

Jurnal

KULTIVASI

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PREFACE

The third issue of 2025, *Kultivasi* Volume 24 No. 3, presents research articles and review papers covering a wide range of agricultural scientific disciplines, including plant production, plant breeding, postharvest technology, seed science, and soil science. This edition also features contributions from authors across the globe, providing diverse perspectives and novel insights into contemporary agricultural research. Such disciplinary and geographical diversity is expected to enrich agricultural knowledge and further support the advancement of scholarly research and publication.

The *Kultivasi* editorial team remains committed to continuously improving article quality to ensure sustainable growth and to strengthen the journal's international presence. We strive to provide the best possible support for authors, editors, reviewers, and stakeholders within the agricultural sector. We sincerely express our gratitude to all scholars and researchers who have contributed to the development of *Kultivasi*, particularly those whose work appears in this issue.

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 supplementary 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 written in detail so that it is repeatable and reproducible. 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:

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Sitompul KN · Mubarak S · Kusumiyati

Role of nano edible coating, packaging, and storage temperature in maintaining color, moisture content, and phenolic compounds of straw mushroom

Abstract. Straw mushrooms are among the most sought-after edible mushrooms, but their harvest is highly perishable due to their high respiration and transpiration rates, leading to a decline in quality during storage. The application of nano edible coating can cover the surface of straw mushrooms and maintain their freshness, while packaging and storage at low temperatures can maintain quality by inhibiting respiration and enzymatic activity. This study aims to evaluate the effects of nano edible coating, packaging types, and different storage temperatures on the color, moisture content, and phenolic compounds of straw mushrooms. The study was conducted at the Horticulture Laboratory, Faculty of Agriculture, Universitas Padjadjaran using a Completely Randomized Design (CRD) consisting of a combination of nano edible coatings (nano sodium alginate and nano aloe vera), packaging types (vacuum packaging, biodegradable, and wrap), and storage temperatures (room temperature (± 25 °C), 10 °C, and 5 °C) so that there are 18 treatment combinations. Each treatment was repeated twice, resulting in 36 experimental units at each observation time. Observations were conducted at 3 and 6 days after storage, resulting in 72 samples observed. The results showed that the combination of nano edible coating, packaging type, and different storage temperatures significantly affected the color, water content, and phenolic compounds of straw mushrooms. The combination of nano aloe vera with vacuum packaging at 10 °C had the best effect on the L^* , b^* , chroma, h° , total phenolic, and total flavonoid values of straw mushrooms during storage.

Keywords: Edible mushroom · Flavonoid · Perishable food

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Introduction

Straw mushroom (*Volvariella volvacea*) is an edible mushroom that is highly sought after due to its taste and nutritional content. Straw mushrooms possess a tasty flavor, so they are used in various foods (Hendrawani & Hulyadi, 2023). Straw mushrooms are also beneficial for health because they contain secondary metabolite compounds such as phenolics and flavonoids (Elawati et al., 2021; Ali et al., 2024). However, the straw mushroom harvest has a high respiration and transpiration rate so it is easily damaged compared to other fresh commodities (Wang et al., 2017). The high respiration and transpiration rates are caused by the mushroom not having a cuticle layer that protects it from mechanical damage and microbial attack during storage (Kumari & Baskaran, 2015; Castellanos-Reyes et al., 2021). According to research by Sakinah et al. (2020), straw mushrooms have a short shelf life at room temperature, for about 1-2 days. This shows that proper post-harvest treatment is necessary to maintain the quality of straw mushrooms.

Nano edible coating is a post-harvest technology that can maintain the quality of straw mushrooms during storage. Nano edible coating has a smaller particle size and a wider contact area, thus providing a better effect (Filho et al., 2021). The use of nano edible coating can maintain freshness and color (Saputra et al., 2023). Sodium alginate and aloe vera are examples of natural polysaccharides that can be used as nano edible coating materials. Nano coatings made from sodium alginate are able to form a gel that is hydrophilic, transparent, and shiny (Puscaselu et al., 2020). With these capabilities, sodium alginate coatings are able to maintain moisture, freshness, and improve the appearance of coated products (Parreidt et al., 2018). Nano coatings made from aloe vera can also maintain product freshness and appearance because they contain antioxidants, antimicrobials, and antifungals (Heř et al., 2019; Mendy et al., 2019).

Straw mushrooms are a storage condition-sensitive food product. Storage temperature affects respiration, microbial growth, and enzyme activity (Claassen et al., 2024). Inappropriate storage temperatures make mushrooms wet or dry, making them unsafe for consumption. Storage at high temperatures enhances mushroom respiration and enzyme

activity (Cliffe-Byrnes & O'Beirne, 2007; Iqbal et al., 2009; Silva et al., 2025). Elevated enzyme activity leads to nutritional degradation and discoloration of mushrooms (Nugraheni et al., 2023). Straw mushrooms can be kept under low-temperature storage to extend their freshness. However, very low temperatures lead to a reduction in quality. Freezing storage temperatures make mushroom cells freeze, leading to cell wall damage that, when thawed, degrades cell structure and tissue (Ilyas & Soeka, 2019; Tan et al., 2021). The deterioration of cells makes the mushrooms discolour and experience chemical changes (Moutia et al., 2024).

The temperature conditions of storage can affect the humidity of the air around the straw mushroom. Excessive humidity can reduce the quality of stored straw mushrooms. High humidity leads to condensation in the packing, especially when the temperature fluctuates (Dehghani et al., 2024). Condensed water in the packaging adheres to the mushroom, inducing the growth of microbes and causing color and texture alterations (Castellanos-Reyes et al., 2021). Excess water vapor from product respiration inside the packaging may condense and cause product shrinkage and deterioration (Basha et al., 2022). Low humidity in storage can deplete the moisture content (Gidado et al., 2024).

The deterioration in the quality of straw mushrooms due to condensation of water vapor within the packaging can be mitigated through the use of appropriate packaging methods. The packaging approach significantly influences the stability of mushroom quality during storage (Kumar et al., 2020). Packaging that effectively regulates humidity and restricts the inflow of external air can help preserve the physicochemical quality of mushrooms (Mahajan et al., 2008; Geyer et al., 2015). Furthermore, suitable packaging minimizes physical damage throughout the distribution chain and contributes to an extended shelf life (Joshi et al., 2018; Sharma et al., 2024).

In selecting appropriate packaging, it is essential to consider the characteristics of the packaging material, storage temperature, and duration. This study employed three types of packaging: vacuum, biodegradable, and wrap. Vacuum packaging is a sealed system with reduced oxygen levels, thereby limiting moisture accumulation resulting from respiration (Chetti et al., 2014; Chen et al., 2022). Wrap packaging helps prevent secondary microbial

contamination and reduces spoilage and weight loss (Dhall et al., 2012). Biodegradable packaging, in addition to being environmentally friendly, offers improved air permeability (Cheng et al., 2024).

Vacuum packaging occupies a smaller internal volume compared to biodegradable and wrap packaging, leading to differences in respiration activity and humidity levels within the package. The volume of the packaging space plays a crucial role in regulating the respiration rate of mushrooms, as the amount of available gas inside the package is directly influenced by this volume (Pogorzelska-Nowicka et al., 2020). Additionally, both storage temperature and duration significantly impact the respiration rate of mushrooms (Joshi et al., 2018). There is still a lack of research on how nano edible coating treatment, packaging type, and storage temperature affect straw mushroom quality. Based on these criteria, this study aims to evaluate the effects of nano edible coating, packaging types, and different storage temperatures on the color, moisture content, and phenolic compounds of straw mushrooms.

Materials and Methods

Time and Location. The research was conducted at the Horticulture Laboratory, Faculty of Agriculture, Universitas Padjadjaran, from September 2024 to March 2025. The straw mushroom samples used were sourced from farmers in Purwakarta. The samples were at the same harvest age, 15 days after sowing the seeds in the growing medium (not blooming/edged), and were free from damage.

Tools and materials. The tools used were a Mettler Toledo analytical balance type AG254 (Switzerland), a Memmert oven type UM300 (Germany), a Konica Minolta reflectance spectrophotometer type CM-600D (Japan), a grinder, a Shimadzu UV-Vis spectrophotometer type UV-1601 (Japan), a centrifuge, a BK-2000 ultrasonic cleaner, a vacuum sealer, a refrigerator, cooling storage, a freezer. The materials used are: semi-edge straw mushrooms, nano aloe vera, nano sodium alginate, methanol, distilled water, folin reagent, sodium carbonate, gallic acid, aluminum chloride, sodium acetate, quercetin, vacuum plastic, biodegradable plastic, styrofoam, and plastic wrap.

Preparation and Application of Nano edible Coating. Sodium alginate and aloe vera were used as coatings on straw mushrooms and subjected to nano-treatment. The nano-sodium alginate and nano aloe vera were produced by the Functional Nano Powder University Center of Excellence (Finder U-CoE) at Universitas Padjadjaran, Indonesia. The particle size of the nano edible coating was approximately 300 nm. A 1% nano edible coating was made by dissolving 1 g of nano-sodium alginate in 100 mL of distilled water, then mixing until uniform. The same process was used to prepare a 1% nano aloe vera solution. Fresh, cleaned straw mushrooms were coated by brushing with nano sodium alginate or nano aloe vera. The coated mushrooms were air-dried before packaging and storage.

Packaging and Storage. Straw mushrooms that have been coated using 1% nano sodium alginate or 1% nano aloe vera are then packaged using vacuum, biodegradable, wrap packaging of ± 100 g each. Packaging using vacuum packaging is done using a vacuum sealer. Wrap packaging uses plastic wrap or cling wrap and Styrofoam. Biodegradable packaging uses biodegradable plastic. The packaged mushrooms are stored at three temperatures, namely room temperature (± 25 °C), 10 °C and 5 °C for 6 days. Observations are made at 3 and 6 days after storage (DAS).

Experimental Design and Analysis. The experimental design used was a Completely Randomized Design (CRD). Treatments consisted of combinations of nano edible coating types (nano sodium alginate, nano aloe vera), packaging types (vacuum, biodegradable, and wrap packaging), and storage temperatures (room temperature (± 25 °C), 10 °C, and 5 °C). Each treatment combination had two replications, resulting in 36 experimental units. Each experimental unit consisted of approximately 100 g of straw mushroom, observed at 3 and 6 days after storage (DAS), resulting in 72 straw mushroom samples. The data obtained were tested for normality and homogeneity, then analyzed using analysis of variance (ANOVA) at a significance level of 5% to determine the effect of the given treatment. When the calculated F-value exceeded the critical F-value at the 5% significance level, mean separation was performed using the Scott-Knott advanced test at a 5% significance level with SmartStatsXL software.

Observation Parameters: Color. Color measurements were performed using a Konica Minolta reflectant spectrophotometer that can measure color quantitatively (Kusumiyati et al., 2022). Measurements were made at two points, namely the front and back of the straw mushroom, and were repeated three times. The data obtained per sample were averaged. The values displayed by the instrument are L^* , a^* , and b^* . The hue angle (h°) and chroma (C) were calculated using the following formula: hue = $\tan^{-1}(b/a)$ and chroma = $(a^2+b^2)^{1/2}$.

Moisture Content. Measurements were made by cutting 4 g of the straw mushroom fruit bodies, referring to SNI, and Kusumiyati et al. (2021). The sample was placed in a cup whose weight had been constant. The cup was placed in an oven at 105 °C for 3 hours. The sample was removed from the oven and cooled for 5 minutes in a desiccator before being weighed. Heating was repeated until the sample weight was constant. The formula for calculating water content is:

$$\text{Moisture content (\%)} = \frac{\text{fresh weight} - \text{dry weight}}{\text{fresh weight}} \times 100$$

Total Phenolic. A 100 mg sample of the dried sample was weighed and transferred into a 10 mL volumetric flask. Methanol solvent was added to the mark. The solution was extracted with a sonicator at room temperature for 60 minutes. The solution was transferred into a centrifuge tube and centrifuged for 10 minutes at 4000 rpm. The supernatant was transferred to a vial. Total phenolics were determined using a modified Folin-Ciocalteu (Sytar et al., 2018). A 0.3 mL sample was extracted, 0.2 mL of methanol and 2.5 mL of 10% Folin reagent were added, then incubated for 5 minutes at room temperature. 2 mL of 7.5% sodium carbonate was added and homogenized. The solution was incubated at room temperature for 60 minutes. A standard solution was prepared by dissolving 10 mg of gallic acid in 10 mL of methanol to obtain a gallic acid concentration of 1000 mg/L and diluting it to 20, 40, 80, 160, and 320 mg/L. The absorbance of the sample and standard was measured at a wavelength of 765 nm using a Shimadzu UV-1601 UV-Vis spectrophotometer (Japan). The total phenolic content in the sample was expressed in GAE mg/100 g using the calculation formula:

$$\text{Total phenolic} \left(\frac{\text{mg GAE}}{100 \text{ g}} \right) = \frac{C \cdot V}{B} \times 100$$

Note: C = Gallic acid concentration (mg/L); V = Volume of test solution (L); B = Sample weight (g)

Total Flavonoid. A 100 mg sample of the dried sample was weighed and transferred into a 10 mL volumetric flask. Methanol solvent was added to the mark. The solution was extracted with a sonicator at room temperature for 60 minutes. The solution was transferred into a centrifuge tube and centrifuged for 10 minutes at 4000 rpm. The total flavonoid measurement procedure refers to Sytar et al. (2018), which has been modified. 1 mL of the extracted sample was added with 2 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M sodium acetate, and 1.8 mL of distilled water. The solution was homogenized, then incubated at room temperature for 30 minutes. A blank was also prepared using the same procedure, but the sample was replaced with methanol. A standard solution was prepared by dissolving 10 mg of quercetin in 10 mL of methanol, obtaining a quercetin concentration of 1000 mg/L, and then diluting it to 4, 8, 16, 32, 64, and 128 mg/L. The absorbance of the sample and standard was measured at a wavelength of 435 nm using a Shimadzu UV-VIS Spectrophotometer (Japan). The absorbance of the standard measurement was used to create a calibration curve. The total flavonoid content in the sample was expressed in QE mg/100 g.

$$\text{Total flavonoid} \left(\frac{\text{mg QE}}{100 \text{ g}} \right) = \frac{C \cdot V}{B} \times 100$$

Note: C = Quercetin concentration (mg/L); V = Volume of test solution (L); B = Sample weight (g)

Results and Discussion

Color (L^* value). The results of the analysis of variance showed that the nano edible coating treatment, packaging type, and storage temperature had a significant effect on mushroom color in terms of L^* values. The combination treatment of nano sodium alginate with wrap packaging at a temperature of 10 °C showed the highest L^* value, which was not significantly different from the combination treatment of nano aloe vera with vacuum packaging at a temperature of 10 °C and significantly different from other combination treatments, especially at a temperature of 5 °C (Table 1). The above treatment also showed a constant L^* value during 6 days of

storage. Semi-fresh straw mushrooms have a bright grayish-light brown color. Color changes indicate a decrease in the quality of straw mushrooms during storage (Khan et al., 2021)

The L* value indicates the lightness and whiteness of the color, with a value of 100, a decrease towards 0 indicates browning (Kamali et al., 2024). A higher and constant L* value indicates freshness and better appearance of the straw mushrooms. A constant L* value during storage indicates that the combination of nano sodium alginate treatment with wrap packaging at a temperature of 10 °C can maintain the freshness and quality of the straw mushrooms during storage. The L* value was positively correlated with other color parameters except the a* value, water content, and total flavonoids during 6 days of storage (Figure 1). Thus, color changes are closely related to changes in the biochemical compounds of straw mushrooms during storage.

The combination treatment of nano edible coating with all packaging at 5 °C showed a

lower L* value compared to the storage temperature of 10 °C. The decrease in the L* value indicates a change in the color of the straw mushrooms during storage. The darker color of the button mushrooms is in line with the decrease in the L* value (Lin & Sun, 2019).

Cold stress can damage membrane integrity and increase cell membrane permeability, which contributes to chilling injury symptoms (Li et al., 2023). Damage to the cell membrane causes the polyphenol oxidase (PPO) enzyme in its bound form to change into its free form, increasing the free form of the PPO enzyme (Zou et al., 2025). The increase in the free form of PPO is in line with the number of PPO enzymes that oxidize phenolic compounds. Oxidation of phenolic compounds by the PPO enzyme forms quinone compounds and ultimately causes melanin clumping. Melanin clumping causes browning in straw mushrooms at a storage temperature of 5 °C. PPO enzyme activity is the main cause of browning (Harisha et al., 2023).

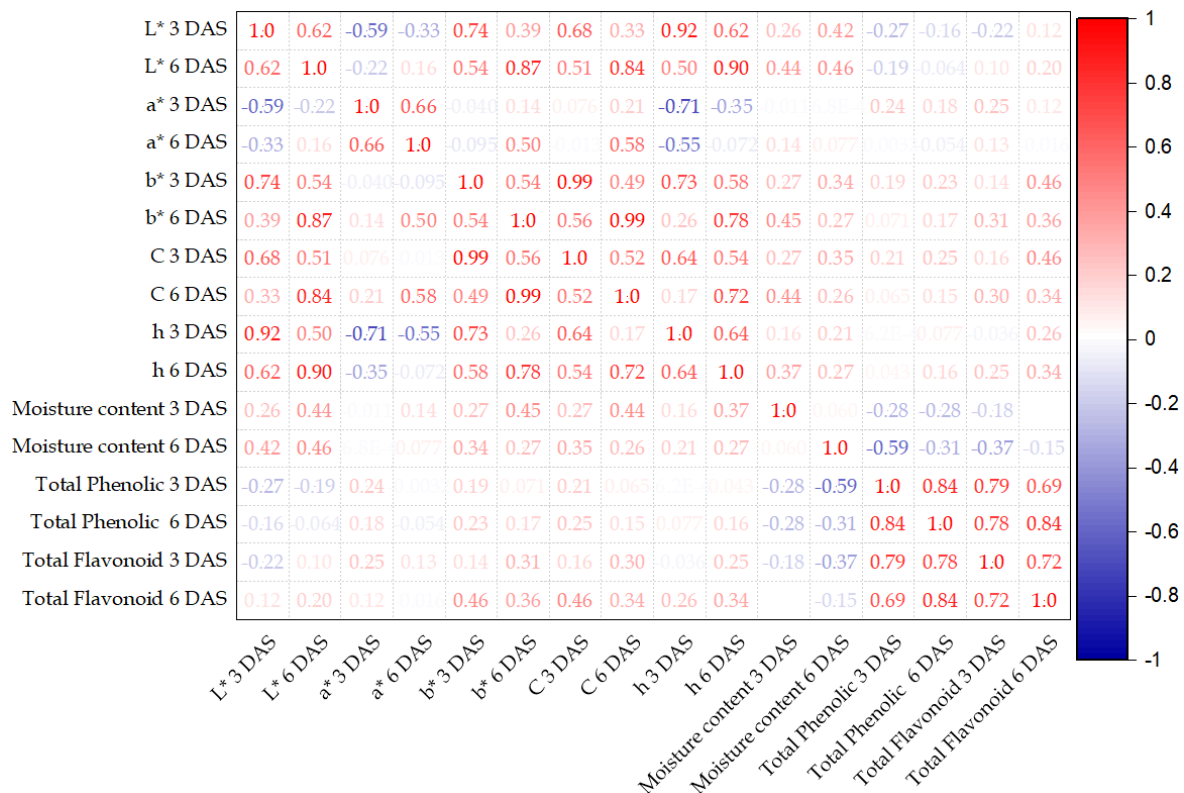


Figure 1. Correlation of all observation parameters during 6 days of storage (DAS)

Table 1. Effect of the combination of nano edible coating, type of packaging, and different temperatures on L* value of straw mushrooms at 3 and 6 days after storage (DAS)

Treatment	L*	
	3 DAS	6 DAS
Nano SA + vacuum + temperature 25 °C	56.27 a	51.06 c
Nano SA + biodegradable + temperature 25 °C	53.55 a	44.07 b
Nano SA + wrap + temperature 25 °C	54.98 a	56.05 d
Nano SA + vacuum + temperature 10 °C	56.88 a	55.53 d
Nano SA + biodegradable + temperature 10 °C	52.62 a	48.88 c
Nano SA + wrap + temperature 10 °C	62.70 a	62.78 d
Nano SA + vacuum + temperature 5 °C	50.66 a	51.04 c
Nano SA + biodegradable + temperature 5 °C	50.12 a	40.80 b
Nano SA + wrap + temperature 5 °C	38.54 a	49.71 c
Nano AV + vacuum + temperature 25 °C	52.13 a	54.19 d
Nano AV + biodegradable + temperature 25 °C	47.42 a	36.69 b
Nano AV + wrap + temperature 25 °C	60.84 a	50.75 c
Nano AV + vacuum + temperature 10 °C	52.01 a	55.03 d
Nano AV + biodegradable + temperature 10 °C	48.34 a	47.91 c
Nano AV + wrap + temperature 10 °C	62.61 a	58.44 d
Nano AV + vacuum + temperature 5 °C	55.94 a	48.34 c
Nano AV + biodegradable + temperature 5 °C	43.63 a	29.04 a
Nano AV + wrap + temperature 5 °C	43.58 a	48.02 c

Note: Means followed by the same lowercase alphabet in the same column are not significantly different based on Scott-Knott Test at 5 %; DAS = days after storage; SA = Sodium Alginate; AV = Aloe Vera.

In this study, straw mushrooms showed symptoms of chilling injury during storage at 5 °C. A temperature of 5 °C is considered extremely cold for straw mushrooms, which grow optimally at high temperatures (35 °C). Extreme cold storage can trigger chilling injury, which increases PPO enzyme activity, resulting in browning (Sogvar et al., 2020). Browning can reduce the visual and commercial quality of straw mushrooms. Nano edible coatings consisting of sodium alginate and aloe vera, packaged in wrap at 10 °C, produced the highest and most consistent L* values during storage. PPO enzyme activity increased with storage time (Zhao et al., 2021). However, the application of nano edible coatings can inhibit PPO enzyme activity during storage.

The application of nano sodium alginate can inhibit browning in straw mushrooms during storage. Sodium alginate has various biological activities, such as antioxidant, antimicrobial, and biocompatibility. In line with research Louis et al. (2021), the sodium alginate coating can maintain the color of button mushrooms during storage. Sodium alginate coating is transparent, biocompatible, biodegradable, and semipermeable (Kenny et al., 2025). Sodium alginate coating can prevent moisture loss by reducing transpiration (Wang et al., 2020).

Sodium alginate coating has low permeability to fat and oxygen (Nair et al., 2020). Thus, sodium alginate coating can slow oxidation that triggers enzymatic browning reactions.

Aloe vera can also inhibit enzymatic browning because it contains natural antioxidants such as phenolics and flavonoids (Takeungwongtrakul et al., 2022). Enzymatic browning is a chemical reaction that occurs when the PPO enzyme oxidizes phenols to quinones (Gupta et al., 2022). Mohebbi et al. (2012) reported that aloe vera coating can reduce browning of button mushrooms during storage.

