ha KCl resulted in the best nutrition, weight of husked grain, and size of seeds.

Keywords: Dosage · Extraction rate · Job's tears · Nutrition · Potassium

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Introduction

Job's tears (*Coix lacryma-jobi* L.) is a cereal plant that has many health benefits and nutritional value (Wicaksono et al., 2024; Aqil et al., 2023). Nutrients such as carbohydrates, proteins, fats, and important minerals, such as calcium and potassium, are found in Job's tears seeds (Ramadhan et al., 2022). Job's tears contain less carbohydrates than rice, so it can be a healthy alternative food (Qosim & Nurmala, 2011). In addition, the high protein content makes Job's tears play a role in the formation and repair of body tissues, enzymes, and hormones, so that it can support the body's metabolic processes (Aminah et al., 2019). Job's tears have a healthy fat content, which supports cell function and body metabolism (Djaja, 2022; Devaraj et al., 2020). Calcium in Job's tears plays a role in maintaining healthy bones and teeth and supporting nerve and muscle function (De & De, 2019; Pravina et al., 2013), while potassium has an important role, especially in maintaining electrolyte balance and nerve function, excretion process and fluid balance in the human body (Aminah et al., 2019). Varieties of job's tears from different regions and habitats may show varied nutritional compounds (Rupitak et al., 2024; Biswas & Das, 2022; Devaraj et al., 2020)

To develop biofortification of Job's tears product, fertilizer is needed to make optimal photosynthesis, macromolecule synthesis, and finally produce high quality product (Wang et al., 2019). One essential nutrient that plays a role in the growth and seed development is potassium (K) (Torabian et al., 2021). Potassium is an essential macronutrient in many physiological processes in plant cells, such as enzyme activation, membrane potential maintenance, and osmotic regulation (Vijayakumar et al., 2021). Appropriate potassium dosing can promote yield formation by promoting photosynthate transport and coordinating biomass accumulation in source organs and storage organs (Wang et al., 2018; Epron et al., 2016). The important role of potassium in determining the quality of agricultural products is related to chemical compositions (Sardans et al., 2021).

Potassium regulates diverse biochemical processes associated with regulating photosynthesis, translocation, protein synthesis, and carbohydrate metabolism (Hasanuzzaman et al., 2018). The application of potassium enhances the efficiency of carbon fixation during photosynthesis by increasing photosynthetic activity. Fructose-1,6-bisphosphatase (FBpase) is a key enzyme in the Calvin cycle, and its activity is closely related to photosynthetic efficiency, as well as the accumulation and distribution of photosynthesis. FBPase has a K^+ binding site, and higher application rates of potassium fertilizer significantly increase FBPase activity, which in turn regulates carbohydrate accumulation and distribution, thereby increasing yield (Hu et al., 2015). Potassium also plays a role in balancing the charge of nitrate ions (NO_3^-) absorbed by plants, thereby facilitating more efficient nitrogen uptake (Blevins et al., 1978). This process is closely related to the role of K^+ in transporting nitrogen, a major component of protein, to the site of protein synthesis (Saktiono et al., 2021). Potassium affects plant quality by increasing lipid content by increasing the activity of enzymes related to lipid synthesis (Chen et al., 2023). Potassium helps regulate the activity of several key enzymes in the lipid biosynthesis pathway, such as Acetyl-CoA carboxylase (ACCase), fatty acid synthase (FAS), glycerol 3-phosphate dehydrogenase (3 GPD), phosphatidyl phosphatase (PPase), and glycerol 3-phosphate acyltransferase (GPAT).

In addition to affecting the chemical composition, potassium also plays a role in the physical quality of seeds in Job's tears. Sufficient potassium levels enable optimal transport of photosynthetic products from leaves to seeds, which can increase size of seeds (Jiaying et al., 2022). Potassium deficiency can result in incomplete seed filling, leading to small and shriveled seeds (Liu et al., 2011). Potassium can increase carbohydrate synthesis and translocation, thereby increasing the thickness of the cell wall, which affects the outermost layer of the seed coat and then increases seed hardness (Mutaqin et al., 2019). Seed hardness influences grain extraction rate from the dehuller machine (Blandino et al., 2010; Reichert & Youngs, 1984). Optimal potassium is expected to produce seeds with a dense structure and good physical quality (Usherwood, 1985).

However, the study of potassium fertilizer on nutritional content and physical characteristics of the local variety of Job's tears seeds is still unknown. This study aims to analyze the effect of K fertilizer dosages on the content and extraction rates of carbohydrate,

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protein, lipid, calcium, and potassium in Job's tears seeds. The research can be useful to provide the best potassium dosage in optimizing the quality of local Job's tears seeds.

Materials and Methods

This research was conducted from January 2024 to May 2024 at Ciparanje Experimental Farm, Faculty of Agriculture, Universitas Padjadjaran, Sumedang, Indonesia. The elevation of the research site is 750 m above sea level, and the agroclimate zone is C3 according to Oldeman classification. The soil has a pH of 5.9, indicating a weakly acidic condition. Nitrogen content is low, phosphorus is high, potassium is low, and the cation exchange capacity is 16.31 $cmol.kg^{-1}$ (Table 1). Analysis of nutrient content was carried out at the Laboratory of the Agency for Agricultural Assembly and Modernization for Vegetable Crops, West Bandung, Indonesia.

The materials used were Job’s tears seeds from the Watani Wado variety, collected from the Laboratory of Crop Production Technology, Faculty of Agriculture, Universitas Padjadjaran; urea; SP-36; KCl fertilizer; cow manure; pesticides; and laboratory materials for nutrition analysis (filter paper, distilled water, etc). The tools used analytical scales, grain dehuller machine, laboratory equipment (oven, analytical scales, digestion tube, test tube, UV-VIS spectrophotometer, microwave digester, dropper, measuring cup, 500 ml Erlenmeyer, 100 ml Kjeldahl flask, distillation equipment, electric heater), and agricultural tools.

This experiment used the Randomized Block Design (RBD) method with six treatments and four replications, so that there were 24 experimental units. The treatment is dosages of KCl fertilizer, consisted of A = no K fertilizer (Control); B = 25% of the recommended dose of K fertilizer (62.5 kg/ha KCl = 3 g/plant); C = 50% of the recommended dose of K fertilizer (125 kg/ha KCl = 6 g/plant); D = 100% of the recommended dose of K fertilizer (250 kg/ha KCl = 11.97 g/plant); E = 150% of the recommended dose of K fertilizer (375 kg/ha KCl = 17.95 g/plant); F = 200% of the recommended dose of K fertilizer (500 kg/ha KCl = 23.94 g/plant).

Table 1. Soil chemical analysis of Ciparanje Experimental Farm

No	Parameters	Unit	Result	Criteria
1.	pH: H_2O	-	5.9	Weakly acidic
2.	pH: KCl 1 N	-	4.6	-
3.	C-organic	(%)	1.94	Low
4.	N-total	(%)	0.21	Low
5.	C/N	-	9	Low
6.	P_2O_5 HCl 25%	(mg/100g)	60.86	High
7.	P_2O_5 (Olsen)	(ppm P)	33.8	High
8.	K_2O HCl 25%	(mg/100g)	13.25	Low
9.	Al-dd	($cmol.kg^{-1}$)	0.00	-
10.	H-dd	($cmol.kg^{-1}$)	0.11	-
11.	CEC	($cmol.kg^{-1}$)	16.31	
	Base saturation	(%)	63	Medium
12.	K: Morgan Venema	(%)	44.4	High
13.	Cations:			
	K-exch	($cmol.kg^{-1}$)	0.11	Low
	Na-exch	($cmol.kg^{-1}$)	0.09	Low
	Ca-exch	($cmol.kg^{-1}$)	7.66	High
	Mg-exch	($cmol.kg^{-1}$)	2.47	Very High
14.	Texture:			
	Sand	(%)	4	
	Dust	(%)	38	
	Clay	(%)	58	

Notes: CEC was cation exchange capacity, exch was exchangeable; source from the Agency for Agricultural Assembly and Modernization for Vegetable Crops (2024)

The size of the plot was 3 x 2 m. The plant spacing was 60 x 40 cm. Basic fertilization used manure 2 t/ha, a week before planting. Seeds were planted after germinating for 4 days. Urea fertilizer 300 kg/ha was applied to the plants 3 times, namely at 3, 6, and 9 weeks after planting (WAP). SP-36 fertilizer 200 kg/ha is given once at 3 WAP, while KCl fertilizer was given twice, at 3 and 6 WAP, with the dose based on each treatment. Plant maintenance includes watering, weeding, and pest and disease control. Harvesting was done at 170 days after planting, when the plants dried, and the seeds reached physiological maturity. Job’s tears seeds are dried under the sun, which aims to reduce the water content in the job's tears seeds. Job’s tears seeds are polished using a dehuller machine.

Chemical analysis was carried out on Job's tears seeds, including protein content by the semi-micro Kjeldahl method; carbohydrate content by the Luff-Schoorl method; fat content by the Soxhlet method; calcium and potassium content by the wet soaking method (AOAC, 2019). Each nutrition extraction rate is measured by multiplying each nutrition content and weight of the husked grain. The size of the seeds is measured using calipers, while the seed hardness is measured using a penetrometer.

Nutrition content data is presented descriptively, while weight of husked grain, extraction yield, size of seeds, and seed hardness data were analyzed using Least Significant Difference (LSD) at a significant level of 5%. The data processing used the SmartstatXL program.

Results and Discussion

Results. The results showed that potassium provision starting from 50% of the recommended dose increased the weight of husked seeds compared to the control (Table 2). The 200% recommended dose gave the best yield, but was not different from 150%.

Potassium greatly affected the nutritional content of Job’s tears seeds (Table 3). Lower doses of potassium (A, B, and C) gave higher carbohydrate content. Increasing potassium doses from 25% to 100% recommended dose gave higher protein compared to the control, but higher doses decreased it. Concerning lipid, calcium, and potassium content, the KCL doses did not show a definite tendency.

Table 2. Effect of potassium dosage on the weight of husked grain of Job’s tears

Treatment	Weight of husked grain (g)
A	31.3 a
B	41.1 ab
C	43.3 b
D	44.2 b
E	51.9 bc
F	56.8 c

Note: A = Control; B = 3 g/plant; C = 6 g/plant; D = 11.97 g/plant; E = 17.95 g/plant; F = 23.94 g/plant. Mean values followed by the same lowercase letter in the same column are not significantly different based on Least Significant Difference (LSD) at a significant level of 5%.

In contrast to the nutrient content, increasing the potassium dose gave a higher nutrient extraction rate compared to the control (Table 4). Increasing the potassium dose to 200% of the recommended dose gave the best carbohydrate and protein rate, although not significantly different from the 150% recommended dose treatment. As much as 200% of the recommended dose gave the best lipid yield, but this dose did not give a significant difference with 150% or 100% in calcium extraction rate, and was not significantly different from the 50 to 150% dose in potassium extraction rate.

Table 3. Effect of potassium dosage on nutrient content of Job’s tears seeds

Treatment	Carbohydrate (%)	Protein (%)	Lipid (%)	Calcium (mg/100g)	Potassium (mg/100g)
A	57.45	14.9	4.01	80	310
B	57.95	15.86	4.27	60	270
C	57.44	16.08	3.58	60	340
D	53.3	16.14	4.14	80	340
E	53.79	15.41	3.75	70	270
F	53.79	15.18	4.14	70	310

Note: A = Control; B = 3 g/plant; C = 6 g/plant; D = 11.97 g/plant; E = 17.95 g/plant; F = 23.94 g/plant.

Table 4. Effect of potassium dosage on nutritional content of Job's tears seeds

Treatment	Carbohydrate (g/plant)	Protein (g/plant)	Lipid (g/plant)	Calsium (mg/plant)	Potassium (mg/plant)
A	18.0 a	4.7 a	1.3 a	25.0 a	97.0 a
B	23.8 b	6.5 b	1.8 bc	24.7 a	64.4 b
C	24.9 b	7.0 bc	1.6 ab	26.0 a	147.4 b
D	23.5 b	7.1 bc	1.8 bc	35.3 b	150.2 b
E	27.9 bc	8.0 cd	1.9 c	36.3b	140.1 b
F	30.6 c	8.6 d	2.4 d	39.8 b	176.2 b

Note: A = Control; B = 3 g/plant; C = 6 g/plant; D = 11.97 g/plant; E = 17.95 g/plant; F = 23.94 g/plant. Mean values followed by the same lowercase letter in the same column are not significantly different based on Least Significant Difference (LSD) at a significant level of 5%.

Tabel 5. The effect of potassium dosage on the physical quality of Job's tears seeds

Treatment	Size of seeds (mm)	Seed Hardness (kgf)
A	6.1 a	4.8 a
B	6.6 b	4.8 a
C	6.7 b	4.9 a
D	6.8 bc	4.9 a
E	6.8 bc	5.0 a
F	7.2 c	5.0 a

Note: A = Control; B = 3 g/plant; C = 6 g/plant; D = 11.97 g/plant; E = 17.95 g/plant; F = 23.94 g/plant. Mean values followed by the same lowercase letter in the same column are not significantly different based on Least Significant Difference (LSD) at a significant level of 5%.

Increasing the potassium dose could increase the seed size of Job's tears. Treatment A yielded the lowest results and was significantly different from the other treatments, while potassium doses ranging from 62.5 kg/ha (B) to 300 kg/ha (E) showed no significant differences among themselves but were significantly different from treatments A and F. The 500 kg/ha dose (F) yielded the largest seed width at 7.2 mm (Table 5). Meanwhile, the seed hardness of Job's tears did not show any significant differences between treatments (Table 5).

Discussion. Potassium is needed by plants, especially for seed formation, through its direct effect on sinks and/or indirectly on source tissue (Sardans & Peñuelas, 2021). This element plays a role in the translocation of photosynthate from leaves to seeds (Rawat et al., 2022). Potassium also helps regulate osmotic pressure and cell turgor, thereby increasing the efficiency of photosynthesis (Mostofa et al., 2022; Jhonson et al., 2022). In terms of direct effect on grain yield, potassium plays a role in increasing the rate and duration of seed filling, thereby reducing small broken grains (Lv et al., 2017; Li-Jun et al., 2011). All these reasons make potassium increase the weight of the husked seeds.

Increasing potassium to a certain limit increased carbohydrate and protein content, but higher doses decreased carbohydrate and protein content. Potassium does not affect starch synthase in seeds significantly, so the effect of potassium is limited to the flow of photosynthate (Hou et al., 2018). Perhaps, there is an interaction between potassium and nitrogen, where higher potassium will reduce nitrogen for the formation of carbohydrates and proteins (The et al., 2021).

Meanwhile, potassium cannot consistently affect lipid, calcium, or potassium content. Previous studies had shown that potassium did not affect lipid synthesis consistently, especially two different effects on the saturated and unsaturated fatty acids synthesis (Gu et al., 2024; Pande et al., 2014). The effect of potassium on calcium content and even potassium itself also did not have a clear pattern. This phenomenon needs to be studied further to ensure the effect of potassium on calcium and potassium contents in grain.

Potassium increased the extraction rate of carbohydrates, proteins, lipids, calcium, and potassium. This increase was due to the multiplier effect of the weight of husked seeds on the nutrient content. Although potassium has various effects on nutrient content, its significant effect on husked seed weight also causes the extraction yield to increase. Potassium plays a role in transporting photosynthetic products from leaves to storage organs such as seeds (Hutagalung et al., 2019).

Larger size of seeds was influenced by increased potassium supply to the seeds (Edy et al., 2021). Potassium dose did not have a significant effect on seed hardness in each treatment. Seed hardness is more influenced by other compounds, such as silica. Previous research has shown that giving liquid silica fertilizer made the skin of Job's tears seeds harder (Nurmala et al., 2017). High doses of potassium (200% of the recommendation) are important to obtain maximum extraction yield of all nutrients.

Conclusion

The 200% recommended dose of potassium resulted in the best extraction rate of carbohydrates, proteins, lipids, calcium, potassium, and seed size of Job's tears. The effects of potassium on lipid, calcium, and potassium content need to be further studied.

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The effect of melatonin and 6-Benzylaminopurine application on the post-harvest quality of cut roses (*Rosa* x *alba*)

Abstract. Roses are known as a high-value commodity frequently used in various important events. However, they are susceptible to postharvest quality deterioration, which can affect their vase life and appearance. In this study, roses with a blooming stage of approximately 25–50% were immersed in melatonin and 6-benzylaminopurine (BAP) solutions at different concentrations. This research aims to analyze the effect of melatonin and BAP application on the freshness of cut roses. The parameters observed included flower vase life, flower wilting angle, increase in flower diameter, fresh weight, solution uptake, and chlorophyll content. The results showed that melatonin and BAP, applied individually or in combination, effectively extended the freshness of cut roses by up to eight days by maintaining solution uptake, flower quality, and chlorophyll content. This study provides new insights for farmers and researchers in improving the quality and longevity of cut roses through the use of plant hormones, particularly cytokinin.

Keywords: Cytokinin · Melatonin · Postharvest · Quality · Rose

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Introduction

Ornamental plants, particularly roses (*Rosa* x *alba*), have become a high-value commodity around the world. Roses are known for their beauty, color, and fragrance, making them one of the most popular ornamental plants (Bilad, 2021). Roses not only have aesthetic value but also significantly contribute to the economy and create opportunities for cultivators (Suparyana *et al.,* 2022). One of the common innovations in rose cultivation is cut roses, as they are widely used in various important events such as weddings and traditional celebrations (Abbas et al., 2024).

Some ornamental plants may experience a loss of quality due to various factors such as wilting or abscission of leaves and petals (Lamsal, 2024). Furthermore, water availability, light quality, ethylene, temperature, and humidity can lower cut flower quality (Verdonk et al., 2023). Environmental factors and proper Treatments have a big impact on the quality of ornamental plants. Cutting flower stems causes oxidative damage, resulting in excess production of reactive oxygen species (ROS). During post-harvest quality degradation of cut roses, ROS levels increase sharply and are accompanied by activities related to the antioxidant system (Jing & Li, 2015).

In response to these challenges, the application of melatonin (MT) and 6-benzylaminopurine (BAP) in cut roses has gained considerable attention from researchers and growers alike. Melatonin, known as a powerful antioxidant, plays an important role in plant aging by reducing oxidative stress and increasing the activity of antioxidant enzymes. This process helps maintain cell integrity and extends the vase life of flowers, and protects the plant from harmful reactive oxygen (Liang et al., 2015; Xu et al., 2019). In a study conducted by Mazrou et al. (2022), it was shown that melatonin with a concentration of 0.2 mM increased the activity of CAT, APX, and GR enzymes until the 10^{th} day in carnation flowers. Based on this study, melatonin delayed carnation aging and extended vase life by maintaining the initial level of fresh weight, membrane stability index, bioactive compounds, and antioxidant activity. This is supported by research from Wang et al. (2024), which found that a 50 µM concentration of melatonin was effective in preserving the shelf life of peony flowers for up to seven days during storage.

Meanwhile, 6-benzylaminopurine application in cut roses can prevent wilting and maintain flower quality by delaying leaf senescence and inhibiting chlorophyll degradation during storage (Man et al., 2016; Zhou et al., 2023). A study conducted by Lone et al. (2021) showed that the application of BAP at a concentration of 50 µM resulted in the best increase in flower vase life and significantly improved the post-harvest performance of calendula cut flowers. Based on the study, cut flowers stored in a 50 µM BAP solution showed the longest vase life, reaching 13 days. This occurs because cytokinins prolong vase life by enhancing photosynthetic output directed toward developing flower buds, which improves water uptake and promotes greater turgidity in the petal tissues (Singh & Tiwari, 2021).

This study was conducted with the aim of analyzing the effect of melatonin and 6-benzylaminopurine on the freshness of cut roses and determining the best concentration of melatonin and 6-benzylaminopurine to maintain the vase life. This study examines the potential benefits of melatonin and 6-benzylaminopurine on cut roses, offering insights for researchers and growers to enhance cut rose production quality and vase life.

Materials and Methods

Research Site. This research was conducted from April to June 2024. The location is situated at the Laboratory of Post-Harvest Analysis of Horticultural Crops, Faculty of Agriculture, Universitas Padjadjaran, Jatinangor District, Sumedang Regency, West Java Province.

Materials. The materials used were cut roses, *Rosa alba* cv. Avalanche with blossom criteria of ± 25–50%, 1% sucrose solution, melatonin (from Nutrilabs), 6-benzylaminopurine (from PhytoTech Lab), methanol, ethanol, HCl, and distilled water solutions. The equipment comprised 460 mL glass bottle, plastic container, cutting scissors, bow, measuring cup, vernier caliper, analytical balance, UV-VIS spectrophotometer, cuvette, volumetric flask, pipette, falcon tube, mortar, magnetic stirrer, and thermohygrometer.

Research Methods. This study used a simple completely randomized design (CRD) with nine treatments, consisting of a control that used sucrose solution (A), melatonin 0,1 mM (B), melatonin 0,5 mM (C), BAP 25 μM (D), BAP 50 μM (E), Melatonin 0,1 mM + BAP 25 μM (F), Melatonin 0,1 mM + BAP 50 μM (G), Melatonin 0,5 mM + BAP 25 μM (H), and Melatonin 0,5 mM + BAP 50 μM (I). There were 9 Treatments with 3 replications, where each treatment consisted of 2 stems for flower freshness observation and 2 stems for deconstruction; the total experiment consisted of 108 flower stems.

Observation variables. The observation parameters consisted of vase life (d), flower angle petals (°), flower diameter increment (mm), flower fresh weight (g), solution uptake (ml), and chlorophyll content (mg/g). Vase life was measured on experimental flowers daily until they deteriorated or lost their freshness. Signs of deterioration included fading color, browning, and changes in the flower curl angle (Mubarok et al., 2018). Flower angle petals were measured daily using a bow, and if the petal angle is more than 120°, the flower is considered deteriorated (Mubarok et al., 2018).

Flower diameter was measured every two days using a caliper, and the increase in diameter was calculated by subtracting the data on day n after treatments from the data on day 0 after treatments (Mubarok et al., 2018). The formula used is:

$$D = \frac{d1 + d2}{2}$$

Description:
D = average diameter
d_1 = longest diameter (mm)
d_2 = shortest diameter (mm)

The study measured the fresh weight of flowers using analytical scales before they lost their freshness or wilted. The solution uptake was calculated by comparing the initial and final amount of solution after the flowers were immersed. The experiment was conducted at the beginning and end of the experiment (Adam & Eldeeb, 2021). The formula used is:

Solution uptake = Amount of initial solution - Amount of final solution

Chlorophyll content was measured on the 4th day after treatments, and the top leaf sample was crushed, extracted with ethanol, and incubated for 15 minutes. The supernatant solution was then measured for absorbance using a UV-VIS spectrophotometer at 647, 664, and 700 nm (Lezoul et al., 2022). Chlorophyll content was calculated using the formula:

$$CC = (5.24 \times (A_{664} - A_{700}) + (22 \times A_{647} - A_{700}))$$

Description:
CC = Chlorophyll content (mg/g)

Data Analysis. Data analysis was conducted using analysis of variance (ANOVA) with a linear model of a completely randomized design (CRD) at a significant level of 5% and followed by Duncan's Multiple Range Test at the 5% as a post hoc test.

Result and Discussion

Vase Life. The study analyzed the length of flower freshness on experimental roses from the first day until they lost their freshness. Treatments H, D, and E had the longest freshness, reaching 8 days, but were significantly different from treatments A, which only reached 5 days. The single and combination treatments of BAP concentrations, MT 0.5 mM + BAP 25 μM, significantly impacted flower freshness. Therefore, the provision of BAP, both single and a combination of BAP and melatonin in the soaking solution, can maintain freshness in cut roses (Table 1).

Melatonin, when applied at a concentration of 0.2 mM, can extend the vase life of carnation flowers by maintaining water relations and antioxidant defense systems (Mazrou et al., 2022). This helps reduce oxidative damage and regulates stomatal opening. Melatonin increases the activity of Superoxide Dismutase (SOD) and Ascorbate Peroxidase (APX) enzymes, which reduce hydrogen peroxide (H_2O_2) radicals. H_2O_2 is the main reactive oxygen species (ROS) in plants, as it can easily penetrate the cell wall and trigger stomatal closure (Jensen et al., 2023). Wang et al. (2024) reported that 50 μM melatonin extended the shelf life of peony flowers by preserving water and inhibiting early opening through modulating ethylene and ABA. Water loss accelerates the aging of cut flowers as it reduces quality, so water absorption must exceed water loss (Chen et al., 2018). This

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research supports the use of melatonin in preserving the health and longevity of plants.

Lone et al. (2021) found that applying BAP at a concentration of 50 µM increased flower vase life by delaying flower aging by increasing water absorption, metabolic content, reducing ethylene hormone production, and preventing membrane lipid peroxidation. Cytokinins provide more photosynthetic products to developing flower buds, increasing water absorption and making the crown tissue more turgid (Singh & Tiwari, 2021). Based on research conducted by Zhou et al. (2023) showed that BAP application can activate aging-related transcription factors and increase gene expression of antioxidant enzymes. Cytokinin increases the activity of antioxidant enzymes, helping to protect cells from excess ROS by converting superoxide ions into water and oxygen (Ul Haq et al., 2022). BAP also increases the enzyme invertase, which delays plant aging (Hönig et al., 2018).

Flower Diameter Increment. The study analyzed the increase in flower diameter every two days; treatments C, D, E, F, and H had the largest change, with values ranging between 12.3 (H) – 16.2 (D), compared to treatment A (Control) with a value of 6.1 mm (Table 1). The flowers were harvested in half-bloom condition, and the flower crown would bloom one by one until perfect bloom. Treatments with flowers soaked in BAP and melatonin solution lasted longer than the control. This is due to BAP's role in cell division and elongation, which increases the growth and expansion of plant tissues, including flower crowns (Lezoul et al., 2022). The availability of sufficient carbohydrates supports the respiration process, allowing flowers to bloom completely (Amiarsi & Tejasarwana, 2016). BAP application can increase flower diameter due to the accumulation of sugars in flower tissue, which increases turgidity through endosmosis, resulting in larger flower size (Ul Haq et al., 2022). Flower decay occurs more rapidly when water loss exceeds water absorption, resulting in plasmolysis. Treatments with BAP can increase water absorption, ensuring the plant's condition remains turgid.

Morphological analysis of cut flowers showed that exogenous application of melatonin increased flower diameter and water absorption (Yang et al., 2025). The rate of change in flower diameter is an important indicator to assess the blooming process and aging rate in cut flowers. Based on research conducted by Wang et al. (2024) on the 'Qi Hua Lu Shuang' variety, melatonin treatments showed an increase in maximum flower diameter by 5.88% and 11.76% respectively, compared to the control group. Melatonin delays the change in water balance value from positive to negative by regulating the opening and closing of stomata so that a balance between transpiration and water absorption is achieved (Wu et al., 2025).

Flower Fresh Weight. Fresh weight measurements were taken on day zero and the last day of storage, and a decrease in fresh weight is characteristic of flower senescence. Treatments B, D, E, F, G, and H had the largest values, ranging between 13.3 (F, G) – 18.3 (D). The lowest average was in the control (6.7 g). The provision of BAP solution, both single and combined with melatonin, can significantly influence the fresh weight of flowers (Table 1).