Wrap and vacuum packaging reduce and inhibit oxygen contact from the outside into the package during storage, compared to biodegradable packaging. This can reduce the oxidation reaction that causes browning in straw mushrooms. The color of enoki mushrooms can be maintained for 6 days of storage using vacuum packaging (Choi et al., 2022). Therefore, the combination of nano sodium alginate or nano aloe vera with wrap packaging at a temperature of 10 °C has been proven to maintain the lightness of the color of straw mushrooms.

Color (a* value). The results of the analysis of variance showed that the nano edible coating treatment, packaging type, and storage temperature had a significant effect on

mushroom color in terms of a^* values. The combination of nano aloe vera with biodegradable packaging at 25 °C and 10 °C showed significantly different a^* values compared to most other treatments, especially at 5 °C (Table 2). This treatment also showed a constant a^* value for 6 DAS. A constant a^* value indicates that the treatment can maintain the freshness of straw mushrooms during storage. A low a^* value indicates the color of fresh straw mushrooms is not striking.

A positive a^* represents red, whereas a negative a^* represents green (Bahreini et al., 2024). The rise in the value of a^* represents a red color, which signifies enzymatic browning of straw mushrooms. Several variables affect enzymatic browning, such as oxygen, phenolic substances, PPO enzymes, and peroxidase (POD), which lead to edible mushroom browning (Sharma et al., 2024). An increase in the a^* value indicates undesirable color changes in straw mushrooms. POD enzymes are associated with undesirable changes in product flavor and color (Singh et al., 2018).

The application of nano aloe vera coating can inhibit the enzymatic browning process by limiting oxygen contact with straw mushrooms at temperatures of 25 °C and 10 °C. Aloe vera coating in antibacterial, protecting the straw mushrooms

from microbial attack that causes colour and appearance changes (Falahati et al., 2024). This agrees with da Silva et al. (2023), finding that aloe vera coating can effectively decrease the discoloration of shiitake mushrooms during storage. Aloe vera coating also contains natural antioxidants that can inhibit the activity of the PPO enzyme. However, its effectiveness decreases at temperatures below 5 °C due to tissue and cell damage caused by cold stress (Figure 2).

Enzymatic browning is linked to membrane breakdown during tissue degeneration (Wang et al., 2024). Extrinsic and intrinsic factors control browning in edible mushrooms. Moisture content, respiration rate, and microbial activity are internal, whereas relative humidity and storage temperature are external (Liang et al., 2024). The decrease in the respiration rate is in line with the lowered metabolic activity that is accountable for straw mushroom browning. Storage temperature significantly affects metabolic processes such as respiration, microbial growth, and enzyme activity, which cause product deterioration (Claassen et al., 2024). Low temperature is capable of retarding metabolic activity, preserving quality (Silva et al., 2025). In line with research by Li et al. (2022), a temperature of 10 °C can inhibit browning of shiitake mushrooms during storage.

Table 2. Effect of the combination of nano edible coating, packaging type, and different temperatures on the a^* value of straw mushrooms at 3 and 6 days after storage (DAS)

Treatment	a^*	
	3 DAS	6 DAS
Nano SA + vacuum + temperature 25 °C	9.12 a	12.16 c
Nano SA + biodegradable + temperature 25 °C	8.08 a	8.23 a
Nano SA + wrap + temperature 25 °C	8.43 a	9.01 a
Nano SA + vacuum + temperature 10 °C	8.89 a	11.32 b
Nano SA + biodegradable + temperature 10 °C	7.81 a	9.60 b
Nano SA + wrap + temperature 10 °C	8.12 a	10.94 b
Nano SA + vacuum + temperature 5 °C	9.32 a	10.66 b
Nano SA + biodegradable + temperature 5 °C	9.66 a	13.84 d
Nano SA + wrap + temperature 5 °C	11.60 b	14.78 d
Nano AV + vacuum + temperature 25 °C	11.45 b	11.43 b
Nano AV + biodegradable + temperature 25 °C	7.97 a	7.81 a
Nano AV + wrap + temperature 25 °C	9.17 a	10.35 b
Nano AV + vacuum + temperature 10 °C	10.50 b	11.67 b
Nano AV + biodegradable + temperature 10 °C	8.05 a	8.53 a
Nano AV + wrap + temperature 10 °C	7.71 a	10.29 b
Nano AV + vacuum + temperature 5 °C	10.13 b	9.89 b
Nano AV + biodegradable + temperature 5 °C	11.18 b	9.88 b
Nano AV + wrap + temperature 5 °C	11.30 b	14.66 d

Note: Means followed by the same lowercase alphabet in the same column are not significantly different based on Scott-Knott Test at 5 %; SA = Sodium Alginate; AV = Aloe Vera.



Figure 2. Color changes of straw mushrooms stored at 5 °C for 3 days: a) nano sodium alginate + vacuum packaging, b) nano sodium alginate + biodegradable packaging, c) nano aloe vera + vacuum packaging, d) nano aloe vera + wrap packaging.

Color (b^*). The results of the analysis of variance showed that the nano edible coating treatment, packaging type, and storage temperature had a significant effect on mushroom color in terms of b^* values. The combination treatment of nano aloe vera with vacuum packaging at a temperature of 10 °C showed the highest b^* value, which was significantly different from other combination treatments, especially the nano sodium alginate combination (Table 3). This combination treatment also showed a constant b^* value during storage. The constant b^* value during storage indicates the treatment's ability to maintain the freshness of straw mushrooms.

The b^* value consists of positive and negative b^* . A positive b^* value indicates a yellow color, while a negative b^* value indicates a blue color (Huo et al., 2024). Nano aloe vera coating treatment can maintain the brownish-yellow color of semi-ripened straw mushrooms during storage. Aloe vera contains flavonoid compounds that can ward off free radicals and act as antioxidants (Tizazu & Bekele, 2024; Wariyah et al., 2024). Mirshekari et al. (2019) also reported that aloe vera coating can reduce the activity of the PPO enzyme, which causes browning in button mushrooms. Several treatments also showed decreases and increases in b^* values during storage.

The decrease and increase in the b^* value during storage are indicators of browning in straw mushrooms. The decrease in the b^* value is a result of the degradation of flavonoid compounds and carotenoid pigments that give the yellow color (Giménez et al., 2015). The oxidation of natural color compounds is exacerbated by temperature and the presence of oxygen, which causes a decrease in quality (Liang et al., 2024). The results of the study showed an increase in the b^* value in several combination treatments, especially at a temperature of 5 °C.

The combination treatment of nano sodium alginate with all packaging at a temperature of 5 °C experienced an increase in the b^* value at 6 DAS (Figure 3). Temperatures that are too low tend to cause post-harvest chilling damage, which actually accelerates browning (Sharma et al., 2024). This temperature accelerates the damage to cell membranes, thereby increasing the oxidation of natural color compounds (Zou et al., 2025). Damage to cell membranes causes an increase in the PPO enzyme, which can oxidize phenolic compounds that form quinone and melanin compounds (Harisha et al., 2023).

A temperature of 5 °C can cause cold stress that triggers the accumulation of free radicals and accelerates cell and tissue damage. Cell and tissue damage causes excessive release of the PPO enzyme, which oxidizes phenolic compounds to quinones through enzyme catalysis. Quinones react with amino acids to form melanin, causing browning in mushrooms (Fu et al., 2023). The increase in PPO enzyme activity increases quinones and melanin, which are indicators of browning of the *Phlebotopus portentosus* fungus (Kong et al., 2025).

Color (Chroma). The results of the analysis of variance showed that the nano edible coating treatment, packaging type, and storage temperature had a significant effect on mushroom color in terms of chroma values. The combination treatment of nano aloe vera with vacuum packaging at 10 °C showed a significantly different chroma value compared to other combination treatments, especially with biodegradable packaging. Biodegradable packaging for all nano edible coatings and storage temperatures showed a lower chroma value and increased during storage. Biodegradable packaging has poor barrier properties, especially against moisture and air (Ivankovic et al., 2017).

Table 3. Effect of the combination of nano edible coating, packaging type, and different temperatures on the b^* value of straw mushrooms at 3 and 6 days after storage (DAS)

Treatment	b^*	
	3 DAS	6 DAS
Nano SA + vacuum + temperature 25 °C	34.52 c	34.21 c
Nano SA + biodegradable + temperature 25 °C	27.60 b	19.20 a
Nano SA + wrap + temperature 25 °C	28.17 b	27.88 b
Nano SA + vacuum + temperature 10 °C	31.99 c	37.10 c
Nano SA + biodegradable + temperature 10 °C	24.91 a	27.18 b
Nano SA + wrap + temperature 10 °C	30.67 c	35.66 c
Nano SA + vacuum + temperature 5 °C	28.14 b	31.56 c
Nano SA + biodegradable + temperature 5 °C	24.66 a	24.96 b
Nano SA + wrap + temperature 5 °C	20.87 a	31.64 c
Nano AV + vacuum + temperature 25 °C	35.78 c	36.36 c
Nano AV + biodegradable + temperature 25 °C	24.10 a	16.01 a
Nano AV + wrap + temperature 25 °C	33.17 c	26.71 b
Nano AV + vacuum + temperature 10 °C	30.99 c	36.65 c
Nano AV + biodegradable + temperature 10 °C	23.16 a	25.03 b
Nano AV + wrap + temperature 10 °C	29.96 c	35.51 c
Nano AV + vacuum + temperature 5 °C	33.14 c	29.88 c
Nano AV + biodegradable + temperature 5 °C	23.73 a	13.21 a
Nano AV + wrap + temperature 5 °C	23.36 a	32.72 c

Note: Means followed by the same lowercase alphabet in the same column are not significantly different based on Scott-Knott Test at 5 %; SA = Sodium Alginate; AV = Aloe Vera.

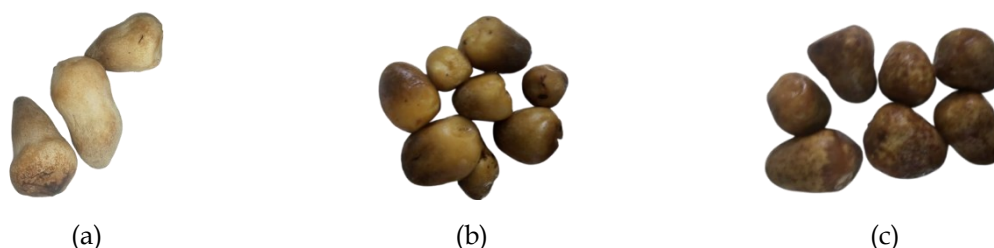


Figure 3. Changes in the color of straw mushrooms during 3 days of storage in the combination treatment of nano sodium alginate at a temperature of 5 °C with various packaging: a) fresh straw mushrooms, b) vacuum packaging, c) biodegradable packaging.

The chroma value indicates color purity or intensity (Pandiselvam et al., 2023). A high and consistent chroma value during storage indicates freshness and good post-harvest visual quality of straw mushrooms. The combination of nano aloe vera with vacuum packaging at 10 °C showed a high and constant chroma value during storage. Aloe vera coating contains natural antioxidant compounds such as aloe-emodin and ascorbic acid, which can maintain the color attributes of the product (Nicolau-Lapeña et al., 2021). The application of aloe vera coating can inhibit color changes in button mushrooms during storage (Mirshekari et al., 2019).

The combination treatment of nano sodium alginate with all packaging and the combination of nano aloe vera with wrap packaging at a temperature of 5 °C showed a low chroma value at 3 DAS and increased at 6 DAS. The increase in chroma value indicates browning in straw mushrooms. This is in line with research by Nunes et al. (2024) that the increase in chroma value is in line with the browning of white shimeji mushrooms. A temperature of 5 °C can cause cell damage, resulting in phenolic compounds and PPO enzymes meeting outside the cells, and enzymatic browning occurs.

Table 4. Effect of the combination of nano edible coating, packaging type, and different temperatures on the chroma value of straw mushrooms at 3 and 6 days after storage (DAS)

Treatment	Chroma	
	3 DAS	6 DAS
Nano SA + vacuum + temperature 25 °C	35.71 b	36.33 c
Nano SA + biodegradable + temperature 25 °C	28.78 a	20.90 a
Nano SA + wrap + temperature 25 °C	29.40 a	29.30 b
Nano SA + vacuum + temperature 10 °C	33.20 b	38.79 c
Nano SA + biodegradable + temperature 10 °C	26.17 a	28.84 b
Nano SA + wrap + temperature 10 °C	31.73 b	37.30 c
Nano SA + vacuum + temperature 5 °C	29.68 a	33.31 c
Nano SA + biodegradable + temperature 5 °C	26.49 a	28.61 b
Nano SA + wrap + temperature 5 °C	23.90 a	34.95 c
Nano AV + vacuum + temperature 25 °C	37.58 b	38.11 c
Nano AV + biodegradable + temperature 25 °C	25.39 a	17.82 a
Nano AV + wrap + temperature 25 °C	34.41 b	28.65 b
Nano AV + vacuum + temperature 10 °C	32.72 b	38.46 c
Nano AV + biodegradable + temperature 10 °C	24.53 a	26.46 b
Nano AV + wrap + temperature 10 °C	30.94 b	36.98 c
Nano AV + vacuum + temperature 5 °C	34.67 b	31.48 c
Nano AV + biodegradable + temperature 5 °C	26.24 a	16.50 a
Nano AV + wrap + temperature 5 °C	25.95 a	35.93 c

Note: Means followed by the same lowercase alphabet in the same column are not significantly different based on Scott-Knott Test at 5 %; SA = Sodium Alginate; AV = Aloe Vera.

Table 5. Effect of the combination of nano edible coating, packaging type, and different temperatures on the h° value of straw mushrooms at 3 and 6 days after storage (DAS)

Treatment	h°	
	3 DAS	6 DAS
Nano SA + vacuum + temperature 25 °C	75.16 b	70.30 c
Nano SA + biodegradable + temperature 25 °C	73.70 b	66.33 b
Nano SA + wrap + temperature 25 °C	73.30 b	72.08 c
Nano SA + vacuum + temperature 10 °C	74.47 b	73.05 c
Nano SA + biodegradable + temperature 10 °C	72.36 b	70.52 c
Nano SA + wrap + temperature 10 °C	75.18 b	72.94 c
Nano SA + vacuum + temperature 5 °C	71.37 b	71.33 c
Nano SA + biodegradable + temperature 5 °C	68.61 b	62.55 b
Nano SA + wrap + temperature 5 °C	60.85 a	64.88 b
Nano AV + vacuum + temperature 25 °C	72.19 b	72.55 c
Nano AV + biodegradable + temperature 25 °C	71.59 b	63.96 b
Nano AV + wrap + temperature 25 °C	74.56 b	68.83 c
Nano AV + vacuum + temperature 10 °C	71.24 b	72.34 c
Nano AV + biodegradable + temperature 10 °C	70.93 b	71.17 c
Nano AV + wrap + temperature 10 °C	75.54 b	73.82 c
Nano AV + vacuum + temperature 5 °C	72.92 b	71.68 c
Nano AV + biodegradable + temperature 5 °C	64.77 a	53.22 a
Nano AV + wrap + temperature 5 °C	64.17 a	67.54 c

Note: Means followed by the same lowercase alphabet in the same column are not significantly different based on Scott-Knott Test at 5 %; SA = Sodium Alginate; AV = Aloe Vera.

Color (h° value). The results of the analysis of variance showed that the nano edible coating treatment, packaging type, and storage temperature had a significant effect on

mushroom color in terms of h° values. The combination treatment of nano aloe vera with vacuum packaging at a temperature of 10 °C showed a hue value (h°) that was significantly

different from other combination treatments, especially the combination with biodegradable packaging at a temperature of 5 °C (Table 5). This treatment also showed a constant value during 6 days of storage. The constant h° value during storage indicates the treatment's ability to maintain color and is one indicator of the freshness of straw mushrooms. Differences in storage temperatures showed different effects on the color of straw mushrooms, especially on the h° value. This indicates that 10 °C can inhibit the activity of the PPO enzyme that causes browning without damaging the straw mushroom tissue. In line with research Kumari & Baskaran (2015) The temperature of 10 °C can reduce the activity of the PPO enzyme in oyster mushrooms.

The h° value indicates the type of color or color angle in the color spectrum (Shameena et al., 2024). A decreasing h° value indicates browning, resulting in a decrease in the visual and commercial quality of straw mushrooms. The combination treatment of nano sodium alginate and nano aloe vera with all packaging at a temperature of 10 °C is able to maintain the h° value during storage. Nano sodium alginate and nano aloe vera can protect mushrooms against a decrease in h° value at a storage temperature of 10 °C. Sodium alginate coating can form a semipermeable protective layer, reducing the rate of respiration and oxidation (Kalita et al., 2025). However, the layer formed is less dense compared to nano sodium alginate. Aloe vera coating has natural antibacterial and antioxidant activity and slows down color changes (Habibi et al., 2022).

Vacuum packaging shows a better effect than biodegradable packaging and wrap packaging in maintaining h° values, especially at a temperature of 5 °C. It can reduce oxygen in the packaging, thereby slowing the oxidation reaction that causes browning. Storage temperature significantly affects the h° value. A temperature of 5 °C indicates a lower h° value. Temperatures that are too low or extreme (≤ 5 °C) can cause chilling injury, which triggers metabolic reactions that cause faster browning (Farcuh, 2025).

Extreme temperatures can also accelerate cell membrane damage and PPO enzyme activity. A decrease in the h° value indicates browning in straw mushrooms. The decrease in the hue value is caused by the activity of the PPO enzyme that causes browning (Liang et al., 2024). Thus, the combination treatment of Nano sodium

alginate and nano aloe vera with wrap packaging at a temperature of 10 °C is the best combination in maintaining the h° value of straw mushrooms.

Moisture Content. The results of the analysis of variance showed that the combination of nano edible coating, packaging type, and different storage temperatures significantly affected the moisture content of straw mushrooms at 3 DAS and did not significantly affect the moisture content at 6 DAS (Table 6). The combination treatment of nano sodium alginate with vacuum packaging at a temperature of 10 °C showed the highest air content, which was significantly different from other combination treatments, especially the combination treatment with biodegradable packaging at 3 DAS. The combination of nano sodium alginate with vacuum packaging at a temperature of 10 °C showed a constant moisture content during storage (3 and 6 DAS). Thus, the combination treatment of nano sodium alginate with vacuum packaging at a temperature of 10 °C can maintain the freshness of straw mushrooms during storage.

Straw mushrooms are an agricultural commodity with a relatively high moisture content. Moisture content is an indicator of the post-harvest technology's ability to maintain freshness, with a constant moisture content being optimal. In this study, the treatment of nano sodium alginate and nano aloe vera coating with vacuum packaging and wrap at temperatures of 10 °C and 5 °C showed a higher water content compared to the combination treatment with biodegradable packaging in 3 DAS. This may be because sodium alginate and aloe vera can inhibit transpiration in mushrooms, which causes water loss. According to Martínez-Cano et al. (2022) The application of sodium alginate edible coating can form a semipermeable layer, thereby reducing water loss and weight loss.

The vacuum packaging used is also more effective in maintaining moisture than biodegradable packaging. Vacuum packaging inhibits mushroom respiration due to controlled atmospheric conditions and minimal air contact with the product (Sakinah et al., 2020). Moradinezhad & Dorostkar (2021) also stated that vacuum packaging can minimize water loss through transpiration and maintain relative humidity within the package. Therefore, vacuum packaging proved more effective in maintaining mushroom moisture during storage than other packaging.

The moisture content of mushrooms in biodegradable packaging showed a lower value than in other packaging. This may be due to the relatively high air and water vapor permeability of biodegradable packaging. This relatively high permeability allows the moisture content in the mushrooms to easily escape, resulting in a decrease in moisture content during storage. Furthermore, maintaining the internal moisture of mushrooms in biodegradable packaging is difficult. The higher and more constant moisture content at a storage temperature of 10 °C also indicates that the respiration and transpiration of straw mushrooms can be inhibited. Iqbal et al. (2009), reported that the respiration rate of button mushrooms decreased at 8 °C and increased at 20 °C. Therefore, the combination of nano sodium alginate with vacuum packaging at 10 °C can be applied to maintain the freshness of straw mushrooms during storage.

Total Phenolic. The results of the analysis of variance showed that the nano edible coating treatment, packaging type, and storage temperature had a significant effect on the total phenolic content of mushrooms. The combination of nano aloe vera with vacuum packaging at temperatures of 10 °C showed a

significantly different total phenolic content compared to other treatments during storage (Table 7). The treatment also showed a constant total phenolic content during storage. A constant total phenolic content indicates the treatment's ability to maintain the quality of straw mushrooms during storage.

Phenolic compounds are bioactive compounds with high antioxidant activity. Phenolic compounds in edible mushrooms act as natural antioxidants (Chun et al., 2021). The total phenolic content indicates the functional value of mushrooms and is related to resistance to oxidation and browning (Liang et al., 2024). The combination treatment of nano aloe vera with vacuum packaging at a storage temperature of 10 °C showed the highest total phenolics. Aloe vera has antioxidant properties because it contains phenolic compounds (Mensah et al., 2025). These phenolic compounds include aloin, aloesin, and aloe emodin (Solaberrieta et al., 2022). These compounds are antioxidants, thus maintaining freshness, inhibiting the activity of the PPO enzyme, and stabilizing phenolic compounds during storage. Mirshekari et al. (2019), reported that aloe vera coating can increase the total phenolic content of button mushrooms during storage.

Table 6. Effect of the combination of nano edible coating, type of packaging, and different temperatures on the moisture content of straw mushrooms at 3 and 6 days after storage (DAS)

Treatment	Moisture content (%)	
	3 DAS	6 DAS
Nano SA + vacuum + temperature 25 °C	90.36 b	91.09 a
Nano SA + biodegradable + temperature 25 °C	86.78 a	91.85 a
Nano SA + wrap + temperature 25 °C	90.80 b	94.75 a
Nano SA + vacuum + temperature 10 °C	90.81 b	89.57 a
Nano SA + biodegradable + temperature 10 °C	88.99 a	89.18 a
Nano SA + wrap + temperature 10 °C	90.84 b	91.24 a
Nano SA + vacuum + temperature 5 °C	90.58 b	89.37 a
Nano SA + biodegradable + temperature 5 °C	87.89 a	88.28 a
Nano SA + wrap + temperature 5 °C	89.90 b	91.16 a
Nano AV + vacuum + temperature 25 °C	88.89 a	90.26 a
Nano AV + biodegradable + temperature 25 °C	90.78 b	82.81 a
Nano AV + wrap + temperature 25 °C	90.33 b	94.90 a
Nano AV + vacuum + temperature 10 °C	89.71 b	88.13 a
Nano AV + biodegradable + temperature 10 °C	88.19 a	89.20 a
Nano AV + wrap + temperature 10 °C	90.67 b	91.86 a
Nano AV + vacuum + temperature 5 °C	90.47 b	90.32 a
Nano AV + biodegradable + temperature 5 °C	88.89 a	89.66 a
Nano AV + wrap + temperature 5 °C	90.69 b	89.94 A

Note: Means followed by the same lowercase alphabet in the same column are not significantly different based on Scott-Knott Test at 5 %; SA = Sodium Alginate; AV = Aloe Vera.

Vacuum packaging can control the atmosphere in the packaging, thereby reducing oxygen availability (Chen et al., 2022). Limited oxygen availability can inhibit phenol oxidation, which causes browning in straw mushrooms. A temperature of 10 °C can maintain total phenolic content because it inhibits PPO enzyme activity and is not too cold to trigger chilling injury.

Total Flavonoid. The results of the analysis of variance showed that the combination of nano edible coating treatment, packaging type, and storage temperature had a significant effect on the total flavonoids of straw mushrooms during storage (Table 8). The combination treatment of nano aloe vera with vacuum packaging at a storage temperature of 10 °C showed the highest total flavonoids, which were significantly different from other treatments. The use of nano edible coating can maintain quality during storage because aloe vera contains polysaccharides such as glucomannan and acemannan (Moghaddasi & Kumar Verma, 2011; Sefrienda et al., 2020). Aloe vera coating also acts as an air barrier for straw mushrooms so that

they can maintain freshness and quality during storage.

Flavonoids are a major group of phenolic compounds that function as natural antioxidants (Huyut et al., 2017). The higher the total flavonoid content, the higher the antioxidant activity of edible mushrooms (Kozarski et al., 2015). However, antioxidant activity can decrease during storage due to the degradation of flavonoid compounds by the PPO enzyme. Based on research results, the use of nano aloe vera with vacuum packaging at a temperature of 10 °C can maintain the total flavonoid content of straw mushrooms during storage.

The stability of total flavonoids during storage is due to the application of aloe vera coating containing natural antioxidants that can inhibit the activity of the PPO enzyme (Ali et al., 2019). Vacuum packaging also supports the performance of nano aloe vera in inhibiting the activity of the PPO enzyme by reducing oxygen, which causes oxidation reactions. Storing straw mushrooms at a temperature of 10 °C can inhibit the activity of the PPO enzyme without causing chilling injury.

Table 7. Effect of the combination of nano edible coating, type of packaging, and different temperatures on total phenolics of straw mushrooms at 3 and 6 days after storage (DAS)

Treatment	Total Phenolic (mg GAE/100g)	
	3 DAS	6 DAS
Nano SA + vacuum + temperature 25 °C	1,615.63 i	3.16 g
Nano SA + biodegradable + temperature 25 °C	1,396.11 h	2.92 e
Nano SA + wrap + temperature 25 °C	270.71 a	2.77 c
Nano SA + vacuum + temperature 10 °C	1,822.54 j	3.34 h
Nano SA + biodegradable + temperature 10 °C	1,184.05 g	3.07 f
Nano SA + wrap + temperature 10 °C	310.48 a	2.47 a
Nano SA + vacuum + temperature 5 °C	1,361.90 h	3.02 f
Nano SA + biodegradable + temperature 5 °C	1,191.59 g	2.88 d
Nano SA + wrap + temperature 5 °C	1,085.00 f	2.71 c
Nano AV + vacuum + temperature 25 °C	1,970.87 k	3.19 g
Nano AV + biodegradable + temperature 25 °C	1,650.71 i	2.74 c
Nano AV + wrap + temperature 25 °C	522.86 c	2.52 b
Nano AV + vacuum + temperature 10 °C	2,042.86 l	3.32 h
Nano AV + biodegradable + temperature 10 °C	1,429.76 h	3.18 g
Nano AV + wrap + temperature 10 °C	414.13 b	2.56 b
Nano AV + vacuum + temperature 5 °C	1,379.13 h	3.07 f
Nano AV + biodegradable + temperature 5 °C	955.95 e	2.94 e
Nano AV + wrap + temperature 5 °C	779.37 d	2.72 c

Note: Means followed by the same lowercase alphabet in the same column are not significantly different based on Scott-Knott Test at 5 %; SA = Sodium Alginate; AV = Aloe Vera.

Table 8. Effect of the combination of nano edible coating, type of packaging, and different temperatures on total flavonoids in straw mushrooms at 3 and 6 days after storage

Treatment	Total flavonoid (mg QE/100g)	
	3 DAS	6 DAS
Nano SA + vacuum + temperature 25 °C	168.16 c	226.06 c
Nano SA + biodegradable + temperature 25 °C	115.99 a	98.19 a
Nano SA + wrap + temperature 25 °C	126.31 b	140.85 b
Nano SA + vacuum + temperature 10 °C	218.05 d	289.04 d
Nano SA + biodegradable + temperature 10 °C	140.21 b	164.65 b
Nano SA + wrap + temperature 10 °C	63.12 a	73.12 a
Nano SA + vacuum + temperature 5 °C	246.77 e	122.48 b
Nano SA + biodegradable + temperature 5 °C	204.22 d	120.85 b
Nano SA + wrap + temperature 5 °C	178.40 c	89.43 a
Nano AV + vacuum + temperature 25 °C	244.82 e	193.58 c
Nano AV + biodegradable + temperature 25 °C	145.82 b	108.26 a
Nano AV + wrap + temperature 25 °C	89.61 a	139.93 b
Nano AV + vacuum + temperature 10 °C	321.42 f	334.75 e
Nano AV + biodegradable + temperature 10 °C	233.40 e	199.50 c
Nano AV + wrap + temperature 10 °C	92.16 a	77.09 a
Nano AV + vacuum + temperature 5 °C	155.67 c	186.91 c
Nano AV + biodegradable + temperature 5 °C	95.57 a	125.00 b
Nano AV + wrap + temperature 5 °C	83.30 a	87.38 a

Note: Means followed by the same lowercase alphabet in the same column are not significantly different based on Scott-Knott Test at 5 %; SA = Sodium Alginate; AV = Aloe Vera.

The results of this study indicate that the combination of nano edible coating, packaging type, and different storage temperatures have important implications in increasing shelf life and maintaining the quality of straw mushrooms, especially for post-harvest applications in perishable commodities. The use of nano aloe vera coating with vacuum packaging at 10 °C has important implications as an effective post-harvest technology in maintaining the color and phenolic components of straw mushrooms, thus potentially increasing their shelf life and commercial value during storage. These findings open up opportunities for future research to evaluate more stable nano edible coating formulations, examine their interactions with various types of packaging, and assess their effectiveness under different storage conditions and at larger production scales. However, this study still has limitations, such as the limited types of nano edible coatings, the limited variation in nano coating concentrations tested, the observation period is too long, and the lack of a more in-depth evaluation of physiological and microbiological changes in mushrooms (e.g., enzymes that play a role in color changes and metabolite compounds of straw mushrooms, as well as disease incidence

parameters), so further research is needed to produce more comprehensive recommendations.

Conclusion

Straw mushroom harvest is easily damaged, so the quality decreases during storage. The results of the study showed that the combination treatment of nano aloe vera with vacuum packaging at a temperature of 10 °C gave the best effect on color parameters (L^* , b^* , chroma, h° values), total phenolics, and total flavonoids of straw mushrooms during storage. The ability of the post-harvest combination treatment above can improve the economy by increasing shelf life, maintaining quality, and reducing post-harvest losses of straw mushrooms. Further research can be done with pre-harvest treatment to improve the quality and shelf life of straw mushroom fruit bodies.

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References

- Ali S, Khan AS, Nawaz A, Anjum MA, Naz S, Ejaz S, Hussain S. 2019. Aloe vera gel coating delays postharvest browning and maintains quality of harvested litchi fruit. *Postharvest Biology and Technology*, 157: 1-7.
- Ali S, Yousaf N, Usman M, Javed MA, Nawaz M, Ali B, Azam M, Ercisli S, Tirasci S, Ahmed AE. 2024. *Volvariella volvacea* (paddy straw mushroom): A mushroom with exceptional medicinal and nutritional properties. *Heliyon*, 10(21):1-14.
- Bahreini Z, Abedi M, Ashori A, Parach A. 2024. Extraction and characterization of anthocyanin pigments from Iris flowers and metal complex formation. *Heliyon*, 10(11): 1-9.
- Basha RK, Zaidy NF, Azmi NS, Tawakkal ISMA, Amin AM, Hasnan NZN. 2022. Effect of packaging materials on antifogging film properties and quality of oyster mushrooms at different storage temperatures. *Advances in Agricultural and Food Research Journal*, 4(2): 1-15.
- Castellanos-Reyes K, Villalobos-Carvajal R, Beldarrain-Iznaga T. 2021. Fresh mushroom preservation techniques. *Foods*, 10(9): 1-25.
- Cheng J, Gao R, Zhu Y, Lin Q. 2024. Applications of biodegradable materials in food packaging: A review. *Alexandria Engineering Journal*, 91: 70-83.
- Chen Q, Qi W, Peng J, Tang R, Liu D, Guo X. 2022. The vacuum and light-avoided packaging ameliorate the decline in quality of whole chili during storage. *Journal of Food Quality*, 2022: 1-9.
- Chetti MB, Deepa GT, Antony RT, Khetagoudar MC, Uppar DS, Navalgatti CM. 2014. Influence of vacuum packaging and long term storage on quality of whole chilli (*Capsicum annuum* L.). *Journal of Food Science and Technology*, 51(10): 2827-2832.
- Choi JW, Lim S, Lee JH, Eum HL, Lee J-S, Park HS, Im J-H, Do KR. 2022. Developed vacuum film packaging method maintains quality of enoki mushrooms (*Flammulina velutipes*) during Simulated Vessel Export to Vietnam. *Korean Journal of Packaging Science and Technology*, 28(2): 133-142.
- Chun S, Gopal J, Muthu M. 2021. Antioxidant activity of mushroom extracts/polysaccharides –their antiviral properties and plausible antiCOVID-19 properties. *Antioxidants*, 10(12): 1-30.
- Claassen GDH, Kirst P, Van ATT, Snels JCMA, Guo X, van Beek P. 2024. Integrating time-temperature dependent deterioration in the economic order quantity model for perishable products in multi-echelon supply chains. *Omega (United Kingdom)*, 125: 1-11.
- Cliffe-Byrnes V, O'Beirne D. 2007. Effects of gas atmosphere and temperature on the respiration rates of whole and sliced mushrooms (*Agaricus bisporus*) – Implications for film permeability. *JFS. Food Engineering and Physical Properties*, 72(4): 197-204.
- Da Silva VG, Dantas TBH, Moitinho D, Usberti FCS. 2023. Xtend® packaging and aloe vera coating benefit shelf life and safety maintenance of fresh shiitake mushrooms. *Revista Ciencia Agronomica*, 54: 1-11.
- Dehghani P, Ami Ahmadi H, Hosseinalipour SM, Akbari H. 2024. Impact of relative humidity, temperature difference, and surface type on humid air condensation heat transfer. *International Journal of Thermal Sciences*, 203: 1-19.
- Dhall RK, Sharma SR, Mahajan BVC. 2012. Effect of shrink wrap packaging for maintaining quality of cucumber during storage. *Journal of Food Science and Technology*, 49(4): 495-499.
- Elawati NE, Lestari CR, Dewi SP. 2021. Proximate analysis of merang mushrooms (*Volvariella volvacea*) cultivated on corncob and rice bran media. *Natural Sciences Engineering and Technology Journal*, 2(2): 92-98.
- Falahati M, Ariaii P, Khoshkhoo Z, Asadi G, Hosseini SE. 2024. Preserving quality of button mushrooms (*Agaricus bisporus*) using nano-chitosan-aloe vera coating with tomato seed protein hydrolyzate. *Research Square*, 1-31.
- Farcuh M. 2025. Understanding chilling injury and sugar metabolism-related genes and metabolites in 'Red Haven' peaches. *Plants*, 14(14): 1-20.
- Filho JG de O, Miranda M, Ferreira MD, Plotto A. 2021. Nanoemulsions as edible coatings: A potential strategy for fresh fruits and vegetables preservation. *Foods*, 10(10): 17.
- Fu Y, Tan H, Wang B, Peng W, Sun Q, Yu Y. 2023. Integrated multi-omic analyses on yellow
- Sitompul KN, Mubarak S, Kusumiyati. 2025. Role of nano edible coating, packaging, and storage temperature in maintaining color, moisture content, and phenolic compounds of straw mushroom. *Jurnal Kultivasi*, 24(3): 249-267.

- Flammulina filiformis* cultivar reveal postharvest oxidative damage responses. *Postharvest Biology and Technology*, 195: 1-12.
- Geyer M, Rux G, Linke M, Mahajan P, Saengerlaub S, Pant A. 2015. Packaging of mushrooms in a humidity-regulating plastic tray for preventing water condensation. *Acta Horticulturae*, 1079: 153-158.
- Gidado MJ, Gunny AAN, Gopinath SCB, Ali A, Wongs-Aree C, Salleh NHM. 2024. Challenges of postharvest water loss in fruits: Mechanisms, influencing factors, and effective control strategies - A comprehensive review. *Journal of Agriculture and Food Research*, 17: 1-13.
- Giménez PJ, Fernández-López JA, Angosto JM, Obón JM. 2015. Comparative thermal degradation patterns of natural yellow colorants used in foods. *Plant Foods for Human Nutrition*, 70(4): 380-387.
- Gupta S, Sood M, Gupta N, Bandral JD, Langeh A. 2022. Food browning, its type and controlling measures: A review article. *Chemical Science Review and Letters*, 11(41): 29-45.
- Habibi A, Yazdani N, Chatrabnous N, Koushesh Saba M, Vahdati K. 2022. Inhibition of browning via aqueous gel solution of Aloe vera: a new method for preserving fresh fruits as a case study on fresh kernels of Persian walnut. *Journal of Food Science and Technology*, 59(7): 2784-2793.
- Harisha R, Singh SK, Ahlawat AK, Narwal S, Jaiswal JP, Singh JB, Kumar RR, Singhal S, Balakrishnan AP, Shukla P, Bhavya B, Agrawal A, Singh SK, Mahendru-Singh A. 2023. Elucidating the effects on polyphenol oxidase activity and allelic variation of polyphenol oxidase genes on dough and whole wheat-derived product color parameters. *International Journal of Food Properties*, 26(2): 2716-2731.
- Hendrawani, Hulyadi 2023. Kondisi ideal tumbuh kembang jamur merang. *Empiricism Journal*, 4(1): 156-162.
- Hęś M, Dzedzic K, Górecka D, Jędrusek-Golińska A, Gujska E. 2019. Aloe vera (L.) Webb.: natural sources of antioxidants - a review. *Plant Foods for Human Nutrition*, 74(3): 255-265.
- Huo J, Zhang N, Gong Y, Bao Y, Li Y, Zhang L, Nie S. 2024. Effects of different light intensity on leaf color changes in a Chinese cabbage yellow cotyledon mutant. *Frontiers in Plant Science*, 15: 1-16.
- Huyut Z, Beydemir Ş, Gülçin I. 2017. Antioxidant and antiradical properties of selected flavonoids and phenolic compounds. *Biochemistry Research International*, 2017: 1-10.
- Ilyas M, Soeka YS. 2019. Accelerated rate storage and viability test of Basidiomycetous fungal strains were cryopreserved at - 80° C. *IOP Conference Series: Earth and Environmental Science*, 308(1): 1-9.
- Iqbal T, Rodrigues FAS, Mahajan PV, Kerry JP. 2009. Effect of time, temperature, and slicing on respiration rate of mushrooms. *Journal of Food Science*, 74(6): 298-303.
- Ivankovic´, I, Zeljko K, Talic´ S, Bevanda AM, Lasic M. 2017. Biodegradable packaging in the food industry Übersichtsarbeit: Biologisch abbaubare Verpackungen in der Lebensmittelindustrie. *Journal of Food Safety and Food Quality*, 68: 23-52.
- Joshi K, Warby J, Valverde J, Tiwari B, Cullen PJ, Frias JM. 2018. Impact of cold chain and product variability on quality attributes of modified atmosphere packed mushrooms (*Agaricus bisporus*) throughout distribution. *Journal of Food Engineering*, 232: 44-55.
- Kalita P, Roy PK, Bhattacharjee B, Goswami A, Pachuau L, Roy S. 2025. Alginate-based coating in post-harvest fruits preservation: A comprehensive review. *International Journal of Biological Macromolecules*, 320: 1-16.
- Kamali M, Shabanpour B, Pourashouri P, Kordjazi M. 2024. Evaluating shelf life and anti-browning of shrimp by chitosan-coated nanoliposome loaded with licorice root extract. *Food Chemistry: X*, 23: 1-11.
- Kenny HM, Reynolds CM, Garcia-Vaquero M, Feeney EL. 2025. Keeping an eye on alginate: innovations and opportunities for sustainable production and diverse applications. *Carbohydrate Polymers*, 366: 1-22.
- Khan BA, Sahoo NR, Pal US, Nayak R, Bakhara CK, Panda MK. 2021. Development of a packaging, storage and transportation cabinet for paddy straw mushroom. *Journal of Food Science and Technology*, 58(6): 2377-2384.
- Kong Q, Lin S, Wang R, Chen S, Ge K, Chen D. 2025. Changes in quality, endogenous

- enzyme activities, and their relationships during post-harvest storage of *Phlebopus portentosus*- an edible fungus. *Food Chemistry*, 474: 1-11.
- Kozarski M, Klaus A, Jakovljevic D, Todorovic N, Vunduk J, Petrović P, Niksic M, Vrvic MM, Van Griensven L. 2015. Antioxidants of edible mushrooms. *Molecules*, 20(10): 19489-19525.
- Kumar N, Kaur P, Devgan K, Attkan AK. 2020. Shelf life prolongation of cherry tomato using magnesium hydroxide reinforced bio-nanocomposite and conventional plastic films. *Journal of Food Processing and Preservation*, 44(4). 2877-2897.
- Kumari A, Baskaran P. 2015. An overview on mushroom technology: cultivation, harvesting, post-harvest management and marketing, 7: 67-82.
- Kusumiyati K, Hadiwijaya Y, Suhandy D, Munawar AA. 2021. Prediction of water content and soluble solids content of "manalagi" apples using near infrared spectroscopy. *IOP Conference Series: Earth and Environmental Science*, 922(1): 1-5.
- Kusumiyati K, Hadiwijaya Y, Sutari W, Munawar AA. 2022. Global model for in-field monitoring of sugar content and color of melon pulp with comparative regression approach. *AIMS Agriculture and Food*, 7(2): 312-325.
- Liang Y, Luo K, Wang B, Huang B, Fei P, Zhang G. 2024. Inhibition of polyphenol oxidase for preventing browning in edible mushrooms: A review. *Journal of Food Science*, 89(11): 6796-6817.
- Lin X, Sun DW. 2019. Research advances in browning of button mushroom (*Agaricus bisporus*): Affecting factors and controlling methods. *Trends in Food Science and Technology*, 90: 63-75.
- Li Q, Lin H, Lin Ht, Lin Ms, Wang H, Wei W, Chen Jy, Lu Wj, Shao Xf, Fan Zq. 2023. The metabolism of membrane lipid participates in the occurrence of chilling injury in cold-stored banana fruit. *Food Research International*, 173: 1-12.
- Li Y, Ding S, Kitazawa H, Wang Y. 2022. Storage temperature effect on quality related with cell wall metabolism of shiitake mushrooms (*Lentinula edodes*) and its modeling. *Food Packaging and Shelf Life*, 32, 1-11.
- Louis E, Villalobos-Carvajal R, Reyes-Parra J, Jara-Quijada E, Ruiz C, Andrades P, Gacitúa J, Beldarraín-Iznaga T. 2021. Preservation of mushrooms (*Agaricus bisporus*) by an alginate-based-coating containing a cinnamaldehyde essential oil nanoemulsion. *Food Packaging and Shelf Life*, 28: 1-10.
- Mahajan PV, Oliveira FAR, Macedo I. 2008. Effect of temperature and humidity on the transpiration rate of the whole mushrooms. *Journal of Food Engineering*, 84(2): 281-288.
- Martínez-Cano B, Mendoza-Meneses CJ, García-Trejo JF, Macías-Bobadilla G, Aguirre-Becerra H, Soto-Zarazúa GM, Feregrino-Pérez AA. 2022. Review and perspectives of the use of alginate as a polymer matrix for microorganisms applied in agro-industry. *Molecules*, 27(13): 1-20.
- Mendy TK, Misran A, Mahmud TMM, Ismail SI. 2019. Antifungal properties of aloe vera through in vitro and in vivo screening against postharvest pathogens of papaya fruit. *Scientia Horticulturae*, 257: 1-7.
- Mensah EO, Adadi P, Asase RV, Kelvin O, Mozhdehi FJ, Amoah I, Agyei D. 2025. Aloe vera and its byproducts as sources of valuable bioactive compounds: Extraction, biological activities, and applications in various food industries. *PharmaNutrition*, 31: 1-26.
- Mirshekari A, Madani B, Golding JB. 2019. Aloe vera gel treatment delays postharvest browning of white button mushroom (*Agaricus bisporus*). *Journal of Food Measurement and Characterization*, 13(2): 1250-1256.
- Moghaddasi S, Verma SK. 2011. Aloe vera their chemicals composition and applications: A review. *International Journal of Biological & Medical Research Int J Biol Med Res*, 2(1): 466-471.
- Mohebbi M, Ansarifar E, Hasanpour N, Amiryousefi MR. 2012. Suitability of aloe vera and gum tragacanth as edible coatings for extending the shelf life of button mushroom. *Food and Bioprocess Technology*, 5(8): 3193-3202.
- Moradinezhad F, Dorostkar M. 2021. Effect of vacuum and modified atmosphere packaging on the quality attributes and sensory evaluation of fresh jujube fruit. *International Journal of Fruit Science*, 21(1): 82-94.
- Moutia I, Lakatos E, Kovács AJ. 2024. Impact of dehydration techniques on the nutritional