Table 1. The effect of melatonin and 6-benzylaminopurine application on flower freshness, flower diameter increment, fresh weight, and solution uptake of cut rose cv. Avalanche

Code	Treatment	Vase life (DAT)	Flower Diameter Increment (mm)	Fresh Weight (g)	Solution Uptake (ml)
A	Control	5.3 a	6.1 a	6.7 a	48.3 a
B	Melatonin 0.1 mM	7.3 bcd	9.3 ab	15.0 bc	73.3 de
C	Melatonin 0.5 mM	6.7 b	13.1 bc	11.7 ab	68.3 cd
D	BAP 25 µM	8.3 de	16.2 c	18.3 c	85.0 f
E	BAP 50 µM	8.0 cde	14.4 bc	15.0 bc	73.3 de
F	Melatonin 0.1 mM + BAP 25 µM	7.0 bc	13.3 bc	13.3 bc	66.7 bcd
G	Melatonin 0.1 mM + BAP 50 µM	7.3 bcd	9.2 ab	13.3 bc	63.3 bc
H	Melatonin 0.5 mM + BAP 25 µM	8.7 e	12.3 abc	16.7 bc	80.0 ef
I	Melatonin 0.5 mM + BAP 50 µM	6.7 b	9.1 ab	11.7 ab	58.3 b

Note: Means followed by the same lowercase alphabet in the same column are not significantly different based on Duncan's multiple range test at 5 %.

Table 2. The effect of melatonin and 6-benzylaminopurine application on the cut flower angle petals of rose cv. Avalanche from day 0 to day 8.

Code	Treatment	Flower Angle Petals (°)				
		day-0	day-2	day-4	day-6	day-8
A	Control	36.7 abc	70.0 d	113.3 d	120.0 c	120.0 b
B	Melatonin 0.1 mM	43.3 cd	56.7 c	86.7 c	116.7 bc	120.0 b
C	Melatonin 0.5 mM	46.7 d	56.7 c	83.3 c	120.0 c	120.0 b
D	BAP 25 µM	30.0 a	33.3 a	56.7 ab	76.7 a	113.3 b
E	BAP 50 µM	30.0 a	36.7 a	53.3 a	76.7 a	113.3 b
F	Melatonin 0.1 mM + BAP 25 µM	33.3 ab	43.3 ab	73.3 bc	103.3 b	120.0 b
G	Melatonin 0.1 mM + BAP 50 µM	43.3 cd	53.3 bc	80.0 c	103.3 b	116.7 b
H	Melatonin 0.5 mM + BAP 25 µM	40.0 bcd	43.3 ab	56.7 ab	73.3 a	106.7 a
I	Melatonin 0.5 mM + BAP 50 µM	43.3 cd	56.7 c	83.3 c	120.0 c	120.0 b

Note: Means followed by the same lowercase alphabet in the same column are not significantly different based on Duncan's multiple range test at 5 %.

Ali et al. (2021) found that the application of BAP produced the highest fresh weight of flowers compared to the control (5.2 g). This is due to BAP is a cytokinin hormone that stimulates cell division and growth, thus increasing the fresh weight of flowers. When damage occurs to the cell membrane, BAP could maintain the stability of the cell membrane, thereby reducing the occurrence of lipid peroxidation (Cubría-Radío et al., 2017).

Research conducted by Hosseini et al. (2025) showed that the administration of 200 µM melatonin produced the highest fresh weight compared to the control. The study found that melatonin maintained water balance during vase storage, a crucial balance between organ dehydration and water absorption index for maintaining cell turgor in cut flowers. Membrane stability by melatonin plays a role in maintaining high tissue water content, enhancing antioxidant defense, and reducing lipid peroxidation, which is significantly affected by stress factors such as aging (Lezoul et al., 2022).

Solution Uptake. Observations were conducted at the beginning and end of the experiment. Results showed that treatments with different types and concentrations of solutions had a significant effect on absorption. Treatments D and H had the highest absorption of 85 ml (D), while treatment H had an absorption of 80 ml. Treatment A (Control) had the lowest absorption at 48.3 ml (Table 1). This aligns with research by Saptorini et al. (2015), which found that the application of BAP caused orchid flower cells to become hypotonic, allowing water to enter and expand. This is an optimal condition for plant cells, as reduced water absorption can accelerate wilting and decrease cell turgor (Nento et al., 2017). Lower transpiration in petals and other plant tissues can also influence solution absorption (Lezoul et al., 2022). Water absorption is crucial in preventing solution dehydration caused by transpiration.

Hosseini et al. (2025) reported that decreased solution uptake in control plants was due to membrane damage, ion leakage, and increased respiration that accelerated dehydration. Melatonin reduces water loss and extends postharvest life in ornamental plants (Wang et al., 2024). The mechanism of melatonin administration in absorbing solutions by reducing ion leakage, balancing hormones, inhibiting ROS production, increasing water absorption, and stimulating protein synthesis (Zhou et al., 2023).

Flower Angle Petals. The angle between petals and stem indicates deterioration in cut flowers, while flower senescence is characterized by wilted petals and an increased stem angle. The flower angle must reach over 120° to indicate the flower is no longer fresh (Mubarok et al. 2018).

Table 2 demonstrates that a combination of BAP and melatonin can maintain the flower angle, with treatment H having the smallest flower angle (106.7°) on the 8th day. Melatonin and 6-benzylaminopurine application can significantly maintain the decay of cut roses, characterized by a smaller droop angle. In line with research conducted by Hu et al. (2023) that the concentration of 0.1 mM melatonin can reduce damage characterized by wilting in

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carnation. Melatonin application also affects water balance, as a decrease in water absorption and loss can cause cut flowers to wilt (Wang et al., 2024; Yu et al., 2024). Melatonin plays a role in increasing aquaporin activity, optimizing water distribution in plants, and maintaining cell turgor. Overall, melatonin can help maintain the angle of cut roses.

Research by Saptorini et al. (2015) found that a 20 ppm dose of BAP can inhibit flower bud wilting in cut flowers of orchid Vanda cv. Douglas. BAP, a cytokinin hormone, regulates the plant life cycle, including flowering, increasing productivity, and reducing wilting. The cytokinin hormone contains nitrogen compounds that play a role in optimizing the synthesis of amino acids and proteins. Amino acids and proteins are utilized by plants for leaf growth (Ayuningtias et al., 2024). It also inhibits ethylene production by inhibiting the enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) synthase, which is crucial for aging and flower senescence. Combining BAP and melatonin can maintain and inhibit flower senescence.

Chlorophyll Content. Observations of leaf chlorophyll content were conducted on the fourth day while the flowers were still fresh. According to Table 3, treatment D had the highest total chlorophyll content at 31.3 mg/g, which was not significantly different from treatment E at 30.4 mg/g, whereas treatment A (control) had the lowest content at only 10.7 mg/g (Table 3). This indicates that treatment D had a significant effect on chlorophyll content and was significantly different from the control solution. The high chlorophyll content in treatments D and E is due to the important role of BAP compounds in inhibiting chlorophyll degradation (Man et al., 2016). Based on research conducted by Rasouli et al. (2015) cut flowers treated with 25 and 75 μM cytokinin showed significant differences in chlorophyll content compared to the control. This occurs because chlorophyll plays a crucial role in photosynthesis; the higher the chlorophyll content, the greater the photosynthetic output, which is then utilized by the plant for growth. Photosynthesis in plants depends on light-dependent reactions, chlorophyll and other pigments capture light energy to drive redox reactions in the electron transport chain in the thylakoid membrane of chloroplasts (Guo et al., 2023). In a study conducted by Yan et al. (2021), it was shown that higher chlorophyll content increased photosynthesis efficiency and increased carbohydrate production. Chlorophyll changes are closely related to photosynthesis; exogenous application or genetic overproduction of cytokinins can significantly delay senescence (Hönig et al., 2018).

Table 3. The effect of melatonin and 6-benzylaminopurine application on the chlorophyll content of cut rose | Cv. Avalanche

Code	Treatment	Chlorophyll Content (mg/g)
A	Control	10.7 a
B	Melatonin 0.1 mM	15.1 ab
C	Melatonin 0.5 mM	17.6 bc
D	BAP 25 μM	31.3 d
E	BAP 50 μM	30.4 d
F	Melatonin 0.1 mM + BAP 25 μM	13.5 ab
G	Melatonin 0.1 mM + BAP 50 μM	11.8 a
H	Melatonin 0.5 mM + BAP 25 μM	21.1 c
I	Melatonin 0.5 mM + BAP 50 μM	11.0 a

Note: Means followed by the same lowercase alphabet in the same columnares not significantly different based on Duncan's multiple range test at 5 %.

Conclusion

The application of melatonin and 6-benzylaminopurine solution, applied individually or in combination, significantly influenced the freshness of cut roses compared to the control. The 25 μM BAP treatments showed the best results in maintaining vase life, maintaining fresh weight, increasing flower diameter growth, total chlorophyll content, and enhancing solution uptake.

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Tini EW · Kurniawan RR

Optimization of rabbit urine liquid organic fertilizer and reduced NPK fertilizer doses for improving growth and physiology of shallot (*Allium ascalonicum* L.)

Abstract. Rabbit urine liquid organic fertilizer (LOF) can be used as an alternative fertilizer to reduce the use of inorganic fertilizers, especially for shallots, as a national food commodity. This study aims to determine the best concentration of rabbit urine LOF, the best dose of NPK fertilizer dose reduction, and a combination of rabbit urine LOF and NPK fertilizer dose reduction on the physiological characters of shallot plants. The observed variables consisted of net assimilation rate, crop growth rate, stomatal density, stomatal aperture, and chlorophyll content. The experimental results showed that a concentration of 200 mL L^{-1} of rabbit urine LOF had the best effect on several physiological characteristics studied. In addition, a 50% reduction in the NPK dose had the best effect on physiological characteristics compared to other treatments. Rabbit urine LOF 200 mL L^{-1} and a 50% reduction in NPK doses had a significant interaction effect on the physiological characters of shallot plants.

Keywords: Organic fertilizer · Rhizomes · Shallot · Sustainable

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Introduction

Horticultural commodities are an important source of providing nutritional needs and have a high potential to increase a country's income through export activities. One of the potential horticultural commodities is shallots (*Allium ascalonicum* L.). Shallot production in Indonesia peaked in July 2023 at 223.17 thousand tons from a harvested area of 18.31 thousand hectares. East Java, Central Java, and West Sumatra were the top-producing provinces. East Java contributed 24.41% to national production (484.67 thousand tons; 51.02 thousand hectares harvested), followed closely by Central Java at 24.13% (479.09 thousand tons; 46.80 thousand hectares) (Badan Pusat Statistik, 2024).

Brebes Regency is the largest shallot production center in Indonesia, providing 75% of the shallot needs in all parts of Central Java Province and supplying 23% of the national needs in Indonesia (Badan Pusat Statistik, 2015). The Bima Brebes shallot variety is an adaptive, superior variety with high production yields compared to other varieties. The results of research by Kartinaty et al. (2019), that of the five superior varieties of shallots tested showed that Bima Brebes and Katumi varieties produced the highest production of 9.37 tons ha^{-1} and 9.09 tons ha^{-1}; besides that the Bima Brebes variety had a high value compared to other varieties in each research variable in bulb diameter 9.55 mm; weight 25 g; yield per plot 68.5 kg followed by Katumi varieties with bulb diameter 9.47 mm; weight 22.5 g; yield per plot 54.5 kg.

The Bima Brebes shallot variety is adaptive to acidic soil. The Bima Brebes shallot variety has a high percentage of survival for planting on acidic soil, compared to other varieties (Ayu, 2022). The Bima shallot variety is adaptive to bulb rot disease, making it a preference for the interests of farmers and markets in Indonesia. Consumer interest in shallots with a strong aroma makes the Bima Brebes variety of shallots one of the preferred choices to be cultivated by farmers in Indonesia. The shallot cultivation carried out by farmers in Indonesia, especially in Brebes, Central Java, uses inorganic fertilizers at a high dose. Shallot farmers in Brebes tend to apply phosphorus (P) and potassium (K) fertilizers in high doses exceeding the recommended doses, although the application does not always increase crop yields (Trisnaningsih et al., 2023). NPK fertilizer supplies the primary macronutrients: nitrogen (N), phosphorus (P_2O_5), and potassium (K_2O) in varying compositional ratios (Sinha & Tandon, 2020).

In fact, long-term excessive chemical fertilizer application induces soil degradation, loss of beneficial soil microorganisms, and associated detrimental impacts (Vitousek et al., 1997). Efforts to reduce the accumulation of chemical residues in inorganic fertilizers are carried out by switching to the use of rabbit urine liquid organic fertilizer integrated with various other organic materials that form a solution medium, making it an innovation that has the potential to overcome the problems of cost and chemical residues.

The use of rabbit urine as an alternative organic fertilizer shows promise, particularly when fermented with additional natural ingredients that work synergistically to enhance nutrient content. The high nitrogen content in rabbit urine supports its use as a very prospective fertilizer. According to Rosniawaty et al. (2018), when compared with other manure, fertilizer from rabbit urine contained three times more nitrogen and urea than other livestock. Nitrogen demand in plants plays a role in plant development in important processes such as growth, leaf expansion, and biomass generation. These processes play a crucial role in the formation of physiological characteristics in plants. When nutrient requirements are met, essential primary macronutrients (N, P, and K) drive plant growth and physiological improvements (Ye et al., 2019; Wang et al., 2021). The few studies that have currently been conducted focus more on the use of rabbit urine as a single element in fertilization. Limited studies have added rhizomes and pineapple peels to the fermentation process of making rabbit urine LOF.

Materials and Methods

Plant material. This research was conducted on experimental land at coordinates 7º21'23 "S 109º15'35" E (Google Earth) at an altitude of 220 meters above sea level in Banjarsari Wetan Village, Sumbang District, Banyumas Regency, Central Java Province, from March to August 2023. The general condition of the average temperature in the morning, afternoon, and evening at the research site ranged from 23.3 – 32.2 °C, 28.9 – 35.3 °C, and 25.4 – 30.9 °C, respectively. Humidity in the morning, afternoon, and evening at the research site ranged from 55 – 99%, 49 – 89%, and 60 – 93%, respectively. Sunlight intensity on experimental land in the morning, afternoon, and evening at the research site ranged from 5900 – 73700 lux, 9920 – 83300 lux, and 11280 – 32700 lux, respectively. Daily rainfall ranged from 0 – 6.12 mm day^{-1}. Seeds of Bima Brebes shallot were collected from Brebes, Central Java. LOF is produced by fermenting for three months a mixture of rabbit urine, water, sambiloto (*Andrographis paniculata*) leaves, turmeric, galangal, temu ireng (*Curcuma aeruginosa* Roxb), brown sugar, pineapple peel waste, and Effective Microorganism 4 (EM4). The inorganic fertilizer used in this study is NPK (16-16-16).

Experimental design and treatments. This research was arranged in a complete randomized group design consisting of two factors. The first factor of rabbit urine concentration (K) consisted of 4 levels, namely 0 mL L^{-1}, 100 mL L^{-1}, 150 mL L^{-1}, and 200 mL L^{-1}. The second factor is NPK inorganic fertilizer (16-16-16) dose (P) consisting of 3 levels, namely 0% (200 kg ha^{-1}), 25% (150 kg ha^{-1}), 50% (100 kg ha^{-1}). The two factors were combined to create 12 treatment combinations, each replicated three times, for a total of 36 experimental units. Each unit contained 45 plants, resulting in 1,620 plants in total. One experimental unit measured 2 x 1.2 m with a spacing of 20 x 20 cm. The observation variables included net assimilation rate (NAR) and crop growth rate (CGR) at 24, 38, and 52 days after planting (DAP). In addition, stomatal density, stomatal aperture, and chlorophyll content were also measured. Observation data were analyzed statistically by analysis of variance (ANOVA) at the 5% level. If the results show a significant effect, continue with Duncan's Multiple Range Test (DMRT) at the 5% level.

Net assimilation rate. Net assimilation rate (NAR) measures the average photosynthetic efficiency of leaves in a plant community. It is calculated using the formula by Gardner et al. (1991):

$$\text{NAR} = \frac{(w2-w1)}{(T2-T1)} \times \frac{(lnA2-lnA1)}{(A2-A1)}$$

Notes:
w_1: Plant dry weight at the first observation
w_2: Plant dry weight at the second observation
T_1: Plant age at the first observation
T_2: Plant age at the second observation
A_1: Leaf area at the first observation
A_2: Leaf area at the second observation

Crop growth rate. Crop growth rate (CGR) quantifies the increase in plant biomass over time and can be calculated using the formula by Rahman et al. (2021):

$$\text{CGR} = \frac{W2-W1}{A(T2-T1)}$$

Notes:
W_1: Total plant dry weight at time T_1
W_2: Total plant dry weight at time T_2
A: Land area
T_1: Initial observation time
T_2: Subsequent observation time.

Stomatal density. Stomatal density was measured using a microscope after determining the field of view area. With a magnification of 40 x 10, the field of view area was 0.1589 mm^2, calculated using the formula:

Density = number of stomata/field of view area (Izza, 2015).

Stomatal aperture. Stomatal aperture was measured at random points using a micrometer, and the average aperture was calculated from all measured points.

Chlorophyll content. A total of 0.1 g of shallot leaves was finely ground and extracted with 10 mL of 80% acetone. The extract was filtered using filter paper until clear, then transferred into a test tube and sealed with cotton. A 3 mL aliquot of the extract was placed in a cuvette for measurement using a UV-Vis spectrophotometer at wavelengths of 645 nm and 663 nm. Chlorophyll content was calculated using the equations from Arnon (1949):

$$\text{Chlorophyll a} = \{(12.7 \times A663) - (2.69 \times A645)\} \times 10^{-1}$$
$$\text{Chlorophyll b} = \{(22.9 \times A645) - (4.86 \times A663)\} \times 10^{-1}$$
$$\text{Total Chlorophyll} = \{(20.2 \times A645) - (8.02 \times A663)\} \times 10^{-1}$$

Notes:
A663: Absorbance at 663 nm
A645: Absorbance at 645 nm

Results and Discussion

Table 1 shows that the effect of rabbit urine LOF significantly affects the NAR at 38 DAP with the highest value of 0.0020 g cm^{-2} day^{-1} at a

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concentration of 200 mL L^{-1} greater 53.85% than the concentration of 0 mL L^{-1} and CGR at 38 DAP with the highest value at 200 mL L^{-1} (38.14 g cm^{-2} day^{-1}) and 150 mL L^{-1} (34.17 g cm^{-2} day^{-1}). Reduction of NPK fertilizer doses had a significant effect on NAR at 24 and 52 DAP with the highest values of 0.0009 g cm^{-2} day^{-1} and 0.0007 g cm^{-2} day^{-1} at 50% dose reduction, respectively, 28.57% and 250% greater than 0% dose reduction, while at CGR at 24 DAP, the highest value was 7.08 g cm^{-2} day^{-1} at 50% reduction, 39.92% greater than 0% dose reduction.

The combination of rabbit urine LOF and reduced doses of NPK fertilizer significantly increased NAR at 24, 38, and 52 DAP. The highest NAR values—0.0012, 0.0022, and 0.0009 g cm^{-2} day^{-1}—were achieved with LOF applications of 150, 200, and 150 mL L^{-1}, combined with NPK reductions of 50%, 0%, and 25%, respectively. These values were 100%, 120%, and 12.5% higher than the control (0 mL L^{-1} LOF and 0% NPK reduction). Rabbit urine LOF combined with reduced NPK doses significantly increased CGR at 24, 38, and 52 DAP. The highest CGR values—10.26, 47.14, and 34.57 g cm^{-2} day^{-1}—were achieved with LOF applications of 100, 200, and 150 mL L^{-1}, respectively, each combined with a 25% NPK reduction. These values were 161.07%, 152.36%, and 54.19% higher than the control (0 mL L^{-1} LOF and 0% NPK reduction).

Table 1. The effect of rabbit urine LOF and reduced dose of NPK fertilizer on NAR and CGR

Treatments	Net Assimilation Rate (NAR) (g cm^{-2} day^{-1})			Crop growth rate (CGR) (g cm^{-2} day^{-1})		
	24 DAP	38 DAP	52 DAP	24 DAP	38 DAP	52 DAP
Rabbit urine LOF concentration (mL L^{-1})						
0	0.0007a	0.0013b	0.0004a	5.48a	24.27b	19.57a
100	0.0009a	0.0013b	0.0002a	7.24a	24.70b	15.34a
150	0.0008a	0.0016b	0.0005a	7.13a	34.17a	20.34a
200	0.0008a	0.0020a	0.0006a	5.14a	38.14a	23.62a
Reduction of NPK fertilizer dosage (%)						
0%	0.0007b	0.0016a	0.0004ab	5.06b	27.75a	19.66a
25%	0.0007b	0.0018a	0.0002b	6.59ab	35.04a	16.09a
50%	0.0009a	0.0014a	0.0007a	7.08a	28.19a	23.41a
Combination of Rabbit urine LOF and Reduction of NPK fertilizer dosage						
0 + 0%	0.0006d	0.0010d	0.0008ab	3.93d	18.68f	22.42ab
0 + 25%	0.0007cd	0.0014bcd	-0.0002ef	6.22bcd	26.27de	8.62bc
0 + 50%	0.0007cd	0.0015bc	0.0007ab	6.29bcd	27.87d	27.65ab
100 + 0%	0.0007cd	0.0012cd	0.0001de	4.32cd	20.44ef	20.75ab
100 + 25%	0.0010ab	0.0015bc	-0.0004f	10.26a	36.62bc	-0.23c
100 + 50%	0.0008bcd	0.0012cd	0.0008ab	7.13bc	17.05f	25.49ab
150 + 0%	0.0008cd	0.0016b	0.0002cd	8.08ab	41.59ab	13.30abc
150 + 25%	0.0003e	0.0016b	0.0009a	5.03cd	30.11cd	34.57a
150 + 50%	0.0012a	0.0015bc	0.0004bcd	8.27ab	30.82cd	13.13abc
200 + 0%	0.0007cd	0.0022a	0.0004bcd	3.91d	30.29cd	22.14ab
200 + 25%	0.0007cd	0.0024a	0.0005abc	4.87cd	47.14a	21.38ab
200 + 50%	0.0009bc	0.0014bcd	0.0007ab	6.64bcd	37.00bc	27.34ab
CV. (%)	16.90	12.39	48.99	24.55	13.79	57.49

Note: The mean values followed by the same letter in the same column are not significantly different according to Duncan's Multiple Range Test at the 5% significance level. CV – coefficient of variance.

Table 2. The effect of rabbit urine LOF and inorganic fertilizer dose reduction on stomatal density (SD), stomatal aperture (SA), chlorophyll a content (CCa), chlorophyll b content (CCb), total chlorophyll content (CCt)

Treatments	Observed variables				
	SD (unit mm^{-2})	SA (µm)	CCa (mg g^{-1})	CCb (mg g^{-1})	CCt (mg g^{-1})
Rabbit urine LOF concentration (mL L^{-1})					
0	83.68a	7.01a	0.59b	0.22a	0.82b
100	80.16a	7.43a	0.67ab	0.24a	0.91ab
150	66.80b	8.89a	0.62ab	0.22a	0.85ab
200	65.40b	8.61a	0.69a	0.22a	0.92a
Reduction of NPK fertilizer dosage (%)					
0%	83.86a	8.07ab	0.55b	0.23a	0.79b
25%	66.45b	8.80a	0.76a	0.24a	1.01a
50%	71.73ab	7.08b	0.61b	0.21a	0.83b
Combination of Rabbit urine LOF and Reduction of NPK fertilizer dosage					
0 + 0%	101.27a	6.67a	0.59cd	0.23a	0.84a
0 + 25%	71.73a	7.71a	0.75abc	0.24a	1.01a
0 + 50%	78.06a	6.67a	0.42e	0.20a	0.63a
100 + 0%	88.61a	7.71a	0.52de	0.24a	0.78a
100 + 25%	78.06a	7.50a	0.80a	0.25a	1.06a
100 + 50%	73.84a	7.08a	0.68abc	0.22a	0.91a
150 + 0%	65.40a	8.75a	0.50de	0.23a	0.74a
150 + 25%	61.18a	10.42a	0.74abc	0.23a	0.97a
150 + 50%	73.84a	7.50a	0.63bcd	0.20a	0.84a
200 + 0%	80.17a	9.17a	0.60bcd	0.19a	0.81a
200 + 25%	54.85a	9.58a	0.75abc	0.24a	1.01a
200 + 50%	61.18a	7.08a	0.72abc	0.23a	0.96a
CV. (%)	17.06	20.04	12.96	25.85	13.24

Note: The mean values followed by the same letter in the same column are not significantly different according to Duncan's Multiple Range Test at the 5% significance level. CV – coefficient of variance.

Table 2. showed that the effect of rabbit urine LOF application had a significant effect on stomatal density with the highest value of 83.68 and 80.16 units mm^{-2} at a concentration of 0 and 100 mL L^{-1}; chlorophyll a content and total chlorophyll content with the highest values of 0.69 and 0.92 mg g^{-1}, respectively, at a concentration of 200 mL L^{-1}, 16.95% and 12.20% greater than the concentration of 0 mL L^{-1}. Reduction of NPK fertilizer dose significantly affected stomatal density with the highest value observed at 0% reduction (83.86 units mm^{-2}), which was statistically not different from 50% reduction (71.73 units mm^{-2}), but significantly higher than 25% NPK reduction (66.45 units mm^{-2}); the highest stomatal aperture was observed at a 25% NPK reduction (8.80 units mm^{-2}), which was statistically no different from a 0% NPK reduction (8.07 units mm^{-2}), but significantly higher than a 50% NPK reduction (7.08 units mm^{-2}), chlorophyll a content, and total chlorophyll content reached the highest values of 0.76 and 1.01 mg g^{-1}, which were 38.18% and 27.85% higher, respectively, than the 0% NPK reduction treatment. The combination of rabbit urine LOF and reduced NPK fertilizer dose significantly affected chlorophyll *a* content, with the highest value of 0.80 mg g^{-1} obtained from 100 mL L^{-1} LOF and a 25% reduced NPK dose—35.59% higher than the control (0 mL L^{-1} LOF and 0% NPK reduction).

NAR and CGR at 38 DAP were significant for the application of LOF rabbit urine as organic fertilizer. The use of organic fertilizer represents an effective technique for improving soil physical attributes through increased soil organic matter content, thereby significantly impacting soil quality improvement (Lasmini et al., 2022). Rabbit urine contains high nutrient values, including 2.72% N, 1.1% P, and 0.5% K. (Setyanto et al., 2014). High nitrogen levels in rabbit urine support shallot growth during the growth phase. The controlling factors of NAR and CGR are centered on photosynthetic performance. The availability of nutrients from rabbit urine LOF, particularly nitrogen as a component of chlorophyll, enhances carbon assimilation, promotes cell division, and drives biomass expansion, consistent with the source-sink model where photosynthates determine growth. Rabbit urine provides high levels of N, primarily as NH_4^+ and urea. Nitrogen is an essential structural component of chlorophyll for photosynthesis, the process of fixing CO_2 into organic compounds for plant growth. Plant roots absorb N in the form of NH_4^+, NO_3^-, amino acids, and urea, with NO_3^- needing to be reduced to NH_4^+ before assimilation (Marschner, 2012).

According to Irianto et al. (2017), the initial reduction in NAR during early growth stages was attributed to underdeveloped young leaves exhibiting suboptimal photosynthetic capacity. Both NAR and CGR subsequently increased until 35 – 42 DAP, driven by progressive leaf expansion and bulb development. This growth phase was characterized by a rising bulb-to-leaf dry weight ratio, reflecting resource allocation shifts toward reproductive structures. Mojaddam and Noori (2015) stated that CGR exhibited a gradual increase during initial vegetative development, followed by a significant acceleration in subsequent growth stages. The initial phase's attenuated CGR was attributed to high meristematic activity and incomplete leaf expansion, limiting photosynthetic capacity. A pronounced CGR surge occurred post-attainment of maximum leaf area index, driven by optimized solar radiation utilization efficiency.