- and microbial profiles of dried mushrooms. *Foods*, 13(20): 1–24.
- Nair MS, Tomar M, Punia S, Kukula-Koch W, Kumar M. 2020. Enhancing the functionality of chitosan- and alginate-based active edible coatings/films for the preservation of fruits and vegetables: A review. *International Journal of Biological Macromolecules*, 164: 304–320.
- Nicolau-Lapeña I, Colàs-Medà P, Alegre I, Aguiló-Aguayo I, Muranyi P, Viñas I. 2021. Aloe vera gel: An update on its use as a functional edible coating to preserve fruits and vegetables. *Progress in Organic Coatings*, 151: 1–11.
- Nugraheni SD, Harijono, Wardani AK. 2023. Optimasi konsentrasi senyawa anti pencokelatan pada penyimpanan jamur kancing (*Agaricus bisporus*) segar. *Agrointek: Jurnal Teknologi Industri Pertanian*, 17(3): 632–643.
- Nunes MPC, Vespucci IL, Rimoli PAR, Morgado CMA, Campos AJde. 2024. Postharvest of fresh white shimeji mushroom subjected to UV-C radiation. *Heliyon*, 10(3): 1–8.
- Pandiselvam R, Mitharwal S, Rani P, Shanker MA, Kumar A, Aslam R, Barut YT, Kothakota A, Rustagi S, Bhati D, Siddiqui SA, Siddiqui MW, Ramniwas S, Aliyeva A, Khaneghah AM. 2023. The influence of non-thermal technologies on color pigments of food materials: An updated review. *Current Research in Food Science*, 6: 1–15.
- Parreidt TS, Müller K, Schmid M. 2018. Alginate-based edible films and coatings for food packaging applications. *Foods*, 7(10): 1–38.
- Pogorzelska-Nowicka E, Hanula M, Wojtasik-Kalinowska I, Stelmasiak A, Zalewska M, Póltorak A, Wierzbicka A. 2020. Packaging in a high O₂ or air atmospheres and in microperforated films effects on quality of button mushrooms stored at room temperature. *Agriculture (Switzerland)*: 10(10): 1–19.
- Puscaselu RG, Lobiuc A, Dimian M, Covasa M. 2020. Alginate: From food industry to biomedical applications and management of metabolic disorders. *Polymers*, 12(10): 1–30.
- Sakinah NMJ, Misran A, Mahmud TMM, Abdullah S, Azhar M. 2020. Evaluation of storage temperature, packaging system and storage duration on postharvest quality of straw mushroom (*Volvariella volvacea*). *Food Research*, 4(3): 679–689.
- Saputra A, Herpandi H, Supriadi A, Saputra D. 2023. The effects of nano-edible coating on shelf life, physicochemical, microbial and sensory properties in food preservation and horticulture: A mini review. *E3S Web of Conferences*, 373: 1–6.
- Sefrienda AR, Amri AF, Ariani D, Susanto A, Nurhikmat A. 2020. The extended shelf life of aloe vera drink with a modified hot-filling practice for the SME. *IOP Conference Series: Earth and Environmental Science*, 462(1): 1–9.
- Shameena S, Lekshmi PRG, Gopinath PP, Gidagiri P, Kanagarajan S. 2024. Dynamic transformations in fruit color, bioactive compounds, and textural characteristics of purple-fleshed dragon fruit (*Hylocereus costaricensis*) across fruit developmental stages under humid tropical climate. *Horticulturae*, 10(12): 1–18.
- Sharma V, Singh P, Singh A. 2024. Shelf-life extension of fresh mushrooms: From conventional practices to novel technologies—A comprehensive review. *Future Postharvest and Food*, 317–333.
- Silva M, Vida M, Ramos AC, Lidon FJ, Reboredo FH, Gonçalves EM. 2025. Storage temperature effect on quality and shelf-life of *hericium erinaceus* mushroom. *Horticulturae*, 11(2): 1–18.
- Singh B, Suri K, Shevkani K, Kaur A, Kaur A, Singh N. 2018. Enzymatic browning of fruit and vegetables: A Review. In In: Kuddus, M. (eds) *Enzymes in Food Technology*. Springer, Singapore.
- Sogvar OB, Rabiei V, Razavi F, Gohari G. 2020. Phenylalanine alleviates postharvest chilling injury of plum fruit by modulating antioxidant system and enhancing the accumulation of phenolic compounds. *Food Technology and Biotechnology*, 58(4): 433–444.
- Solaberrieta I, Jiménez A, Garrigós MC. 2022. Valorization of aloe vera skin by-products to obtain bioactive compounds by microwave-assisted extraction: antioxidant activity and chemical composition. *Antioxidants*, 11(6): 1–25.
- Sytar O, Hemmerich I, Zivcak M, Rauh C, Brestic M. 2018. Comparative analysis of bioactive phenolic compounds composition from 26

- medicinal plants. *Saudi Journal of Biological Sciences*, 25(4): 631–641.
- Takeungwongtrakul S, Sai-Ut S, Waraput T, Supapvanich S. 2022. Thermal processes improving antibrowning potential of mixed aloe vera and pineapple core extract solution on browning inhibition of fresh-cut apples. *International Journal of Food Science and Technology*, 57: 6881–6889.
- Tan M, Mei J, Xie J. 2021. The formation and control of ice crystal and its impact on the quality of frozen aquatic products: A review. *Crystals*, 11(1): 1–17.
- Tizazu A, Bekele T. 2024. A review on the medicinal applications of flavonoids from aloe species. *European Journal of Medicinal Chemistry Reports*, 10: 1–12.
- Wang A, Siddique B, Wu L, Ahmad I, Liu X. 2020. Sodium alginate edible coating augmented with essential oils maintains fruits postharvest physiology during preservation: A review. *International Journal of Multidisciplinary Research and Development*, 7(8): 135–140.
- Wang C, Meng L, Zhang G, Yang X, Pang B, Cheng J, He B, Sun F. 2024. Unraveling crop enzymatic browning through integrated omics. *Frontiers in Plant Science*, 15: 1–8.
- Wang HJ, An DS, Rhim JW, Lee DS. 2017. Shiitake mushroom packages tuned in active CO₂ and moisture absorption requirements. *Food Packaging and Shelf Life*, 11: 10–15.
- Wariyah C, Riyanto, Slamet A. 2024. Chemical changes of aloe vera gel during the preparation of instant powder and its antioxidant activity. *Food Research*, 8(2): 1–7.
- Zhao K, Xiao Z, Zeng J, Xie H. 2021. Effects of different storage conditions on the browning degree, ppo activity, and content of chemical components in fresh liliun bulbs (*Lilium brownii f.e.brown var. viridulum baker*). *Agriculture (Switzerland)*: 11(2): 1–18
- Zou H, Xiao Q, Li G, Wei X, Tian X, Zhu L, Ma F, Li M. 2025. Revisiting the advancements in plant polyphenol oxidases research. *Scientia Horticulturae*, 341: 1–11.

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A review of bioactive compounds of *Caesalpinia sappan*: Pre- and post-harvest effect

Abstract. Sappanwood (*Caesalpinia sappan* L.) is a shrub or small tree that thrives in tropical regions and has been widely distributed across various areas. It has long been used as a natural dye in textiles, cosmetics, and herbal beverages due to its content of various bioactive compounds. This literature review discusses the secondary metabolites in sappanwood, their health benefits, and strategies to enhance product quality through pre-harvest and post-harvest treatments. This literature review was conducted by searching for relevant journals on Google Scholar using relevant keywords. Sappanwood contains diverse secondary metabolites, including flavonoids, phenolics, anthraquinones, triterpenoids, steroids, alkaloids, and tannins, with the heartwood generally having the highest concentration, particularly of brazilin. Various production techniques have been studied to optimize morphological and physiological traits as well as secondary metabolite content, such as adjusting planting density and applying different types of fertilizers. Post-harvest treatments, including drying and blanching, are also critical as they influence product quality and bioactive compound retention.

Keywords: Antioxidant · Drying · Fertilizer · Planting density · Secondary metabolites

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Introduction

Sappanwood (*Caesalpinia sappan* L.) is a shrub or small tree that thrives in tropical regions and has been widely distributed across various regions, including Asia, Africa, America, and Europe (Uddin et al., 2015). This species is commonly found in Southeast Asia (Thanayutsiri et al., 2023) and is native to, as well as still grows wild in, several regions of South and Southeast Asia, such as southern India, West Bengal, Orissa, Madhya Pradesh, the Malay Peninsula, Sri Lanka (Ahmed et al., 2024), and the Pacific Islands (Dapson & Bain, 2015). Sappanwood has been utilized as a medicinal plant in various countries, including India, Sri Lanka, Myanmar, Vietnam, the Malay Peninsula (Ahmed et al., 2024), and Thailand (Srisaikham & Rupitak, 2020). Sappanwood serves as a natural red dye and has been extensively utilized in textile dyeing (Kannathasan & Kokila, 2021), cosmetic formulations (Agustin & Pratiwi, 2023), and herbal beverages, which are traditionally consumed either as a sole ingredient or in combination with other medicinal herbs (Septiyani et al., 2024). In Indonesia, wedang uwuh is a traditional herbal beverage that contains sappanwood as one of its ingredients and has been traditionally consumed (Setyowati et al., 2023).

Indonesians are very interested in traditional medicine. In Indonesia, herbal medicine (jamu) remains an integral part of traditional healthcare practices and is widely available in traditional markets across the country (Mardiyanto et al., 2023). A recent study reported that the prevalence of herbal medicine use reached 68% (431 out of 634 respondents), with 40% (219 out of 549 individuals who reported illness) practicing self-medication using herbal remedies during the past six months (Rahayu et al., 2020). Sappanwood is widely recognized as a promising plant for pharmaceutical development due to its rich content of bioactive compounds and diverse pharmacological activities.

Pharmacological activity is derived from a plant's bioactive compounds, which are typically classified as secondary metabolites. Secondary metabolites are organic molecules derived from primary metabolites through physiological processes in plants (Twaij & Hasan, 2022). Secondary metabolites are produced and regulated through primary metabolic pathways

(Caretto et al., 2015), which not only supply the energy essential for plant development but also generate the carbon skeletons required for their biosynthesis (Liu et al., 2017). While primary metabolites—such as carbohydrates, lipids, and proteins—play a direct role in plant growth and development, secondary metabolites primarily function in defense mechanisms and facilitating interactions with the surrounding environment (Jan et al., 2021).

Secondary metabolites in sappanwood can be classified into groups such as flavonoids, phenolics, anthraquinones, triterpenoids, steroids, alkaloids, and tannins (Vij et al., 2023). These secondary metabolites also include antioxidant compounds such as homoiso-flavonoids, notably brazilin and protosappanin, which exhibit strong antioxidant, anti-inflammatory, and anticancer activities (Nirmal et al., 2015). The brazilin compound, which gives the red color to sappanwood, is known to have benefits in pharmacology as an antioxidant, antibacterial, anti-inflammatory, antihyperglycemic, and analgesic properties (Settharaksa et al., 2019).

Sappanwood naturally occurs in regions characterized by clay, limestone, or sandy soils, predominantly at low to moderate altitudes (ICRAF, 2023). However, the lack of proper and sustainable cultivation practices, including adequate nutrient management and controlled agronomic interventions, may lead to a gradual decline in its natural populations within native habitats. On the other hand, sappanwood possesses considerable potential as a source of secondary metabolites, whose biosynthesis and accumulation can be enhanced through specific cultivation strategies, such as phytohormone application and nutrient management. Applying mineral nutrients as supplements can potentially promote plant growth and regulate the accumulation of secondary metabolites (Yang et al., 2018). Most previous review articles have primarily focused on phytochemical composition and pharmacological activities, while discussions on cultivation strategies that regulate secondary metabolite accumulation remain limited. Therefore, this review aims to discuss cultivation strategies that can be applied to sappanwood, particularly phytohormone application, and nutrient supplementation to enhance secondary metabolite production.

Materials and Methods

In conducting this literature review, relevant keywords were first identified in English, which served as the primary search method to locate related journal articles on Google Scholar. Keywords in Indonesian were also used as a supplementary search strategy to ensure broader coverage of locally published studies. The journal search was conducted from May 15, 2024, to December 24, 2025. The keywords used included: "Origin of the sappanwood plant", "benefits of the sappanwood", "sappanwood as a natural dye", "utilization of sappanwood in traditional medicine in Indonesia", "pharmacological activities of sappanwood", "secondary metabolites of sappanwood", "benefits of secondary metabolites in sappanwood", "relationship between secondary metabolites and antioxidant activity", "antioxidant activity of sappanwood", "antioxidant potential of sappanwood", "brazilin and brazilein as the major characteristic compounds of sappanwood", "mechanisms of brazilin and brazilein as antioxidants in sappanwood", "mechanisms of brazilin and brazilein in the human body as therapeutic agents", "secondary metabolites from different parts of sappanwood", "preharvest of sappanwood", "crop pattern of sappanwood", "phytohormones in plants", "functions of phytohormones", "utilization of phytohormones as plant growth regulators", "application of phytohormones in various medicinal plants", "application of phytohormones

in sappanwood cultivation", "nutrient application as a plant cultivation technique", "role of mineral nutrients in enhancing secondary metabolites and antioxidant activity", "use of fertilizers to increase antioxidant activity and secondary metabolite production", "fertilizer application and nutrient management in medicinal plants", "fertilizer application to enhance secondary metabolite production in sappanwood", "post harvest technique for sappanwood", "post harvest technique for woody plant", "effect of drying for sappanwood product", and "effect of blanching for sappanwood product". Most of the articles were published within the past decade. This review discusses a total of 111 publications, written mostly in English.

Results and Discussion

Secondary metabolites in *Caesalpinia sappan* L.

The secondary metabolites contained in sappanwood provide numerous medicinal benefits. Multiple studies confirm that sappanwood extracts are rich in polyphenols and flavonoids, which are responsible for their potent antioxidant effects (Artati et al., 2025). Extracts with higher total phenolic (TPC) and total flavonoid content (TFC) consistently show stronger free radical scavenging activity (Masturi et al., 2021). The secondary metabolite content in sappanwood is presented in more detail in Table 1.

Table 1. Secondary metabolites found in sappanwood

Class	Compounds	Medicinal uses	References
Flavonoid	brazilin, hematoxylin, protosappanin, brazilein, 3'-O-metilbrazilin, sappanin, chalcone, and sappanalcone	Antioxidant, anti-inflammatory, anticancer, analgesic	(Niu et al., 2020; Vij et al., 2023)
Phenolic	Chlorogenic acid, caffeic acid, and gallic acid	Antioxidant and anti-inflammatory	(Sakti et al., 2019)
Anthraquinone	Brazilin, brazilein, and sappanone-A	Antimicrobial, anticancer, and anti-inflammatory	(Ahmad et al., 2020b)
Triterpenoid	Lupeol, β -amyrin, and cycloartenol	Antimicrobial, anticancer, and anti-inflammatory	(Rajput et al., 2022)
Steroid	Stigmasterol and β -sitosterol	Anticancer and anti-inflammatory	(Tu et al., 2022)
Alkaloid	Sappan chalcone and sappanone B	Anticancer and anti-inflammatory	(Tamburini, 2019)
Tannin	Gallic acid and ellagic acid	Provides astringent taste	(Vij et al., 2023)

Table 2. Secondary metabolite profile of different plant parts of sappanwood

Plant Part	Identified Secondary Metabolites	Key findings	References
Branch (Sapwood & Heartwood)	alkaloids, triterpenoids (except branch sapwood), Tannins, saponin, phenolic	The total phenolic content and antioxidant activity in branch sapwood were higher than those in branch heartwood.	(Arsiningtyas, 2021)
Middle Stem (Sapwood & Heartwood)	Alkaloid, tannins, saponins, triterpenoid, phenolic	The total phenolic content and antioxidant activity in middle stem sapwood were higher than those in middle stem heartwood.	(Arsiningtyas, 2021)
Main Trunk (Sapwood & Heartwood)	alkaloids, triterpenoids, tannins, saponins, phenolic	The total phenolic content in main trunk sapwood was lower than that in main trunk heartwood; however, the antioxidant activity of main trunk sapwood was higher than that of main trunk heartwood.	(Arsiningtyas, 2021)
Bark	Flavonoid, antioxidant activity	Flavonoid total 0.170 ± 0.10 mg QE/100 g extract.	(Nurulita & Harahap, 2025)
Seed	Flavonoid, antioxidant activity	Flavonoid total 0.032 ± 0.12 mg QE/100 g extract.	(Nurulita & Harahap, 2025)
Leaves	Flavonoid, antioxidant activity	Flavonoid total 0.147 ± 0.002 mg QE/100 g extract.	(Nurulita & Harahap, 2025)
Sappanwood product (samples from local markets in Bandung, Indonesia)	Alkaloid, triterpernoid, phenolic, flavonoid	Flavonoids play a major role in the extract's antioxidant activity, contributing to an effectiveness of up to 80%.	(Artati et al., 2025)
Seed	Caesalsappanin R and caesalsappanin S	Two new cassane-type diterpenoids (1(R) and 2(S)) were isolated from <i>Caesalpinia sappan</i> , with compound 1 showing in vitro antiplasmodial activity ($IC_{50} = 3.60 \mu M$).	(Zhu et al., 2017)

Brazilin and brazilin represent the key secondary metabolites characteristic of sappanwood. Brazilin is the major flavonoid found in sappanwood and has shown potential for medicinal applications (Septiani et al., 2022). The secondary metabolites in sappanwood are predominantly concentrated in the stem region, including the bark, wood, and heartwood (Vij et al., 2023), with the pith being identified as the primary site of brazilin accumulation (Rosniawaty, et al., 2024b). Figure 1 illustrates the stem region where secondary metabolites are concentrated.

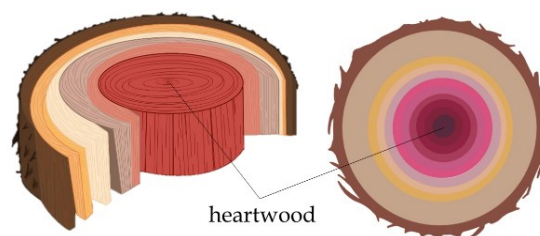


Figure 1. Illustration of the Stem of Sappanwood (*Caesalpinia sappan* L.) Showing the Presence of Secondary Metabolites in the Inner Tissues

Previous studies consistently report that the heartwood is the richest source of bioactive secondary metabolites, particularly phenolic compounds such as brazilin and brazilein, which are responsible for the characteristic red color and strong antioxidant activity of sappanwood. The extraction of sappanwood wood involves felling the trees, irrespective of whether they grow wild in forests or are planted in domestic gardens (Arsiningtyas, 2021). Conservation of sappanwood can be achieved by harvesting wood selectively from branches or the central portions of the tree, rather than felling it entirely (Arsiningtyas, 2021). This practice promotes regrowth and mitigates the risk of extinction. Additionally, the distinction between heartwood and sapwood is important, as these compartments possess different secondary metabolites that may influence their respective antioxidant activities (Miranda et al., 2017). Table 2 shows that different parts of sappanwood contain diverse secondary metabolites with varying concentrations. Table 2 indicates that heartwood tends to produce higher levels of secondary metabolites, particularly phenolic compounds that contribute to the tree's natural durability. The higher yield in heartwood compared to the middle and branch sections is likely due to the increasing extractive content with wood age (de Paula et al., 2019).

Scientific studies have validated many of sappanwood for traditional uses, brazilin and brazilein are proven to reduce inflammation, enhance blood circulation (Mueller et al., 2016), and protect against oxidative stress (Vij et al., 2023), supporting their potential for modern therapeutic applications. Brazilin, in particular, has demonstrated potential in lowering blood glucose, improving glucose metabolism, and even inhibiting the formation of toxic protein fibrils, which may help in treating infections and promoting wound healing (Septiani et al., 2022; Xuan et al., 2022). Brazilein, meanwhile, is noted for its stability as a dye and its medicinal effects, including cytotoxic activity against cancer cells and the ability to suppress melanin synthesis, making it useful in medicine (Ngamwonglumlert et al., 2020).

Brazilin is a yellow-orange crystalline compound found in sappanwood. Brazilin is readily oxidized to brazilein, which produces the vivid red color characteristic of sappanwood extracts, a transformation that is particularly enhanced in alcoholic and aqueous solutions under air exposure or alkaline conditions (Vij et al., 2023). Brazilin and brazilein are two primary

bioactive compounds isolated from sappanwood, both of which exhibit a wide range of pharmacological properties with well-studied mechanisms of action. Table 3 shows the mechanism of brazilin and brazilein as a medicinal treat.

Brazilin demonstrates anticancer properties by inducing apoptosis (programmed cell death) through the upregulation of proteins such as Bcl-2, p21, caspase-3/-7, and PARP, and by inhibiting cancer cell growth through ROS-mediated activation of NF- κ B (Nava-Tapia et al., 2022; He et al., 2017). Brazilin exerts anti-inflammatory effects by inhibiting NF- κ B activation, leading to reduced expression of pro-inflammatory cytokines and suppression of TACE enzyme activity (Tumor Necrosis Factor- α Converting Enzyme) (Amarawati et al., 2019), along with mitigating tissue oxidative stress (He et al., 2017). Brazilin's antioxidant activity is associated with the activation of the SIRT3/GPX4 signaling pathway, which plays a crucial role in protecting cells from oxidative stress and ferroptosis—a type of cell death driven by the accumulation of iron-dependent lipid peroxides (Yan et al., 2025). Brazilin also demonstrates neuroprotective effects by preventing amyloid beta ($A\beta$) aggregation, a key pathological feature of both Alzheimer's (Cui et al., 2024) and Parkinson's diseases (Nava-Tapia et al., 2022). This mechanism involves hydrophobic interactions and hydrogen bonding with $A\beta$, facilitating the remodeling of amyloid plaques. Brazilin has also been reported to exhibit antidepressant and anxiolytic effects by mitigating oxidative stress and inflammation in the central nervous system (Jamaddar et al., 2023).

Brazilein, the oxidized derivative of brazilin, also exhibits strong anticancer activity by inducing apoptosis, inhibiting epithelial-mesenchymal transition (EMT), and downregulating PD-L1 expression, a key molecule in cancer immune evasion (Wudtiwai et al., 2023). Brazilein exerts its biological effects through the modulation of several signaling pathways, such as AKT, NF- κ B, and GSK3 β / β -catenin, and additionally suppresses the activity of matrix metalloproteinases (MMPs), enzymes that contribute to extracellular matrix degradation during the metastatic process (Wudtiwai et al., 2023). Its anti-inflammatory effects are mediated through mechanisms similar to those of brazilin, including TACE inhibition, reduction of pro-inflammatory cytokines, and immunomodulation (Amarawati et al., 2019).

Table 3. Mechanisms of action of brazilin and brazilein as therapeutic agents in humans

Compound	Therapeutic Effects	Main Mechanisms	References
Brazilin	Anticancer (induces apoptosis, inhibits proliferation, autophagy in various cancers)	Induces apoptosis (Bcl-2, p21, caspases, PARP), inhibits proliferation, triggers autophagy via ROS-NF-κB, Blocks JNK, regulates Nrf2, chelates iron.	(He et al., 2017; Jamaddar et al., 2023; Nava-Tapia et al., 2022; Raptania et al., 2024)
	Anti-inflammatory (inhibits NF-κB, NLRP3 inflammasome, reduces cytokines, protects tissues)	Inhibits NF-κB pathway, suppresses pro-inflammatory cytokines, inhibits TACE, reduces oxidative stress	(Amarawati et al., 2019; Jamaddar et al., 2023; Nava-Tapia et al., 2022; Yan et al., 2025)
	Antioxidant (protects against oxidative stress, reduces ferroptosis)	Enhances SIRT3/GPX4 pathway, reduces mitochondrial oxidative stress and ferroptosis	(Jamaddar et al., 2023; Nava-Tapia et al., 2022; Yan et al., 2025)
	Neuroprotective (inhibits amyloid β aggregation, potential for Alzheimer's and Parkinson's disease)	Inhibits Aβ fibrillogenesis, remodels amyloid fibrils, binds Aβ via hydrophobic and hydrogen bonding	(Cui et al., 2024; Nava-Tapia et al., 2022)
	Antidepressant/Anxiolytic (reduces oxidative stress and inflammation in neural tissues)	Inhibits oxidative stress and inflammation in neural tissues	(Jamaddar et al., 2023)
Brazilein	Anticancer (induces apoptosis, inhibits EMT and PD-L1, reduces metastasis in breast cancer)	Induces apoptosis, suppresses EMT and PD-L1 via inhibition of AKT, NF-κB, GSK3β/β-catenin, inhibits MMPs	(Wudtiwai et al., 2023)
	Anti-inflammatory (inhibits TNF-α converting enzyme, potential for rheumatoid arthritis)	Inhibits TACE, reduces pro-inflammatory cytokines, immunomodulation	(Amarawati et al., 2019; Wudtiwai et al., 2023)

The use of chemical-based medicines is generally effective in treating various diseases; however, their application may be associated with serious side effects in certain cases (Kaushik et al., 2021). Consequently, plant-based medicines and herbal remedies are increasingly regarded as healthier, more natural, and safer alternatives due to their origin from natural resources (Wyk & Prinsloo, 2020). In addition to producing essential primary metabolites, plants are capable of synthesizing a wide range of secondary or specialized metabolites that play crucial roles in plant-environment interactions (Pagare et al., 2015). These secondary metabolites are typically produced in low quantities (less than 1% of dry weight), and their accumulation varies depending on the physiological status, developmental stage, and environmental conditions of the plant (Madani et al., 2021).

Importantly, secondary metabolites serve as key indicators of the therapeutic quality of medicinal plants and contribute significantly to their pharmacological properties (Pant et al., 2021). Moreover, these compounds function as defense mechanisms against biotic and abiotic stresses while simultaneously possessing substantial therapeutic potential for the treatment of various human diseases (Hilal et al., 2024).

The distribution and sustainability of medicinal plants are strongly influenced by environmental factors such as climate, altitude, and seasonal fluctuations (Kaushik et al., 2021), highlighting the necessity of appropriate agronomic interventions to ensure their continued availability. In this context, the cultivation of sappanwood as a medicinal plant represents a promising strategy to support the growth of plant-based pharmaceutical industries.

Production techniques involving the application of plant growth regulators (phytohormones) and optimized fertilization practices have the potential to enhance the plant's capacity to synthesize essential secondary metabolites. The characteristic bioactive compounds of sappanwood, including brazilin and brazilin, are responsible for its therapeutic activity and are closely associated with environmental and agronomic factors that regulate their biosynthesis.

Pre-Harvest Effect on Bioactive Compounds. In sappanwood, applying phytohormones holds the potential to boost the production of secondary metabolites. An overview of phytohormone applications is provided in Table 4. The combination of auxins and cytokinins of the induction callus of sappanwood was more effective in inducing callus formation than the use of a single phytohormone (Bukke & Shankar, 2014). Methyl jasmonate and salicylic acid functioned as elicitors that influenced the growth of sappanwood plants (Rosniawaty et al., 2024b). The results indicated that the separate application of methyl jasmonate at 50 μ M and salicylic acid at 50 μ M was effective in increasing the number of leaves during the 12 and 14 weeks after planting (WAP) (Rosniawaty et al., 2024b). Treatment with 50 μ M salicylic acid resulted in the highest dry weight of sappanwood seedlings compared to other treatments. However, these elicitor applications did not significantly affect plant height or stem diameter. Further investigation by Rosniawaty et al. (2025a) on sappanwood leaves remains inconclusive. However, salicylic acid application has shown particular effectiveness in promoting stem diameter growth, whereas methyl jasmonate, especially when applied under close spacing conditions (1 \times 1 m), results in the highest accumulation of phenolic and flavonoid compounds (Rosniawaty et al., 2025a). Additional studies are required to assess the levels of secondary metabolites and antioxidant capacity of sappanwood at harvest, considering its significant potential as a medicinal plant with a wide range of pharmacological activities. A potential interaction exists between plant growth regulators and reactive oxygen species (ROS) in sappanwood, given that ROS can act as signaling molecules that enhance antioxidant activity.

Metabolic activities in plants, including photosynthesis and respiration, result in the formation of various compounds, among them

reactive oxygen species (ROS), these ROS are persistently generated in mitochondria, chloroplasts, peroxisomes (Torun et al., 2020), plasma membranes, apoplast and the endoplasmic reticulum and cell walls (Mansoor et al., 2022). Excessive levels of ROS can damage cellular components such as DNA, proteins, and lipids; however, plants employ both enzymatic and non-enzymatic systems to neutralize ROS (Dvořák et al., 2021; Janku et al., 2019; Mansoor et al., 2022). Beyond their role as toxic byproducts, ROS also act as key signaling molecules that modulate plant growth, development, and adaptive responses to environmental stresses (Mhamdi & Van Breusegem, 2018; Wang et al., 2024b; Waszczak et al., 2018). ROS play a signaling role in processes such as cell proliferation, differentiation, programmed cell death, and environmental adaptation, while also interacting with phytohormones and other signaling pathways (Mhamdi & Van Breusegem, 2018; Singh et al., 2016b).

The role of phytohormones for plants is as a regulator of plant growth and development, and also plays a role in responding to stress. Phytohormones, synthesized in small quantities within plant tissues, function as critical regulators of diverse biological processes such as embryogenesis, reproductive development, defense mechanisms, and tolerance to environmental stress (Kashchenko et al., 2021). Based on their chemical structure, phytohormones are classified into several groups, such as auxins, cytokinins (CK), ethylene (ET), gibberellins (GA), brassinosteroids (BR), abscisic acid (ABA), strigolactones, and jasmonates (JA)—all of which have the potential to enhance plant defense mechanisms (Wani et al., 2016).

Gibberellin is one of the phytohormones naturally present in plants, but it can also be applied exogenously through the use of plant growth regulators (PGRs). The application of gibberellin in the form of GA₃ at a concentration of 2 mg/L on *Stevia rebaudiana* resulted in the most effective treatment for enhancing secondary metabolite parameters, such as total phenolic content (TPC: 9.84 mg GAE/g dry weight), total phenolic production (TPP: 147.6 mg/L), total flavonoid content (TFC: 5.12 mg QE/g dry weight), and total flavonoid production (TFP: 76.91 mg/L). This treatment also improved antioxidant activity, reaching 77.2% (Ahmad et al., 2020a). In the medicinal plant *Tinospora*

cordifolia, gibberellic acid (GA) treatment resulted in a marked increase in total alkaloid content, reaching 9,454 mg/g dry weight (DW) in cell extracts, thereby demonstrating GA's stimulatory effect on alkaloid biosynthesis, the total phenolic content peaked at 3 mg/L GA, with 20,5 mg/g DW in cell extracts, confirming GA's regulatory role in phenolic metabolic pathways (Patel et al., 2024). The increasing concentrations of GA₃ significantly enhance total flavonoid content. GA₃ plays a role in facilitating the formation of secondary metabolites, particularly in flavonoid production (Ahmadi et al., 2020). In ramie (*Boehmeria nivea* L.), exogenous application of gibberellin significantly reduces lignin content in the leaves but not in the stems, and overall, gibberellin treatment effectively decreases lignin accumulation while enhancing flavonoid and chlorogenic acid contents in the forage (Jie et al., 2023). In plantation crops such as tea (*Camellia sinensis*), gibberellin is also known to suppress dormant buds, thereby improving tea quality, including increasing catechin content as an antioxidant (Anjarsari, 2023).

In general, phytohormones function as natural signaling compounds in plants to regulate growth and development, while the application of elicitors as exogenous compounds aims to trigger defense reactions in plants. Various types of elicitors have been investigated for their effects on enhancing the secondary metabolite content in plants. Elicitation is a production technique used to stimulate the production of secondary metabolites in plants. When plants are treated with elicitors (inducing compounds), signaling molecules from the elicitor bind to receptors in the plant under in vitro conditions (Kumari et al., 2020). Elicitors play several aspects, such as : 1) elicitors are considered ecologically safe because they involve activating the innate (endemic) immune potential of plants through gene expression; 2) elicitors provide protective effects against pathogens; 3) when elicitors induce resistance in plants, they activate multiple defense systems rather than relying on a single mechanism; and; 4) elicitors can trigger a broad-spectrum resistance response, protecting plant against various types of pathogens, including fungi, bacteria, and viruses (Kumari et al., 2020). This resistance is considered non-specific because it is not targeted to a particular pathogen but instead enhances the overall plant defense mechanisms.

Based on their source, elicitors are categorized as either abiotic or biotic. Abiotic elicitors are non-living chemical or physical agents such as metal ions including oxalate, cadmium, calcium, europium, and lanthanum (Kumari et al., 2020; Naik & Al-Khayri, 2016). Biotic elicitors are compounds derived from living organisms, including polysaccharides (such as alginate, pectin, chitosan), oligosaccharides (such as guluronate, mannan, galacturonide), peptides (including glutathione), and various molecules derived from bacteria, fungi, algae, and yeast (Bhaskar et al., 2022; Jain et al., 2024; Naik & Al-Khayri, 2016). By activating plant defense responses, elicitors stimulate gene expression and metabolic pathways involved in the production of secondary metabolites such as alkaloids, flavonoids, phenolics, and terpenoids. (Bhaskar et al., 2022).

Phytohormones such as brassinosteroids, jasmonic acid, salicylic acid, strigolactones, and peptide hormones play vital roles in both plant development and stress response regulation (Zhao et al., 2021). Various production techniques involving abiotic and biotic stress treatments have the potential to enhance antioxidant levels in sappanwood. In general, when plants are exposed to stress, genes associated with antioxidant defense are activated, leading to enhanced activity of antioxidant systems to neutralize ROS, which simultaneously accumulate at elevated levels (Sanam et al., 2020). These responses collectively enhance the plant's resilience under stress conditions.

Abiotic stress triggers phytohormone-mediated signaling pathways in plants, leading to the activation of MYB genes, which in turn upregulate antioxidant-related genes and help mitigate stress-induced damage (Zhao et al., 2021). Under high-temperature stress, WRKY genes act as essential components of the jasmonic acid signaling pathway, while ethylene plays a pivotal role in mediating the plant's response to waterlogging stress. Ethylene biosynthesis involves two major enzymes—ACC synthase (ACS) and ACC oxidase (ACO)—both of which are critically involved in the plant's adaptive response to waterlogging conditions. Ethylene signaling is known to modulate physiological responses, including changes in phenolic compounds in tea leaves (Ahammed & Li, 2022). Additionally, antioxidant enzyme activities exhibited differential responses following ACC treatment: the activities of superoxide dismutase

(SOD), peroxidase (POD), and catalase (CAT) decreased, while the activity of ascorbate peroxidase (APX) increased (Ke et al., 2018).

As a phytohormone, salicylic acid (SA) participates in multiple physiological functions and serves as a key signal in stimulating the biosynthesis of secondary metabolites (Gorni et al., 2020). Salicylic acid has synergistic and antagonistic effects with various other plant phytohormones in responding to environmental stress (Wang et al., 2020a). Jasmonic acid and salicylic acid often act synergistically or through overlapping pathways in plant defense mechanisms. Salicylic acid contributes significantly to plant immunity by promoting resistance to diverse pathogens such as fungi, viruses, and bacteria (Singh et al., 2017a), Jasmonic acid (JA), on the other hand, modulates signaling pathways involved in the biosynthesis of compounds essential for plant responses to both biotic and abiotic stresses (Kumari et al., 2020).

Nutrients are an important factor in plant cultivation to achieve optimal quantity and quality of yields. Plants require a balanced supply of nutrients, including both macro- and micronutrients, to support their growth and productivity. Fertilizers provide key nutrients as a vital for plant growth and metabolic function. As key macronutrients, nitrogen (N) and phosphorus (P) significantly contribute to the regulation and enhancement of antioxidant defenses in plants. Mineral nutrients, including C, N, P, K, S, and various micronutrients, function not only as essential resources for plant growth but also as signaling molecules and metabolic substrates that reshape primary metabolic pathways and, consequently, regulate the biosynthesis of phenolics, flavonoids, terpenes, alkaloids, saponins, and other secondary metabolites in plants and microorganisms (Bhat et al., 2020). In plants, nutrient limitation or imbalance redirects metabolic resources from growth toward defense- and quality-related secondary metabolites, increasing secondary metabolites, whereas optimal or high nutrient supply generally favors biomass accumulation at the expense of some carbon-based defenses, although it may enhance nitrogen-containing metabolites or specific essential oils depending on the species and biosynthetic pathway (El-Nakhel et al., 2019). Research shows that the combined application of N and P can significantly increase the activities of

key antioxidant enzymes such CAT, peroxidase (PX), SOD, and APX, which help neutralize reactive oxygen species and protect plants from oxidative stress, especially under stress conditions (Jia et al., 2025; Ma et al., 2024; Zhang et al., 2024). Balanced N and P fertilization enhances plant growth and nutrient uptake while simultaneously strengthening antioxidant defenses, leading to improved health, productivity, and resilience across various plant species and environments (Jia et al., 2025; Ma et al., 2024).

The balance of nitrogen plays a crucial role in determining the accumulation of secondary metabolites in medicinal plants. In basil, prolonged low mineral nutrition, with variations in nitrogen level and form, enhanced phenylalanine ammonia-lyase (PAL) activity, increased total phenolic and flavonoid contents, and improved antioxidant capacity, showing strong specificity depending on cultivar and nitrogen form, while roots under nutrient limitation accumulated particularly high levels of caffeic and rosmarinic acids (Jakovljević et al., 2019). In *Lonicera japonica*, nitrate nutrition promotes phenolic metabolite accumulation via enhanced photosynthesis, carbon flux, and phenylpropanoid enzyme activity, while ammonium nutrition shifts metabolism toward amino acids and lignin-related compounds (Cao et al., 2025). In *Panax notoginseng*, nitrogen deficiency increased the C/N ratio, amino acids, and sugars while upregulating genes in the mevalonate (MVA) and methylerythritol phosphate (MEP) pathways, leading to higher total triterpenoid saponins despite decreases in some flavonoids, whereas in ginseng, moderate nitrogen optimized root yield and total ginsenosides and modulated root-associated microbes and soil metabolites, including plant secondary metabolite pathways (Cun et al., 2024; Li et al., 2025).

The use of organic fertilizers, such as compost or biofertilizers, is believed to increase antioxidant activity in sappanwood plants. The nutrient content in organic fertilizers varies depending on their source materials, and easily decomposed materials tend to become more effective sources of nutrients (Purba et al., 2021). Raw rice husk decomposition enriches the soil with N, P, and Potassium (K), contributing to improved nutrient status and fertility (Thiyageshwari et al., 2018). The application of rice husk improves soil physical properties,

increases organic matter content, and enhances nutrient availability, which collectively contributes to enhanced root growth, greater biomass accumulation, and elevated chlorophyll levels in sunflower plants (Bashir et al., 2021; Rupngam et al., 2025). Additionally, rice husk amendments can help sunflowers cope with environmental stresses, such as heavy metal contamination, by reducing oxidative stress and increasing the uptake of essential nutrients like N, P, and K (Bashir et al., 2021). Production of *Brassica rupestris* in nitrogen-rich soil resulted in the enhanced accumulation of key bioactive constituents, including total antioxidants, chlorophyll, carotenoids, and vitamin C (ascorbic acid) (Muscolo et al., 2019).

Vermicompost application under drought stress significantly improved the antioxidant profile and yield components of *Thymus vulgaris* by increasing chlorophyll content, carotenoids, essential oils, nutrient absorption (N, P, K), and relative water content (Rahimi et al., 2023). Light water stress increased the amount of oil and oil yield of thyme, with the highest values of 2.61% and 3.68 g/m², respectively (Rahimi et al., 2023). Water stress enhances antioxidant activity by activating enzymes such as SOD, APX, and CAT, which play a role in protecting plants against lipid peroxidation (as indicated by malondialdehyde, MDA, and content). Organic fertilizer application appears to improve antioxidant capacity and thyme yield by increasing water-use efficiency under drought conditions.

The application of chicken, cow, and goat manure had no significant effect on the growth performance of sappanwood plants (Rosniawaty, et al., 2024c). In general, solid or liquid animal manure contains approximately 0.5% N, 0.25% P, and 0.5% K, which serve as essential nutrient sources for plant growth (Purba et al., 2021). The application of cattle manure had no significant effect on branch and leaf growth in purslane (*Portulaca grandiflora*). However, the highest levels of total phenolics (0.1532 mg GAE/g), flavonoids (0.1529 mg GAE/g), and antioxidant

activity (0.6440 μmol TE/g fresh) were observed in plants treated with 10 g of cattle manure per polybag (Liwanda et al., 2023).

Applications of organic fertilizers as nutrient supplements in sappanwood production are summarized in Table 4. The use of rice husk as a growing medium of sappanwood showed the greatest stem diameter and the highest total flavonoid content, it increased stem diameter by 45.93% and total flavonoid content by 20.12% compared to the control (Srisaikham & Rupitak, 2020). The use of compost-based growing media can influence both the growth and the content of bioactive compounds in sappanwood (Srisaikham & Rupitak, 2020). The combined application of compost and NPK fertilizer (N:P:K = 21:17:17) at a 1:1 ratio significantly improved the biochemical attributes and antioxidant activity of *Moringa oleifera*, as evidenced by increased carbohydrate levels, phenolic content, and flavonoid accumulation, compared to the sole application of either compost or NPK fertilizer alone (Sarwar et al., 2020).

Although plants produce a wide variety of secondary metabolites, not all of these compounds necessarily confer health benefits to humans. Many secondary metabolites function primarily in plant defense, pigmentation, or stress responses, and their effects on human physiology can vary depending on chemical structure, dosage, and bioavailability. Some secondary metabolites, such as certain flavonoids, phenolics, and saponins, have been reported to exhibit antioxidant properties, which allow them to scavenge ROS and potentially contribute to human health. However, the antioxidant activity of plant secondary metabolites cannot be assumed for all compounds. Further research is therefore needed to identify which secondary metabolites exert health benefits through ROS modulation, similar to the mechanism of conventional antioxidants. Such studies are essential to clarify the specific compounds and mechanisms responsible for the beneficial effects of secondary metabolites in humans.

Table 4. Pre-harvest conditions of sappanwood

No	Plant Material	Treatment	Experimental conditions	Effect for Sappanwood	References
1	Sappanwood seeds	Indole-3-acetic acid (IAA), α -naphthalene acetic acid (NAA), 6-benzylaminopurine (BAP)	The use of topsoil and cow manure as a growing medium.	Soaking in NAA (1.25 ppm) and BAP (1.25 ppm) produced the best results for leaf number and stem growth of sappanwood seedlings.	Al-Adawiah et al. 2023
2.	Sappanwood seeds	IAA, NAA, BAP	The addition of cow manure (2:1) to the sappanwood growing medium.	Auxin and cytokinin affected the seedling height, stem diameter, number of leaves, leaf area, root length, root dry weight, and stem dry weight of sappanwood seedlings.	Rosniawaty et al., 2023d
3.	Callus of sappanwood	Auxins + cytokinins	The cultures maintained at a temperature of 28 ± 2 °C. at a 16-hours light/8-hours dark cycle	Inducing callus formation than the use of a single phytohormone	Bukke & Shankar, 2014
4	Sappanwood seedlings	Cytokinins and gibberelins	Foliar spray on sappanwood seedlings	The application of BAP at 60 ppm and gibberellin at 150 ppm produced the highest root length response.	Rosniawaty et al., 2023e
5.	Sappanwood seedlings	Methyl jasmonate at 50 μ M and salicylic acid at 50 μ M	Foliar spray at two-week intervals	The separate application of phytohormone was effective in increasing the number of leaves during the 12 and 14 weeks after planting (WAP)	Rosniawaty, et al., 2024b
6.	Fresh foliage sample from 16-month-old plants	Salicylic acid 200 ppm and methyl jasmonate 300 ppm	Foliar spray; spacing 1x1 m, 2x2 m, 3x3 m	Salicylic acid increase stem diameter, and interactions of 1 x 1 m planting distance with methyl jasmonates improved phenolic and flavonoid total in sappanwood	Rosniawaty et al., 2025a
7.	12 months sappanwood seedlings	Application of chicken, cow, and goat manure	Planting distance levels 1 x 1 m, 2 x 2 m, 3 x 3 m.	An interaction between planting distance and manure application influenced stem diameter of sappanwood at 3 months after planting, manure application showed no significant effect on the measured parameters.	Rosniawaty, et al., 2024c
8.	Fresh foliage samples from 3.5 years old sappanwood plants	Rice husk, coconut coir dust	Different growing media	Rice husk-based growing media produced the greatest stem diameter and highest total flavonoid content in sappanwood compared to the control Similarly, compost-based growing media were shown to influence both plant growth and bioactive compound.	Srisaikhram & Rupitak, 2020

Post-Harvest Effect on Bioactive Compound. Post-harvest handling is critical for preserving the quality and bioactive compounds of sappanwood, particularly brazilin, the major phenolic pigment responsible for its red color and antioxidant activity. Improper handling can lead to degradation of bioactive compounds, color loss, and reduced pharmacological efficacy. High drying or storage temperatures accelerate oxidation and hydrolysis of vitamin C, phenols, flavonoids, glycosides, and pigments, reducing antioxidant activity and therapeutic potential (ElGamal et al., 2023). Exposure to air and light promotes oxidation of polyphenols, carotenoids, and anthocyanins, driving color fading and loss of radical-scavenging capacity (Ndhlala et al.,

2025). Insufficient drying or storage in a humid environment can promote the growth of mold and microorganisms, which may change the plant's chemical composition and reduce product safety (Patil et al., 2024).

In plants with wood as the target organ, such as cinnamon, wood sorting can determine quality classes based on color, flavor, moisture content, ash content, and oil yield (Izhar & Jendri, 2022). Drying is necessary to prevent mold and to ensure the product can be stored safely for a long period (Baiqi et al., 2019). Drying can be carried out using traditional methods by sun-drying or using controlled heat dryers such as ovens (Baiqi et al., 2019). Post-harvest handling of sappanwood can be seen in Table 5.