The application of rabbit urine LOF affected the NAR at 38 DAP, but not at 52 DAP, due to the reduced effectiveness of plant light absorption due to higher leaf density and density at 52 DAP than at 38 DAP. NAR peaks under direct leaf sunlight exposure but declines during subsequent growth periods due to leaf area index expansion-induced mutual shading. Enhanced nitrogen availability promotes leaf area expansion and biomass accumulation, but mutual shading by older leaves with diminished photosynthetic capacity reduces NAR during plant aging (Islam et al., 2019). NAR in Table 1 at 24 and 52 DAP was significantly different at reduced NPK doses because the sunlight shaded by the leaves on the same plant was not as shaded during the vegetative peak towards the bulb formation phase. The leaves at 24 DAP were not yet shady, while at 52 DAP, they began to dry out because the plants focused on tuber formation. This is in accordance with the statement of Fauziah et al. (2016), that at 35 DAP to 56 DAP, shallot plants enter the phase of bulb formation and development, and the growth of shallot height and leaves begins to senescence because the energy of photosynthesis is used to form and fill the bulbs.

CGR at 38 DAP is significantly different, but at other ages it is not significantly different because at this age the plants are in the phase after the vegetative peak, which has the highest plant growth activity compared to other phases that have focused on tuber filling and plant yield. CGR at 52 DAP is also not significantly different from the application of rabbit urine LOF because at that age, the plants are already in the bulb enlargement phase. CGR at 24 DAP has a real effect, because the plants are in the vegetative phase, so they are more focused on the growth process by performing photosynthesis. Optimal plant growth depends critically on efficient photosynthetic light-to-chemical-energy conversion and precise photo assimilate management (Sonnewald & Fernie, 2018).

The main factor in reduced yields is due to leaves covering each other at certain phases. Sunlight is an important factor in the photosynthesis process and determines CGR, so that the intensity, duration of irradiation, and quality affect the photosynthesis process. Solar movement generates diurnal light redistribution within canopies, altering spectral composition and creating three radiation components: direct (85% under full sun), diffuse, and scattered/transmitted light (Durand et al., 2021). Geographic latitude critically influences plant performance by modulating solar position and day length, which interact with canopy architecture. Specifically, leaf inclination angle and leaf area determine radiation flux efficiency; erect leaves enhance light capture at low solar angles (e.g., high latitudes/dawn/dusk) but reduce interception at zenith positions (Ezcurra et al., 1991; Falster & Westoby, 2003; Murchie & Burgess, 2022).

Research conducted by Safrina et al. (2023) stated that the provision of biourin had a very significant effect on the relative growth rate and net assimilation rate of corn plants at the age of 4-6 weeks after planting, with the highest value of 1.819 at a concentration of 250 mL L^{-1}. Nitrogen application can increase the number of stomata, which affects the density of stomata, to increase the rate of transpiration and absorption of CO_2 for photosynthesis. Stomatal density shows the number of stomata per unit area of the observation field, where if the number of stomata on the leaves is large, the level of CO_2 absorption will be greater, to increase the rate of photosynthesis (Regazzoni et al., 2014). The results of research conducted by Nisa & Rahayu (2022), showed that the provision of LOF-Si on soybean plants was significantly different and resulted in a higher number of stomata in LOF Si 20 mL LOF mixed with 1 L of water with the addition of Si 1 g L^{-1} the value of stomatal density reached 111.77 while the lowest number of stomata was found in plants treated with control LOF 20 mL LOF mixed with 1 L of water with a value of 89.50.

Shallot's stomatal density

0 mL L^{-1} | 150 mL L^{-1} | 100 mL L^{-1} | 200 mL L^{-1}

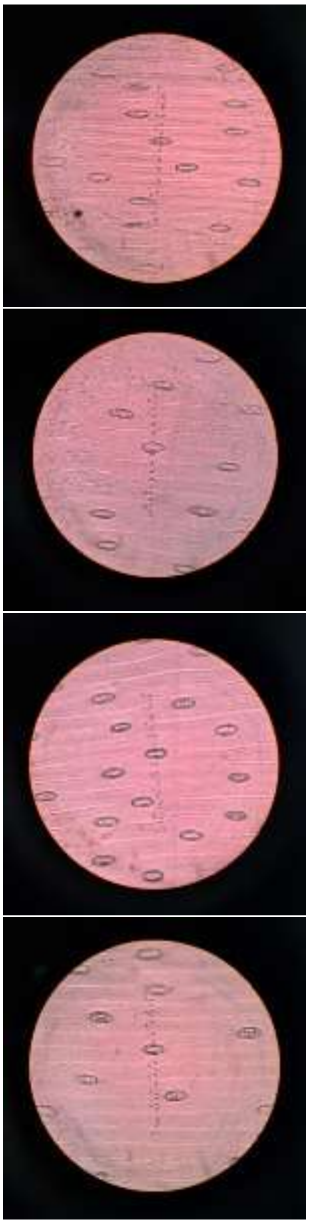

Figure 1. Shallot's stomatal density in the treatment of rabbit urine liquid organic fertilizer

Stomatal mechanism occurs due to changes in the volume of the guard cell controlled by ion exchange and cytoskeleton modifications (Golec & Szarejko, 2013). Regulation of stomatal opening is also influenced by physiological and environmental factors, including CO_2 concentration, the hormone abscisic acid (ABA), humidity levels, drought, pathogen attack, and ozone exposure (Shemer, 2015). Research by Zhou et al. (2010), showed that plants respond quickly to changes in temperature through physiological adjustments, one of which is by regulating the opening and closure of stomata. K^+ controls stomatal aperture to facilitate gas exchange and water flux (Andrés et al., 2014; Tränker et al.,2018), while adequate chloroplast K^+ concentrations maintain stroma lamella structure, supporting chloroplast integrity and photosynthetic efficiency (Jia et al., 2008; Sustr et al., 2019). Reducing the dose of NPK fertilizer has a significant effect on stomatal openings. Research by Rahayu et al. (2023), that the stomatal openings of barley plants are influenced by the dose of fertilizer; 100% fertilization of N, P, and K produces lower stomatal openings, while 50% doses show optimal openings. At 4-5 cm of waterlogging, 25% doses produce the best stomatal openings. Stomatal openings are influenced by leaf anatomy, whose growth is influenced by nutrients, where the application of NPK fertilizers provides nutrient supplies for plants; besides, potassium nutrients are known to affect stomatal openings. Stomatal opening is influenced by various factors, including potassium (K^+) and chloride (Cl^-) ions, humidity, temperature, sunlight intensity, pH, and CO_2 levels.

The application of rabbit urine LOF has a low effect, but it is suspected that other factors, affect chlorophyll levels in plants in the form of NPK fertilizer as a nitrogen source. Providing additional nitrogen to the leaves can produce wider leaves and a higher chlorophyll content. Leaf chlorophyll content increased with nitrogen application and irrigation compared to unfertilized and rainfed conditions (Wang et al., 2021). Reducing the dose of NPK fertilizer has no significant effect on chlorophyll b. This is in accordance with the research of Same (2019), that the provision of NPK fertilizer has no significant effect on the chlorophyll content of pepper seedlings, the highest average result is 42.62 at 3 g $polybag^{-1}$, and the lowest average is 37.31 at 0 g $polybag^{-1}$.

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Shallot's stomatal aperture

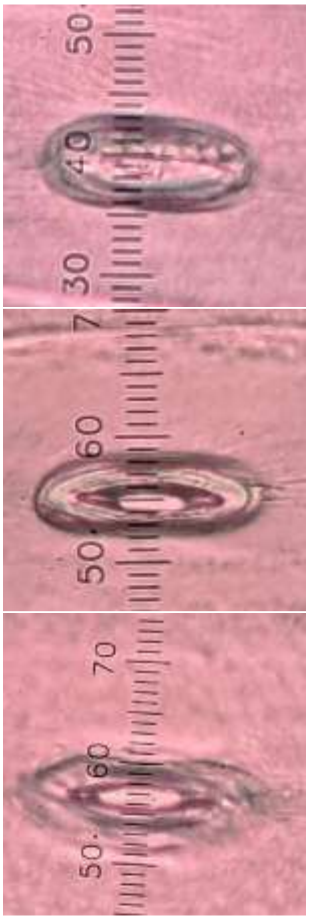

Figure 2. Shallot's stomatal aperture in the treatment of the reduction of NPK fertilizer dosage

Plant chlorophyll levels are influenced by the availability of nutrients in the soil, where NPK fertilizers are thought to be able to provide nutrient requirements for chlorophyll levels, but the provision of nutrients is needed in optimal amounts that are not excessive to saturation. Factors that limit soil fertility include N, P, K, and Mg (Marchesi, 2020). This shows that the provision of a certain amount of plant nutrients increases growth to an optimal limit, which then cannot increase again and even tends to decrease. Liebig's minimum law states "that the yield of a crop is limited by the nutrient that is present in the least quantity in that environment" (Liebig 1943; Tang & Riley 2021; Baio et al., 2024). The combination of rabbit urine LOF and reduced NPK fertilizer dose interacted with the chlorophyll a level. Rabbit urine LOF increases enzyme activity that plays a role in chlorophyll synthesis, while the reduction of NPK avoids excess nutrients that inhibit photosynthesis. N fertilization under low irrigation water elevated chlorophyll content and antioxidant enzyme activity, which preserved photosynthetic integrity and enzymatic function, optimizing the production of assimilates as organic compounds, increased plant yield (Muhammad et al., 2022).

Nutrient deficiencies critically impair plant physiology: Nitrogen (N) limitation induces anthocyanin accumulation (manifested as purple leaves) and reduces chlorophyll/carotenoid content by 34-36% in rice (Huang et al., 2004), while suppressing energy metabolism enzymes in photosynthesis and respiration (Lin et al., 2011). Phosphorus (P) deficiency decreases $NADP^+$ substrate for NADPH synthesis, diminishing ATP synthase activity and limiting proton export to the chloroplast stroma, thereby acidifying the thylakoid lumen (Goltsev et al., 2016; Karlsson et al., 2015). Concurrently, potassium (K) deficiency triggers necrotic lesions via chlorophyll degradation, disrupting carbon translocation and plant-water homeostasis (Abbas et al., 2021). Reducing the dose of NPK fertilizer can reduce the risk of stress due to chemical nutrient overload, while rabbit urine LOF helps plants to still get enough nutrients, so that the chlorophyll a level remains optimal.

Rabbit urine contains high levels of salt and ammonia, which trigger the accumulation of Na^+ and NH_4^+ ions in the root zone. The increase in pH and EC values after the addition of composted rabbit manure is due to its high salt content (Greco, 2021), which has the potential to cause salinity stress in plants (Ceccarini et al., 2019). Similar findings were reported in rabbit and goat manure compost with a pH of 9.41 and EC of 11.33 dS m^{-1} (Paredes et al., 2015; Li et al., 2022). In salt-sensitive shallots, this reduces soil water potential, causing osmotic stress. Plants under osmotic stress respond by increasing abscisic acid (ABA) synthesis. Stomatal responses to hydraulic/non-hydraulic signals confirm ABA's key role in plant signaling adaptation to environmental dynamics (Cai et al., 2017). In contrast, in the control without rabbit urine, no osmotic pressure occurred, so stomata remained optimally open.

The conversion of urea in rabbit urine produces excess ammonia (NH_3) in the soil. This compound inhibits the activity of the enzyme glutamine synthetase in guard cells, disrupting glutamate synthesis. As a result, there is an accumulation of reactive oxygen species (ROS) that damage the guard cell membrane, reducing the ability of the stomata to open. Guard cell plasma membranes contain anion channels activated by key stimuli, including ABA, NADPH metabolism, and voltage-gated K^+ channel-mediated anion fluxes (Murata et al., 2015; Roelfsema et al., 2012). ROS functions as an essential regulator of stomatal closure (Song et al., 2014). During stomatal movement regulation, ROS generated initially in the guard cell apoplast subsequently activate anion channels through sensing and signaling pathways (Singh et al., 2017). This toxic mechanism does not occur in controls (concentration 0 mL L^{-1}) at stomatal aperture parameter, so stomatal function remains normal.

Conclusion

Application of rabbit urine at a concentration of 200 mL L^{-1} increased the net assimilation rate, crop growth rate at 38 DAP, chlorophyll a content, and total chlorophyll content. A 0% reduction in NPK fertilizer dosage increased stomatal density. A 50% reduction in NPK fertilizer dosage increased net assimilation rate and growth rate at 24 DAP, and net assimilation rate at 52 DAP. The combination of rabbit urine LOF and reduced dose of NPK fertilizer on shallot plants was 100 mL L^{-1} and 25% highest in crop growth rate 24 DAP and chlorophyll a content; 150 mL L^{-1} and 25% highest in net assimilation rate 52 DAP and crop growth rate 52 DAP; 150 mL L^{-1} and 50% highest in net assimilation rate 24 DAP; 200 mL L^{-1} and 0% highest in net assimilation rate 38 DAP; 200 mL L^{-1} and 25% highest in net assimilation rate 38 DAP and crop growth rate 38 DAP.

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Effect of eco-enzyme application on soil nutrient and plant productivity of green mustard-peanut in inceptisol

Abstract. Appropriate land management has a long-term impact on soil performance and is believed to improve soil fertility. This study investigated the effect of eco-enzymes on soil nutrients and plant productivity of green mustard-peanut in inceptisol. The research was conducted from February to April 2025. The experiment used a randomized block design (RBD) consisting of 5 treatments and replicated 3 times, so that there were 15 units, including N0 = without Eco-enzyme, N1 = Eco-enzyme 2cc/L. N2= Eco-enzyme 4cc/L, N3= Eco-enzyme 6cc/L, and N4= Eco-enzyme 8cc/L. The commodities used were green mustard (*Brassica juncea* L) and peanut (*Arachis hypogaea* Linn). Soil properties variables included soil pH, N-total, and P-available, while plant productivity variables included plant height, number of leaves, leaf area, fresh weight, and pod production. The results showed that the concentration of eco-enzymes had a significant effect on N-total, P-available, green mustard plant height, and peanut plant height. However, eco-enzyme concentration had no significant effect on soil pH, number of green mustard leaves, green mustard fresh weight, green mustard leaf area, number of peanut branches, and peanut pod production. The lowest production of green mustard was 0.85 tons/ha, and the highest was 3.29 tons/ha. While the lowest production of Peanut pods was 4.3 tons/ha and the highest was 6.67 tons/ha.

Keywords: Eco-enzyme · Inceptisol · Nitrogen and phosphorus nutrients · Productivity

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Introduction

The primary objective of sustainable agriculture is to enhance crop productivity while preserving ecosystem balance. A significant challenge in implementing a sustainable agricultural system is the limited soil fertility, particularly regarding the availability of essential nutrients such as nitrogen (N) and phosphorus (P), which are crucial for plant growth. Improper land management methods, especially the overapplication of synthetic fertilizers, may result in soil degradation (Aji et al., 2020), therefore diminishing soil fertility (Hartati et al., 2023). Notwithstanding the extensive endeavor for agricultural intensification, its effects have been minimal in various locations, leading to detrimental outcomes including soil compaction, reduced organic carbon levels, increased evapotranspiration, and heightened erosion rates (Lal, 2004; Rofita et al., 2022). Inceptisol soil, prevalent in Indonesia, demonstrates fluctuating fertility levels. Its presence in Indonesia is predicted to be 52 million hectares (Kasno, 2013), indicating a potential for agricultural development (Swardana et al., 2023). However, the level of development of this immature soil is indicated by problems with its fertility level (Hartati et al., 2020). Consequently, the utilization of organic fertilization technology emerges as a pivotal strategy to foster environmentally sound and sustainable agricultural systems, capable of enhancing soil fertility through natural means (Soobhany, 2019; Xiang et al., 2022; Zhang et al., 2023).

The use of eco-enzymes is one of the alternative approaches that is considered more environmentally friendly in organic-based land management. Eco-enzyme is derived from the fermentation of organic waste materials, comprising a range of bioactive components (Bashir et al., 2023). Eco-enzyme provides clear advantages for plantations by improving plant growth, soil health, pest management, and environmental sustainability. Its use supports both higher productivity and eco-friendly agricultural practices. In rice, eco-enzyme application increased plant height up to 25% in some cultivars, accelerated harvest time by 2 –3 weeks, and improved tiller number and grain yield compared to commercial liquid fertilizers (Defiani & Astarini, 2023). In soil, eco-enzyme enriches soil with essential nutrients (N, P, K, organic carbon) and beneficial enzymes such as amylase, protease, lipase, promoting nutrient cycling and root uptake (Kriswantoro et al., 2022). Its use in composting accelerates organic matter decomposition, resulting in high-quality compost with neutral pH and improved plant growth (Narang et al., 2023). They provide a sustainable way to recycle organic waste, reducing landfill burden and transforming waste into valuable products for cleaning, agriculture, and wastewater treatment (Hasanah, 2021). These components include enzymes, natural growth hormones, organic acids, and microorganisms. The combined action of these components has been proven to accelerate the breakdown of organic matter while also boosting the availability of nutrients in the soil. A substantial body of prior research has shown that the use of eco-enzymes improves soil nutritional quality (Hemalatha & Visantini, 2020; Rochyani et al., 2020) and has the potential to increase plant productivity (Yuliandewi et al., 2018; Salsabila & Winarsih, 2023).

Green mustard (*Brassica juncea* L.) and peanuts (*Arachis hypogaea* Linn.) are crops in high demand. National demand for both has been rising annually due to population growth, which increases food requirements, nutritional needs, food diversification, and industrial demand (Mahyiddin et al., 2025). Besides the source is easily obtained, this horticultural plant has a lot of content. According to data 100 g of peanuts provide approximately 567 kilocalories of energy, with a nutrient composition including 49 g of total fat, 7 g of saturated fat, and no cholesterol content. Additionally, peanuts contain 18 mg of sodium, 16 g of total carbohydrates, including 9 g of dietary fiber and 4 g of sugar. Notably, peanuts' protein content is estimated at 26 g, accompanied by notable micronutrient contributions, including 4.6 mg of iron, 0.3 mg of vitamin B6, and an impressive 168 mg of magnesium (USDA, 2019; Yeri et al., 2024). Meanwhile, 100 grams of green mustard contain the following nutrients: fat 0.3 g, protein 2.3 g, carbohydrate 4.0 g, Ca 220 mg, P 38 mg, Fe 2.9 mg, vitamin A 1,940 mg, vitamin B 0.09 mg, and vitamin C 120 mg (Haryanto et al., 2007; Sulistyawati et al., 2024). Despite the implementation of various initiatives aimed at enhancing the productivity of horticultural crops, empirical evidence in the field indicates the persistent utilization of the monoculture system by farmers. Intercropping systems have been implemented to enhance crop productivity and yield. A substantial body of research has emerged on the subject, and its findings are quite robust. For instance, intercropping systems have been shown to enhance crop productivity by 62% (Nurhayati et al., 2013), suppress weed growth (Pujisiswanto, 2011), and increase the yield of plant growth (Murdiono et al., 2016). While eco-enzymes and related organic amendments show promise for improving soil health and crop productivity, there is a clear research gap regarding their application in green mustard-peanut rotations on Inceptisol soils. Direct, system-specific studies are needed to validate and optimize these benefits for this context.

This study aims to study the effect of eco-enzymes on soil nutrient and on soil nutrients and plant productivity of green mustard-peanut in Inceptisol. The study's findings are expected to provide empirical evidence for the efficacy of eco-enzymes in improving soil fertility and crop yields, incorporation as a component of a sustainable agricultural system.

Materials and Methods

This field experiment research was conducted from February to April 2025 in Sasa Village, South Ternate, Ternate City. The analysis of soil properties was carried out at the Chemistry and Soil Fertility Laboratory, Department of Soil Science, Faculty of Agriculture, Universitas Hasanuddin, Makassar. Materials used in the study were soil, green mustard (*Brassica juncea* L) seeds cv. Shinta, peanut (*Arachis hypogaea* Linn) seeds cv. Talam 1, water, chicken manure, and eco-enzyme. The tools used are treatment signboard, hand sprayer, hoe, shovel, meter, crossbar, camera, stationery. This study used a Randomized Block Design (RBD) consisting of 5 treatments and repeated 3 times, so that there were 15 experimental units, including (1) N0 = without eco-enzyme; (2) N1 = eco-enzyme 2 cc/L; (3) N2 = eco-enzyme 4 cc/L; (4) N3 = eco-enzyme 6 cc/L; and (5) N4 = eco-enzyme 8 cc/L.

The research variables measured soil property analysis, with soil pH (1:5) measured using the electrometric method and a pH meter; total nitrogen (N-total) measured using the Kjeldahl titrimetric method; and available phosphorus (P-available) determined by the Olsen method and measured with a spectrophotometer. All soil chemical analyses followed the laboratory measurement standards of the Agricultural Instrument Standardization Agency, Ministry of Agriculture. In addition to edaphic factor analysis, plant responses were measured. For green mustard, plant height (cm) and leaf number were recorded at 7, 14, 21, and 28 days after planting (DAP). Leaf area was calculated using the L × W × CF method, where L is leaf length, W is leaf width, and CF is the correction factor. Fresh weight was measured at harvest. For peanuts, plant height and branch number were recorded at 7, 14, 21, and 28 DAP, and pod number was counted at harvest. The data analysis technique used was analysis of variance (ANOVA) and followed by Tukey's HSD post hoc test.

Results and Discussion

Initial Soil Chemical Properties. As illustrated in Table 1, Inceptisol exhibits a soil pH range of 5.84 to 5.96, within the criteria for slight acidity. Furthermore, the soil total nitrogen content ranges between 0.16% and 0.22%, falling within the low-to-medium range, as defined by the criteria. Soil pH values affect nitrogen levels in the soil. Imbalanced pH can reduce TN by affecting microbial activity and nitrogen retention (Tian et al., 2024). Similarly, the phosphorus availability spans from 12.97 ppm to 19.51 ppm, categorizing it as very low or low, as delineated by the specified criteria.

Table 1. Results of initial soil chemical analysis

Soil Properties	Value	Criteria
Soil pH (1:5)	5.84 – 5.98	Slightly Acidic
Total Nitrogen (%)	0.16 – 0.22	Low - Moderate
P - Availability (ppm)	12.97 – 19.51	Very Low - Low

Source: Soil Chemistry Laboratories, Faculty of Agriculture, Universitas Hasanudin (2025)

Various concentrations of eco-enzyme exerted no significant effect on soil pH, as illustrated in Figure 1. As shown in Figure 1, the soil pH analysis indicates that the N0 treatment typically yields a higher soil pH value in comparison to the other treatments. In general, low pH is caused by strong acids, such as sulfuric acid, dissolved carbon dioxide, or organic compounds, including humic acid (Bolan et al., 2021). Furthermore, the ion concentration of the soil solution can vary significantly depending on the soil's characteristics. The fluctuation in hydrogen ion concentration within the soil solution affects its pH level. An increase in hydrogen ion concentration typically leads to a decrease in soil pH, indicating an acidic condition. Conversely, a decrease in hydrogen ion concentration increases soil pH, indicating an alkaline condition (Nopriani et al., 2023).

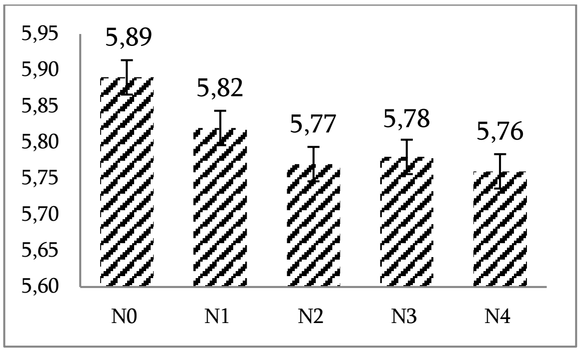

Figure 1. Effect of eco-enzyme concentration on soil pH

Note: N0: without eco-enzyme; N1: eco-enzyme 2cc/L; N2: eco-enzyme 4 cc/L; N3: eco-enzyme 6 cc/L; N4: eco-enzyme 8 cc/L

Various concentrations of eco-enzymes had a significant effect on the N-total and P-availability of Inceptisol (Table 2). The N-total and P-availability levels of Inceptisol revealed a marked difference in response to the N4 treatment in comparison to the other treatments. The results showed that the concentration of eco-enzyme increased on soil total nitrogen, where the lowest initial soil total nitrogen range was 0.16% and the highest 0.26%, while the lowest final soil total nitrogen analysis was 0.11 and the highest 0.26 (%). Increased enzyme activity in the soil can increase the availability of total nitrogen through its role in nutrient cycling and soil biogeochemical processes (Yang et al., 2023). Eco-enzymes can produce bacterial sources of enzymes such as nitrogenase and reductase, in addition to playing a role in the process of nitrogen fixation. These enzymes also act as biocatalysts to accelerate the breakdown of Nitrogen compounds into macro nutrients, Nitrogen needed for soil fertility (Lubis et al., 2022). The analysis showed that eco-enzyme concentration affected soil P-availability, with 8 cc/L (N4) having a significant effect (Table 2). Eco-enzyme, produced from organic waste through a fermentation process, increases phosphorus levels in soil by helping with decomposition and nutrient uptake, including phosphorus. This process produces CO_2, which affects soil phosphate solubility (Nopriani et al., 2023). Organic matter influences soil phosphate availability, especially through weathering products like organic acids and CO_2. These acids can metals like Al, Fe, and Ca from the soil solution (Pasaribu et al., 2014).

Table 2. Effect of eco-enzyme concentration on soil total nitrogen and P-availability

Treatment	Average	
	Soil Total Nitrogen Total (%)	P-Availability (ppm)
N0 (Without Eco-enzyme)	0.11 d (l)	11.12 d (vl)
N1 (Eco-enzyme 2 cc/L)	0.16 c (l)	13.18 c (vl)
N2 (Eco-enzyme 4 cc/L)	0.19 bc (l)	15.33 b (l)
N3 (Eco-enzyme 6 cc/L)	0.22 ab (m)	16.37 ab (l)
N4 (Eco-enzyme 8 cc/L)	0.26 a (m)	17.09 a (l)
HSD 0.05	0.04	1.39

Note: Mean numbers followed by the same letter in the same column mean not significantly different at the Tukey's HSD test α 0.05 level; (vl) = very low; (l) = low; (m) = moderate.

Green mustard plant height. Various concentrations of eco-enzyme gave a significant effect on the height of green mustard plants at 7 and 28 DAP, but the eco-enzyme treatment gave no significant effect at 14 and 21 DAP. Table 3 shows plant height at 7 days after planting (DAP), where the N2 treatment did not differ significantly from N1 but was significantly different from the other treatments. At 28 DAP, the N4 treatment showed a significant difference compared to all other treatments.

Tabel 3. Effect of eco-enzyme concentration on green mustard plants height (cm)

Treatment	Plant Height	
	7 DAP	28 DAP
N0 (Without Eco-enzyme)	4.21 c	19.93 e
N1 (Eco-enzyme 2cc/L)	5.83 ab	24.99 b
N2 (Eco-enzyme 4 cc/L)	5.85 a	22.75 c
N3 (Eco-enzyme 6 cc/L)	5.47 bc	20.65 d
N4 (Eco-enzyme 8 cc/L)	3.27 d	25.56 a
HSD α 0.05	1.43	4.28

Note: Mean numbers followed by the same letter in the same column mean not significantly different at the Tukey's HSD test α 0.05 level.