Table 5. Post harvest technique of sappanwood

Post-harvest	Purpose	Methods	Key findings	References
Drying	To evaluate the effects of foaming agents and drying temperature on the drying kinetics and phytochemical properties of sappanwood extract powder	The use of foaming agents (egg albumin and gum Arabic) and drying temperatures (40, 60, and 80 °C).	Optimal drying of sappanwood extract was achieved using 5% egg albumin and 25% gum Arabic at 64.1 °C for 64.7 minutes, resulting in a drying process approximately seven times faster than without a foaming agent, while retaining 87.25% of total phenolic compounds.	Utari et al., 2023
Blanching & drying	To identify the optimal method to preserve brazilin, the phytochemical responsible for the red coloration, in a stable and effective form	Steam blanching, water blanching, sun drying, and cabinet drying were evaluated, and the traditional method without blanching and with sun drying was used as the control.	The combination of water blanching and cabinet drying (BCD) produced the highest red color intensity, representing a 48% increase compared to the control. This increase in color intensity was consistent with the higher brazilin content, which was 376% greater than that of the control. In addition, the BCD treatment exhibited the highest antioxidant activity and total phenolic content, with an IC ₅₀ value of 98.99 ppm and a total phenolic content of 213.12 mg GAE/g. These results indicate that brazilin plays an important role as an antioxidant compound.	Septiyani et al., 2024

Table 5 shows that the drying process affects the secondary metabolite content of sappanwood. Brazilin is highly susceptible to oxidation when exposed to air, heat, light, or pH changes, converting its hydroxyl groups (-OH) into carboxyl groups (-COOH) (Utari et al., 2023). The stability of brazilin also can be influenced by metal ion interactions (Ngamwonglumlert et al., 2020) and temperature of processing the product (Septiyani et al., 2024). Sappanwood is widely used in beverages without pre-treatment, though pH, temperature, and light can significantly affect drink quality and brazilin stability during storage (Putri et al., 2024).

Foam mat drying is applied to stabilize such unstable compounds through the addition of foaming agents. The foam, a gas dispersion in a liquid or solid, is stabilized by surfactants or surface-active agents (Utari et al., 2023; Hardy & Jideani, 2017). Egg albumin, as a foaming agent, enhances lamellar interfacial viscoelasticity and generates small initial bubbles, resulting in a more stable foam (Utari et al., 2023). In contrast, the role of gum Arabic is to form a highly viscous solution due to its large polysaccharide structures, which hinders foam formation and leads to uneven pore distribution in the dried extract (Utari et al., 2023).

Blanching can inactivate enzymes, which may cause undesirable color changes in dried products, while also helping to stabilize bioactive compounds; however, phenolic losses may still occur due to thermal degradation or leaching, reducing antioxidant activity (Deylami et al., 2016). During drying, prolonged heat can degrade pigments and diminish color intensity, and oxidation reactions can further contribute to color changes (ElGamal et al., 2023). In addition, drying affects antioxidant activity through the loss of water-soluble antioxidants, the retention of lipid-soluble compounds, and the Maillard reaction, which is a heat-induced reaction between amino acids and reducing sugars that can lead to browning and alter bioactive compounds (Calín-Sánchez et al., 2020).

Optimized post-harvest strategies – including blanching, controlled drying, and careful extract processing – are essential to maintain the quality, stability, and functional properties of sappanwood. Proper handling not only enhances its pharmacological potential but also supports its use as a standardized raw material for medicinal, functional food, and natural colorant applications.

Conclusion

Sappanwood contains a wide range of secondary metabolites, including flavonoids, phenolics, anthraquinones, triterpenoids, steroids, alkaloids, and tannins, which are suggested to have significant health benefits. These metabolites are distributed in various parts of the plant, with the heartwood generally containing the highest concentration, particularly of brazilin. Numerous production techniques have been studied to optimize morphological and physiological traits as well as the secondary metabolite content that governs antioxidant activity. Practices such as adjusting planting density and applying different types of fertilizers have been shown to enhance these parameters. In addition to pre-harvest treatments, post-harvest processes are also critical, as they determine the quality and properties of sappanwood as an herbal drink. Post-harvest methods such as drying and blanching play a key role in preserving brazilin content and ensuring product quality.

References

- Agustin E, Pratiwi A. 2023. The Utilization of Secang Wood (*Caesalpinia sappan* L.) Extract Nanoemulsion as Natural Pigment in Lip Cream Preparations. *Journal of Drug Delivery and Therapeutics*, 13(10): 54–58. <https://doi.org/10.22270/jddt.v13i10.6242>
- Ahmed GJ, Li X. 2022. Hormonal regulation of health-promoting compounds in tea (*Camellia sinensis* L.). *Plant Physiology and Biochemistry*, 185: 390–400. <https://doi.org/10.1016/j.plaphy.2022.06.021>
- Ahmad A, Ali H, Khan H, Begam A., Khan S, Ali SS, Ahmad N, Fazal H, Ali M, Hano C, Ahmad N, Abbasi BH. 2020a. Effect of gibberellic acid on production of biomass, polyphenolics and steviol glycosides in adventitious. *Plants*, 9: 420–434.
- Ahmad I, Arifianti AE, Sakti AS, Saputri FC, Mun'im A. 2020b. simultaneous natural deep eutectic solvent-based compounds of Cinnamon bark and Sappan wood as a Dipeptidyl Peptidase IV inhibitor. *Molecules*, 25: 1–11.

- Ahmadi AM, Wulandari RA, Taryono. 2020. Performance of three clones tea (*Camellia sinensis* L.) seedling on the two different seedling media. *Vegetalika*, 9(2): 359–372.
- Ahmed R, Jeyabalan, G, Jeyabalan JB. 2024. Pharmacognostical Studies on the Leaves of *Caesalpinia sappan* Linn (Fabaceae). *International journal of pharmaceutical quality assurance*, 15(02): 917–923. <https://doi.org/10.25258/ijpqa.15.2.59>
- Al-Adawiah AR, Rosniawaty S, Anjarsari IRD. 2023. Respons peningkatan perkecambah benih dan pertumbuhan bibit tanaman secang (*Caesalpinia sappan* L.) terhadap penggunaan zat pengatur tumbuh dan media tanam yang berbeda. *Jurnal Agrikultura*, 66(1): 66–73.
- Amarawati GAK, Susanti NMP, Laksmiani NPL. 2019. Aktivitas anti-rheumatoid arthritis dari brazilin dan brazilin secara in silico. *Jurnal Kimia*, 13(2): 153–158. <https://doi.org/10.24843/jchem.2019.v13.i02.p05>
- Anjarsari IRD. 2023. Potensi penggunaan zat pengatur tumbuh sitokinin dan giberelin pada budidaya teh (*Camellia sinensis* (L.) O. Kuntze) di Indonesia. *Agronomika*, 21(1): 20–24.
- Arsiningtyas IS. 2021. Antioxidant profile of heartwood and sapwood of *Caesalpinia sappan* L. tree's part grown in Imogiri Nature Preserve, Yogyakarta. *IOP Conference Series: Earth and Environmental Science*, 810(1). <https://doi.org/10.1088/1755-1315/810/1/012040>
- Artati A, Pratama R, Nurisyah N, Asyikin A, Abdullah T, Daswi DR, Dewi R. 2025. Phytochemical testing, antioxidant activity and determination of specific and non-specific parameters of secang wood extract (*Caesalpinia sappan* L.). *Journal of Research in Science Education*, 11(2):918–929. <https://doi.org/10.29303/jppipa.v11i2.10563>
- Baiqi AU, Utami PP, Anugrah D, Fauzan AA, Ningsih WS, Rusydi MI. 2019. Improving the quality and quantity of cinnamon drying process using art cave in Lambung Bukit West Sumatra. *IOP Conference Series: Materials Science and Engineering*, 602(1). <https://doi.org/10.1088/1757-899X/602/1/012023>
- Bashir S, Qayyum MA, Husain A, Bakhsh A, Ahmed N, Hussain MB, Elshikh MS, Alwahibi MS, Almunqedhi BMA, Hussain R, Wang YF, Zhou Y, Diao ZH. 2021a. Efficiency of different types of biochars to mitigate Cd stress and growth of sunflower (*Helianthus*; L.) in wastewater irrigated agricultural soil. *Saudi Journal of Biological Sciences*, 28(4): 2453–2459. <https://doi.org/10.1016/j.sjbs.2021.01.045>
- Bhaskar R, Xavier LSE, Udayakumaran G, Kumar DS, Venkatesh R, & Nagella P. 2022. Biotic elicitors: a boon for the in-vitro production of plant secondary metabolites. *Plant Cell, Tissue and Organ Culture*, 149(1-2): 7–24. <https://doi.org/10.1007/s11240-021-02131-1>
- Bhat B, Islam S, Ali A, Sheikh B, Tariq L, Islam S, Dar T. 2020. Role of micronutrients in secondary metabolism of plants, 311–329. https://doi.org/10.1007/978-3-030-49856-6_13
- Bukke AN, Shankar PC. 2014. Effect of different plant growth regulators on callus induction in *Caesalpinia Sappan* L. a medicinal plant. *International Journal of Recent Scientific Research*, 5(11): 1991–1994.
- Calín-Sánchez Á, Lipan L, Cano-Lamadrid M, Kharaghani A, Masztalerz K, Carbonell-Barrachina AA, Figiel A. 2020. Comparison of traditional and novel drying techniques and its effect on quality of fruits, vegetables and aromatic herbs. In *Foods* 9(9). MDPI AG. <https://doi.org/10.3390/foods9091261>
- Cao Y, Yang Y, Tan Z, Feng X, Tian Z, Liu T, Pan Y, Wang M, Su X, Liang H, Guo S. 2025. Metabolomics combined with photosynthetic analysis reveals potential mechanisms of phenolic compound accumulation in *Lonicera japonica* induced by nitrate nitrogen supply. *International Journal of Molecular Sciences*, 26. <https://doi.org/10.3390/ijms26094464>
- Caretto S, Linsalata V, Colella G, Mita G, Lattanzio V. 2015. Carbon fluxes between primary metabolism and phenolic pathway in plant tissues under stress. *International Journal of Molecular Sciences*, 16(11): 26378–26394. <https://doi.org/10.3390/ijms161125967>
- Cui Z, Qu L, Zhang Q, Lu F, Liu F. 2024. Brazilin-7-2-butenoate inhibits amyloid β -protein aggregation, alleviates cytotoxicity, and protects *Caenorhabditis elegans*. *International Journal of Biological Macromolecules*, 264: 1–11.

- <https://doi.org/10.1016/j.ijbiomac.2024.130695>
- Cun Z, Zhang J, Hong J, Yang J, Gao L, Hao B, Chen J. 2024. Integrated metabolome and transcriptome analysis reveals the regulatory mechanism of low nitrogen-driven biosynthesis of saponins and flavonoids in *Panax notoginseng*. *Gene*. <https://doi.org/10.1016/j.gene.2024.148163>.
- Dapson RW, Bain CL. 2015. Brazilwood, sappanwood, brazilin and the red dye brazilin: From textile dyeing and folk medicine to biological staining and musical instruments. *Biotechnic and Histochemistry*, 90(6): 401-423. <https://doi.org/10.3109/10520295.2015.1021381>
- de Paula PT, Scatolino MV, de Araújo ACC, de Oliveira ACF, de Figueiredo ICR, de Assis MR, Trugilho PF. 2019. Assessing proximate composition, extractive concentration, and lignin quality to determine appropriate parameters for selection of superior Eucalyptus Firewood. *Bioenergy Research*, 12(3): 626-641. <https://doi.org/10.1007/s12155-019-10004-x>
- Deylami MZ, Abdul, Tan CP, Bakar J, Olusegun L. 2016. Effect of blanching on enzyme activity, color changes, anthocyanin stability and extractability of mangosteen pericarp: A kinetic study. *Journal of Food Engineering*, 178: 12-19. <https://doi.org/10.1016/J.JFOODENG.2016.01.001>
- Dvořák P, Krasnylenko Y, Zeiner A, Šamaj J, Takáč T. 2021. Signaling toward reactive oxygen species-scavenging enzymes in plants. In *Frontiers in Plant Science*, 11: 1-24. <https://doi.org/10.3389/fpls.2020.618835>
- ElGamal R, Song C, Rayan AM, Liu C, Al-Rejaie S, ElMasry G. 2023. Thermal degradation of bioactive compounds during drying process of horticultural and agronomic products: A comprehensive overview. In *Agronomy* 13(6). MDPI. <https://doi.org/10.3390/agronomy13061580>
- El-Nakhel C, Pannico A, Kyriacou M, Giordano M, De Pascale S, Roupael Y. 2019. Macronutrient deprivation eustress elicits differential secondary metabolites in red and green-pigmented butterhead lettuce grown in closed soilless system. *Journal of the science of food and agriculture*. <https://doi.org/10.1002/jsfa.9985>.
- Gorni PH, Pacheco AC, Moro AL, Silva JFA, Moreli RR, de Miranda GR, Pelegrini JM, Spera KD, Bronzel JL, & da Silva RMG. 2020. Salicylic acid foliar application increases biomass, nutrient assimilation, primary metabolites and essential oil content in *Achillea millefolium* L. *Scientia Horticulturae*, 270: 1-9. <https://doi.org/10.1016/j.scienta.2020.109436>
- Hardy Z, Jideani VA. 2017. Foam-mat drying technology: A review. *Critical Reviews in Food Science and Nutrition*, 57(12): 2560-2572. <https://doi.org/10.1080/10408398.2015.1020359>
- He ZJ, Zhu FY, Li SS, Zhong L, Tan HY, Wang K. 2017. Inhibiting ROS-NF-κB-dependent autophagy enhanced brazilin-induced apoptosis in head and neck squamous cell carcinoma. *Food and Chemical Toxicology*, 101: 55-66. <https://doi.org/10.1016/j.fct.2017.01.002>
- Hilal B, Khan MM, Fariduddin Q. 2024. Recent advancements in deciphering the therapeutic properties of plant secondary metabolites: phenolics, terpenes, and alkaloids. *Plant Physiology and Biochemistry*, 211. <https://doi.org/10.1016/J.PLAPHY.2024.108674>
- ICRAF.2023. Agroforestry Tree Database: *Caesalpinia sappan*. World Agroforestry Centre. <https://apps.worldagroforestry.org>
- Izhar L, Hendri J. 2022. Postharvest standard practices for improving Cinnamon quality product in Kerinci. *IOP Conference Series: Earth and Environmental Science*, 1024(1). <https://doi.org/10.1088/1755-1315/1024/1/012078>
- Jain D, Bisht S, Parvez A, Singh K, Bhaskar P, Koubouris G. 2024. Effective biotic elicitors for augmentation of secondary metabolite production in medicinal plants. *Agriculture (Switzerland)*, 14(796): 1-24. Multidisciplinary Digital Publishing Institute (MDPI). <https://doi.org/10.3390/agriculture14060796>
- Jakovljević D, Topuzović M, Stanković M. 2019. Nutrient limitation as a tool for the induction of secondary metabolites with antioxidant activity in basil cultivars. *Industrial Crops and Products*.

- <https://doi.org/10.1016/j.indcrop.2019.06.025>
- Jamaddar S, Sarkar C, Akter S, Mubarak MS, El-Nashar HAS, El-Shazly M, Islam MT. 2023. Brazilin: An updated literature-based review on its promising therapeutic approaches and toxicological studies. In *South African Journal of Botany*, 158: 118-132. Elsevier B.V. <https://doi.org/10.1016/j.sajb.2023.04.053>
- Jan R, Asaf S, Numan M, Lubna, Kim KM. 2021. Plant secondary metabolite biosynthesis and transcriptional regulation in response to biotic and abiotic stress conditions. *Agronomy*, 11(5): 1-31. <https://doi.org/10.3390/agronomy11050968>
- Janku M, Luhová, L, Petrivalský M. 2019. On the origin and fate of reactive oxygen species in plant cell compartments. In *Antioxidants*, 8(14): 1-15. MDPI. <https://doi.org/10.3390/antiox8040105>
- Jia S, Zhao X, Huang J, Yao X, Xie F. 2025. Phosphorus alleviates cadmium damage by reducing cadmium accumulation and enhancing antioxidant enzymes at the vegetative phase in Soybean. *Agronomy*, 15(637): 1-19. <https://doi.org/10.3390/agronomy15030637>
- Jie H, Zhao L, Ma Y, Rasheed A, Jie Y. 2023. Integrated transcriptome and metabolome analysis reveal that exogenous gibberellin application regulates lignin synthesis in ramie. *Agronomy*, 13(6). <https://doi.org/10.3390/agronomy13061450>
- Kannathasan K, Kokila P. 2021. Dyeing of cotton fabric by *Caesalpinia sappan* aqueous extract at different temperatures and mordants. *Current Botany*, 12: 188-191. <https://doi.org/10.25081/cb.2021.v12.7277>
- Kashchenko NI, Olennikov DN, Chirikova NK. 2021. Phytohormones and elicitors enhanced the ecdysteroid and glycosylflavone content and antioxidant activity of *Silene repens*. *Applied Sciences (Switzerland)*, 11:1-14. <https://doi.org/10.3390/app112311099>
- Kaushik B, Sharma J, Yadav K, Kumar P, Shourie A. 2021. Phytochemical properties and pharmacological role of plants: secondary metabolites. *Biosciences Biotechnology Research Asia*, 18(1): 23-35. <https://doi.org/10.13005/bbra/2894>
- Ke SW, Chen GH, Chen CT, Tzen JTC, Yang CY. 2018. Ethylene signaling modulates contents of catechin and ability of antioxidant in *Camellia sinensis*. *Botanical Studies*, 59(1). <https://doi.org/10.1186/s40529-018-0226-x>
- Kumari N, Varghese BA, Devi S, Khan MA, Jangra S, Kumar A. 2020. Abiotic elicitation: A tool for producing bioactive compounds. *Plant Archives*, 20: 2683-2689.
- Li K, Wan M, Han M, Yang L. 2025. The response of *Panax ginseng* root microbial communities and metabolites to nitrogen addition. *BMC Plant Biology*, 25. <https://doi.org/10.1186/s12870-025-07031-6>
- Liu J, Liu Y, Wang Y, Abozeid A, Zu YG, Tang ZH. 2017. The integration of GC-MS and LC-MS to assay the metabolomics profiling in *Panax ginseng* and *Panax quinquefolius* reveals a tissue- and species-specific connectivity of primary metabolites and ginsenosides accumulation. *Journal of Pharmaceutical and Biomedical Analysis*, 135: 176-185. <https://doi.org/10.1016/j.jpba.2016.12.026>
- Liwanda N, Nurinayah I, Mubayyinah H, Pratiwi ARR, Wahyuningrum T, Ashari RZ, Aisyah SI, Nurcholis W. 2023. Effect of cow manure fertilizer on growth, polyphenol content, and antioxidant activity of purslane plants. *International Journal of Chemical and Biochemical Sciences*, 23(1): 43-54.
- Ma J, Xin X, Cao Y, Zhao L, Zhang Z, Zhang D, Fu Z, Sun J. 2024. Root growth characteristics and antioxidant system of *Suaeda salsa* in response to the short-term nitrogen and phosphorus addition in the Yellow River Delta. *Frontiers in Plant Science*, 15:1-11. <https://doi.org/10.3389/fpls.2024.1410036>
- Madani H, Escrich A, Hosseini B, Sanchez-Muñoz R, Khojasteh A, Palazon J. 2021. Biomolecule's effect of polyploidy induction on natural metabolite production in medicinal plants. 11. <https://doi.org/10.3390/biom>
- Mansoor S, Wani OA, Lone JK, Manhas S, Kour N, Alam P, Ahmad A, Ahmad P. 2022. Reactive oxygen species in plants: From source to sink. *Antioxidants*, 11(2): 1-14. MDPI. <https://doi.org/10.3390/antiox11020225>
- Mardiyanto MB, Foresty RS, Arlysia V, Chorunissa ZFN, NugrohoGD, Yasa A, Naim DM, Setyawan AD. 2023. Plants as herbal medicine at Nguter Traditional Market, Sukoharjo, Central Java, Indonesia.

- Asian Journal of Ethnobiology, 6(1):65–74. <https://doi.org/10.13057/asianjethnobiol/y060108>
- Masturi, Alighiri D, Edie SS, Hanisyifa U, Drastisianti A. 2021. Determination of total phenol and flavonoid contents and antioxidant activity from extract fraction of sappan wood (*Caesalpinia sappan* L.) by liquid-liquid extraction and vacuum liquid chromatography. Asian Journal of Chemistry, 33(8):1729–1735. <https://doi.org/10.14233/ajchem.2021.23029>
- Mhamdi A, Van Breusegem F. 2018. Reactive oxygen species in plant development. Development (Cambridge), 145: 1-12. <https://doi.org/10.1242/dev.164376>
- Miranda I, Sousa V, Ferreira J, Pereira H. 2017. Chemical characterization and extractives composition of heartwood and sapwood from *Quercus faginea*. PLoS ONE, 12(6). <https://doi.org/10.1371/journal.pone.0179268>
- Mueller M, Weinmann D, Toegel S, Holzer W, Unger FM, Viernstein H. 2016. Compounds from *Caesalpinia sappan* with anti-inflammatory properties in macrophages and chondrocytes. Food and Function, 7(3):1671–1679. <https://doi.org/10.1039/c5fo01256b>
- Muscolo A, Sidari M, Settineri G, Papalia T, Mallamaci C, Attinà E. 2019. Influence of soil properties on bioactive compounds and antioxidant capacity of *Brassica rupestris* Raf. Journal of Soil Science and Plant Nutrition, 19(4): 808–815. <https://doi.org/10.1007/s42729-019-00080-5>
- Naik PM, Al-Khayri JM. 2016. Abiotic and biotic elicitors-role in secondary metabolites production through in vitro culture of medicinal plants. In Abiotic and Biotic Stress in Plants - Recent Advances and Future Perspectives. InTech, 10: 247-277. <https://doi.org/10.5772/61442>
- Nava-Tapia DA, Cayetano-Salazar L, Herrera-Zúñiga LD, Bello-Martínez J, Mendoza-Catalán MA, Navarro-Tito N. 2022. Brazilin: Biological activities and therapeutic potential in chronic degenerative diseases and cancer. In Pharmacological Research, 175: 1-14. Academic Press. <https://doi.org/10.1016/j.phrs.2021.106023>
- Ndhlala AR, Ngobeni GT, Mulaudzi R, Lebelo SL. 2025. Different temperature storage conditions and packaging types affects colour parameters, amino acid composition, microbial contamination, and key bioactive molecules of *Moringa oleifera* Lam. Powder. Molecules, 30(20). <https://doi.org/10.3390/molecules30204048>
- Ngamwonglumlert L, Devahastin S, Chiewchan N, Raghavan GSV. 2020. Color and molecular structure alterations of brazilin extracted from *Caesalpinia sappan* L. under different pH and heating conditions. Scientific Reports, 10: 1-10. <https://doi.org/10.1038/s41598-020-69189-3>
- Nirmal NP, Rajput MS, Prasad RGSV, Ahmad M. 2015. Brazilin from *Caesalpinia sappan* heartwood and its pharmacological activities: A review. Asian Pacific Journal of Tropical Medicine, 8(6): 421–430. <https://doi.org/10.1016/j.apjtm.2015.05.014>
- Niu Y, Wang S, Li C, Wang J, Liu Z, Kang W. 2020. Effective compounds from *Caesalpinia sappan* L. on the Tyrosinase in vitro and in vivo. Natural Product Communications, 15(4): 1–8. <https://doi.org/10.1177/1934578X20920055>
- Nurulita U, Harahap ZA. 2025. 3rd Lawang Sewu International Symposium on Medical and Health Sciences. 1. Spinger Nature. Available at: https://books.google.co.id/books?hl=id&lr=&id=qo9qEQAAQBAJ&oi=fnd&pg=PA304&dq=sappanwood+seedlings&ots=d6Lg4hUMR5&sig=5w_XHz96DH8yqn42PMKGij1yYME&redir_esc=y#v=onepage&q=sappanwood%20seedlings&f=false
- Pagare S, Bhatia M, Tripathi N, Pagare S, Bansal YK. 2015. Secondary metabolites of plants and their role: Overview. Current trends in biotechnology and pharmacy, 9(3): 293-304.
- Pant P, Pandey S, Dall'Acqua S. 2021. The influence of environmental conditions on secondary metabolites in medicinal plants: A literature review. In Chemistry and Biodiversity, 18(11). John Wiley and Sons Inc. <https://doi.org/10.1002/cbdv.202100345>
- Patel RM, Patel KH, Adhvaryu MR. 2024. Enhancement of secondary metabolite production in *tinospora cordifolia* suspension cultures using gibberellic acid elicitation: A Comprehensive analysis of alkaloids and phenols. International Journal of Innovative Science and Research Technology (IJISRT), 910–917.

- <https://doi.org/10.38124/ijisrt/ijisrt24nov500>
- Patil M, Sharma S, Sridhar K, Anurag RK, Grover K, Dharni K, Mahajan S, Sharma M. 2024. Effect of postharvest treatments and storage temperature on the physiological, nutritional, and shelf-life of broccoli (*Brassica oleracea*) microgreens. *Scientia Horticulturae*, 327. <https://doi.org/10.1016/J.SCIENTA.2023.112805>
- Purba T, Situmeang R, Rohman HF, Mahyati, Arsi, Firgiyanto R, Junaedi AS, Saadah TT, Junairiah, Herawati J, Suhastyo AA. 2021. Pemupukan dan Teknologi Pemupukan. In R. Watrionthos (Ed.), *Yayasan Kita Menulis* (1st ed.).
- Putri EA, Rahmadhia SN, Septiyani R. 2024. Estimation of the shelf life of wedang uwuh ready to drink with blanching and non blanching treatments. *Jurnal Al-Azhar Indonesia Seri Sains dan Teknologi*, 9(1). <https://doi.org/10.36722/sst.v9i1.2133>
- Rahayu YYS, Araki T, Rosleine D. 2020. Factors affecting the use of herbal medicines in the universal health coverage system in Indonesia. *Journal of Ethnopharmacology*, 260. <https://doi.org/10.1016/j.jep.2020.112974>
- Rahimi A, Gitari H, Lyons G, Heydarzadeh S, Tuncturk M, Tuncturk R. 2023. Effects of vermicompost, compost and animal manure on vegetative growth, physiological and antioxidant activity characteristics of *Thymus vulgaris* L. under water stress. *Yuzuncu Yil University Journal of Agricultural Sciences*, 33(1): 40-53. <https://doi.org/10.29133/yyutbd.1124458>
- Rajput MS, Nirmal NP, Nirmal SJ, Santivarangkna C. 2022. Bio-actives from *Caesalpinia sappan* L.: Recent advancements in phytochemistry and pharmacology. *South African Journal of Botany*, 151: 60-74. <https://doi.org/10.1016/j.sajb.2021.11.021>
- Raptania CN, Zakia S, Fahira AI, Amalia R. 2024. Article review: Brazilin as potential anticancer agent. *Frontiers in Pharmacology*, 15: 1-9. *Frontiers Media SA*. <https://doi.org/10.3389/fphar.2024.1355533>
- Rosniawaty S, Ariyanti M, Mubarak S, Sudirja R, Rachman AL, Septiani D, Hamis ZM. 2025a. Impact of elicitor applications and planting distances on growth and phytochemical properties of Sappanwood (*Caesalpinia sappan* L.). *Research on Crops*, 26(1): 132-137. <https://doi.org/10.31830/2348-7542.2025.ROC-1152>
- Rosniawaty S, Bahjatien ID, Soleh MA, Ariyanti M, Sudirja R. 2024b. Potensi metil jasmonat dan asam salisilat sebagai elisitor dalam mempengaruhi pertumbuhan bibit tanaman secang (*Caesalpinia sappan* L.). *Agroland*, 31(1): 55-62.
- Rosniawaty S, Prasasta FP, Ariyanti M, Anjarsari IRD, Soleh MA, Mubarak S, Sudirja R. 2024c. Effect of crop pattern and types of manure on the growth of Sappan wood (*Caesalpinia Sappan* L.) in Jatinangor, West Java, Indonesia. *Research on Crops*, 25(1): 122-127. <https://doi.org/10.31830/2348-7542.2024.ROC-1042>
- Rosniawaty S, Anjarsari IRD, Sudirja R, Mubarak S, Fatmawati D. 2023d. Effect of growth regulators and organic matter on the growth of Sappan wood seedlings (*Caesalpinia sappan* L.). *Research on Crops*, 24(1): 198-209. <https://doi.org/10.31830/2348-7542.2023.ROC-896>
- Rosniawaty S, Al-Adawiah AR, Mubarak S, Sudirja R, Ariyanti M. 2023e. Respons pertumbuhan akar bibit secang (*Caesalpinia sappan* L.) di dataran rendah terhadap sitokinin dan giberelin. *Agrisaintifika*, 7(1).
- Rupngam T, Udomkun P, Boonupara T, Kaewlom P. 2025. Enhancing soil health, growth, and bioactive compound accumulation in sunflower sprouts using agricultural byproduct-based soil amendments. *Agronomy*, 15: 1-33. <https://doi.org/10.3390/agronomy15051213>
- Sakti AS, Saputri FC, Mun'im A. 2019. Optimization of choline chloride-glycerol based natural deep eutectic solvent for extraction bioactive substances from *Cinnamomum burmannii* barks and *Caesalpinia sappan* heartwoods. *Heliyon*, 5: 1-9. <https://doi.org/10.1016/j.heliyon.2019.e02915>
- Sanam SC, Marzvan S, Khiavi SJ, Rahimi M. 2020. Changes in growth, biochemical, and chemical characteristics and alteration of the antioxidant defense system in the leaves of tea clones (*Camellia sinensis* L.) under drought stress. *Scientia Horticulturae*, 265: 1-10.

- <https://doi.org/10.1016/j.scienta.2020.109257>
- Sarwar M, Patra JK, Ali A, Maqbool M, Arshad MI. 2020. Effect of compost and NPK fertilizer on improving biochemical and antioxidant properties of *Moringa oleifera*. *South African Journal of Botany*, 129: 62–66. <https://doi.org/10.1016/j.sajb.2019.01.009>
- Septiani D, Suryadi H, Mun'im A. 2022. Improving enzyme-assisted extraction of brazilin from Sappanwood (*Caesalpinia Sappan* L.) extract by fungal cellulase. *Pharmacognosy Journal*, 14(1): 21–28. <https://doi.org/10.5530/pj.2022.14.4>
- Septiyani R, Wikandari R, Santoso U, Raharjo S. 2024. Brazilin content, color stability, and antioxidant activity of sappan wood (*Caesalpinia Sappan* L.) traditional drink by different blanching and drying methods. *Trends in Sciences*, 21(12). <https://doi.org/10.48048/tis.2024.8535>
- Settharaksa S, Monton C, Charoenchai L. 2019. Optimization of *Caesalpinia sappan* L. heartwood extraction procedure to obtain the highest content of brazilin and greatest antibacterial activity. *Journal of Integrative Medicine*, 17(5): 351–358. <https://doi.org/10.1016/j.joim.2019.05.003>
- Setyowati N, Masyhuri, Mulyo JH, Irham, Yudhistira B. 2023. The hidden treasure of wedang uwuh, an ethnic traditional drink from Java, Indonesia: Its benefits and innovations. *International Journal of Gastronomy and Food Science*, 31. <https://doi.org/10.1016/j.ijgfs.2023.100688>
- Singh J, Mirza A, Singh S, Singh P. 2017a. Impact of phytohormones on physio-chemical properties of tropical and subtropical fruits: A review. *Plant Archives*, 18(1): 12–18.
- Singh R, Singh S, Parihar P, Mishra RK, Tripathi DK, Singh V., Chauhan DK, Prasad SM. 2016b. Reactive oxygen species (ROS): Beneficial companions of plants' developmental processes. In *Frontiers in Plant Science*, 7: 1-19. *Frontiers Media S.A.* <https://doi.org/10.3389/fpls.2016.01299>
- Srisaikhram S, Rupitak Q. 2020. Growth of *Caesalpinia sappan* L. by using different growing media and evaluation of antioxidant activity of foliage. *Science Technology and Engineering Journal (STEJ)*, 6(2): 50–61. <https://stej.msu.ac.th/wp-content/uploads/2021/01/JOURNAL-STEJ6-2-0005-56-67.pdf>
- Tamburini D. 2019. Investigating Asian colourants in Chinese textiles from Dunhuang (7th-10th century AD) by high performance liquid chromatography tandem mass spectrometry – Towards the creation of a mass spectra database. *Dyes and Pigments*, 163: 454–474. <https://doi.org/10.1016/j.dyepig.2018.12.025>
- Thanayutsiri T, Patrojanasophon P, Opanasopit P, Ngawhirunpat T, Laiwattanapaisal W, Rojanarata T. 2023. Rapid and efficient microwave-assisted extraction of *Caesalpinia sappan* Linn. heartwood and subsequent synthesis of gold nanoparticles. *Green Processing and Synthesis*, 12:1–9. <https://doi.org/10.1515/gps-2022-8109>
- Thiyageshwari S, Gayathri P, Krishnamoorthy R, Anandham R, Paul D. 2018. Exploration of rice husk compost as an alternate organic manure to enhance the productivity of blackgram in typic haplustalf and typic rhodustalf. *International Journal of Environmental Research and Public Health*, 15: 1–14. <https://doi.org/10.3390/ijerph15020358>
- Torun H, Novák O, Mikulík J, Pěňčík A, Strnad M, Ayaz FA. 2020. Timing-dependent effects of salicylic acid treatment on phytohormonal changes, ROS regulation, and antioxidant defense in salinized barley (*Hordeum vulgare* L.). *Scientific Reports*, 10(1): 1–17. <https://doi.org/10.1038/s41598-020-70807-3>
- Tu WC, Ding LF, Peng LY, Song LD, Wu X-De, Zhao QS. 2022. Cassane diterpenoids from the seeds of *Caesalpinia bonduc* and their nitric oxide production and α -glucosidase inhibitory activities. *Phytochemistry*, 193: 1–11. <https://doi.org/10.1016/j.phytochem.2021.112973>
- Twaij BM, Hasan MN. 2022. Bioactive secondary metabolites from plant sources: Types, synthesis, and their therapeutic uses. *International Journal of Plant Biology*, 13(1): 4–14. <https://doi.org/10.3390/ijpb13010003>
- Uddin GM, Kim CY, Chung D, Kim KA, Jung SH. 2015. One-step isolation of sappanol and brazilin from *Caesalpinia sappan* and their effects on oxidative stress-induced retinal death. *BMB Reports*, 48(5): 289–294. <https://doi.org/10.5483/BMBRep.2015.48.5.189>

- Utari FD, Sari DA, Kurniasari L, Kumoro AC, Djaeni M, Hii CL. 2023. The enhancement of sappanwood extract drying with foaming agent under different temperature. *AIMS Agriculture and Food*, 8(1): 214–235. <https://doi.org/10.3934/AGRFOOD.2023012>
- Vij T, Anil PP, Shams R, Dash KK, Kalsi R, Pandey VK, Harsányi E, Kovács B, Shaikh AM. 2023. A comprehensive review on bioactive compounds found in *Caesalpinia sappan*. *Molecules*, 28: 1-22. Multidisciplinary Digital Publishing Institute (MDPI). <https://doi.org/10.3390/molecules28176247>
- Wang J, Song L, Gong X, Xu J, Li M. 2020a. Functions of jasmonic acid in plant regulation and response to abiotic stress. *International Journal of Molecular Sciences*, 21: 1-17. <https://doi.org/10.3390/ijms21041446>
- Wang P, Liu WC, Han C, Wang S, Bai MY, Song CP. 2024b. Reactive oxygen species: Multidimensional regulators of plant adaptation to abiotic stress and development. In *Journal of Integrative Plant Biology*, 66(3): 330-367. John Wiley and Sons Inc. <https://doi.org/10.1111/jipb.13601>
- Wani SH, Kumar V, Shriram V, Sah SK. 2016. Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. *Crop Journal*, 4(3): 162–176. <https://doi.org/10.1016/j.cj.2016.01.010>
- Waszczak C, Carmody M, Kangasjärvi J. 2018. Reactive oxygen species in plant signaling. *Annual Review of Plant Biology*, 1: 209–236. <https://doi.org/10.1146/annurev-arplant-042817>
- Wudtiwai B, Kodchakorn K, Shwe TH, Pothacharoen P, Phitak T, Suninthaboonrana R, Kongtawelert P. 2023. Brazilein inhibits epithelial-mesenchymal transition (EMT) and programmed death ligand 1 (PD-L1) expression in breast cancer cells. *International Immunopharmacology*, 118: 1-12. <https://doi.org/10.1016/j.intimp.2023.109988>
- Wyk ASV, Prinsloo G. 2020. Health, safety and quality concerns of plant-based traditional medicines and herbal remedies. In *South African Journal of Botany*, 133: 54-62. Elsevier B.V. <https://doi.org/10.1016/j.sajb.2020.06.031>
- Xuan Q, Zhou JF, Jiang F, Zhang W, Wei A, Zhang W, Zhang Q, Shen H, Li H, Chen C, Wang P. 2022. Sappanwood-derived polyphenolic antidote of amyloid toxicities achieved detoxification via inhibition/reversion of amyloid fibrillation. *International Journal of Biological Macromolecules*, 214: 446–458. <https://doi.org/10.1016/j.ijbiomac.2022.06.141>
- Yan X, Xu L, Qi C, Chang Y, Zhang J, Li N, Shi B, Guan B, Hu S, Huang C, Wang H, Chen Y, Xu X, Lu J, Xu G, Chen C, Li S, Chen Y. 2025. Brazilein alleviates acute lung injury via inhibition of ferroptosis through the SIRT3/GPX4 pathway. *Apoptosis*, 30: 768–783. <https://doi.org/10.1007/S10495-024-02058-W/METRICS>
- Yang L, Wen KS, Ruan X, Zhao YX, Wei F, Wang Q. 2018. Response of plant secondary metabolites to environmental factors. In *Molecules*, 23: 1-26. MDPI AG. <https://doi.org/10.3390/molecules23040762>
- Zhang J, Shoaib N, Lin K, Mughal N, Wu X, Sun X, Zhang L, Pan K. 2024. Boosting cadmium tolerance in *Phoebe zhennan*: The synergistic effects of exogenous nitrogen and phosphorus treatments promoting antioxidant defense and root development. *Frontiers in Plant Science*, 15: 1-17. <https://doi.org/10.3389/fpls.2024.1340287>
- Zhao B, Liu Q, Wang B, Yuan F. 2021. Roles of phytohormones and their signaling pathways in leaf development and stress responses. *Journal of Agricultural and Food Chemistry*, 69(12): 3566–3584. <https://doi.org/10.1021/acs.jafc.0c0790>
- Zhu NL, Sun ZH, Hu MG, Wu TY, Yuan JQ, Wu HF, Tian Y, Li PF, Yang JS, Ma GX, Xu XD. 2017. New cassane diterpenoids from *Caesalpinia sappan* and their antiplasmodial activity. *Molecules*, 22(10). <https://doi.org/10.3390/molecules22101751>

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Comparative analysis of color, texture, and water content in three shallot cultivars at different harvest ages

Abstract. Shallots are a major horticultural commodity with high economic value. The quality of shallots is determined not only by the quantity of the harvest but also by physical characteristics such as bulb color, texture, and water content. This study aims to evaluate the differences in these physical parameters in three popular local cultivars: Brebes, Sumenep, and Tuktuk, harvested at 56, 63, and 70 days after planting (DAP), respectively. This research uses a Randomized Block Design consisting of 9 treatment combinations and analyzed using the SmartstatXL application. Color measurements are conducted using the CIELAB method and converted to CIELCH and RGB for color type identification. The texture of the tuber is measured using the Texture Analyzer MCT-2150W, while the water content is analyzed using the oven method. The study's results demonstrate a negative correlation between bulb texture and water content. Sumenep cultivar showed the lowest water content (77.57%) and highest texture (17.97 N) compared to others. Additionally, each cultivar displays significant color variations that correspond to differences in harvest maturity. The Brebes cultivar tuber changes from dark coffee to dark taupe, the Sumenep cultivar from mocha to beaver, and the Tuktuk cultivar from rose taupe to dark coffee. This means that the color has changed from dull and gray to bright and full. These findings provide practical guidance for determining the optimal harvest timing to preserve shallot quality during postharvest handling.

Keywords: CIELAB · Cultivar · Harvest age · Shallots · Texture · Water content

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Introduction

Shallot (*Allium cepa* var. *aggregatum* L.) is one of the leading horticultural commodities with high economic value. Market demand for shallots is determined not only by the quantity of the harvest but also by quality in terms of physical attributes including bulb color, texture (Kurniawan et al., 2020), and water content (Kaveri et al., 2015). These parameters are very important as they affect consumer preference, shelf life, and post-harvest processing efficiency. Studies on shallot quality often focus on single physical parameters or individual cultivars, while comparative analyses across cultivars and harvest ages, as well as the interrelationships among key physical traits, remain limited.

The physical quality of shallot can be influenced by various factors, including genetic variation in the form of cultivars and harvest age. In Indonesia, there are various local cultivars with distinct characteristics, including the Brebes, Sumenep, and Tuktuk cultivars. Each cultivar has its own unique characteristics, such as bulb color, bulb texture, and water content, which affect quality and storage durability. According to previous study, the Brebes cultivar is characterized by its uniform shape, bright red color, and harder texture (Kurniawan et al., 2020). The Tuktuk cultivar typically has a rounder shape, good adaptability, and lower dry weight (Wijoyo et al., 2020). Meanwhile, the Sumenep cultivar has a relatively low water content (Kusumiyati et al., 2024) which makes it widely used for fried onions.

Harvest age is a significant determinant of shallots' physical quality in addition to genetic considerations. Shallots with a high water content are typically produced by harvesting them too early, whereas those with a lower water content can be harvested (Ansar et al., 2022). Shallots' texture after harvesting is influenced by their water content. A tougher texture may be the result of low water content (Sukorini, 2024).

Shallot quality can also be assessed by looking at the bulb, especially the color of its skin. Color changes may be influenced by how long shallot skin is exposed to sunlight (Ghazali et al., 2023). Shallots' skin color is associated with the compounds they contain, such as flavonoids, phenolics, anthocyanins, and antioxidants (Vojnović et al., 2025). Therefore, measuring shallot color using CIELAB-based technology can be useful for a variety of purposes, including determining harvest age in the field, setting

market standards for product quality, and monitoring the distribution and storage processes. Color parameter measurements using the CIE Lab* and LCh* systems, as well as texture testing, provide an objective picture of the visual and mechanical quality of shallot bulbs.

Shallot bulb quality can be objectively assessed through texture testing and color parameter measurements using the CIE Lab* and LCh* systems. Meanwhile, water content analysis provides important information regarding potential shelf life and weight loss during storage. Nevertheless, there are currently few studies that compare these three quality parameters in-depth while taking harvest age and cultivar genetic factors into account. This study aims to evaluate and compare color, texture, and water content among three local shallot cultivars (Brebes, Sumenep, and Tuktuk) harvested at different maturity stages, to determine the optimal harvest age for maintaining posharvest quality. The results of this study are expected to provide information for farmers and industry participants in determining optimal harvesting strategy in different cultivar to maintain the quality of shallots.

Materials and Methods

Time and Location. This experiment was conducted in open fields in Sindanggalih Village, Cimanggung District, Sumedang Regency, West Java, from October 2024 to December 2025. The experimental site was located at an altitude of 746 m above sea level with Latosol and Alluvial soil types and a temperature range of 26-30 °C (Guswita et al., 2020). The research was conducted at the Horticulture Laboratory, Faculty of Agriculture, Universitas Padjadjaran University from January to March 2025.

Material. The seeds came from farmers in Brebes for the Brebes cultivar, while the Sumenep and Tuktuk cultivars came from Garut, each of which had been stored for at least 2 months. The cultivated land was then fertilized with 20 tons/ha of chicken manure and 2 tons/ha of dolomite. Planting was carried out in open fields using a monoculture system with a planting distance of 15 x 15 cm. Follow-up fertilization used NPK 16:16:16 and KCL at 14, 28, and 42 days after planting.

Experimental Design. The experiment used a Randomized Block Design with different

treatment combinations of three cultivars (Brebek, Sumenep, and Tuktuk) and three harvest ages: 54, 63, and 70 days after planting (DAP). There were 9 treatment combinations, each with 3 replicates, and each replicate consisted of 3 samples, which were then analyzed using the SmartstatXL application with a Scott-Knott post hoc test at a 5% level.

Texture Measurement. Texture measurements refer to the method of Devgan et al., (2019) which has been modified and adapted to the product being tested. Measurement of onion bulb texture was carried out using a Texture Analyzer (Material Testing Machine-2150W from Japan). The tool was calibrated before being used for testing. The probe used was a cylindrical probe with a diameter of 2 mm, a compression mode type, a 50N force sensor and a pressing speed of 300 mm/min. Measurements were carried out by operating the tool connected to the software. The probe will automatically insert into the onion sample being tested. Measurements were carried out at three points: on the right side, left side, and center of the onion bulb. The texture value of the onion bulb is displayed in units of g/force or Newton.

Water Content Determination. To measure water content, a method referring to Ansar et al., (2022) was used. Onion samples were cut into small pieces and weighed 1-4 g. Then, the onion pieces were placed in aluminum foil cups whose weight had been homogenized. The cups were arranged on a baking sheet and then oven-dried for 3 hours at 105 °C. The cups were placed in a desiccator for 10 minutes. The samples were then weighed until they had a constant dry weight. The water content measurement was calculated using the following formula:

$$\text{Water content (\%)} = \frac{\text{fresh weight} - \text{dry weight}}{\text{fresh weight}} \times 100\%$$

Color Measurement. Color measurement using a reflectant Spectrophotometer (Konica, Minolta, Tokyo, Japan) the values displayed on the device are L*, a*, b*. Sample measurements were carried out at three measurement points, namely on the right, left, and center of the bulb. The color value is then visualized in the measurement of L* (Lightness), a* (green-red color), and b* (blue-yellow color) (Kusumiyati et al., 2018). Furthermore, these values are used to determine C* and H° which are calculated using the equation by Askin et al., (2021):

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

$$\text{Hue angle value (H°)} = \text{Tan}^{-1} \frac{b^*}{a^*}$$

The Chroma value is used to determine the intensity or brightness of a color. The higher the Chroma value, the higher the color intensity perceived by humans (Pathare et al., 2013). Meanwhile, the Hue value produces an angle whose number determines the color type of an object. The results of the L*, C*, and H° values are then analyzed using the Lch.oklch and ColorXs websites to determine the color type and compare RGB (Red, Green, Blue) and Hex values (Azetsu et al., 2021).