The concentration of eco-enzyme had no significant effect on the green mustard plant height at 14 and 21 DAP (Figure 2). However, N2 treatment tended to show a higher height than others at 14 DAP. While at 21 DAP, N1 treatment gave the highest plant height compared to other treatments. Plant height is also influenced by the availability of nitrogen in the soil to support plant growth. Using an eco-enzyme concentrate on green mustard plants provides nutrients for the plants. The nitrate in eco-enzyme liquid organic fertilizer helps the growth phase and development phase of green mustard plants (Novianto & Bahri, 2023). Nitrogen nutrients are important for plant growth and development, especially when the plant is growing new cells and making food for itself. Several plant nutrients play crucial roles in cell development and enlargement. Nitrogen, phosphorus, potassium, calcium, and magnesium are essential for cell growth and division, while micronutrients like zinc and boron are also vital for specific aspects of cell development, such as root growth and elongation. The process will happen quickly as more carbohydrates are added to promote plant growth, including plant height, the number of leaves, and leaf area (Suwandi et al., 2024).

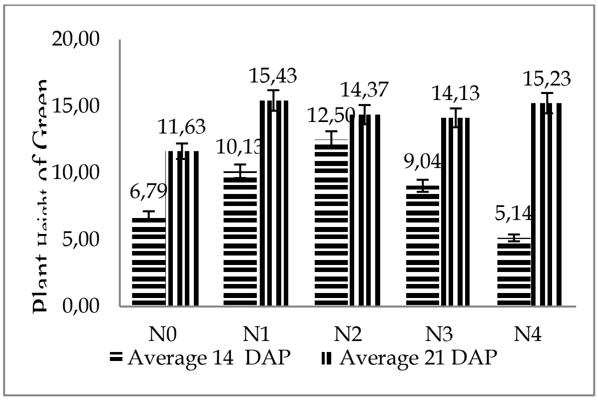

Figure 2. Effect of eco-enzyme concentration on green mustard plant height

Note: N0: without eco-enzyme; N1: eco-enzyme 2cc/L; N2: eco-enzyme 4 cc/L; N3: eco-enzyme 6 cc/L; N4: eco-enzyme 8 cc/L

Number of Leaves of Green Mustard Plants. Various concentrations of eco-enzyme exerted no significant effect on the parameter of the number of leaves of green mustard at 7, 14, 21, and 28 DAP. At 7 and 14 DAP, the N4 treatment tended to produce the highest number of leaves compared to other treatments. By 21 and 28 DAP, the N3 treatment resulted in a greater number of leaves than the other treatments.

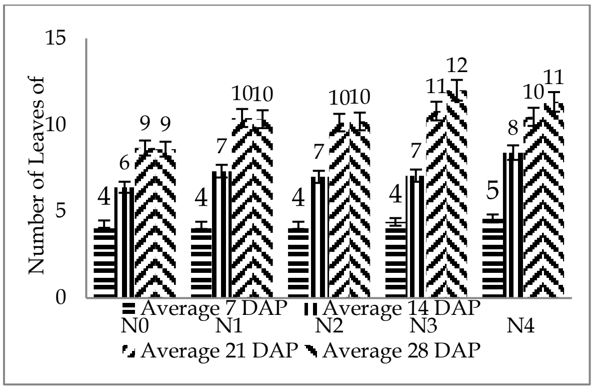

Figure 3. Effect of Eco-enzyme Concentration on the Number of Leaves of Green Mustard

Note: N0: without eco-enzyme; N1: eco-enzyme 2cc/L; N2: eco-enzyme 4 cc/L; N3: eco-enzyme 6 cc/L; N4: eco-enzyme 8 cc/L

Figure 3 shows that there is a relationship between eco-enzyme concentration and plant growth parameters, like plant height, number of leaves, root length, and wet biomass of green mustard plants. The more eco-enzymes you give to plants, the more valuable their growth will be. The same thing happened with the results of mustard plants. The highest dose had a positive impact on the growth and yield of pakcoy (Salsabila & Winarsih, 2023). Nitrogen plays an important role in plant growth. It is needed for every bud formation, stem development, and leaf development in plants. When there is enough N supply, plant leaves will grow large and increase the surface area for photosynthesis (Syifa et al., 2020).

Fresh Weight of Green Mustard Plants. The results of the analysis of variance showed that the concentration of eco-enzyme on the fresh weight of green mustard plants exerted no significant effect; however, the N1 treatment exhibited a tendency to yield the optimal effect on the fresh weight, as illustrated in Figure 4. The N1 treatment exhibited a tendency to possess the highest fresh weight in comparison to the N0, N2, N3, and N4 treatments.

Fresh weight is generally related to stem diameter. Water content in the stem affects the uptake of nutrients during the metabolic process because water plays a role in cell turgidity, so that leaf cells will enlarge and increase in number (Novianto & Bahri, 2023). From the results of plot/hectare conversion production, the lowest fresh weight production was treatment N0 = 266.67 g/plot or equivalent to 0.93 tons/ha. While the highest weight production was treatment N1 = 1028.67 g/plot or equivalent to 3.57 tons/ha. This condition illustrates that there is an influence from the provision of eco-enzymes, even though it is not significant, but the provision of eco-enzymes tends to increase the fresh weight of green mustard plants. Previous research mentioned that the application of liquid organic fertilizer has a positive impact on increasing the fresh weight of flowers by 137% (Widnyana et al., 2023), pakcoy plants up to 83.33 g/plant (Mustofa et al., 2022), and green spinach plants (Nurseha, et al., 2023).

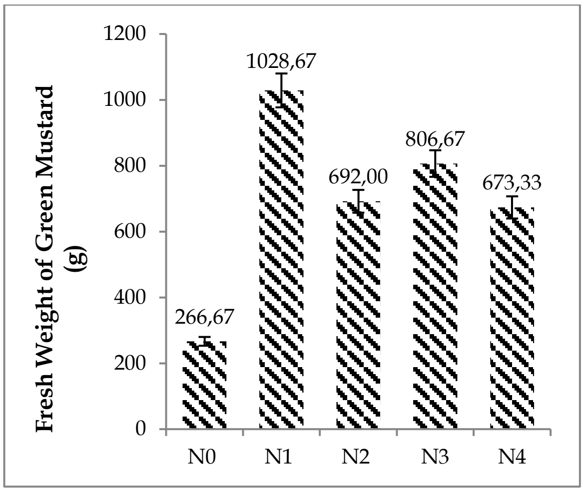

Figure 4. Effect of eco-enzyme concentration on fresh weight of green mustard plants
Note: N0: without eco-enzyme; N1: eco-enzyme 2cc/L; N2: eco-enzyme 4 cc/L; N3: eco-enzyme 6 cc/L; N4: eco-enzyme 8 cc/L

Leaf Area of Green Mustard Plants. The results of the analysis of variance showed that the concentration of eco-enzyme had no significant effect on the leaf area of green mustard plants, but the N1 treatment was able to produce a larger leaf area than the others, as shown in Figure 5. The N1 treatment tended to provide the widest leaf area compared to the N0, N2, N3, and N4 treatments. Generally, vegetative parts of plants, such as leaves, stalks, and roots, have a very high mineral composition compared to fruits, tubers, and seeds. The N1 treatment has optimal absorption of N by the roots, so that it affects the growth of leaf area, while plants that do not meet their nitrogen needs well will grow stunted and have a small leaf area. The provision of nutrients should be appropriate and balanced because nutrient deficiencies or excess nutrients can cause non-optimal growth in plants (Syifa et al., 2020).

Furthermore, several studies have revealed that proper application of liquid organic fertilizer can have a positive effect on plants, though their effects can vary by crop type, fertilizer source, and application rate, and are sometimes less pronounced than those of chemical fertilizers. Previous research states that liquid organic fertilizer can increase the leaf area of lettuce plants by 5% to 40% (Shaik *et al*., 2022) and potato plants (Zhuravel *et al*., 2023).

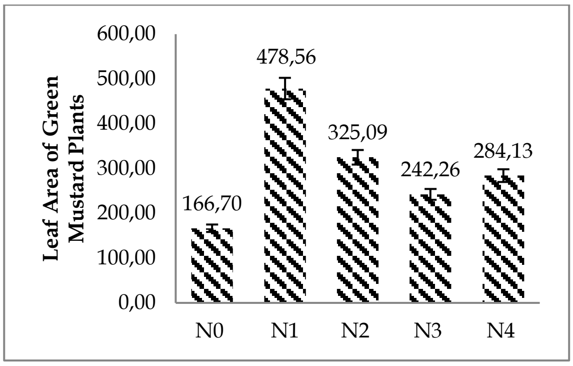

Figure 5. Effect of eco-enzyme concentration on leaf area of green mustard plants.
Note: N0: without eco-enzyme; N1: eco-enzyme 2cc/L; N2: eco-enzyme 4 cc/L; N3: eco-enzyme 6 cc/L; N4: eco-enzyme 8 cc/L

Peanut Plant Height. Various concentrations of eco-enzyme exhibited a significant effect on peanut plant height at 28 DAP. At 28 DAP, treatments N4 and N3 exhibited higher plant height compared to N2, N1 and N0.

Table 5. Effect of eco-enzyme concentration on peanut plant height (cm)

Treatment	Average (cm)
N0 (Without Eco-enzyme)	26.67 c
N1 (Eco-enzyme 2cc/L)	27.99 bc
N2 (Eco-enzyme 4 cc/L)	30.82 ab
N3 (Eco-enzyme 6 cc/L)	33.44 a
N4 (Eco-enzyme 8 cc/L)	33.51 a
HSD α 0.05	3.06

Note: Mean numbers followed by the same letter in the same column mean not significantly different at the Tukey's HSD test α 0.05 level; (vl) = very low; (l) = low; (m) = moderate.

As illustrated in Figure 6, the concentration of eco-enzymes exhibited no substantial impact on the height of peanut plant. However, the N3 treatment tended to show a higher plant height at 7 DAP in comparison to the other treatments. the N4 treatment exhibited the highest plant height compared to the other treatments at 14 and 21 DAP. The application of liquid organic fertilizer with different doses will have a different effect on plant growth and yield, while the right dose will have a significant effect on plant growth and yield (Silalahi et al., 2024). Eco-enzyme functions to fertilize the soil. Because the element nitrogen is very important during the growth of a plant, so that by giving eco-enzymes can accelerate the breakdown of N needed for soil fertility (Lubis et al., 2022).

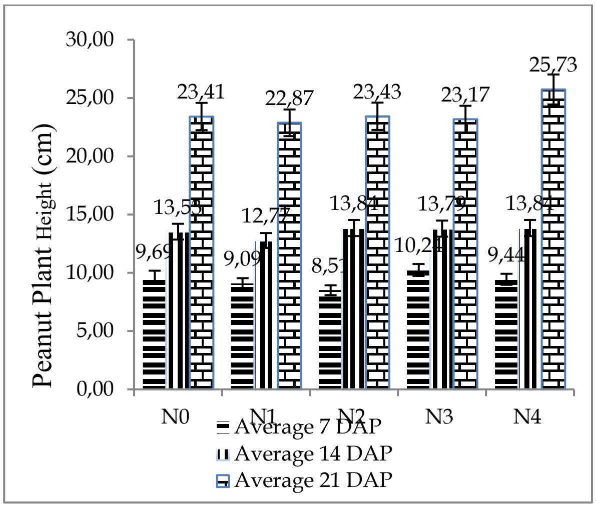

Figure 6. Effect of eco-enzyme concentration on peanut plant height

Note: N0: without eco-enzyme; N1: eco-enzyme 2cc/L; N2: eco-enzyme 4 cc/L; N3: eco-enzyme 6 cc/L; N4: eco-enzyme 8 cc/L

Number of Branches of Peanut Plants. The analysis of variance indicated that eco-enzyme concentration had no significant effect on the number of peanut branches at 7, 14, 21, or 28 days after planting (DAP) (Figure 7). However, trends were observed: N1 tended to produce more branches at 7 DAP, N2 at 21 DAP, and N4 at 14 and 28 DAP. These differences are likely related to the adequate and balanced availability of macro-nutrients, which are essential for cell division and contribute to increased growth during the generative phase. Additionally, macro-nutrients present in the planting medium are believed to support both cell division and enlargement (Ngantung et al., 2018).

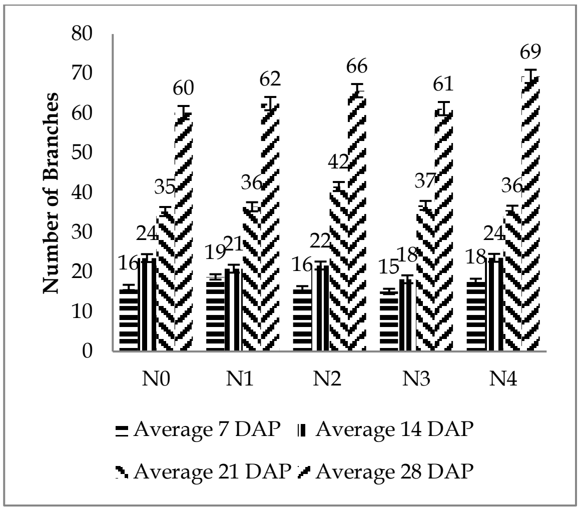

Figure 7. The effect of eco-enzyme concentration on the number of branches of peanut plants

Note: N0: without eco-enzyme; N1: eco-enzyme 2cc/L; N2: eco-enzyme 4 cc/L; N3: eco-enzyme 6 cc/L; N4: eco-enzyme 8 cc/L

Number of Peanut Pods Production. The results of the analysis of variance showed that the concentration of eco-enzyme had no significant effect on peanut pod production. However, the N4 treatment gave the highest pod production compared to the other treatments, as shown in Figure 8. The N4 treatment tended to give more peanut pod weight compared to the other treatments.

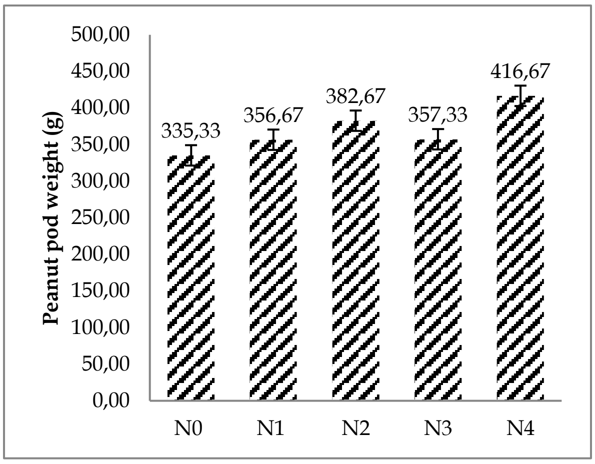

Figure 8. Effect of Eco-enzyme Concentration on Peanut Pod Weight

Note: N0: without eco-enzyme; N1: eco-enzyme 2cc/L; N2: eco-enzyme 4 cc/L; N3: eco-enzyme 6 cc/L; N4: eco-enzyme 8 cc/L

The application of eco-enzyme with a concentration of 8 cc/L(N4) was able to provide sufficient phosphate, where the P element plays a role in the formation of flowers, fruits, seeds, and pods. This is in line with the statement of Salsabila and Winarsih (2023), which states that eco-enzyme given directly to plants by spraying can also increase P-availability in the soil. In addition, the application of liquid organic fertilizer can increase pod production, seed yield, and mineral composition in peanut plants (Ozaktan and Doymaz, 2022), okra plants (Chotaliya, 2020), and long bean plants (Rusnaini et al., 2023).

Conclusion

The application of eco-enzyme concentration had a significant effect on soil total nitrogen, P-available, plant height of green mustard, and peanut. However, the concentration of eco-enzymes had no significant effect on soil pH, number of green mustard leaves, green mustard leaf area, green mustard fresh weight, number of peanut branches, and peanut pod production. Furthermore, the concentration of eco-enzyme treatment of 8 cc/L (N4) gave a significant effect on soil total nitrogen available, P-available, green mustard plant height at 7 and 28 DAP, and peanut plant height at 28 DAP. Meanwhile, the lowest production of green mustard was 0.85 tons/ha, and the highest was 3.29 tons/ha.

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Enhancing sustainable rice production through organic plus fertilizer in irrigated paddy fields

Abstract. Sustainable rice farming is increasingly threatened by declining soil fertility, excessive reliance on chemical fertilizers, and environmental degradation from intensive agricultural practices. There is an urgent need for innovative organic fertilizer products that combine organic materials with macro and micronutrient enhancements to restore soil health effectively. This research aimed to evaluate the effect of organic plus fertilizer (OPF) as a sustainable soil amendment to improve soil nutrient status, increase paddy productivity, and enhance overall soil health. The experiment was conducted using a Randomized Block Design (RBD) with nine treatments: one recommended OPF dose, six combinations of NPK (75 – 100%) and OPF (75 – 150%), one recommended conventional NPK dose, and one control. Variables observed included plant growth, yield, and yield components, total soil nitrogen, and plant uptake of N, P, and K. Results indicated that OPF combined with NPK significantly increased plant height (29.13 – 31.38%) and number of panicles (57.89%) compared to the control. Nutrient uptake improved for nitrogen (23.68%) and potassium (15.96 – 21.28%), although no significant improvement was observed for phosphorus. Yield parameters showed an 81.97%–118.50% increase over the control. The combinations of 75% NPK + 150% OPF, 100% NPK + 75% OPF, and 100% NPK + 100% OPF produced taller plants, higher yields, greater nutrient uptake, and lower residual nitrogen in the soil. For optimal rice yield and soil health, integrated application of OPF with either a full or 75% NPK dose is recommended.

Keywords: Environment · Fertilizer · Organic ·Residue · Soil health

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Introduction

Indonesia is one of the world's biggest rice producers, and it largely relies on paddy farming to maintain a safe food supply. In recent years, the application of chemical fertilizers, extensive monoculture practices, and a general disregard for the addition of organic materials have put at risk the future of rice farming (Bhattacharyya et al., 2015). These harmful practices have increased soil acidity, reduced organic carbon levels, decreased cation exchange capacity (CEC), and damaged essential microbial populations important to nutrient cycling (Anisuzzaman et al., 2021). Watering and flooding rice fields often also speed up the leaching of critical micronutrients like zinc (Zn) and iron (Fe), which makes the nutrient imbalances even worse (Nishimura & Itani, 2013). Organic fertilizers are popular because they are a long-term way to restore damaged soils and make rice harvests more productive. Organic fertilizers are made from decomposing plant and animal waste or organic compounds added to the soil. They enhance soil health by stabilizing pH levels, improving water retention, improving soil structure quality, and enhancing organic carbon content (Koorneef et al., 2024). Their slow-release nutrient composition makes sure that potassium (K), phosphorus (P), and nitrogen (N) are always available in paddy systems. This reduces the need for synthetic fertilizers and the loss of nutrients (Paramesh et al., 2023). Using organic fertilizers also helps to reach bigger agroecological goals. Research shows that adding organic matter to the soil makes it more diverse, helps plants absorb nutrients more effectively, and sometimes leads to higher yields (Paramesh et al., 2023). These benefits are achieved by the goals of agricultural systems that are good for the environment and can withstand climate change. These systems are vital for the future of farming in Indonesia. Using organic fertilizers on rice fields is not only a way to increase production, but it is also a vital step toward managing land in a way that is good for the environment and ensuring there is enough food for everyone. Adding organic fertilizer to rice fields, especially paddy fields, increases crop yields and follows ecological principles and food safety standards (Susanti et al., 2024). As worries about the environment and soil degradation grow, using organic fertilizer is an important way to make farming more sustainable and able to withstand climate change.

Despite the growing promotion of organic fertilizers as environmentally friendly substitutes for chemical inputs, many Indonesian commercial products still have several drawbacks. First, many organic formulations have relatively low nutrient content, especially in terms of potassium (K_2O), phosphorus (P_2O_5), and nitrogen (N), which frequently fall short of the nutritional requirements of staple crops like rice (Romadhon et al., 2024). According to Cao et al. (2021), many products also have an unbalanced C/N ratio, which can alter nitrogen dynamics by immobilizing or quickly mineralizing nitrogen in the soil. The existence of heavy metals, especially in fertilizers made from sludge or urban waste, is another serious issue that could endanger crop and soil safety in the long run (Su et al., 2025). Additionally, a public health concern is the presence of pathogenic microbes, like *Salmonella* or *Escherichia Coli*, due to insufficient composting (Xu et al., 2022).

Furthermore, many organic fertilizers currently on the market do not address micronutrient deficiencies like iron (Fe) and zinc (Zn), essential for rice metabolism and yield performance. The Organic Plus Fertilizer (OPF) formulation signifies an advance over traditional fertilization methods by synergizing organic and mineral nutrient sources. In contrast to conventional inorganic fertilizers that mainly provide immediately accessible nutrients while offering few benefits to long-term soil health, OPF integrates humic substances, agricultural products, and composted animal waste with specific macro and micronutrient enhancements. This dual strategy immediately provides vital nutrients, including potassium from ash or mineral deposits, phosphorus from natural phosphate rock, and nitrogen from targeted supplementation, while improving soil organic matter, microbial diversity, and nutrient cycling efficiency. The integration of humic compounds enhances nutrient chelation and accessibility, whereas composted manure offers slow-release nutrient savings, mitigating the risk of leaching and loss of nutrients. This formulation technique meets immediate crop nutrient requirements while ensuring long-term soil fertility, establishing OPF as an innovative solution that reconciles high-yield agriculture with ecological stewardship. Because of that, there is a growing need for organic fertilizer products that offer enhanced and consistent nutrient availability and contribute significantly to soil rehabilitation and

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increased crop productivity sustainably. This research focuses on exploring the potential of organic fertilizer as a sustainable soil amendment solution for increasing soil nutrients, increasing paddy productivity, and supporting long-term soil fertility restoration and environmentally friendly rice cultivation.

Materials and Methods

Time, Place, and Methods of The Experiment. The research was conducted from February to July 2024 in the experimental field of the Soil Chemistry and Plant Nutrition Laboratory, Universitas Padjadjaran, Jatinangor. A randomized block design (RBD) was implemented in the experiment, consisting of nine treatments, namely one dose OPF recommendation, six combination dose NPK (75 – 100%) and dose OPF (75 – 150%), one recommended fertilizer dose (conventional), and one control treatment for comparison. Each treatment was given three replications, producing twenty-seven experimental plots. Plant growth data were observed every two weeks starting from 14 DAP to 56 DAP, yield components, content of total N in the soil during the maximum vegetative stage, sorption of macro elements (N, P, and K) by rice plants during the maximum vegetative stage, and initial soil analysis were observed. The data obtained from the results of laboratory and field analysis tested the difference in the average effect of treatment with the F test at the 5% significance level based on analysis of variance (ANOVA) to determine the effect of treatment on the observed parameters of each treatment given, and the difference in the average treatment continued with Duncan's multiple range test at the 5% significance level. The linear model equation for RBD is as follows (Gutpa et al., 2016). For analyzing the relationship between two continuous variables for all variables, the Pearson correlation coefficient was used, calculated by(Teng & Chen, 2024)**:**

$$r = \frac{\Sigma\,(X_i - X)(Y_i - Y)}{\sqrt{\Sigma(X_i - X)^2\,.\Sigma(Y_i - Y)^2}}$$

Which: r is Pearson correlation; X_i is the value of variable X at observation i, Y_i is the value of variable Y at observation i, X is the mean of X, Y is the mean of Y, and Σ is the summation symbol.

Sample Preparations. The plant sample includes components of unpolluted, healthy plants, including leaves and shoots. Thoroughly washing samples with distilled water eliminates dust or soil particles. To eliminate surface moisture, samples are initially rinsed with tap water and then dried gently with a paper towel or left to air dry. The organic material is placed in an oven at 65 – 70 °C for 48 to 72 hours, or until it starts to lose weight. A 0.5 mm sieve is employed to homogenize the ground dried plant tissue to ensure uniform particle size. Store ground samples in clean, sealed paper bags or containers. The sample information must be visible on the bags or containers (Balitan, 2005).

Samples of soil were collected at the Jatinangor region, 6° 55' 20,376" S dan 107° 46' 27,748" E as one of the Inceptisols soil type. The composite soil was collected from the area to reflect the local conditions. GPS (Garmin 585) was used to record the coordinates of each location point, and ArcGIS Desktop 10.2 software was used to plot the data on a map. Purposive sampling was used for the sampling process (Balitan, 2005).

Soil and Plant Analysis. Soil and plant analysis used reference materials (Balitan, 2005). Quantifying total nitrogen content in soil by converting all nitrogen in the sample into ammonium (NH_4^+), then liberating it as ammonia gas (NH_3), capturing it in a known amount of trapping solution, and determining the amount of nitrogen through acid–base titration. In plant sorption analysis, such as nitrogen sorption, all organic nitrogen in plant tissue is converted to ammonium sulfate through digestion with concentrated H_2SO_4 in the presence of a catalyst mixture (K_2SO_4 + $CuSO_4$ or selenium). The ammonium is released as NH_3 upon alkalization with NaOH, distilled, trapped in boric acid, and titrated with standardized H_2SO_4 or HCl. For phosphorus analysis, P in plant tissue is released by wet digestion. The orthophosphate reacts with ammonium molybdate under acidic conditions to form phosphomolybdic acid, which is reduced with ascorbic acid to produce a blue complex. Absorbance is measured at 882 nm using a spectrophotometer. K in plant tissue is released using the same digestion method as for P. The digested sample is aspirated into a flame photometer, where the excitation of potassium atoms produces light at 766.5 nm. The light intensity is proportional to the K concentration (Balitan, 2005).

Organic Fertilizer Formula. The first step in the formulation process is the selection of good quality of organic base materials, such as humic substances, agricultural residues, and composted livestock manure (20:20:60). These materials are supplemented with vital macro and micronutrients during or after maturation, such as potassium from ash or mineral sources, phosphorus from natural phosphate rock, and occasionally nitrogen. After enrichment, the mixture is made into granules using a pan or drum granulator and natural binders such as bentonite or molasses. This granulation, with particle sizes between 3 and 5 mm, yields an effective and homogeneous product that is easy to manage and utilize. Subsequently, the granules are desiccated to achieve less than 10% moisture content to enhance their structural integrity and longevity, and a selective coating procedure utilizing materials utilized to improve durability. The completed product is stored in dry, well-ventilated settings after being wrapped in moisture-resistant bags (Figure 1).

Figure 1. Morphological form of organic plus fertilizer (OPF)

Organic Fertilizer Analysis. For the quality of organic plus fertilizer, the present work follows the authorized Indonesian method for evaluating the safety and quality of solid organic fertilizers, as outlined in SNI 7763:2018. As part of this recommendation, the fertilizer's physical, chemical, and biological properties are thoroughly tested to ensure it is safe for crop use. To obtain uniform granules, the sample is then air-dried and sieved. Two crucial physical parameters examined are the moisture content, which cannot exceed 20%, and the particle size, which must be at least 80% able to pass through a 5 mm sieve. A minimum of 15% organic carbon (organic C) must be present in the product. Total nitrogen (N), phosphorus (P_2O_5), potassium (K_2O), pH, and the C/N ratio are all measured using standard laboratory techniques such as the Walkley-Black and Kjeldahl techniques. According to the guideline, levels of heavy metals such as chromium (Cr), lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg) must remain below specific safety thresholds. Additionally, it states that testing for micronutrients like zinc and iron is necessary. Microbial safety is also crucial; no dangerous microorganisms, such as *Salmonella* species or *Escherichia Coli*, should be present in the fertilizer, referring to the regulations in force at the Ministry of Agriculture. When a product satisfies all the requirements, it can be certified and labeled, allowing it to be used in Indonesian agriculture without compromising crop nutrition, soil fertility, or the environment (National Standardization Agency, 2018).

Results and Discussion

Quality of Organic Fertilizer. Based on the analysis of the product of OPF (Table 1), the chemical content of the analysis followed SNI 7763:2018, the Indonesian National Standard for solid organic fertilizers. The product exhibited a high organic carbon (organic C) content of 23.22%, indicating a strong potential to improve soil organic matter and enhance microbial activity. The C/N ratio was measured at 21.30, within an ideal range to support balanced nitrogen mineralization and organic matter decomposition in the soil. The moisture content was relatively low at 8.80%, suggesting good storage stability and ease of handling. The fertilizer is enriched with both macro and micronutrients, featuring 1.09% total nitrogen (N), 4.90% phosphorus (P_2O_5), a notably high value that supports early root development, and 0.36% potassium (K_2O), essential for plant water regulation and stress tolerance.

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Table 1. Chemical characteristics, macro- and micro-elements, heavy metals, and contaminant microorganisms in organic plus fertilizer

No.	Parameters	Unit	Result	Indonesian National Standard Criteria
1.	Organic C	%	23.22	meet the criteria
2.	C/N Ratio	-	21.30	meet the criteria
3.	By Products	%	-	
4.	Water Content	%	8.80	meet the criteria
5.	Macro Elements:			
	N	%	1.09	meet the criteria
	P_2O_5	%	4.90	meet the criteria
	K_2O	%	0.36	meet the criteria
6.	Micro Elements:			
	Total Fe	ppm	6938.89	meet the criteria
	Available Fe	ppm	6.55	meet the criteria
	Zn	ppm	403.14	meet the criteria
7.	pH	-	8.40	meet the criteria
8.	Heavy Metals:			
	As	ppm	0.00	meet the criteria
	Hg	ppm	0.00	meet the criteria
	Pb	ppm	9.71	meet the criteria
	Cd	ppm	0.62	meet the criteria
	Ni	ppm	44.28	meet the criteria
	Cr	ppm	104.05	meet the criteria
9.	Contaminant Microorganisms:			
	E. Coli	CFU/g	Negative	meet the criteria
	Salmonella sp.	CFU/g	Negative	meet the criteria
10.	Grain Size	%	89.42	meet the criteria

Micronutrient analysis indicated a substantial iron (Fe) concentration of 6938.89 ppm (total) and 6.55 ppm (available), with zinc (Zn) at 403.14 ppm, both of which are essential for enzymatic processes and photosynthesis in crops. This fertilizer, with a pH value of 8.40, is classified as moderately alkaline, making it appropriate for acidic soils by increasing the pH. Significantly, heavy metal analysis verified undetectable concentrations of arsenic (As) and mercury (Hg), with permissible levels of lead (Pb), cadmium (Cd), nickel (Ni), and chromium (Cr), all conforming to safe limits as per SNI 7763:2018 standards. Microbial safety was confirmed, with negative findings for *E. Coli* and *Salmonella sp.*, validating its hygiene and appropriateness for agricultural application as organic fertilizer. The product exhibited exceptional granule homogeneity, with 89.42% of particles meeting the specified size, facilitating uniform distribution during field application. This organic fertilizer plus product adheres to the rigorous quality and safety standards specified in the standard, rendering it a dependable resource for sustainable soil fertility and crop productivity improvement. This material serves not only as a soil conditioner but also as a nutrient source and booster of soil health. Its elevated phosphorus and micronutrient levels, acceptable heavy metal concentrations, and carbon-to-nitrogen ratio set it apart from traditional organic inputs, especially for intensive paddy rice farming.

Preliminary Soil Analysis. Table 2 comprehensively describes the physicochemical parameters of the soil sample used to conduct this study. The parameters being analyzed are critical indicators of soil health, fertility, and capacity to optimize crop growth, particularly for rice, which depends on pH and nutrient imbalances (Mulyani et al., 2019). The pH of this soil is slightly acidic, as is familiar with many tropical soils, which was classified as slightly acidic to moderately acidic (Tariq et al., 2020). While rice grows well in slightly acidic conditions, excessive acidity, as measured by the KCl reading, may inhibit the absorption of nutrients, especially phosphorus (P) and molybdenum (Mo) (McCauley et al., 2017).

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Table 2. Physicochemical parameters in preliminary soil analysis

No.	Parameters	Value	Result	Criteria *
1.	pH (H_2O)	-	6.25	Slightly
	pH (KCl)		4.59	Acidic
2.	Organic C	%	2.35	Medium
3.	Total N	%	0.23	Medium
4.	C/N	-	10	Low
5.	P_2O_5 (HCl 25 %)	mg.100 g^{-1}	24.93	Medium
6.	P_2O_5 (Bray I)	ppm P	9.92	Medium
7.	K_2O (HCl 25 %)	mg.100 g^{-1}	23.88	Medium
8.	Cation Exchangeable Capacity (CEC)	cmol.kg^{-1}	17.87	Medium
9.	Cations:			
	Ca	cmol.kg^{-1}	4.50	Low
	Mg	cmol.kg^{-1}	1.77	Medium
	K	cmol.kg^{-1}	0.44	Medium
	Na	cmol.kg^{-1}	0.06	Low
10.	Base Saturation	%	37.92	Low
11.	Texture:			
	Clay	%	11	
	Dust	%	34	Clay
	Sand	%	55	

According to the soil research centre criteria, the soil's total nitrogen content is 0.23%, which falls into the medium category, and its organic carbon content is moderate at 2.35%. Enhancing soil structure, water retention, and microbial diversity depends heavily on organic matter. However, the low carbon-to-nitrogen ratio of 10 indicates fast mineralization, potentially leading to an immediate release of nutrients and a rapid depletion (Kumar et al., 2016). The phosphorus levels are in the medium range, at 9.92 ppm (Bray I) and 24.93 mg.100 g^{-1} (HCl 25%). These levels may be inadequate for later stages of rice development unless supplemented, despite being sufficient for early growth. The pH level significantly influences phosphorus availability; in acidic soils such as this, phosphorus combines with aluminium and iron, reducing its bioavailability (Akter et al., 2018). The measured potassium level of 23.88 mg.100 g^{-1} is also within the moderate fertility range. K is necessary for rice grain filling, disease resistance, and water regulation. Although K may not immediately limit at the level seen, supplementation would help guarantee steady uptake throughout the crop cycle (Ragel et al., 2019). The CEC value of 17.87 cmol.kg^{-1} indicates a moderate ability to retain and exchange nutrients. Soils that balance clay and organic matter tend to have a moderate CEC. The low base saturation of 37.92% suggests that acidic cations like Al^{3+} and H^+ occupy a sizable portion of the exchange sites. Without fertilization, this exchange level indicates a reduced nutrient reserve that could impact plant growth (Silva & Uchida, 2000). The content of Ca is 4.5 cmol.kg^{-1} (low), Mg 1.77 cmol.kg^{-1} (moderate), K moderate, 0.44 cmol.kg^{-1}, and Na extremely low, 0.06 cmol.kg^{-1}, given the importance of calcium for root development and cell wall strength, low calcium and sodium levels point out the need for nutrient amendment. Although not necessary, high sodium can be a sign of salinity problems, so this matter must be paid close attention to (Shrivastava & Kumar, 2015).

The soil texture is sandy loam, with 55% sand, 34% silt, and 11% clay. Although this texture encourages excellent drainage and aeration, it may have a low capacity to hold water and nutrients, consistent with the noted mild CEC. Adding organic matter greatly enhances the structure and retention of these soils (Paramesh et al., 2023). The soil profile, which is sandy loam, moderately acidic, and medium in organic matter and nutrients, indicates that productivity can be increased by using organic fertilizers to increase microbial activity and nutrient retention. In particular, NPK supplementation addresses deficiencies in P and Ca. Using integrated nutrient management techniques, this approach balances short-term agricultural requirements with long-term soil health. This aligns with contemporary sustainable agriculture practices in tropical areas, where chemical dependence and soil degradation are significant issues (Massoukou Pamba et al., 2023).

Height of Plant. The results clearly show that using OPF alone or inorganic NPK fertilizer made the rice plants grow taller at every growth stage compared to the control (Table 3). Plant height is a standard metric for assessing rice plants' nutrient uptake and overall health throughout the initial stages of growth (Pandey et al., 2017). The stage of early growth is 14 days after planting (DAP). At 14 DAP, treatments with 100% dose of OPF through 100% NPK + 150% dose of OPF worked far better than the usual NPK treatment of standard NPK and the control.

Table 3. Effect of various combinations of organic plus fertilizer (OPF) on the plant height (cm)

Code	Treatments	Days After Planting (DAP)			
		14	28	42	56
A	Control	28.64 a	49.02 a	68.29 a	75.89 a
B	100% NPK Standard	35.79 b	61.36 b	76.19 ab	85.97 b
C	100% Dose of OPF	43.26 c	66.85 bc	78.79 b	86.51 b
D	75% NPK + 75% OPF	44.53 c	60.22 b	84.50 bc	86.56 b
E	75% NPK + 100% OPF	43.65 c	59.13 b	82.84 bc	86.54 b
F	75% NPK + 150% OPF	44.48 c	62.91 b	89.72 c	87.45 b
G	100% NPK + 75% OPF	42.90 c	62.23 b	88.24 c	87.12 b
H	100% NPK + 100% OPF	44.40 c	66.43 bc	88.18 c	87.09 b
I	100% NPK + 150% OPF	37.00 b	72.01 c	88.18 c	85.53 b

Note: Means followed by the same letter within the same column do not show significant differences based on Duncan's Multiple Range Test at the 5% significance level.

Treatments 75% NPK + 75% OPF and 75% NPK + 150% OPF showed the most notable early growth, indicating that OPF can support rapid early vegetative development even with lower NPK input. This could be because OPF has nutrients and beneficial microbial metabolites that are easy for plants to access and help grow roots (Iqbal et al., 2025). Compared to the control group, all the fertilized treatments at this time made the plants much taller. Treatment 100% NPK + 150% OPF had the tallest plants (72.01 cm), which suggests that a high dose of OPF and full NPK may work together to make plants grow taller. This backs up the findings of previous studies that show how organic matter improves the soil's ability to hold water, microbes, and nutrients, all of which help plants grow better (Iqbal et al., 2025; Mulyani et al., 2017). OPF is probably a slow-release nutrient source, especially in tropical soils with low amounts of organic matter. This reduces the possibility of nutrient leaching and enhances their availability to plants during the vegetative stage. Stages 42 and 56 of the late vegetative to reproductive cycle treatment 75% NPK + 150% OPF resulted in the tallest plants (89.72 cm) by 42 DAP.

Treatments 100% NPK combined with 75% OPF, 100% NPK with 100% OPF, and 100% NPK paired with 150% OPF followed closely behind. In these treatments, varying quantities of OPF were given with either complete or almost complete NPK. This outcome demonstrates the essential role of organic inputs in maintaining a consistent nutrition supply during crucial growth stages, particularly during panicle development and grain filling. The lack of notable variations between treatments that combined NPK with OPF and those utilizing OPF exclusively at 14, 28, and 56 DAP indicates that the nutrient provision from OPF was adequate to satisfy the plant's initial growth needs.

Organic plus fertilizer, comprising humic substances, composted manure, and enhanced macro- and micronutrients, offers immediately accessible nutrients and slow-release variants. During the initial growth stages (2 and 4 weeks), rice plants predominantly depend on the nitrogen reserves present in the soil and on the starting fertilizers. The mineralization of organic matter in OPF gradually releases nitrogen, phosphorus, and potassium, which can sustain optimal growth without the immediate requirement for more NPK. This aligns with the findings of Shu et al. (2025), which showed that well-balanced organic formulations can equal the efficacy of complete chemical fertilizers during the initial vegetative stages. At 8 weeks post-planting, the plant height measurements for both OPF-only and OPF with NPK treatments were statistically comparable, suggesting that the nutrient release from OPF consistently facilitated growth without acting as a limiting factor. This indicates that supplementary NPK inputs may not result in proportional growth enhancements when nutrient availability is sufficient, an effect commonly referred to as "diminishing returns" in fertilizer response.

Furthermore, the organic component in OPF enhances soil nutrient retention and the root-zone microenvironment, facilitating effective nutrient absorption. This can diminish reliance on elevated dosages of chemical NPK during the vegetative stage, using sustainable agriculture principles that seek to optimize rather than maximize fertilizer application. At 56 DAP, all fertilizer treatments significantly outperformed

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the control group. Nonetheless, treatments: One dose of OPF was given with one NPK, and one and a half doses of OPF exhibited no meaningful difference. This convergence indicates that the internal redistribution of nutrients becomes increasingly crucial as the plant matures. The prevailing trend remains in favor of OPF-enriched therapies. This indicates that they can offer a more consistent and enduring supply of nutrients throughout the crop's life cycle (Gamage et al., 2023).

Number of Tillers. Observation of the number of tillers (Table 4) showed interesting data to discuss, as each treatment can give different responses at different DAP. Because it directly affects the number of grains produced per plant, the number of tillers is essential to rice productivity. Important patterns about the effects of organic and inorganic fertilizer combinations on tiller growth can be seen in the data presented. In the early stage (14 and 28 DAP), the treatments did not differ significantly at 14 DAP.

All treatments, including the control, showed similar numbers of tillers (8 – 10), meaning that the tillers' growth had not fully reacted to the early fertilizer applications. The differences between the treatments were not statistically significant by 28 DAP, meaning the plants were still in the early stages of taking up nutrients and directing them to their reproductive parts. These findings are consistent with those of Huanhe et al. (2024), who found that vegetative biomass accumulation has a greater impact on early growth stages than reproductive. A more precise pattern appeared during growth from mid to late (42 and 56 DAP) at 42 DAP. In this situation, the treatments that used both NPK and OPF, especially at higher amounts (treatments 75% NPK + 150% OPF, 100% NPK + 75% OPF, 100% NPK + 100% OPF, and 100% NPK + 150% OPF), significantly raised the number of tillers compared to the control. The treatments 75%NPK + 150% OPF and 100% NPK + 75% OPF, 100% NPK + 100% OPF, and 100% NPK + 150% OPF (with full NPK and more OPF) had the highest number of tillers (30 tillers), which was much better than NPK only (21 tillers) and the control (19 tillers). The lack of notable differences between NPK with OPF combinations and OPF alone in weeks 2, 4, and 8 regarding the number of tillers likely arises from the notion that early tiller initiation is predominantly affected by the availability of balanced nutrients and the plant's physiological preparation rather than by excessive fertilizer application. The OPF comprises both macro and micronutrients in organic and mineral forms and humic compounds that enhance nutrient availability, soil microbial activity, and root health. When OPF sufficiently fulfils the crop's nutritional requirements, supplementary NPK may not result in a discernible alteration in yield components throughout that growth cycle. A further contributing element is that the number of panicles is established very early during tillering. However, nutrient absorption from OPF is gradual and persistent, enabling plants to achieve comparable tiller counts to those receiving combined NPK + OPF treatments. In inundated rice systems, nutrient losses by leaching or volatilization may diminish the advantages of additional NPK, rendering the efficacy of OPF-only treatments equivalent. This outcome demonstrates how chemical and organic fertilizers work in concert. Through slow-release mechanisms, organic amendments improve nutrient availability, microbial activity, and soil structure. These improvements help tillers' growth and development by encouraging better root growth and nutrient uptake. The trend persisted at 56 DAP. The number of tillers remained higher than the controls in all OPF treatments. The preservation of high values indicates that the beneficial effects of OPF are maintained throughout the reproductive stage, even though the increase in tiller numbers was less abrupt than at 42 DAP. Organic fertilizers are crucial not only for improving the physical and biological properties of soil but also for the long-term support of flowering and grain filling, according to claims (Wang et al., 2019).

Straw weight, Number of Panicles, and Panicle Length. The results indicate that adding OPF, either alone or in combination with NPK, improved straw weight, number of panicles per plant, and panicle length compared to the control. The analysis of yield components is shown in Table 5 below.

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Table 4. Effect of various combinations of organic plus fertilizer (OPF) on the number of tillers

Code	Treatments	Days After Planting			
		14	28	42	56
A	Control	9 a	25 a	19 a	20 a
B	100% NPK Standard	9 a	23 a	21 a	22 b
C	100% Dose of OPF	8 a	23 a	22 ab	22 b
D	75% NPK + 75% OPF	9 a	25 a	24 bc	23 b
E	75% NPK + 100% OPF	10 a	24 a	27 cd	23 b
F	75% NPK + 150% OPF	10 a	23 a	28 d	24 b
G	100% NPK + 75% OPF	10 a	28 a	30 d	24 b
H	100% NPK + 100% OPF	10 a	27 a	30 d	22 b
I	100% NPK + 150% OPF	10 a	27 a	30 d	23 b

Note: Means followed by the same letter within the same column do not show significant differences based on Duncan's Multiple Range Test at the 5% significance level.

Table 5. Effect of various combinations of organic plus fertilizer (OPF) on straw weight, number of panicles, and panicle length

Code	Treatments	Straw Weight/plot (kg)	Number of panicles/plant	Panicle length (cm)
A	Control	10.27 a	12 a	23.74 a
B	100% NPK Standard	10.91 a	16 d	24.67 a
C	100% Dose of OPF	12.63 a	13 ab	24.31 a
D	75% NPK + 75% OPF	10.49 a	14 bc	25.17 ab
E	75% NPK + 100% OPF	11.50 a	14 bc	25.89 ab
F	75% NPK + 150% OPF	13.35 a	15 cd	26.00 ab
G	100% NPK + 75% OPF	13.79 a	17 d	26.49 ab
H	100% NPK + 100% OPF	15.96 a	17 d	27.69 b
I	100% NPK + 150% OPF	10.27 a	12 a	23.74 a

Note: Means followed by the same letter within the same column do not show significant differences based on Duncan's Multiple Range Test at the 5% significance level.

Based on data in Table 5, the statistical differences were insignificant ($p > 0.05$). The highest straw biomass was recorded in treatment 100% NPK + 100% OPF at 15.96 kg/plot, representing a 55.4% increase compared to the control (10.27 kg/plot). Treatments containing a higher proportion of OPF, such as 75% NPK + 150% OPF and 100% NPK + 75% OPF, also showed higher straw weights. This suggests that OPF contributes to enhanced vegetative growth, possibly through improved soil structure and nutrient retention, in line with previous findings by Liu et al. (2023) that organic amendments boost biomass accumulation. For the number of panicles/plant, the highest values (17 panicles) were obtained in treatments 100% NPK + 75% OPF and 100% NPK + 100% OPF, significantly higher than the control (12 panicles). This improvement of about 41.7% indicates that balanced nutrient availability from combined organic and inorganic sources can stimulate tiller development and reproductive branching (Khambalkar et.al, 2025). The longest panicles (27.69 cm) were recorded in 100% NPK + 100% OPF, significantly higher than the control (23.74 cm). Longer panicles are typically associated with higher grain-bearing capacity, suggesting potential yield advantages (IRRI, 2013). While some differences were not statistically significant for straw biomass, the trend shows that combining NPK with OPF at optimal doses (particularly 100% NPK + 100% OPF) produces the best results in yield component parameters. The likely mechanism involves synergistic effects: NPK provides immediate nutrient availability, and OPF enhances soil fertility, microbial activity, and nutrient release over time.

Sorption of N, P, and K in Plants. The analysis of macro elements such as N, P, and K sorption by plants is shown in Table 6.

Table 6. Effect of various combinations of organic plus fertilizer (OPF) on the sorption of plant

Code	Treatments	N	P	K
		%		
A	Control	1.14 a	0.69 a	0.94 ab
B	100% NPK Standard	1.34 b	0.85 a	1.08 bcd
C	100% Dose of OPF	1.47 b	0.78 a	0.88 a
D	75% NPK + 75% OPF	1.35 b	0.75 a	0.99 abc
E	75% NPK + 100% OPF	1.14 a	0.73 a	0.88 a
F	75% NPK + 150% OPF	1.46 b	0.80 a	0.99 abc
G	100% NPK + 75% OPF	1.41 b	1.00 a	1.09 cd
H	100% NPK + 100% OPF	1.41 b	0.92 a	1.14 d
I	100% NPK + 150% OPF	1.39 b	0.98 a	1.05 bcd

Note: Means followed by the same letter within the same column do not show significant differences based on Duncan's Multiple Range Test at the 5% significance level.

Table 7. Effect of various combinations of organic plus fertilizer (OPF) on soil N

Code	Treatments	Soil N (%)
A	Control	0.35 a
B	100% NPK Standard	0.38 a
C	100% Dose of OPF	0.40 ab
D	75% NPK + 75% OPF	0.44 ab
E	75% NPK + 100% OPF	0.40 a
F	75% NPK + 150% OPF	0.38 a
G	100% NPK + 75% OPF	0.51 b
H	100% NPK + 100% OPF	0.44 ab
I	100% NPK + 150% OPF	0.38 a

Note: Means followed by the same letter within the same column do not show significant differences based on Duncan's Multiple Range Test at the 5% significance level.

Nitrogen is necessary for the production of chlorophyll and for vegetative growth. While several treatments, such as 100% OPF, 75% NPK + 150% OPF, 100% NPK + 75% OPF, 100% NPK + 100% OPF, and 100% NPK + 150% OPF, recorded significantly higher levels (≥1.39%), suggesting effective nitrogen uptake, the data show that the control treatment had the lowest N content (1.14%). Treatments employing full OPF alone or in conjunction with NPK (75% NPK + 150% OPF and 100% NPK + 150% OPF) markedly increased the nitrogen content compared to control or NPK alone. This is in line with research by Eghball et al. (2002), which showed that in addition to providing a slow-release form of nitrogen, organic fertilizers also promote nutrient mineralization by increasing microbial activity and organic matter decomposition. Additionally, Ghosh et al. (2022) found that combining organic and inorganic sources improved nitrogen availability and uptake in rice systems.

Phosphorus is essential for root growth and energy transfer. This dataset showed no appreciable differences between treatments, ranging from 0.69% to 1.00%. Despite statistically similar P levels, the numerically higher P content in treatments 100% NPK + 75% OPF and 100% NPK + 150% OPF suggested a potential synergistic effect of combining NPK with organic inputs. Organic materials boost soil pH buffering and microbial activity, enhancing phosphorus uptake and solubilization (Suvendran et al., 2025). This corroborates the results of Mahmood et al. (2017), who found that organic manure increased P availability by releasing organic acids that mobilized the insoluble phosphates in the soil. Potassium is necessary for both water regulation and enzyme activation. The K content of treatment 100% NPK + 100% OPF was the highest at 1.14%, considerably higher than that of the control (0.94%) and OPF-only treatments. Treatments 100% NPK + 75% OPF and 100% NPK + 150% OPF also had relatively high K values. These findings support the notion that integrated nutrient management offers a long-term and immediate supply of K. Chemical fertilizers offer readily available K. However, organic amendments boost cation exchange capacity (CEC), ultimately enhancing K availability and retention (Morash et al., 2024).

Soil Analysis of Total N. Table 7 displays the effect of various combinations of organic fertilizer on N in soil, gives different results depending on the treatments.

Regarding applying nitrogen from OPF in one cultivation period, the nitrogen residue in the soil is still at a reasonable level. This is because organic fertilizer does not produce undesirable residue in the soil, as opposed to nitrogen derived

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from inorganic materials. Enhancing soil organic matter (SOM) and microbial activity through organic fertilizers is essential for improving soil nitrogen retention. (Bingham & Cotrufo, 2016) claim that organic matter aids in binding nitrogen in more stable organic forms, minimizing leaching immediately, but gradually mineralizing over time. The higher N residue in treatment 100% NPK + 75% OPF can also be explained by (Govindasamy et al., 2023), who found that adding organic amendments to NPK improves nitrogen use efficiency (NUE) by improving soil structure, boosting microbial activity, and buffering nutrient release. It may have been best to apply OPF partially to prevent "microbial N immobilization", which stimulates microbial demand that can compete with plants for nitrogen, or excessive nitrogen immobilization. Furthermore, (Govindasamy et al., 2023) stressed that N loss is frequent in flooded paddy soils and that methods that enhance N retention, like incorporating organic inputs, can significantly enhance the sustainability of soil fertility. This is consistent with recent research showing that the best results were obtained when combining mineral and organic fertilizers.

In agronomic and practical consequences, the greater N conservation is indicated by treatment 100% NPK + 75% OPF, which has high residual N, which may encourage early growth in the following cropping season and lessen the need for initial fertilization. N residue may not always rise in treatments containing only OPF or high concentrations of OPF (75% NPK + 150% OPF, 100% NPK + 150% OPF). This could be because of increased microbial activity that consumes available N or because N release and plant demand are not sufficiently synchronized. It is crucial to maximize the proportion of inorganic to organic fertilizer. Better results might not always result from excessive organic input that is not balanced. A promising strategy for sustaining higher soil nitrogen levels after harvest is integrating 100% NPK + 75% OPF. This will improve soil fertility resilience and support sustainable nutrient management in rice-based systems. This approach aligns with the larger trend toward integrated nutrient management (INM), which recent studies have shown enhances soil health and productivity (Amanullah et al., 2023).

Yield Parameter. Parameters of the plant yield showed different responses for each treatment (Table 8). Several treatments with OPF combinations saw a significant increase in grain yield, with the treatment 100% NPK + 100% OPF having the highest yield at 9.33 kg, which was significantly higher than the control (4.27 kg).

Table 8. Effect of various combinations of organic plus fertilizer (OPF) on grain weight

Code	Treatments	Grain Weight/plot (kg)
A	Control	4.27 a
B	100% NPK Standard	6.46 ab
C	100% Dose of OPF	4.95 ab
D	75% NPK + 75% OPF	5.75 ab
E	75% NPK + 100% OPF	6.80 ab
F	75% NPK + 150% OPF	7.47 ab
G	100% NPK + 75% OPF	7.77 b
H	100% NPK + 100% OPF	9.33 b
I	100% NPK + 150% OPF	7.65 ab

Note: Means followed by the same letter within the same column do not show significant differences based on Duncan's Multiple Range Test at the 5% significance level.

Treatments 100% NPK + 75% OPF and 75% NPK + 150% OPF produced high grain yields (7.77 kg and 7.47 kg, respectively). This is consistent with previous studies by Zhao et al. (2016), which highlighted how combining organic and inorganic nutrients promotes balanced nutrient availability, enhancing grain filling and overall productivity. More straw biomass means better plant growth and photosynthesis, which are affected by more nutrients available (Rahman et al., 2020). The availability of potassium and nitrogen, which enhance vegetative growth, is frequently linked to straw yield. The pattern shows that mixtures enriched with OPF help keep strong plant growth, even though the results were similar across different treatments. Mulyani et al. (2017) stated that the application of OPF promotes the growth of additional shoots in plants by slowly supplying nutrients, which improves overall growth and the development of productive shoots. In systems combining organic and inorganic materials, the number of particles strongly influences nitrogen availability and how well it meets the plants' needs. Because micronutrients and organic matter support cellular development and hormone regulation, this trait is frequently improved when available (Rahman et al., 2020). Despite not being substantially different from most treatments, the steady rise across OPF-enriched treatments illustrates the compound's cumulative impact on plant physiology.

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Conclusion

According to the study's findings, applying Organic Plus Fertilizer (OPF), especially when combined with inorganic NPK fertilizer, significantly enhances rice plant growth, nutrient uptake, soil fertility, and yield performance. Treatments that combined a full or partial dose of NPK with OPF consistently outperformed the control and NPK-only treatments in key agronomic parameters like plant height, panicle count, nutrient absorption, and grain production. The increase in nutrient uptake and improved nitrogen residue in the soil under OPF treatments further illustrate its role in improving soil health and nutrient efficiency. These findings show that integrated nutrient management, which uses organic and inorganic fertilizers, is a practical and effective way to increase rice yields while maintaining soil fertility. Also, a well-balanced mix of high-quality organic raw materials improves the organic fertilizer product. This synergy makes important nutrients more readily available right away and helps keep soil healthy in the long run by improving soil structure and keeping nutrient cycling going. These mixes provide both short-term and long-term benefits.

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Evaluation of color, water content, and antioxidant properties of wood ear mushroom with nano edible coating, packaging, and storage temperature

Abstract. Wood ear mushrooms are often consumed in Asia and Tropical America for their jelly-like texture, health, and freshness. However, they are easily damaged due to microbes and poor postharvest handling, which affects quality. The combination of nano edible coating, packaging, and storage temperature offers a solution to these issues. This study aims to evaluate the effect of nano edible coating, packaging, and storage temperature on the color (L^*, a^*, and b^*), water content, and antioxidant properties (total phenolic and flavonoid) of wood ear mushrooms. The study was conducted at the Horticulture Laboratory, Faculty of Agriculture, Universitas Padjadjaran. The experimental design used was a Completely Randomized Design with a total of 18 combinations of nano edible coating (sodium alginate and aloe vera), packaging (biodegradable, wrap, and vacuum plastic), and storage temperature (±25 °C, 10 °C, and 5 °C). Each treatment was replicated twice, resulting in 36 experimental units, with 3 mushrooms per unit for a total of 108 mushrooms. The results showed a significant effect of the combination treatments on L^* at 9 days after treatment (DAT), a^* at 6 DAT, water content at 6 and 9 DAT, total phenolics, and total flavonoids during the storage period. Nano aloe vera with vacuum packaging at 5 °C gave the best effect on L^* value at 9 DAT, water content at 6 and 9 DAT, total phenolic at 3 and 6 DAT, and total flavonoid at 6 DAT. These results indicated the potential of the treatment in maintaining the quality of the wood ear mushroom during storage.

Keywords: *Auricularia auricula* · Nano sodium alginate · Nano aloe vera · Vacuum packaging · Yield quality

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Introduction

Mushrooms have been widely used as both food and medicine do to their rich nutritional content. The wood ear mushroom (*Auricularia auricula-judae* (Bull.) Quél.) is the third most cultivated among the four major edible mushrooms and is considered highly important globally (Rawiningtyas et al., 2023; Zhou et al., 2023). It contains secondary metabolites, such as total phenolics and flavonoids (Herawati et al., 2021). A high content of phenolics and flavonoids indicates strong antioxidant activity (Indriyah et al., 2023), enhancing the functional value of wood ear mushrooms as a healthy food ingredient.

Global production and market demand for wood ear mushrooms are significantly high. In China, it ranks as the second most-produced edible mushroom (Guan et al., 2024), with the total production reaching 7.06 million tons in 2020, making China the world's largest producer (Feng et al., 2024). In 2022, mushroom production in Indonesia, including wood ear mushrooms, reached 63.16 tons, and the largest production is in Central Java (Arista et al., 2024). Wood ear mushrooms are often consumed because of their jelly-like character, dark greyish-brown color, and flavorless taste (Faridah et al., 2023). High market demand requires the mushroom industry to supply sustainably (Nur Sakinah et al., 2020). Their quality and safety increase consumer preference (Fu et al., 2020).

Mushrooms are more perishable than other commodities due to their high respiration and transpiration, as well as weak epidermis structure (Lu et al., 2016). After harvest, mushrooms continue to grow (Nur Sakinah et al., 2020), which causes high catabolic activity, which is 200–500 mg/kg/hour at 20 °C (Kim et al., 2006). Wood ear mushrooms have a high water content, ranging from 85–95% (Khaskheli et al., 2015; Zhu et al., 2024), which makes the mushrooms more easily damaged by microorganisms and have a short shelf-life (Lestari et al., 2023). If not treated in time, it will cause a high possibility of rotting and damage due to the growth of microorganisms (Castellanos-Reyes et al., 2021; Zhu et al., 2024).

Various factors can affect the chemical composition and nutritional value of wood ear mushrooms, including the environment and storage conditions (Fan et al., 2023; Ma et al., 2015). Optimal post-harvest technology can extend the shelf-life of wood ear mushrooms and maintain the yield quality. The common post-harvest treatment for wood ear mushrooms is drying, but this treatment can increase the purine content, which can cause gout if often consumed by humans (Kaneko et al., 2014). Therefore, we used fresh wood ear mushrooms in this research.

Edible coating is an eco-friendly solution to extend the shelf-life of crop yield (Tahir et al., 2019). Sodium alginate, an edible coating made from algae, acts as a barrier to reduce gas transfer by reducing the respiration rate and is effective in controlling the enzyme process that causes browning (Díaz-Mula et al., 2012). Color changes were inhibited in plums coated with sodium alginate at concentrations of 1% and 3% (Valero et al., 2013). Aloe vera, edible coating made from leaves of aloe vera contain carbohydrates, saccharides, and others, can protect the fruit body surface, so that it can reduce respiration, decay, and water loss (Misir et al., 2014). The color decrease was higher in the uncoated fruit body by aloe vera (Ates et al., 2022).

Coating materials with larger particle sizes may inhibit absorption, making them less effective. Nano edible coatings tend to be more easily absorbed by agricultural products due to their very small particle size, which allows for more effective penetration and adhesion (Gidagiri et al., 2025). Nano edible coatings have been developed to improve the adhesion of coating materials to the surface of fresh-cut fruits (Bassey et al., 2021; Sánchez et al., 2020). Alginate-based ZnO (Alg-ZnO) nanoparticles have been shown to preserve fruit quality, reduce decay, and extend the shelf life of mangoes (Hmmam et al., 2023). Similarly, nano-formulated aloe vera has proven effective in prolonging the shelf life of mangosteen and fresh-cut mangoes (Suriati et al., 2020, 2021).

Various types of packaging have been widely used to store fresh products, including mushrooms. Currently, most food products are packaged using plastic materials (Mohamad et al., 2022; Shimazu, 2018). However, the varying permeability of plastic to gas, light, and moisture is considered one of its major limitations (Opara & Mditshwa, 2013). Biodegradable plastic offers an alternative for short-term storage and helps reduce the environmental risk associated with conventional, non-degradable plastics (Shaikh et al., 2021). In modern markets, wood ear mushrooms are commonly packaged using styrofoam trays and plastic wrap; however, this packaging often traps air inside. Vacuum

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packaging provides a more efficient alternative by reducing oxygen levels to less than 1% (Meena et al., 2022), thereby inhibiting the growth of aerobic bacteria and fungi and improving food preservation (Quaglia et al., 2020). It also prevents the evaporation of volatile compounds (Galli et al., 2024), reduces weight loss and total soluble solids, lowers the browning index, and increases the total phenolic content, as observed in jujube fruit (Moradinezhad & Dorostkar, 2021).

Temperature fluctuations during transportation and storage cause mushrooms to undergo physiological changes such as softening, discoloration (browning), off-flavor, and nutrient loss, thereby reducing their postharvest quality (Xia et al., 2024; Zhang et al., 2018). Storage temperature also significantly affects plant secondary metabolites. Flavonoids and phenolic acids increase markedly after low-temperature storage treatments (Liu et al., 2023a; Liu et al., 2023b). For example, the total flavonoid content of shiitake mushrooms increased one week after storage at 3 °C, while the total phenolics increased after two weeks at 5 °C (Kim et al., 2023). In general, increased phenolic and flavonoid levels reflect high antioxidant activity. However, such increases can also indicate oxidative stress or tissue damage due to environmental stress, so stable levels are more ideal during storage.

The combination of nano edible coating and packaging, with the right storage temperature, is important in maintaining the quality of post-harvest wood ear mushrooms during storage. However, there is still few research about various types of packaging and nano edible coating in post-harvest handling of wood ear mushrooms, as well as the appropriate temperature that can maintain the quality of the mushrooms. Therefore, this study aims to evaluate the effect of combinations of different types of nano edible coating, packaging, and storage temperatures on the quality of wood ear mushrooms, and to identify the most effective treatment for preserving their quality.

Materials and Methods

The research was conducted at the Horticulture Laboratory, Faculty of Agriculture, Universitas Padjadjaran, from January to April 2025. The materials used in this study included wood ear mushrooms, nano sodium alginate, nano aloe vera, biodegradable plastic, wrap plastic, vacuum plastic, Folin-Ciocalteu reagent, 7.5% sodium bicarbonate solution, gallic acid, methanol, 10% aluminum chloride, 2 M sodium acetate solution, distilled water, and quercetin. The tools used included oven (Memmert, UM300, Germany); analytical balance (Mettler Toledo, Switzerland); spectrophotometer reflectance (Konica Minolta, CM-600D, Japan); digital scales; thermo recorder; spectrophotometer UV-Vis (Shimadzu, Uv-1601, Japan); centrifuge; sonicator; pipette; aluminum cup; measuring flasks; 50 mL test tubes; vacuum and sealer; knife; chopping board; grinder; water bath; and 10 mL glass bottles, cooling storage, refrigerator, and freezer.

The research design used was a Completely Randomized Design (CRD) with a combination of nano edible coating treatments—Nano Sodium Alginate (NSA) and Nano Aloe Vera (NAV)—packaging (biodegradable, wrap, and vacuum plastic), and storage temperature (±25 °C, 10 °C, and 5 °C). A total of 18 treatment combinations were each replicated twice, resulting in 36 experimental units. Each unit, consisting of 3 mushrooms, was observed at 3, 6, and 9 days after treatment (DAT), for a total of 108 mushrooms.

Wood ear mushroom samples were sourced from farmers in the Sukatani Village, Garut, West Java. Mushrooms were selected based on uniform harvest age (4 weeks after pinhead emergence), with fully bloomed fruit bodies weighing 20–60 g each, free from visible signs of disease or blemishes. Samples were grouped in portions of approximately 100 g (3–4 fruit bodies). The 1% nano edible coating, made from aloe vera and sodium alginate (300 nm), was provided by the Functional Nano powder University Center of Excellence (FiNder U-CoE), Universitas Padjadjaran.

The samples were coated by brushing (Momin et al., 2021), air-dried (10 min), and then packaged with vacuum, wrap, and biodegradable. After being packaged, the mushrooms were stored at ±25 °C (room temperature), 10 °C (cooling storage), and 5 °C (refrigerator). Measurements were conducted on 3, 6, and 9 DAT, using two samples per treatment (two replications).

The observation parameters consisted of color characteristics, water content, total phenolic, and total flavonoid. Color was measured on both upper and lower parts of wood ear mushrooms (3 fruit bodies/samples) using a spectrophotometer reflectance (Konica Minolta,

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CM-600D, Japan) to obtain the L*, a*, and b* values (Cavusoglu et al., 2021). Chroma was calculated using the formula from Ates et al. (2022):

$$\text{Chroma}=\sqrt{(a^*)^2+(b^*)^2}$$

Water content determination refers to SNI 01-2891-1992. Samples (4 g) were weighed to obtain the initial weight, dried in an oven (105 °C, 3 hours), cooled in a desiccator, and reweighed until constant weight. Water content (%) was calculated as:

$$\text{Water content (\%)}=\frac{(W_0-W_1)}{W_0} \times 100$$

W_0 = weight before drying (g), W_1 = weight after drying (g).

Dried samples were used for total phenolic and flavonoid measurement. Samples were oven-dried at 60 °C for 24 hours, ground using a grinder. 500 mg of powder was extracted in 10 mL of methanol, sonicated (60 min), and centrifuged (4000 rpm, 10 min). The supernatant was transferred into a 10 mL glass bottle. The total phenolic measurement based on Sytar et al. (2018) with modifications. A 0.5 mL extract was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent and incubated (5 min). Next, 2 mL of 7.5% sodium bicarbonate solution was added, homogenized, incubated (60 min), and the absorbance was measured at 765 nm. The standard solution was prepared using gallic acid concentration (8–128 ppm). Results were expressed as Gallic Acid Equivalent (GAE) mg/100 g:

$$\text{Total Phenolic (mg GAE/100 g)}=\frac{C.V}{B} \times 100$$

Total flavonoid measurement refers to Sytar et al. (2018), modified. 1 mL of extract was mixed with 2.0 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 2 M sodium acetate, and 1.8 mL of distilled water, incubated (30 min), and measured at 435 nm. Quercetin standards (2–128 ppm) were used for calibration. Results were expressed as mg quercetin equivalent (QE)/100 g:

$$\text{Total Flavonoid (mg QE/100 g)}=\frac{C.V}{B} \times 100$$

Where: C = Concentration (ppm), V = Volume (L), B = Sample weight (g).

Data were analyzed using the F-test to determine the effect of the treatments, followed by the Scott-Knott test to compare the treatments at a 5% significance level. The analysis was performed using Microsoft Excel software equipped with the SmartstatXL add-in.

Results and Discussion

Lightness (L*). The surface color of the mushroom was evaluated based on the L* value (lightness). Visually, the surface of the wood ear mushroom appeared dark red. The color of the wood ear mushroom is influenced by both external and internal factors. External factors include toxins, mechanical damage, and the development of pathogens that cause damage to the intracellular membrane (Hanula et al., 2021). Internal factors involve enzymatic reactions with substrates, for example, tyrosinase, the main PPO enzyme responsible for browning in mushrooms (Jolivet et al., 1998; Oms-Oliu et al., 2008). Tyrosinase catalyzes the conversion of phenolic substrates into intermediate compounds that initiate melanin synthesis (Lin & Sun, 2019; Zhang et al., 2018). The results of this study indicate that the combination of nano edible coating, packaging type, and storage temperature did not significantly affect the L* value of the wood ear mushroom during the storage period, except on 9 DAT. On days 3 and 6 of storage, the Scott-Knot test showed that all treatments belonged to the same group (denoted by letter a) (Table 1). This suggests that there was no significant difference in the L* among the treatment combinations, and the treatments were effective in maintaining the brightness of the wood ear mushroom up to 6 DAT. Li et al. (2022) observed that the color change, indicated by browning degree, showed no significant differences across treatments (untreated and treated) during 0–4 days of storage.

On day 9 after treatment (DAT), a significant difference was observed among the treatment combinations. Treatment using nano sodium alginate and nano aloe vera at approximately 25 °C with all types of packaging— except nano sodium alginate with vacuum packaging at that temperature —were classified into group b, exhibiting a higher L* value. This increase in the L* value indicates greater brightness, which did not align with the visually optimal appearance of the wood ear mushroom. The elevated brightness suggests discoloration, likely caused by the high respiration rate at ±25 °C. Storing mushrooms at such temperatures (20–25 °C) leads to quality deterioration, including stem elongation, cap opening, texture softening, and discoloration (Zhang et al., 2021).

Table 1. Effect of various combinations of nano edible coating, packaging, and storage temperature on the lightness (L*) of wood ear mushroom

Treatment	L*		
	3 DAT	6 DAT	9 DAT
NSA+ ±25 °C + vacuum	22.78 a	23.46 a	23.93 a
NSA+ ±25 °C + wrap	26.42 a	25.83 a	29.50 b
NSA+ ±25 °C + biodegradable	22.43 a	26.91 a	30.17 b
NSA+ 10 °C + vacuum	23.12 a	25.43 a	23.11 a
NSA+ 10 °C + wrap	20.28 a	26.94 a	22.41 a
NSA+ 10 °C + biodegradable	21.92 a	23.37 a	23.64 a
NSA+ 5 °C + vacuum	21.39 a	21.29 a	26.80 b
NSA+ 5 °C + wrap	24.09 a	20.15 a	23.05 a
NSA+ 5 °C + biodegradable	22.27 a	25.12 a	25.15 a
NAV+ ±25 °C + vacuum	24.07 a	24.00 a	25.87 b
NAV+ ±25 °C + wrap	25.50 a	28.13 a	27.61 b
NAV+ ±25 °C + biodegradable	31.67 a	29.93 a	30.44 b
NAV+ 10 °C + vacuum	22.75 a	24.92 a	26.96 b
NAV+ 10 °C + wrap	24.88 a	25.29 a	22.45 a
NAV+ 10 °C + biodegradable	24.19 a	22.73 a	23.01 a
NAV+ 5 °C + vacuum	24.71 a	21.00 a	23.36 a
NAV+ 5 °C + wrap	24.23 a	21.48 a	22.69 a
NAV+ 5 °C + biodegradable	23.05 a	24.32 a	22.42 a

Note: Means followed by the same lowercase alphabet in the same column are not significantly different based on the Scott-Knot test at 5%, DAT: Day After Treatment, NSA: Nano Sodium Alginate, NAV: Nano Aloe Vera.

Storage temperature has a direct impact on respiration rate, with higher temperatures accelerating respiration (Zhang et al., 2018). Srivastava et al. (2020) reported that when the storage temperature decreased from 16 to 4 °C, the respiration rate dropped from 96% to 61%. Wang et al. (2021) also found that edible mushrooms respiration rate, ranged from 132–158 mL CO_2 at 20 °C and 20–30 mL CO_2 kg/h at 5 °C. This high-respiration activity is attributed to the thin and porous epidermal layer of the mushrooms (Sharma et al., 2024). During storage, respiration leads to the release of large amounts of CO_2 and heat, which contribute to softening, weight loss, cap opening, rotting, wrinkling, water soaking, and browning of the mushroom fruit body (Gong et al., 2025). Water soaking results in the tissue appearing more translucent compared to healthy tissue, which corresponds to a higher L* value in the wood ear mushroom. This discoloration may also influence the a* and b* color parameters, as changes in red and yellow tones are associated with browning processes (Walkowiak-Tomczak et al., 2020).

Nano sodium alginate with vacuum packaging at ±25 °C yielded consistent results. This is due to the low oxygen content in the packaging (Moradinezhad & Dorostkar, 2021), minimizing color changes in the form of increased lightness and maintaining a stable L* value. In addition, the high lightness value in nano sodium alginate treatment with vacuum packaging at 5 °C is likely due to the coating effect of nano sodium alginate, which makes the surface of the mushroom appear brighter or slightly shiny, thereby increasing the lightness value (Ning et al., 2025; Song et al., 2011).

a* Value. The Scott-Knot test showed that all treatments on the 3rd day of storage were grouped into the same category (denoted by letter a) (Table 2). This indicates that at the early stage of storage, the combination of nano edible coating, packaging, and storage temperature did not result in a significant difference in the a* value of the wood ear mushroom. This may be because color changes were not yet prominent at the beginning of the storage period. This finding is consistent with the study by Yeo et al. (2022), which reported that noticeable color changes in horticultural products, such as mango, typically begin to occur around the 7th day after storage. This is due to enzymatic activity and pigment oxidation reactions. The a* value defines greenness when negative and redness when positive (Nakilcioğlu-Taş & Ötleş, 2020).

Entering the 6th day after storage, the separation of the groups was clearer. Nano sodium alginate treatment at ±25 °C and 10 °C using vacuum packaging, as well as nano aloe vera at ±25 °C and 10 °C with vacuum packaging, are included in group c with higher a* values. This indicates that the combination of nano-based edible coating and vacuum packaging is effective in maintaining the color of the wood ear mushroom better than plastic wrap or biodegradable packaging. The color change to brown on the mushroom cap increases due to enzymatic reactions (Mohapatra et al., 2008). The enzymes responsible for the browning process react with the substrate, causing brown pigmentation (Cavusoglu et al., 2021). Vacuum packaging plays a role in creating an environment with lower oxygen levels, so that it can slow down the activity of the polyphenol oxidase (PPO), which triggers browning and degradation of color pigments (Perera et al., 2010). Polyphenol oxidase activity increases in the fruit body after harvest, and phenol activity is closely related to the browning development (Abou-Elwafa et al., 2023). Browning occurs due to two definite mechanisms of phenol oxidation, namely through tyrosinase activation or spontaneous oxidation (Jolivet et al., 1998; Vamos-Vigyázó, 1981).

Meanwhile, treatments with plastic wrap and biodegradable tend to be included in groups a and b with lower a^* values, indicating faster color loss. Lower a^* values indicate that the color tends to change towards greenish/brown. This can be caused by the high gas permeability of plastic wrap and biodegradable materials, so that oxygen can enter more easily, which results in faster respiration rates and phenolic oxidation. The presence of oxygen in uncontrolled packaging can accelerate the formation of oxidation compounds that cause browning/greening (decrease in a^*). Modifying the packaging atmosphere can be an effective strategy to limit gas exchange, thereby reducing oxygen availability (Cavusoglu et al., 2021). Vacuum packaging, by minimizing oxygen exposure, has been shown to effectively inhibit browning (Martha & Daniel, 2025).

On the 9th day of storage, the results of the Scott-Knot test once again showed that all treatments were classified into group a, indicating that the treatment combinations no longer had a statistically significant effect on the a^* value. Although the highest a^* value was recorded for the nano sodium alginate treatment with vacuum packaging at 5 °C (7.94), the difference was not statistically significant. This suggests that after 9 days, the color degradation process had progressed uniformly across all treatments, potentially due to the reduced activity of the nano edible coatings, the stabilization of the internal atmosphere within the packaging, or the accumulation of metabolic byproducts during storage. This finding aligns with the study by Garnida et al. (2022), which reported that the effectiveness of edible coatings in maintaining the visual quality of fresh products is optimal within the first 5-10 days of storage and tends to diminish thereafter.

Table 2. Effect of various combinations of nano edible coating, packaging, and storage temperature on the a^* and b^* value of wood ear mushroom

Treatment	a^*			b^*		
	3 DAT	6 DAT	9 DAT	3 DAT	6 DAT	9 DAT
NSA+ ±25 °C + vacuum	5.72 a	6.49 c	6.22 a	5.89 a	6.09 a	5.38 a
NSA+ ±25 °C + wrap	5.06 a	4.77 b	5.11 a	6.60 a	6.70 a	8.20 a
NSA+ ±25 °C + biodegradable	4.91 a	3.69 a	3.50 a	5.38 a	5.67 a	6.65 a
NSA+ 10 °C + vacuum	6.16 a	5.90 c	5.51 a	6.45 a	6.42 a	6.23 a
NSA+ 10 °C + wrap	5.24 a	6.13 c	5.56 a	6.08 a	7.69 a	6.89 a
NSA+ 10 °C + biodegradable	4.77 a	5.16 b	4.67 a	6.06 a	6.05 a	6.17 a
NSA+ 5 °C + vacuum	4.61 a	4.55 b	7.94 a	4.52 a	4.79 a	9.82 a
NSA+ 5 °C + wrap	6.61 a	4.02 a	5.15 a	7.55 a	3.09 a	5.09 a
NSA+ 5 °C + biodegradable	5.12 a	6.68 c	7.11 a	5.32 a	8.13 a	8.29 a
NAV+ ±25 °C + vacuum	6.31 a	5.97 c	6.89 a	7.19 a	6.39 a	8.56 a
NAV+ ±25 °C + wrap	4.18 a	4.71 b	4.13 a	6.10 a	7.77 a	6.31 a
NAV+ ±25 °C + biodegradable	5.86 a	5.25 b	5.20 a	8.78 a	8.11 a	8.67 a
NAV+ 10 °C + vacuum	5.85 a	5.93 c	5.90 a	7.04 a	7.11 a	8.18 a
NAV+ 10 °C + wrap	6.31 a	5.07 b	5.47 a	7.67 a	7.00 a	6.44 a
NAV+ 10 °C + biodegradable	6.67 a	4.76 b	5.35 a	7.36 a	5.76 a	7.43 a
NAV+ 5 °C + vacuum	6.76 a	4.66 b	6.50 a	8.36 a	4.53 a	6.35 a
NAV+ 5 °C + wrap	4.96 a	4.29 a	6.00 a	5.87 a	5.07 a	6.53 a
NAV+ 5 °C + biodegradable	5.14 a	5.01 b	5.61 a	5.92 a	6.39 a	6.00 a

Note: Means followed by the same lowercase alphabet in the same column are not significantly different based on the Scott-Knot test at 5%, DAT: Day After Treatment, NSA: Nano Sodium Alginate, NAV: Nano Aloe Vera.

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Overall, the results of this study showed the importance of combination of edible coatings, packaging types, and storage temperatures, especially in the first 6 days after harvest. The combination of nano aloe vera and nano sodium alginate with vacuum packaging at 10 °C is an effective choice in maintaining the color of wood ear mushrooms during storage. Nanomaterials have specific characteristics including antimicrobial, oxygen absorbers, and as a barrier to gas or moisture (Rai et al., 2019).

b* Value. The results showed that nano edible coatings, packaging, and storage temperatures did not affect the b* value of the wood ear mushroom during the entire storage period. At 3, 6, and 9 DAT all treatments were in the same group (a), which indicates a relatively constant value during the storage period (Table 2). The b* value represents the level of yellowness or bluishness, where positive values indicate a tendency towards yellowish color, while negative values indicate a bluish color (Ali et al., 2014). The constant b* value during storage indicates that the color changes that occur are more dominant in the L* and a* components, while the yellowish component of the wood ear mushroom does not experience significant fluctuations. Mushroom pigments that contribute to the b* value (yellow-blue) in the CIELAB color space are generally more stable to oxidation than the a* value (red-green) (Toma et al., 2023; Hinsch et al., 2022). This is because the yellow to blue pigments in mushrooms, such as isoprenoid-based carotenoids, have conjugated double bonds that can be more resistant to oxidative damage compared to other chemical structures (Toma et al., 2023; Hinsch et al., 2022).

In addition, the stable b* value can also be caused by the low content of carotenoids or yellow phenolic compounds (Meléndez-Martínez et al., 2006), which are susceptible to degradation during storage. Thus, even though there are changes in lightness (L*) or in the red-green color balance (a*), the yellowish color component of the mushroom remains relatively stable. The stability of the b* value during storage can also indicate that the nano edible coatings, packaging, and storage temperatures treatments are effective in suppressing non-enzymatic browning reactions, which generally also affect the b* value. This treatment can play a role in maintaining the visual quality of wood ear mushrooms, especially in maintaining the natural color characteristics expected by consumers.

Table 3. Effect of Various Combinations of Nano Edible Coating, Packaging, and Storage Temperature on the Chroma of Wood Ear Mushroom

Treatment	Chroma		
	3 DAT	6 DAT	9 DAT
NSA+ ±25 °C + vacuum	8.28 a	9.04 a	8.33 a
NSA+ ±25 °C + wrap	8.55 a	8.30 a	9.87 a
NSA+ ±25 °C + biodegradable	7.41 a	7.21 a	7.81 a
NSA+ 10 °C + vacuum	8.98 a	8.80 a	8.38 a
NSA+ 10 °C + wrap	8.11 a	9.89 a	8.98 a
NSA+ 10 °C + biodegradable	7.74 a	8.00 a	7.86 a
NSA+ 5 °C + vacuum	6.55 a	6.69 a	12.73 a
NSA+ 5 °C + wrap	10.19 a	5.09 a	7.34 a
NSA+ 5 °C + biodegradable	7.52 a	10.70 a	11.04 a
NAV+ ±25 °C + vacuum	9.65 a	8.85 a	11.04 a
NAV+ ±25 °C + wrap	7.65 a	9.23 a	7.76 a
NAV+ ±25 °C + biodegradable	10.70 a	9.93 a	10.24 a
NAV+ 10 °C + vacuum	9.18 a	9.30 a	10.26 a
NAV+ 10 °C + wrap	9.99 a	8.74 a	8.54 a
NAV+ 10 °C + biodegradable	9.96 a	7.58 a	9.21 a
NAV+ 5 °C + vacuum	10.85 a	6.54 a	9.15 a
NAV+ 5 °C + wrap	7.85 a	6.71 a	8.97 a
NAV+ 5 °C + biodegradable	7.92 a	8.31 a	8.33 a

Note: Means followed by the same lowercase alphabet in the same column are not significantly different based on the Scott-Knot test at 5%, DAT: Day After Treatment, NSA: Nano Sodium Alginate, NAV: Nano Aloe Vera.

Chroma. The chroma value indicates the degree of color saturation and is proportional to the intensity of the color (Gupta et al., 2011). Chroma or saturation is defined as the property of chromatic content in color perception. Chroma is also the degree of difference from the gray of the same lightness. The higher the chroma value, the clearer the color will appear, while the lower the color will look faded. A high chroma value indicates that the dominant colors of the pumpkin, namely yellow and red, are pure and concentrated (Onwude et al., 2017). In this study, nano edible coatings, packaging types, and storage temperatures did not have a significant effect on the chroma value at any storage period (Table 3).

The chroma values remained stable over time, with no significant fluctuations observed. In other mushrooms, such as enoki mushrooms, an increase in chroma value has been associated with color changes from pale white to brownish, indicating a shift in color intensity (Kusumiyati et al., 2025). However, the stability of the chroma value in this study implies that the color saturation of wood ear mushrooms remained consistent, despite minor variations in the a^* parameter. This condition shows that the post-harvest treatment, nano edible coatings, packaging, and storage temperatures are effective in suppressing excessive color changes, both due to enzymatic and non-enzymatic reactions. Chroma stability also reflects that the pigments that make up the color of the wood ear mushroom are relatively stable against oxidation or degradation during storage.

Thus, the stability of the chroma value in wood ear mushrooms in this study can be one indicator of the success of post-harvest treatment in maintaining the visual quality of the product. Chroma stability is important because color is one of the main factors that influence consumer purchasing decisions for fresh products, including wood ear mushrooms. A consistent chroma value suggests that the intensity of the mushroom's natural color remains visually appealing throughout the storage period, thereby contributing to an extended commercial shelf life.

Water Content. The results of this study indicate that nano edible coatings, packaging types, and storage temperatures had no significant effect on the water content of wood ear mushroom at 3 DAT, but significant effects were observed at 6 and 9 DAT. Water content is a critical factor determining the quality of edible mushrooms during storage. High water content increases susceptibility to deterioration, leading to water soaking, rapid rotting, undesirable odors, and off-flavor. Based on the Scott-Knot test, all treatments were grouped into the same category (a) on day 3 (Table 4), suggesting that, at the early stage of storage, the combination of nano edible coating, packaging, and storage temperature did not significantly influence the mushrooms' water content.

Table 4. Effect of various combinations of nano edible coating, packaging, and storage temperature on the water content of wood ear mushroom

Treatment	Water Content (%)		
	3 DAT	6 DAT	9 DAT
NSA+ ±25 °C + vacuum	91.95 a	91.18 c	91.22 c
NSA+ ±25 °C + wrap	91.74 a	91.69 c	90.98 c
NSA+ ±25 °C + biodegradable	88.11 a	84.58 a	84.53 b
NSA+ 10 °C + vacuum	91.37 a	91.16 c	91.57 c
NSA+ 10 °C + wrap	92.28 a	91.71 c	90.35 c
NSA+ 10 °C + biodegradable	89.14 a	88.23 b	88.29 c
NSA+ 5 °C + vacuum	91.54 a	91.32 c	91.97 c
NSA+ 5 °C + wrap	89.89 a	91.90 c	90.23 c
NSA+ 5 °C + biodegradable	89.10 a	88.78 b	82.81 b
NAV+ ±25 °C + vacuum	90.42 a	92.43 c	92.60 c
NAV+ ±25 °C + wrap	90.90 a	91.27 c	92.85 c
NAV+ ±25 °C + biodegradable	87.61 a	83.09 a	82.41 b
NAV+ 10 °C + vacuum	91.72 a	91.20 c	92.21 c
NAV+ 10 °C + wrap	91.53 a	91.61 c	91.14 c
NAV+ 10 °C + biodegradable	89.12 a	87.79 b	88.22 c
NAV+ 5 °C + vacuum	90.41 a	91.82 c	91.13 c
NAV+ 5 °C + wrap	91.90 a	91.60 c	90.96 c
NAV+ 5 °C + biodegradable	90.29 a	88.86 b	75.95 a

Note: Means followed by the same lowercase alphabet in the same column are not significantly different based on the Scott-Knot test at 5%, DAT: Day After Treatment, NSA: Nano Sodium Alginate, NAV: Nano Aloe Vera.

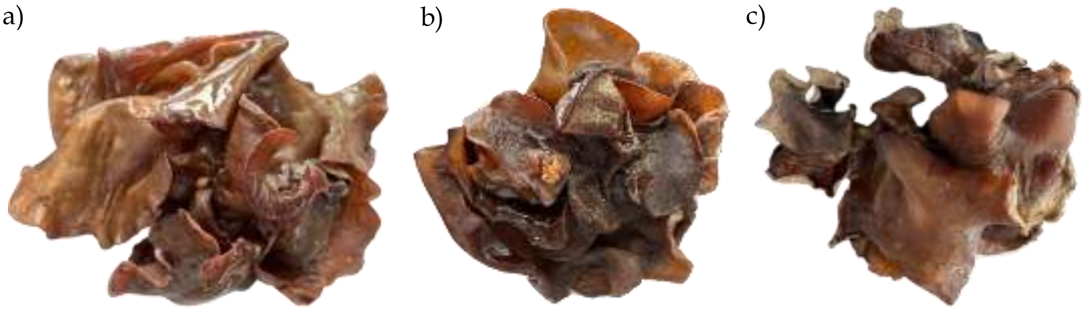

Figure 1. Visual comparison of the freshness of wood ear mushrooms at 9 dat at 5 °C with nano sodium alginate and different packaging treatments: a) Vacuum, b) Wrap, and c) Biodegradable

Under normal conditions, the water content of wood ear mushrooms ranges from 85–95% (Lestari et al., 2023). In this study, the water content of the wood ear mushroom in all treatments was within the normal range, except in several treatments with nano sodium alginate and nano aloe vera combined with biodegradable plastic, especially at temperatures of ±25 °C and 5 °C at 6 and 9 DAT, were classified a and b, which showed lower water content. This is likely due to the higher permeability of biodegradable plastic to water vapor and oxygen, compared to vacuum packaging and wrap plastic (Kumari et al., 2023; Mensitieri et al., 2011). Visually, mushrooms packaged in biodegradable plastic appeared less fresh by the end of the observation period, with a rough texture and dryness (Figure 1c), compared to vacuum packaging (Figure 1a), or plastic wrap (Figure 1b). Low water content during postharvest storage is a major contributor to quality deterioration in mushrooms (Silva et al., 2025). The reduction in water content is often caused by cellular damage and internal water redistribution within the mushroom tissues (Zhang et al., 2018).

Vacuum and wrap packaging are more stable in maintaining the water content of mushrooms at 6 and 9 DAT. This is because of the low oxygen content in the packaging, so that the respiration of wood ear mushrooms can be suppressed and does not produce water vapor, which will increase the water content (Kusumiyati et al., 2025). Fresh mushroom fruit bodies generally contain more than 88% water content after harvest. However, due to the absence of an effective network structure on the surface to prevent water loss, there is rapid spread and loss of water through the transpiration process (Gong et al., 2025). Biodegradable plastic produces lower water content in mushrooms than other packaging, indicating that the packaging has not been able to suppress the transpiration process of the wood ear mushroom, even when coated with a nano edible coating.

The water content in all nano edible coating treatments, storage temperatures, with vacuum packaging and wrap showed optimal values, although there were significant differences between parameters throughout the observation time. Stable water content during storage can also be caused by the use of edible coating, which can reduce respiration in mushrooms, which produce the final product in the form of water vapor. In line with Louis et al. (2021) that the respiration rate of coated mushrooms was significantly lower than uncoated mushrooms. Stable water content during storage can also be caused by the use of edible coating, which can reduce respiration and the final product in the form of water vapor. Respiration rate also could be reduced by modifying natural atmospheric conditions as needed (Kandasamy, 2022). Vacuum packaging can isolate oxygen and water vapor, thereby slowing down the respiration process (Othman et al., 2021).

Total Phenolic. Mushrooms contain phenolic compounds, which are secondary metabolites with dual functions (Hanula et al., 2021). Browning in mushrooms is primarily caused by postharvest stress, which triggers the oxidation of phenolic compounds mediated by PPO. However, phenolics also possess strong antioxidant capacity that can inhibit oxidation chain reactions and prevent lipid peroxidation (Dokhanieh & Aghdam, 2016; Gao et al., 2014). The results of this study indicate that nano edible coatings, packaging types, and storage temperatures had a significant effect on the total phenolic content of wood ear mushrooms throughout the storage period (Table 5). This suggests that postharvest treatments can influence the biochemical stability of mushrooms by modulating the retention or degradation of phenolic compounds during storage.

At the beginning of storage (3 DAT), treatments using nano aloe vera across all packaging types and storage temperatures—except nano aloe vera with vacuum packaging at ±25 °C—exhibited higher total phenolic content compared to those using nano sodium alginate. The same thing also happened at 3 and 9 DAT, where the treatment of nano aloe vera with plastic wrap at 5 °C showed a higher phenolic content compared to nano sodium alginate under the same conditions. This showed that bioactive compounds in nano aloe vera, such as aloin, emodin, and other phenolic compounds, have better antioxidant stability during storage. However, at 6 DAT, there was a decrease in phenolic content in the treatment with nano aloe vera at ±25 °C and 10 °C. In line with research by Abou-Elwafa et al. (2023) that the extension of shelf-life causes a decrease in total phenolic content. This can be caused by the phenolic oxidation process due to oxygen exposure and phenolase activity during storage. The decrease in total phenolic content can be associated with the oxidation of the PPO enzyme, which produces colored quinones, and quercetin is directly oxidized by PPO (Abou-Elwafa et al., 2023).

In contrast, at both 6 and 9 DAT, nano aloe vera treatments stored at 5 °C, regardless of packaging types, were classified into group d, showing higher total phenolic content. This indicated that lower storage temperatures are more effective in preserving phenolic compounds, as they suppress the activity of phenol-degrading enzymes and slow the oxidation of bioactive molecules. Edible coatings also contribute to the preservation of total phenolics (Abou-Elwafa et al., 2023) by forming a barrier that limits oxygen diffusion. Interestingly, at 6 DAT, an increase in total phenolic content was observed in treatments using nano sodium alginate, compared to the initial measurement. This aligns with the findings of Hanula et al. (2021), that total phenolic levels may increase at subsequent storage days relative to day 0. This potentially occurs due to a stress-induced accumulation of secondary metabolites as part of the mushroom's defense response.

Table 5. Effect of various combinations of nano edible coating, packaging, and storage temperature on the total phenolic and total flavonoid of wood ear mushroom

Treatment	Total Phenolic (mg GAE/100 g)			Total Flavonoid (mg QE/100 g)		
	3 DAT	6 DAT	9 DAT	3 DAT	6 DAT	9 DAT
NSA+ ±25 °C + vacuum	42.47 a	59.17 b	76.72 d	17.50 b	33.88 b	28.29 c
NSA+ ±25 °C + wrap	45.23 a	56.21 b	53.48 b	11.68 a	13.20 a	10.13 a
NSA+ ±25 °C + biodegradable	43.11 a	35.10 a	32.79 a	18.28 b	17.80 a	11.52 a
NSA+ 10 °C + vacuum	47.95 a	97.41 d	71.13 d	16.12 b	18.70 a	19.23 b
NSA+ 10 °C + wrap	46.82 a	63.70 b	51.21 b	10.70 a	12.18 a	11.03 a
NSA+ 10 °C + biodegradable	56.72 b	59.59 b	36.61 a	18.06 b	15.95 a	11.80 a
NSA+ 5 °C + vacuum	62.60 b	78.19 c	55.83 b	26.39 c	51.99 e	21.58 b
NSA+ 5 °C + wrap	54.13 a	72.83 c	45.57 b	32.14 d	41.34 c	13.78 a
NSA+ 5 °C + biodegradable	59.14 b	77.40 c	58.45 c	56.36 g	45.73 d	26.68 c
NAV+ ±25 °C + vacuum	40.02 a	84.21 d	70.32 d	17.20 b	35.51 b	27.85 c
NAV+ ±25 °C + wrap	58.19 b	48.13 a	62.62 c	16.58 b	15.36 a	10.56 a
NAV+ ±25 °C + biodegradable	62.97 b	42.16 a	44.67 b	18.55 b	11.62 a	11.25 a
NAV+ 10 °C + vacuum	62.89 b	50.26 a	63.12 c	13.32 a	11.71 a	9.44 a
NAV+ 10 °C + wrap	75.52 b	48.16 a	49.96 b	10.11 a	10.33 a	9.85 a
NAV+ 10 °C + biodegradable	80.10 b	74.24 c	64.56 c	19.80 b	12.23 a	13.30 a
NAV+ 5 °C + vacuum	70.25 b	90.29 d	65.52 c	38.67 e	54.56 e	20.24 b
NAV+ 5 °C + wrap	62.81 b	70.00 c	77.20 d	36.23 e	31.19 b	47.67 d
NAV+ 5 °C + biodegradable	70.71 b	57.74 b	55.11 b	50.83 f	31.38 b	31.36 c

Note: Means followed by the same lowercase alphabet in the same column are not significantly different based on the Scott-Knot test at 5%, GAE: Gallic Acid Equivalent, QE: Quercetin Equivalent, DAT: Day After Treatment, NSA: Nano Sodium Alginate, NAV: Nano Aloe Vera.

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Total Flavonoid. This study demonstrates that the total flavonoid content of wood ear mushrooms was significantly influenced by all treatments and storage periods. On the third day after treatment (3 DAT), the combination of plastic wrap, nano sodium alginate, and storage temperatures of ±25 °C and 10 °C were classified into group a, indicating the lower total flavonoid content (Table 5). In contrast, the combination of nano sodium alginate with biodegradable plastic at 5 °C was classified into group g, showing the highest total flavonoid levels. Nano sodium alginate with plastic wrap at 25 °C and 10 °C, as well as nano aloe vera with vacuum packaging and wrap at 10 °C, showed stable total flavonoid values from day 3 to day 9 after treatment (DAT). This indicates that the treatment was able to maintain flavonoid content until the end of the storage period.

The high flavonoid content in biodegradable plastic packaging may be due to the higher total flavonoid and phenolic content in starch-based packaging compared to conventional plastic packaging (Lopes et al., 2021; Vieira et al., 2024). However, at 6 DAT, the total flavonoid content was higher in vacuum packaging with nano sodium alginate and aloe vera at 5 °C. This also shows that vacuum packaging can reduce oxidation reactions that can damage flavonoids due to the lack of oxygen in the packaging. The higher total flavonoid content is not because the mushroom fruit body produces more flavonoids, but because the flavonoids contained in the mushrooms are not damaged during storage. Vacuum packaging can also limit exposure to aerobic microorganisms that can produce enzymes that destroy phenolic compounds, including flavonoids, such as polyphenol oxidase (PPO) and peroxidase (POD) (Singh et al., 2018). At low temperatures, these enzymes are inactive or very slow, so flavonoids remain stable.

Throughout the storage period, mushrooms stored at 5 °C consistently exhibited higher total flavonoid content compared to other storage temperatures, indicating a strong effect of temperature and packaging type on the total flavonoid retention. This may be attributed to abiotic stress induced by low temperature. When exposed to abiotic stress, mushrooms will activate various metabolic pathways to produce protective compounds, one of which is flavonoids. Flavonoids have antioxidant properties that can ward off free radicals and reduce cell damage due to oxidative stress, including that resulting from cold stress (Banjarnahor & Artanti, 2014; Chandimali et al., 2025; Hassanpour & Doroudi, 2023). Low temperatures can also trigger the expression of genes involved in flavonoid biosynthesis, such as the enzymes chalcone synthase (CHS) and flavonoid synthase (FNS) (Dao et al., 2011; Li et al., 2025; Peng et al., 2019; Yu et al., 2024; Zhao et al., 2024). These enzymes become more active at low temperatures, producing more flavonoids. At low temperatures, cell metabolism can also be disrupted, producing more free radicals (ROS). Flavonoids function as antioxidants that neutralize ROS, thereby helping to maintain the stability of cell membranes and other internal components.

Conclusion

The results showed a significant effect of the combination treatments on L^* at 9 DAT, a^* at 6 DAT, water content at 6 and 9 DAT, total phenolics, and total flavonoids during the storage period. Nano aloe vera with vacuum packaging at 5 °C gave the best effect on L^* value at 9 DAT, water content at 6 and 9 DAT, total phenolic at 3 and 6 DAT, and total flavonoid at 3 and 6 DAT. These results indicated the potential of the treatment in maintaining the quality of the wood ear mushroom during storage.

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Phenotypic evaluation of F10 soybean generations from Grobogan x Slamet cross for large seed size selection

Abstract. Soybeans are an important crop with high nutritional value and diverse uses. Current consumer and industry preference is for soybean varieties with large seeds and high productivity. To meet these demands, one effective approach is through hybridization. This study aims to evaluate the phenotype of F10 soybean line from a cross between Grobogan and Slamet for large-seeded and high-yield. The study was conducted in the screenhouse and Plant Breeding and Biotechnology Laboratory, Faculty of Agriculture, Jenderal Soedirman University. The experiment used a Randomized Complete Block Design (RCBD) with three replications, testing genotypes from Slamet x Grobogan crosses and three check varieties: Slamet, Grobogan, and Wilis. Observational data were analyzed using analysis of variance (ANOVA) with a 5% error rate and continued with LSD to select lines with high-performing genotype. The results of the ANOVA showed that the tested lines affected growth parameters and plant yield components. LSD analysis showed that the highest leaf length and number of leaves were in GS 7. In seed weight per plant, all lines were below Grobogan, followed by GS 7 and GS 47. In 100 seed weight, all lines were below Grobogan, but there were lines with large seed categories (> 14 g/100 seeds), namely GS7, GS 12, GS 36, and GS 39. Correlation analysis showed that the number of pods and 100 seed weight were positively correlated with seed weight per plant. Therefore, these traits can be selection indicators to identify high-yielding soybean genotype.

Keywords: Characterization · Correlation · Hybridization · Seed Size

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Introduction

Soybeans (*Glycine max*) are one of the food crops that play an essential role in meeting human nutritional needs, especially as a source of vegetable protein. In addition, soybeans are also the primary raw material in the food industry, such as tempeh, tofu, and soybean oil (Qin et al., 2022). According to data from the Central Statistics Agency (BPS), in 2024, the Central Statistics Agency (BPS) data noted that domestic soybean production in the latest year only reached around 555,000 tons, while national demand is estimated to reach 2.7 million tons per year. This indicates a fairly large deficit between soybean production and consumption in Indonesia, with a shortfall of around 2.1 million tons that must be met through imports from major soybean-producing countries such as the United States, Brazil, and Argentina (Sibuea et al., 2024). The high volume of imports reflects the lack of domestic soybean production capacity to meet market demand, which in turn requires efforts to increase domestic productivity through various innovations. Apart from its low productivity, the small size of local soybean seeds makes processed soybean food producers prefer imported soybeans with large seeds to local soybeans.

Seed size is one of the most important factors determining soybean yield. Seed size is a complex quantitative trait influenced by genetic and environmental factors during seed development. The composition and quantity of seed storage reserves directly influence seed size. Furthermore, seed size is an agronomic trait correlated with soybean oil and protein content. Therefore, increasing seed size helps increase soybean yield and likely improves seed quality (Duan et al., 2023).

One solution to increase soybean production and quality was through the hybridization method. This technique is carried out by crossing different soybean genotypes to combine the superior traits of both parents. Through this process, it is hoped that new lines can be obtained that have a better combination of characters, such as high seed yields, large seed size, resistance to pests and diseases (Lin et al., 2022), and better adaptation to diverse environments (Biliavska et al., 2021).

The parents used in this cross are Grobogan soybeans and Slamet soybeans. Grobogan soybeans are local soybeans from Grobogan Regency which have yellowish-white seeds and large seed size ranging from 16 -20 g per 100 seeds; plant productivity was quite high, ranging from 2 to 3.5 tons per ha, short plant age, the pods are large, and the level of maturity of the pods and leaves is the same, so when harvested the soybean leaves fall off (Puspitasari & Elfarisna, 2017). Meanwhile, Slamet soybeans are from a cross between Dempo and Wilis, which are resistant to leaf rust disease and tolerant to acidic soil. The average production yield can reach 2.26 tons/ha (Husen et al., 2022). So, the offspring that are expected are of high productivity, have large seeds according to consumer preferences and are resistant to acidic soil.

The diversity of soybean cross-breeding results can be seen from the differences in physical and genetic characteristics of the resulting plants, which in turn affect the production results and quality of the soybean seeds produced. In this context, the diversity of soybean cross-breeding results can be influenced by several factors, such as genetic factors from the crossed parents, the environment in which the plants grow, and the breeding techniques applied (Guo et al., 2022). This study aims to evaluate the phenotype of F10 soybean lines from a cross between Grobogan and Slamet for large-seeded and high-yield.

Materials and Methods

This research was conducted at the screenhouse of the Faculty of Agriculture and at the Plant Breeding Laboratory, Faculty of Agriculture, Jenderal Soedirman University, Purwokerto. The research was conducted in November 2020 - February 2021 during the rainy season. Daytime temperatures ranged from 26 - 30 °C, night time temperatures ranged from 24 - 26 °C, and humidity was 70 - 80%.

The plant materials were seeds, in form of 15 lines from Grobogan x Slamet, namely GS 6, GS 7, GS 12, GS 14, GS 24, GS 30, GS 36, GS 37, GS 38, GS 39, GS 41, GS 47, GS 49, GS 55, GS 58, and three check varieties, namely Slamet, Grobogan, and Wilis. In addition, present study also used urea, SP-36, KCl fertilizer, and growing media in form of inceptisol soil. Plant materials were arranged in a Randomized Complete Block Design (RCBD) with three replications. The treatments included soybean genotypes derived from Slamet and Grobogan crossess, along with three check

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varieties: Slamet, Grobogan and Wilis. The observed variables were plant height (cm), number of leaves (blades), leaf length (cm), leaf width (cm), number of nodes, number of pods, weight of 100 seeds (g), seed weight per plant (g), average number of pods per node, dry weight of the plant. Qualitative variables included hypocotyl color, flower color, seed color, leaf shape, leaf hairs, and leaf hair color.

The selection method used is the direct pedigree method based on agronomic characters, especially seed weight per plant and seed size (based on the weight of 100 seeds).

Data analysis

The observation data were analyzed using analysis of variance (ANOVA) with a 5% error rate, then continued with LSD to selecting high performance lines. The observation data to determine the relationship between quantitative characters using simple correlation analysis with the formula

$$r = \frac{n\sum xy - (\sum x)(\sum y)}{\sqrt{[n\sum x^2 - (\sum x)^2][n\sum y^2 - (\sum y)^2}}$$

r = Represents the correlation coefficient, which indicates the strength and direction of a linear relationship between two variables.
x and y = Represent the variables whose relationship (closeness) is being measured.

Results and Discussion

Qualitative characteristics. The results of qualitative characterization (Table 1) of the F10 soybean population derived from the Grobogan × Slamet cross revealed notable phenotypic variation in several morphological traits, including leaf shape, but uniform with the parental characters in hypocotyl color, seed color, flower color, and trichomes color. These variations indicate the presence of considerable genetic diversity within the population, which is essential for selection in breeding programs. The qualitative traits, although not directly linked to yield, serve as important indicators of genetic variability and can assist in the identification and selection of promising lines in early-generation breeding programs.

Hypocotyl color. Based on the observation results in Table 1, it can be seen that the hypocotyl color of the F10 line resulting from the cross between Grobogan soybeans and Slamet soybeans all produce purple hypocotyl colors. All hypocotyl colors in the promising lines are the same color with their parents and the comparison varieties. Hypocotyl color is related to flower color. Soybeans that have purple hypocotyl color will have purple flower color. Soybeans that have white hypocotyl color will have white flower color. This occurs because of the genetic relationship between hypocotyl color and flower color caused by the influence of pleiotropy (Wang et al., 2023).

Table 1. Qualitative observations of hypocotyl color, seed color, flower color, leaf shape, trichome density, and trichome color of F10 from Grobogan x Slamet cross

Treatment	Hypocotyl color	Seed Color	Flower color	Leaf shape	Trichome density	Trichomes color
Slamet	Purple	Yellow	Purple	Lanceolate	Not dense	White
Grobogan	Purple	Yellow	Purple	Lanceolate	Not dense	White
Wilis	Purple	Yellow	Purple	Rounded ovate	Not dense	White
GS 6	Purple	Yellow	Purple	Triangular ovate	Not dense	White
GS 7	Purple	Yellow	Purple	Lanceolate	Not dense	White
GS 12	Purple	Yellow	Purple	Triangular ovate	Not dense	White
GS 14	Purple	Yellow	Purple	Lanceolate	Not dense	White
GS 24	Purple	Yellow	Purple	Lanceolate	Not dense	White
GS 30	Purple	Yellow	Purple	Lanceolate	Not dense	White
GS 36	Purple	Yellow	Purple	Lanceolate	Not dense	White
GS 37	Purple	Yellow	Purple	Lanceolate	Not dense	White
GS 38	Purple	Yellow	Purple	Lanceolate	Not dense	White
GS 39	Purple	Yellow	Purple	Lanceolate	Not dense	White
GS 41	Purple	Yellow	Purple	Triangular ovate	Not dense	White
GS 47	Purple	Yellow	Purple	Lanceolate	Not dense	White
GS 49	Purple	Yellow	Purple	Lanceolate	Not dense	White
GS 55	Purple	Yellow	Purple	Lanceolate	Not dense	White
GS 58	Purple	Yellow	Purple	Lanceolate	Not dense	White

Seed color. It is known that soybean seeds come in various colors, but in the results of this study (Table 1), all genotypes showed yellow pigmented seeds. All soybean seed colors in these lines should have the same color as their parent varieties, namely Slamet, Grobogan, and Wilis. This character is in line with consumer preferences as stated by Jia et al., (2020), commercial soybean cultivars typically exhibit a yellow color (Choi et al., 2021). Soybean seed color was the result of a dominant allele at the I locus. This genetic factor controls the pigmentation of the seed coat, with the dominant allele causing the production of yellow pigment in the seeds. The presence of this dominant allele overrides any recessive allele at the same locus that might lead to a different color (Mau et al., 2023).

Flower color. Based on the observation results in Table 1, it can be seen that the flower color of the F10 line resulting from a cross between Grobogan and Slamet all produced purple flowers. All flower colors in the desired lines are similar to the parents and comparison variety. Flower pigmentation in soybeans is mainly controlled by six independent loci, namely W1, W2, W3, W4, Wm, and Wp. Among the six loci that control flower color (W1, W2, W3, W4, Wm, and Wp) in soybeans, all loci encode enzymes involved in flavonoid biosynthesis, except W2 (Park et al., 2015). W1 is responsible for the synthesis of delphinidin-3-glucose and the production of purple flowers in soybean (Sundaramoorthy et al., 2015).

Leaf shape. The leaves are compound, meaning they consist of three leaflets (trifoliate), and the leaflets have a smooth or slightly serrated edge. The leaves are usually arranged alternately along the stem. Based on the observation results in Table 1, it can be seen that soybean leaves in the F10 line resulting from the cross between Grobogan and Slamet produce pointed leaf shapes like their two parents, except for accessions GS 12, GS 41, and GS 6 which have triangular, oval leaf shapes. The diversity of leaf shapes in GS12, GS41, and GS6 is possible due to the interaction between genes in Slamet, which is a progeny of Dempo with a pointed leaf shape, and Wilis with a rounded oval leaf shape with the Grobogan variety with a pointed leaf shape. The results of this cross then produce more varied leaf shapes, such as triangular ovals in GS12, GS41, and GS6. Previous studies have found that leaf shape is related to one gene: dominant homozygous genotype (LnLn) and recessive (lnln), which phenotypically show wide leaves and narrow leaves. Heterozygotes (Lnln) experience intermediate leaflets. Bernard and Weiss assigned a new gene symbol, ln, for the narrow leaf trait (Krisnawati & Adie, 2017).

Trichome Density and Color. Trichomes are small, hair-like structures that cover the surface of the leaves. These trichomes can vary in density and length depending on the soybean variety. Based on the observation results in Table 1, it can be seen that the hairs on soybean leaves have a density level that is not dense on the leaves of the F10 soybean line, which is a cross between Grobogan and Slamet. The F10 line has white leaf hairs. All soybean leaf hairs have characteristics similar to the check varieties and parents, namely the Slamet, Grobogan, and Wilis. According to Abywijaya et al., (2024) soybean leaves generally have brightly colored trichomes and their numbers vary. The density of the hairs on the surface of soybean plants is a factor that determines resistance to pests, both in the antixenosis and antibiosis types. (Adie & Krisnawati, 2017).

Quantitative Traits. Quantitative traits in plants are important characteristics that contribute directly to plant productivity and performance. These traits are controlled by many genes (polygenic). The ANOVA results showed significant differences in all characters as shown in Table 2.

The characters of plant height, number of leaves, leaf length, and leaf width showed significant variations, indicating a strong genetic influence on vegetative growth. Similarly, the characters of the number of nodes, number of pods, and the average number of pods per node also showed significant differences, reflecting the diversity of productivity potential between lines. In addition, the weight of 100 seeds and the weight of seeds per plant also showed significant differences, so they can be used as important indicators in the selection of high-yielding lines. The character of plant dry weight also showed significant differences, which illustrates the variation in biomass accumulation between genotypes. Overall, these results indicate that the Grobogan × Slamet cross lines have genetics, so they have the potential to be further developed through selection of superior agronomic characters.

Table 2. Summary of analysis of variance (ANOVA) for characters of crossed lines of Grobogan × Slamet soybeans

No.	Observation characters	Treatments	Coefficient of Variation (%)
1.	Plant height (cm)	**	4.80
2.	Leaf length (cm)	**	9.09
3.	Leaf width (cm)	*	9.47
4.	Number of leaves	**	16.51
5.	Number of segments	*	11.48
6.	Dry Weight of Plant (g)	*	16.86
7.	Number of pods per segment	**	8.56
8.	Number of pods	**	15.34
9.	Weight of 100 seeds (g)	*	16.55
10.	Seed Weight per plant (g)	**	18.21

Note: * = significantly different at the 5% level ($p < 0.05$); ** = very significantly different at the 1% level ($p < 0.01$)

Table 3. Plant height, leaf length, and leaf width of F10 from Grobogan x Slamet cross

Treatment	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Number of leaves	Number of segments	Dry Weight of Plant (g)
Slamet	66.73 def	9.36 a	6.23 bc	72.73 a	6.33 abc	20.77 ab
Grobogan	55.15 g	8.26 ab	6.51 b	57.16 bcd	8.00 ab	17.23 bc
Wilis	49.45 h	6.60 b	5.53 c	31.00 f	8.66 ab	15.01 c
GS 6	72.85 bc	8.93 ab	6.10 bc	52.83 cde	8.66 ab	17.87 bc
GS 7	75.50 b	9.70 a	6.06 bc	74.00 a	8.00 ab	20.34 bc
GS 12	82.65 a	9.13 ab	6.68 b	47.00 de	4.66 c	21.11 ab
GS 14	69.63 cde	8.25 ab	5.80 bc	52.00 cde	9.33 a	18.81 bc
GS 24	74.90 bc	8.48 ab	6.21 bc	53.50 cde	9.33 a	20.81 ab
GS 30	65.50 def	9.26 ab	6.23 bc	70.33 ab	7.33 abc	20.23 bc
GS 36	64.33 ef	9.78 a	7.82 a	61.50 abcd	6.83 abc	26.20 a
GS 37	75.16 b	8.81 ab	5.98 bc	55.00 cde	6.50 abc	20.44 bc
GS 38	70.85 bcd	8.51 ab	5.95 bc	46.50 de	6.83 abc	19.77 bc
GS 39	64.86 ef	8.75 ab	5.90 bc	61.66 abcd	6.83 abc	20.01 bc
GS 41	63.73 f	9.45 a	5.96 bc	60.00 abcd	8.66 ab	17.97 bc
GS 47	65.80 def	8.03 ab	6.08 bc	51.00 cde	6.83 abc	19.47 bc
GS 49	72.93 bc	8.35 ab	5.83 bc	47.83 cde	6.16 abc	19.04 bc
GS 55	67.13 def	9.13 ab	6.23 bc	62.50 abc	5.83 bc	18.07 bc
GS 58	75.93 b	8.20 ab	6.23 bc	40.00 ef	8.50 ab	17.09 bc

Note: Means followed by the same letter in a column indicate no significant difference based on LSD at $\alpha < 0.05$.

The cross between soybean varieties Slamet and Grobogan exhibited a range of quantitative traits (Table 3) that are valuable for breeding and selection. This hybrid combination showed variability in pod formation and seed development, with certain individuals demonstrating higher seed weight and overall biomass compared to the parent lines. The transgressive segregation observed in some traits suggests the potential for selecting superior progenies.

Plant height. Plant height is a significant indicator of a plant's vigor and its ability to compete for resources such as light, water, and nutrients. Taller plants often have a more extensive root system and better access to sunlight, which enhances their growth. The results of the LSD analysis (Table 3) showed that the plant height of all lines exceeded that of the Grobogan and Wilis varieties. Lines with plant heights exceeding the three check varieties were GS 12 (82.65 cm), GS 7 (75.50 cm), GS 37 (75.16 cm), GS 49 (72.93 cm), GS 58 (75.93 cm), GS 6 (72.85 cm), GS 24 (74.90 cm), and GS 38 (70.85 cm). Taller plants have a better ability to capture sunlight because their higher canopy position allows them to access sunlight more directly and avoid shade from other plants. This can support more efficient photosynthesis. Tivoli et al., (2013) also stated that increasing plant height contributes to better air circulation within the canopy, as the more open canopy structure allows for optimal air circulation between the leaves and stems. This helps reduce micro-humidity levels around leaf surfaces, which typically create an ideal environment for the growth of fungi and other pathogens.

Leaf length. The results of the LSD analysis (Table 3) showed that the lines with the longest leaves were GS 7 (9.70 cm), GS 36 (9.78 cm), GS 41 (9.45 cm) which were not significantly different from the Slamet variety, besides that GS 6, GS 12, GS 14, GS 24, GS 30, GS 37, GS 38, GS 39, GS 47, GS 49, GS 55, and GS 58 lines were not significantly different from the Grobogan variety. All lines showed longer leaf lengths than the Wilis variety. Plants will develop and grow well if the leaves which function in the photosynthesis process have the appropriate number and size. A larger leaf surface allows more chloroplasts to be exposed to light, enhancing the plant's ability to absorb sunlight and convert it into energy through photosynthesis. Photosynthesis produces primary metabolites used for plant metabolism that support essential physiological functions, including cell division, elongation, and differentiation, which drive overall plant growth and development. Therefore, plants with larger leaves can maintain higher metabolic activity, resulting in optimal biomass accumulation and yields (Raza et al., 2022).

Leaf width. The wide leaf morphology indicates that the leaf is capable of optimal photosynthesis. The results of the LSD analysis (Table 3) showed that the line with the widest leaves, namely GS 36 (7.82 cm), exceeded the three check varieties, while the line that had a leaf width that was not significantly different from Grobogan was the GS 12 line (6.68 cm), and all lines had leaf widths that were wider than the Wilis variety. Wider leaf sizes tend to have more surface area to absorb sunlight. This is important because the wider the leaf surface area, the more leaf cells can contain chloroplasts, which contain chlorophyll. Increasing the amount of chlorophyll in leaves can increase photosynthesis efficiency because more light can be absorbed and converted into chemical energy. These energy-rich compounds are essential for carbon fixation during the Calvin cycle, which results in greater production of sugars and other primary metabolites that support plant growth and development. (Zhou et al., 2023).

Number of leaves . The leaves of soybean plants are essential to their structure and function, which is the main part of photosynthesis. The results of the LSD analysis (Table 3) showed that the line with the highest number of leaves was GS 7 (74.00), which was not significantly different from Slamet (72.73), several lines showed a higher number of leaves than Grobogan, namely GS 30 (70.33), GS 36 (61.50), GS 39 (61.66), GS 41 (60.00), and GS 55 (62.50), in addition, all lines showed a higher number of leaves than the Wilis variety. The number of leaves is important, because the increase in the number of leaves, the light needed for plant photosynthesis will increase. The increase in seed weight per plant and yield (tons/ha) are also related to the rise in soybean leaves. This can happen because the light that can be captured increases with the increase in the number of leaves. Hence, it has the opportunity to increase the photosynthesis process, and the potential for assimilates translocated to the seeds will also be greater. However, the disadvantage of having many leaves is that the chance of overlapping will increase, so little light is received (Chiozza et al., 2024). However, the more leaves there are, the greater the possibility that the leaves will overlap so that the light the leaves receive becomes limited. The limited light received by the leaves will cause the leaves to be inefficient in producing photosynthate because the photosynthesis process is not optimal (Burgess et al., 2017).

Number of segments. The segments are essentially the internodal sections between leaf nodes, play a key role in determining the overall plant height and the distribution of reproductive structures such as flowers and pods. In soybean, a higher number of segments often correlates with increased potential for branching and pod formation, which can ultimately influence yield. The results of the LSD analysis (Table 3) showed a greater number of segments than Grobogan and Slamet, namely GS 14 (9.33) and GS 24 (9.33). In addition, there were numbers of segments that were not significantly different from Grobogan and Wilis, namely GS 6 (8.66), GS 7 (8.00), GS 41 (8.66), and GS 58 (8.50). There were lines that were not significantly different from Slamet, namely GS 30, GS 36, GS 37, GS 38, GS 39, GS 47, and GS 49, and other lines had a lower number of segments than the three check varieties. Therefore, number of segments determines of a plant's reproductive potential, as each node can produce inflorescences that produce flowers and pods. Increasing node number generally results in a higher capacity for flower and pod formation, which positively contributes to overall seed yield.

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This trait is influenced by genetic factors and environmental conditions that regulate vegetative growth and node differentiation.

Dry Weight of Soybean. The dry weight of soybean plants is the amount of organic matter produced by the plant. Based on Table 2, the dry weight of the F10 line exceeded the Slamet variety; some exceeded the Grobogan variety, some exceeded the Wilis variety, and some were less than check varieties. The results of the LSD analysis (Table 3) showed that the GS 36 line (26.20 g) had a higher dry plant weight compared to the other lines and comparison varieties, namely Slamet (20.77 g), Grobogan (17.23 g) and Wilis (15.01 g) varieties. These results indicate that the GS 36 line has better dry biomass formation potential than the three comparison varieties. This advantage is caused by genetic factors that support more considerable plant growth. Li et al. (2024) stated that plant lines exhibiting high dry matter weight tend to have more efficient nutrient uptake and utilization capacities. This increased efficiency is associated with a well-developed root system that increases access to nutrient uptake, as well as more effective internal transport and metabolic systems for distributing and assimilating nutrients throughout the plant. Higher dry matter weight generally reflects optimal plant physiological performance, including photosynthetic activity, nutrient utilization efficiency, and biomass allocation.

Number of pods per segment. The number of pods per soybean plant node can vary depending on genetic factors, environment, and agronomic treatments applied. The results of the LSD analysis (Table 4) on the number of pods per segment (Table 4) showed that the lines with the high number of pods per segment were GS 6 (7.58), GS 38 (7.58), and GS 58 (7.25) which was higher than Grobogan (5.66 pods) and Wilis (4.83 pods) but lower than Slamet (8.25 pods). Each soybean line has a different genetic potential for pod formation at each stem segment. However, none of the selected lines has inherited the characteristic of high pod number per segment, which is comparable to the Slamet variety. The number of pods per segment is an important quantitative trait in determining the yield potential of soybean plants. This trait is controlled by several genes (polygenic), which act additively and are influenced by genetic interactions and environmental factors. Therefore, it is not a single gene that determines pod number, but rather a combination of many genes, each making a small contribution to the expression of the trait. This polygenic trait leads to wide variation among genotypes, as seen in the results of this study (Kumar et al., 2023).

Table 4. Number of pods per segment, total number of pods, weight of 100 seeds (g), seed weight per plant (g) of F10 from Grobogan x Slamet cross

Treatment	Number of pods per segment	Number of pods	Weight of 100 seeds (g)	Seed Weight per plant (g)
Slamet	8.25 a	70.33 a	13.19 abc	11.31 bc
Grobogan	5.66 fg	59.83 abc	15.63 a	17.71 a
Wilis	4.83 g	29.33 f	12.47 abc	7.30 d
GS 6	7.58 ab	56.66 abcd	11.31 bc	9.34 cd
GS 7	6.83 bcde	51.00 bcde	14.09 abc	13.51 b
GS 12	7.08 bcd	41.83 ef	14.83 ab	12.63 bc
GS 14	7.16 bcd	57.50 abcd	14.21 abc	12.37 bc
GS 24	7.41 abc	52.16 bcde	10.73 c	11.50 bc
GS 30	6.91 bcde	63.16 ab	11.19 c	12.08 bc
GS 36	6.66 bcde	59.66 abc	14.19 abc	12.45 bc
GS 37	7.16 bcd	47.16 cde	12.71 abc	10.80 bcd
GS 38	7.58 ab	54.66 bcde	13.74 abc	11.78 bc
GS 39	6.50 cdef	44.33 de	14.26 abc	9.98 bcd
GS 41	6.33 def	46.83 cde	12.84 abc	10.26 bcd
GS 47	6.00 ef	64.66 ab	13.26 abc	13.48 b
GS 49	6.33 bcde	61.83 ab	11.84 bc	11.89 bc
GS 55	6.66 bcd	55.66 bcd	13.53 abc	12.60 bc
GS 58	7.25 ab	54.66 bcde	12.91 abc	12.71 bc

Note: Means followed by the same letter in a column indicate no significant difference based on LSD at $\alpha < 0.05$.

Number of pods. The LSD analysis (Table 4) results showed that the Slamet variety had the highest number of pods (70.33). This was followed by the GS 30 (63.16), GS 47 (64.66), and GS 49 (61.83) lines, each with a higher number of pods than Grobogan (59.83). All tested lines had a higher number of pods than Wilis. The diversity in the number of pods reflects the genetic potential of each line in producing pods. The GS 30, 47, and 49 lines can be categorized as fairly productive, considering the number of pods they produce is higher than the other two comparison varieties (Grobogan and Wilis) but have not reached the maximum potential like the Slamet variety. This indicates that the number of pods in soybeans is controlled by a complex and quantitative group of genes (Tayade et al., 2023). Because it is controlled by many genes, each with a relatively small effect, the process of genetic segregation in the resulting cross-breeding generation results in a wide variety of phenotypes, encompassing trait values that can be found between the two parents. This diversity reflects the varying combinations of alleles of the genes responsible for the trait. In addition, interactions between genes and interactions between genes and the environment also contribute to expanding the observed phenotypic diversity.

Weight of 100 seeds. The weight of 100 seeds indicates how large or small a soybean seed is. In Indonesia, soybean seed sizes are classified into three categories: small (< 10 g/100 seeds), medium (10-14 g/100 seeds), and large (> 14 g/100 seeds) (Krisnawati & Adie, 2015). The Grobogan variety was included in the large-seeded category, and the Slamet variety was included in the medium-seeded category. The results of the LSD (Table 4) analysis on the weight of 100 seeds per plant (Table 4) showed that the line with the highest value was the Grobogan variety (15.63), followed by GS 12 line (14.83 g per 100 seeds), but several lines had large seed sizes, namely GS7 (14.09 g), GS 12 (14.83 g), GS 36 (14.19 g), and GS 39 (14.26 g). Besides that, there were the diversity in each genotype; some were superior to the Slamet variety, some were superior to the Wilis variety, some lines were under three comparison varieties, and all lines were below the Grobogan variety. Large soybean seeds are a desirable trait in the tempeh industry, as they align with consumer preferences for firmer, more textured, and higher-quality tempeh. In Indonesia, a shift in preference among soybean farmers and the tempeh industry from medium-sized seeds to larger soybean seeds (14 g/100 seeds) has been a significant factor in driving genetic improvement of soybean varieties (Kuswantoro et al., 2020).

Seeds Weight per Plant. The results of the LSD analysis (Table 4) on seed weight per plant (Table 4) The highest yield was found in the Grobogan variety (17.71 g), while the line with the high seed weight, namely GS 7 (13.51 g) and GS 47 (13.48 g), but still below Grobogan's yield and higher than the comparison varieties, namely Slamet and Wilis. Besides that, there were variations in the results obtained in the seed weight variable per plant; 11 lines exceed the seed weight above the Slamet variety and all lines have seed weights higher than the Wilis variety. Tayade et al., (2023) stated that the difference in seed weight is due to the genetic characteristics of the plant related to the size and number of seeds. Genetically, some plants have a tendency to produce large seeds with higher mass, while others produce seeds in greater numbers but with relatively small sizes such as the Slamet variety. This variation reflects differences in the regulation of genes involved in embryo development, seed filling, and the efficiency of photosynthates allocation to reproductive organs. The GS 7 line has the highest weight compared to other lines. This is supported by the weight of 100 seeds, which is included in the large seed category.

Principal Coordinate Analysis. Figure 1 showed that the result of Principal Coordinate Analysis (PCoA) which illustrates the genetic relationship between soybean genotypes resulting from a cross between the Grobogan and Slamet varieties. Based on the analysis results, the genotypes are divided into two main groups. The first group (in green) consists of genotypes GS 7.n, GS 12.n, GS 14.n, GS 36.n, and GS 39.n which are genetically close to each other. This closeness is based on qualitative traits and also the main character that is the goal of this study, namely large seed size and high production. Meanwhile, the second group (in red) includes other genotypes such as GS 6.n, GS 24.n, GS 30.n, and GS 58.n which are lines with medium seed size (less than 14 g/100 seeds). However, GS 7 is located at different coordinates, this shows that apart from having large seed size, GS 7 is also a high-yielding line. This indicates the presence of the main traits that are the purposes of this soybean breeding, namely large seeds and higher production compared to other lines. Increasing seed size is a crucial way to increase soybean yield. Seed composition and reserve content directly determine this. Soybeans with larger seeds have higher oil content, while soybeans with smaller seeds typically have lower oil content than cultivated soybeans. However, seed protein content does not increase in large-seeded soybean cultivars. Therefore, soybean quality improvement involves increasing seed size, which correlates with oil accumulation and possibly accompanying changes in protein content (Wang et al., 2020).

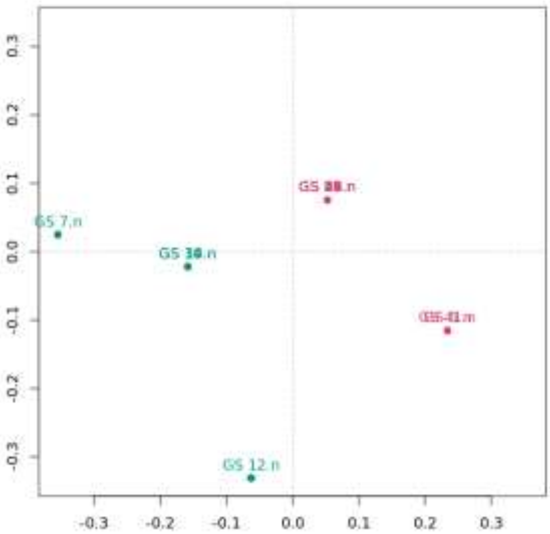

Figure 1. Principal Coordinate Analysis of F10 Lines of Grobogan x Slamet Cross

Correlation Analysis. Growth components are closely related to yield. The relationships between different plant traits are a common phenomenon and understanding these correlations is valuable, particularly as a basis for selection programs. The type of correlation used is Pearson correlation. Correlation analysis is useful for identifying the relationships between two or more variables; however, it cannot precisely distinguish the direct and indirect effects of each trait (Khomphet et al., 2022).

The results of the correlation analysis in Table 5 showed that not all characters are correlated with each other. The analysis results show that the variable plant height is related to leaf length and the number of pods per segment. The level of correlation between plant height, leaf length, and pods per pod is strongly positive, with coefficient figures of 0.621 and 0.695. This correlation is indirect, as taller plants tend to have more stem segments, which could produce more pods overall or, through more optimal photosynthesis, could increase the number of pods per segment. According to Miao et al. (2024), plant height is an important agronomic trait related to apical dominance, plant architecture, cultivation, harvesting, yield, and lodging. Yu et al. (2020) adding that ideal plant height will open up space to obtain more sunlight than non-ideal plants. With more exposure to light, plants can photosynthesize more efficiently, converting light energy into chemical energy stored in sugar. This sugar provides the energy needed for growth, reproduction, and pod formation. So tall plants allow for high yields.

Table 5. Correlation matrix between soybean characters

Karakter	PH	LL	LW	TNS	NP	NL	W100S	SWP	NPS	DWP
PH	1	0.621**	0.089	-0.270	0.122	0.074	-0.132	-0.154	0.695**	0.344
LL		1	0.223	-0.202	0.424*	0.746**	-0.158	0.068	0.693**	0.488*
LW				-0.295	0.290	0.278	0.316	0.323	0.139	0.746**
TNS				1	-0.123	-0.165	-0.313	-0.036	-0.113	-0.389
NP					1	0.493*	-0.065	0.386	0.438*	0.324
NL						1	0.097	0.192	0.380	0.476*
W100S							1	0.553**	-0.189	0.135
SWP								1	-0.182	-0.017
NPS									1	0.404*
DWP										1

Note: PH = Plant height; LL = Leaf length; LW = Leaf width; TNS = Total number of segments; NP = Number of pods; NL = Number of leaves; W100S = Weight of 100 seeds; SWP = Seed weight per plant; NPS = Number of pods per segment; DWP = Dry weight of plant; ** = Highly significant difference; *= Significant difference

The leaf length character is related to the number of pods, the number of leaves, the number of pods per segment, and the dry weight of the plant. The level of closeness of the relationship between leaf length and the number of pods is weakly positive, with a correlation of 0.424, but the number of pods per segment is strongly positive, with a correlation of 0.693. In addition, there is a weakly positive relationship with a correlation of 0.488 against the dry weight of the plant. The character of leaf width is related to the dry weight of the plant. The level of closeness of the relationship between leaf width and plant dry weight is strongly positive, with a correlation of 0.746. The number of leaves is related to the plant's dry weight; the relationship's level of closeness is 0.476. Leaf length is indirectly correlated with pod number in soybean plants. This is because leaf length is a vegetative morphological trait related to the plant's ability to capture light for photosynthesis. Optimal photosynthesis can increase growth and yield, including pod number. However, leaf length does not directly increase pod number; instead, it supports plant physiological processes contributing to yield increases. Therefore, the relationship between leaf length and pod number is indirect through intermediary mechanisms such as photosynthetic efficiency and biomass accumulation (Shi et al., 2019).

There is a positive correlation between the number of pods and the number of pods per segment. However, the strength of this relationship is weak, with a correlation coefficient of 0.438. The total number of pods per plant reflects the plant's capacity to produce pods. This number is influenced by the distribution of resources, as the products of photosynthesis, along with available water and nutrients, must be shared among a greater number of pods (Zewide & Ademe, 2025).

The number of pods per segment is related to the plant’s dry weight. However, the strength of this relationship is only weakly positive, with a correlation coefficient of 0.404. The indirect correlation between the number of pods per segment and the dry weight of the plant shows that in plants with a high number of pods, larger photosynthetic organs likely supply them, which indicates that the dry weight of the plant is greater. Each pod formed on the plant requires energy and nutrients produced through photosynthesis. A higher number of pods per segment is typically supported by the abundant supply of energy and resources provided by the plant through its photosynthetic organs. Plants with a greater number of pods per segment generally also have more biomass, which is reflected in a higher dry weight (Vogel et al., 2021).

The weight of 100 seeds shows a strong positive correlation with the total seed weight per plant, with a correlation coefficient of 0.553. The relationship between 100-seed weight and total seed weight per plant is a direct correlation. This is because 100-seed weight reflects the average seed size, one of the main components determining the total seed yield per plant. Assuming the number of seeds is relatively constant or does not vary significantly between plants, increasing the weight per 100 seeds will directly increase the total seed weight per plant. In this context, the relationship between the two characteristics is causal and linear: the larger the seed size (100-seed weight), the greater the total seed weight produced by the plant. According to Wang et al. (2025), the weight of 100 seeds largely depends on seed size. This was also supported by Baek et al. (2020), who explained that while the maximum potential seed size is genetically determined, the actual size of the seeds is influenced by environmental conditions during the seed-filling stage. Similarly, Mai et al. (2023) found that seed production per plant is correlated with the weight of 100 seeds. The total seed weight per plant reflects the plant's ability to utilize assimilates during seed filling, which is also related to the number of pods produced. So, the higher 100-seed weight tends to result in a higher total seed weight per plant.

The GS 7 line is a line that meets the criteria for breeding objectives, namely having a seed weight per plant that exceeds the comparison variety Slamet and is also the highest among other lines, which is 13.51 g. In addition, GS 7 also has a seed size that is included in the large category, with a weight of 100 seeds exceeding 14 g. However, the dry weight of the GS 7 plant is not higher than the Slamet variety. This condition indicates that GS 7 has a higher efficiency in translocating the results of photosynthesis to generative organs, especially pods, compared to vegetative parts or others biomass. This efficiency is an important indicator in plant breeding because it shows the ability of plants to optimize the final result without having to increase the total biomass.

Conclusion

The morphological characteristics of the F10 lines resulting from the Grobogan × Slamet soybean cross showed traits namely flower color, hypocotyl color, seed color, leaf hairiness, and leaf hair color that were consistent with those of the parent varieties. There was diversity in quantitative traits, but GS 7 line was potential as a candidate variety due to its leaf length and number of leaves, so that had high seed weight per plant (13.51 g), high number of pods (51 pods), and large seed size, with a 100-seed weight of 14.09 g. However, there was no strain that has a higher number of seeds per plant and a weight of 100 seeds than Grobogan. There were also correlations observed among several traits, notably between the number of pods and 100-seed weight with the seed weight per plant.

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