Photographic documentation was conducted using a smartphone camera (Samsung Galaxy A02s, 13 MP; 4128 × 3096 pixels). Photos were captured under natural daylight without flash, at a distance of approximately 20 cm above the sample at a perpendicular angle. The sample was placed on aluminum foil. The images were saved in JPEG format without post-processing. Each treatment was photographed in three replicates. Shallot color analysis with a digital approach uses a Hexadecimal (Hex) color representation system converted into Red, Green, Blue (RGB) format. The initial data is a color code in the format #RRGGBB, where the character pairs RR, GG, and BB indicate the intensity of red, green, and blue colors, respectively, in the base 16 (hexadecimal) number system (Drofova et al., 2024). Each Hex digit pair consists of a first digit (high) and a second digit (low) (Matumoto et al., 2023). According to the digital color system standard, each Hex code can therefore be interpreted into an RGB value within 0-255 (Yoo et al., 2023). Additionally, the color characteristics of shallots for each cultivar and harvest age are interpreted using the RGB values that were obtained. The hexadecimal to RGB conversion formula is as follows (Mohammadi et al., 2024):

$$X_{dec} = (X_{high} \times 16) + X_{low}$$

X_{dec} = decimal value (0-255)

X_{high} = first digit in Hex (base 16)

X_{low} = second digit in Hex (base 16)

Conversions letters to numbers: A=10, B=11,

C=12, D=13, E=14, F=15

Results and Discussion

This research generally demonstrates that the quality of red onion bulbs is impacted by genetic variation manifested in cultivar differences and harvest age. The physical characteristics of the bulbs, such as their color, texture, and water content, clearly show these variations. Each variety has its own characteristics, and the maturity level affects how these characteristics change based on the harvest date. According to the findings, growing red onions that are both market-ready and storage-ready requires selecting the right varieties and timing the harvest.

Table 1. The effect of the combination of shallot cultivars and harvest age on texture and water content

Cultivar + Harvest Age (DAP)	Average Texture (N)	Average Water Content (%)
Brebes + 56 DAP	12.13 a	82.67 d
Brebes + 63 DAP	11.74 a	85.13 f
Brebes + 70 DAP	11.20 a	86.79 g
Sumenep + 56 DAP	12.55 a	80.08 c
Sumenep + 63 DAP	14.93 b	78.91 b
Sumenep + 70 DAP	17.97 c	77.57 a
Tuktuk + 56 DAP	11.13 a	87.97 h
Tuktuk + 63 DAP	12.51 a	86.83 g
Tuktuk + 70 DAP	15.95 b	84.13 e

Notes: Means followed by the same letter indicate values that are not significantly different based on the Scott-Knott test at the 5% level.

Texture and Water Content. The results of the study showed that the water content of shallots has an inverse relationship with texture. Low water content correlates with increased bulb texture. Based on Table 1, the Brebes cultivar has a different pattern from the Sumenep and Tuktuk cultivars, where the longer the harvest age, the more it shows a decrease in texture value and an increase in water content ($r = -0.98$). However, for Sumenep ($r = -1.00$) and Tuktuk ($r = -1.00$), the longer the harvest age, the higher the texture and lower the water content. This indicates a negative correlation between texture and water content, even though the pattern of change in both at harvest age differs between cultivars. The local Sumenep variety, which tends to have a lower water content physiologically, is expected to

produce a denser and firmer bulb texture than other cultivars such as Brebes or Tuktuk.

An earlier study by (Mutia, 2019) clearly demonstrated a negative correlation between these two parameters, based on Table 1. found that shallots with an initial water content of 76.69% has a higher level of texture 4.46 N than samples with higher water content (85.25%) has lower level of texture (4.01 N). The lower the initial water content of shallots during storage, the higher their firmness (Mutia, 2019). High water content accelerates the decline in firmness during storage. The negative correlation between water content and texture suggests that reduced water content increases bulb firmness due to cell wall dehydration and pectin polymerization, consistent with (Lawal et al., 2025). The drying process, or evaporation of water within the bulb tissue cells, causes the cells to shrink, the pectin structure to harden, and the intercellular layers to fuse, resulting in a harder and stiffer texture.

Color is one of the most important visual quality parameters in post-harvest evaluation of shallots. In this study, color characteristics were analyzed using the CIELAB color space system which consists of three main parameters: L^* (lightness), a^* (green-red color direction), and b^* (blue-yellow color direction). These values were then converted into C^* (chroma) and H° (hue angle), and translated into RGB and hex codes for color visualization (Sandoval et al., 2018).

Lighness (L^*) Values. The results in Table 2 showed significant that there are significant variations in L^* values between cultivars and harvest ages. The highest L^* value is found in the Sumenep cultivar at 56 days after planting (54.56), indicating a brighter surface color of the bulb. Meanwhile, the lowest L^* value is found in the Brebes cultivar harvested at 63 days after planting (35.62), indicating a darker color. The L^* value shows a decrease in the Sumenep and Tuktuk cultivars; on the other hand, the L^* value in the Brebes cultivar tends to increase at the 70 DAP harvest age. The increased L^* value indicates a fading process of anthocyanin pigments while physiological processes are still ongoing (Sharma et al., 2015).

The a^* Values. For all samples show positive numbers indicating a dominance of red color in all treatments. The Brebes and Tuktuk cultivars produced higher a^* values compared to the Sumenep cultivar. The highest a^* value was found in the Tuktuk cultivar harvested at 56 DAP (20.20), resulting in a stronger red color.

Meanwhile, the lowest a^* value was produced by the Sumenep red onion harvested at 56 DAP (9.55), resulting in a fainter red color. The a^* value tends to decrease with increasing harvest age. This indicates degradation of red pigments such as anthocyanins (Enaru et al., 2021).

The b^* Values. Experiences an increase over the harvest time, showing a tendency to change color towards yellow. The highest b^* value occurs in the Sumenep cultivar harvested at 56 DAP (14.05), while the lowest value is in the Brebes cultivar harvested at 56 DAP (4.42). The increase in b^* value indicates the occurrence of phenolic oxidation and the formation of yellowish-brown colored compounds during the storage period or differences in harvest timing. (Pathare et al., 2013) and (Sharma et al., 2015).

Chroma (C^*) Values. Chroma values integrate changes in both a^* and b^* , providing an overall indicator of color intensity. The higher C^* value observed in the Tuktuk cultivar at 70 DAP (21.88) reflects a combination of relatively high a^* values and moderate b^* values, indicating sustained pigment saturation despite advancing maturity. Conversely, the lower C^* values in the Sumenep cultivar at early harvest stages (17.29) suggest reduced pigment concentration and lower color intensity. These findings indicate that Tuktuk exhibits greater pigment stability during maturation, whereas Sumenep undergoes faster pigment degradation, resulting in diminished color saturation.

Overall, the coordinated changes in L^* , a^* , b^* , and C^* values demonstrate that bulb color development is closely linked to physiological

maturation and cultivar-specific pigment metabolism. Cultivars with higher water content and softer texture, such as Sumenep at earlier harvest stages, tended to exhibit higher L^* and lower a^* values, indicating lighter and less intense red coloration. In contrast, cultivars with lower water content and firmer texture, such as Brebes and Tuktuk at later harvest ages, showed darker and more saturated red color. This relationship highlights the interaction between water content, texture, and pigment stability in determining shallot bulb color quality.

Hue Angle (H°) Values. The hue angle (H°) values quantitatively describe color hue based on the relative contributions of a^* and b^* . The lower H° values observed in the Brebes cultivar (12.76–34.06) indicate dominance of red pigments, whereas the higher H° values in the Sumenep cultivar (27.80–54.89) reflect increased yellow-brown components. These numerical trends are consistent with the RGB and hex color conversions derived from the same CIELAB data, which show color transitions from deep coffee to mocha, deep taupe, and rose taupe depending on the cultivar and harvest age (Table 3).










These color changes are closely related to post-harvest biochemical processes. Degradation of anthocyanin pigments causes the red color to fade, while the Maillard reaction and phenolic oxidation produce yellow to brownish pigment compounds (Sharma et al., 2015). Previous studies have also shown that temperature and humidity during storage significantly influence the direction and intensity of color changes (Demissew, 2018).

Table 2. The effect of the combination of shallot cultivars and harvest age on lightness, a^* , b^* , chroma, and hue values

Cultivar + Harvest Age (DAP)	L^*	a^*	b^*	C^*	H°
Brebes + 56 DAP	37.06 a	20.13 c	4.42 a	20.81 b	12.76 a
Brebes + 63 DAP	35.62 a	17.32 c	6.24 a	18.81 a	20.21 a
Brebes + 70 DAP	43.24 c	15.23 b	10.26 c	18.96 a	34.06 c
Sumenep + 56 DAP	54.56 d	9.55 a	14.05 d	17.29 a	54.89 e
Sumenep + 63 DAP	46.41 c	15.05 b	8.08 b	17.64 a	27.80 b
Sumenep + 70 DAP	44.61 c	13.68 b	13.87 d	19.60 a	45.16 d
Tuktuk + 56 DAP	40.97 b	20.20 c	6.14 a	21.21 b	16.57 a
Tuktuk + 63 DAP	36.92 a	18.40 c	5.93 a	19.41 a	18.02 a
Tuktuk + 70 DAP	37.55 a	19.61 c	8.90 b	21.88 b	25.15 b

Notes: Means followed by the same letter indicate values that are not significantly different based on the Scott-Knott test at the 5% level.

Table 3. RGB, hex, and color type values in various shallot cultivars with different harvest age

Cultivar	Harvest Age (DAP)		
	56	63	70
Brebis			
	Deep Coffee RGB (119, 74, 81) Hex (#774a51)	Deep Coffee RGB (113, 73, 74) Hex (#71494a)	Deep Taupe RGB (131, 92, 85) Hex (#835C55)
Sumenep			
	Mocha RGB (154, 124, 107) Hex (#9a7c6b)	Deep Taupe RGB (138, 100, 97) Hex (#8A6461)	Beaver RGB (133, 97, 83) Hex (#856153)
Tuk Tuk			
	Rose Taupe RGB (130, 84, 88) Hex (#825458)	Deep Coffee RGB (117, 75, 78) Hex (#754b4e)	Deep Coffee RGB (121, 76, 74) Hex (#794c4a)

Color Type Values. Overall, shallot color is influenced by a combination of genetic (cultivar) and physiological (harvest age) factors, as well as post-harvest processes involving pigment degradation and the formation of new compounds. Research on the changes and

dynamics of color is important to determine the optimal harvest time. This can lead to the development of a digital quality system through objective and standardized color identification. Color is one of the parameters that serves as an indicator of visual quality in the post-harvest

evaluation of shallots. Color transformation indicates visual changes depending on genetic variation and differences in harvest age. The Brebes cultivar shows a transition from deep coffee color to deep taupe at 70 DAP. The Sumenep cultivar tends to produce brighter colors, namely mocha (56 DAP), deep taupe (63 DAP), and beaver (70 DAP). Meanwhile, the Tuktuk cultivar produces rose taupe color (56 DAP) and becomes darker with a deep coffee color at 70 DAP.

Hex and RGB Values. RGB and hex color visualization (Table 4) can help with a digital color quality classification system. Applications for image-based quality standardization, such as automatic sorting and grading in the post-harvest sector, can be developed using this color model as a foundation (Azetsu et al., 2021). The color changes caused by L^* , C^* , and H° values show that the primary causes of color transformation during growth and storage are biochemical processes like anthocyanin degradation, enzymatic oxidation, and Maillard reactions. Furthermore, the rate of these changes is strongly influenced by temperature and humidity (Chope et al., 2012).

The Brebes cultivar shallot produces a red (R) value in the range of 113–131, indicating moderate to strong red dominance. When the R value is at its lowest point, namely 113 at 63 days after planting, the color appears darker and duller. Conversely, when R increases to 131 at 70

days after planting, the red hue becomes more pronounced and gives the impression of a brighter color. The green (G) value is in the range of 73–92, low values at 56 and 63 days after planting make the color tend to be deep towards red-brown, while the increase in G at 70 days after planting adds a slight greenish nuance that creates a neutral or ashy effect on the red hue. The blue (B) value which ranges from 74–85 also plays a role in brightness, the low B value at 63 days after planting strengthens the depth of the red-brown color, while its increase at 70 days after planting gives the impression of a softer color, shifting towards taupe. These changes indicate fading of red pigments (anthocyanins) and an increased contribution of gray-brown pigments resulting from phenolic oxidation. The Hex color code of the Brebes cultivar shows a clear transition between harvest ages. At 56 days after harvest, the identified color is #774a51, which is categorized as deep coffee, depicting a deep red-brown hue. At 63 days after harvest, the code changes to #71494a, still categorized as deep coffee, but darker and duller due to decreased brightness caused by anthocyanin pigment degradation. Entering 70 days after harvest, the Hex code changes to #835c55, which is categorized as deep taupe. This shift indicates an increase in brightness and a slight shift towards gray-brown, in line with the fading of the red color and the formation of secondary pigments through phenolic oxidation.

Table 4. Conversion results from hex to RGB values

Cultivar	Harvest Age	Hex	R (pair)	Count R	R Decimal	G (pair)	Count G	G Decimal	B (pair)	Count B	B Decimal
Brebes	56 DAP	#774a51	77	$(7 \times 16) + 7 = 112 + 7$	119	4A	$(4 \times 16) + 10 = 64 + 10$	74	51	$(5 \times 16) + 1 = 80 + 1$	81
Brebes	63 DAP	#71494a	71	$(7 \times 16) + 1 = 112 + 1$	113	49	$(4 \times 16) + 9 = 64 + 9$	73	4A	$(4 \times 16) + 10 = 64 + 10$	74
Brebes	70 DAP	#835c55	83	$(8 \times 16) + 3 = 128 + 3$	131	5C	$(5 \times 16) + 12 = 80 + 12$	92	55	$(5 \times 16) + 5 = 80 + 5$	85
Sumenep	56 DAP	#9a7c6b	9A	$(9 \times 16) + 10 = 144 + 10$	154	7C	$(7 \times 16) + 12 = 112 + 12$	124	6B	$(6 \times 16) + 11 = 96 + 11$	107
Sumenep	63 DAP	#8a6461	8A	$(8 \times 16) + 10 = 128 + 10$	138	64	$(6 \times 16) + 4 = 96 + 4$	100	61	$(6 \times 16) + 1 = 96 + 1$	97
Sumenep	70 DAP	#856153	85	$(8 \times 16) + 5 = 128 + 5$	133	61	$(6 \times 16) + 1 = 96 + 1$	97	53	$(5 \times 16) + 3 = 80 + 3$	83
Tuktuk	56 DAP	#825458	82	$(8 \times 16) + 2 = 128 + 2$	130	54	$(5 \times 16) + 4 = 80 + 4$	84	58	$(5 \times 16) + 8 = 80 + 8$	88
Tuktuk	63 DAP	#754b4e	75	$(7 \times 16) + 5 = 112 + 5$	117	4B	$(4 \times 16) + 11 = 64 + 11$	75	4E	$(4 \times 16) + 14 = 64 + 14$	78
Tuktuk	70 DAP	#794c4a	79	$(7 \times 16) + 9 = 112 + 9$	121	4C	$(4 \times 16) + 12 = 64 + 12$	76	4A	$(4 \times 16) + 10 = 64 + 10$	74

Notes: Columns R (pair), G (pair), B (pair) are hex digit pairs for each color value.

In the Sumenep cultivar, a high R value at 56 DAP (154) produces a bright, warm light brown hue. A decrease in R to 133 at 70 DAP makes the color appear more intense and dark. The G value, which was initially 124 at 56 DAP, gives a bright and neutral impression, but when it drops to 97 at 70 DAP, the color loses its brightness and shifts to reddish brown. The B value also shows a similar pattern, from 107 at 56 DAP to 83 at 70 DAP, which eliminates the bluish nuances, making the color warmer and more intense. These changes illustrate the shift from a bright, neutral color to reddish brown due to the degradation of blue/green pigments and the dominance of red-brown pigments during the ripening process. In the Sumenep cultivar, the color at 56 DAP has the code #9a7c6b or mocha, which describes a bright light brown hue. As the harvest age increases at 63 DAP, the code shifts to #8A6461 (deep taupe), indicating a darkening of the color and a shift towards reddish gray. At 70 DAP, the code changes again to #856153 (beaver), indicating a deeper reddish brown color. These changes indicate a loss of brightness due to a decrease in G and B values in the RGB composition, followed by a dominance of red-brown tones due to the degradation of blue/green pigments.

Meanwhile, in the Tuktuk cultivar, the R value is relatively stable at 117–130, maintaining a soft to deep red hue. The lowest R value at 63 DAP makes the color slightly darker, and a further increase at 70 DAP warms the hue. The G value, which ranges from 75–84, is consistently low, so the color remains intense and does not experience much shift in brightness. The B value, which ranges from 74–88, maintains a stable red-gray hue, the highest B value at 56 DAP gives a slightly pale impression, while the lowest value at 70 DAP reinforces the warm and deep impression of the color. This stability of the RGB composition indicates that the Tuktuk cultivar tends to maintain its red-brown character with relatively small changes in intensity, likely due to the pigment's resistance to degradation during ripening.

Overall, the results indicate a clear interaction between genotype and harvest age in determining the physical quality of shallot bulbs. Each cultivar responded differently to increasing maturity, as shown by the contrasting trends in water content, texture, and color parameters. For instance, the Sumenep cultivar consistently exhibited decreasing water and increasing texture

with later harvest ages, while Brebes and Tuktuk showed more moderate changes in firmness but stronger shifts in color attributes such as a and hue angle. These findings highlight that cultivar-specific physiological characteristics interact with harvest timing to produce distinct quality profiles. Recognizing this interaction is essential for identifying the most suitable cultivar–harvest age combinations to achieve desired postharvest traits. The Tuktuk shallot cultivar at 56 DAP showed the code #825458 (rose taupe), which exhibits a pale red hue with ash undertones. At 63 DAP, the code shifted to #754b4e (deep coffee), indicating a darkening of the color due to a decrease in light intensity across all RGB values. Interestingly, at 70 DAP, the code changed to #794c4a, still in the deep coffee category, but slightly warmer than at 63 DAP. This reflects the stability of the red-brown hue of this cultivar, with small variations in brightness and color saturation.

Conclusion

This study demonstrates that cultivar and harvest age significantly affect shallot bulb color (L^* , a^* , b^* , C^* , H°), texture, and water content. A strong negative relationship was observed between water and texture. Among all combinations, Sumenep harvested at 70 DAP showed the highest texture (17.97 N) with the lowest water (77.57%), indicating superior postharvest firmness and potential for longer shelf life. Brebes and Tuktuk showed stronger red tones (higher a^*), while Sumenep 56 DAP exhibited the brightest surface (highest L^*). Practically, Sumenep around 70 DAP is recommended when firmness and low water are prioritized, while harvest timing for Brebes and Tuktuk can be adjusted to meet specific color preferences. These findings provide actionable guidance for selecting cultivar–harvest age combinations to maintain shallot quality during handling and storage.

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References

- Ansar M, Bahrudin, Maemunah, Paiman. 2022. Effect of harvest age and storage duration on viability and vigor of shallot (*Allium cepa* L.) tubers. *Research on Crops*, 23(4): 815–821. doi: 10.31830/2348-7542.2022.ROC-909
- Askin B, Yazici SO. 2021. Bioactive Compounds, Antioxidant Potential and Color Properties of Dried Red Pepper (*Capsicum annum* L.). *International Journal of Innovative Approaches in Agricultural Research*, 5(2): 158–171. doi: 10.29329/ijiaar.2021.358.1
- Azetsu T, Suetake N. 2021. Chroma enhancement in cielab color space using a lookup table. *Designs*, 5(2). doi: 10.3390/designs5020032
- Chope GA, Cools K, Hammond JP, Thompson AJ, Terry LA. 2012. Physiological, biochemical and transcriptional analysis of onion bulbs during storage. *Annals of Botany*, 109(4): 819–831. doi: 10.1093/aob/mcr318
- Demissew A. 2018. Effect of Storage Temperature on Physicochemical Quality Attributes of Fresh Bombay Red Onion Bulb (*Allium cepa* L.). doi: 10.20944/preprints201803.0111.v1
- Devgan K, Kaur P, Kumar N, Kaur A. (2019). Active modified atmosphere packaging of yellow bell pepper for retention of physicochemical quality attributes. *Journal of Food Science and Technology*, 56(2): 878–888. doi: 10.1007/s13197-018-3548-5
- Drofova I, Adamek M. 2024. Color Models in the Process of 3D Digitization of an Artwork for Presentation in a VR Environment of an Art Gallery †. *Electronics (Switzerland)*, 13(22). doi: 10.3390/electronics13224431
- Enaru B, Dreţcanu G, Pop TD, Stănilă A, Diaconeasa Z. 2021. Anthocyanins: Factors affecting their stability and degradation. In *Antioxidants* (Vol. 10, Issue 12). MDPI. doi: 10.3390/antiox10121967
- Ghazali NS, Mohd W, Ikwan R, Hussin W, Salleh RM, Basir MH, Rozita W, Engah W, Rosali N. 2023. Curing Technique Affects Physicochemical Changes of Shallot. *International Journal of Agriculture*, 13.
- Guswita D, Makhmudi M, Kusmiyati. 2020. Keberdayaan Anggota Kelompok Tani dalam Menerapkan Good Handling Practices (GHP) Padi Sawah di Desa Sindanggalih Kecamatan Cimanggung Kabupaten Sumedang. *Jurnal Inovasi Penelitian*, 1: 303–316.
- Kaveri G, Thirupathi V. 2015. Studies on Geometrical and Physical Properties of CO 4 Onion Bulb (*Allium cepa* L. Var. *Aggregatum* Don.). *International Journal of Recent Scientific Research*, 6(3): 2897–2902. Retrieved from <http://www.recentscientific.com>
- Kurniawan YR, Hidayat DD, Luthfiyanti R, Andriansyah RCE, Indriati A. 2020. A comparative study on engineering properties of three varieties of shallots. *IOP Conference Series: Earth and Environmental Science*, 462(1). doi: 10.1088/1755-1315/462/1/012025
- Kusumiyati K, Farida F, Sutari W, Mubarak S. 2018. Kualitas Buah Mangga Selama Penyimpanan pada Keranjang Anyaman Bambu dengan Identifikasi Ruang Warna L*,a* dan b*. *Kultivasi*, 17.
- Kusumiyati K, Manurung GP, Hamdani JS. 2024. Growth, yield, and secondary metabolite responses of three shallot cultivars at different watering intervals. *Open Agriculture*, 9(1). doi: 10.1515/opag-2022-0255
- Lawal HM, Salau IA, Sufiyanu S. 2025. Comparative Study on Flavor, Texture, and Quality Preservation in Onion Bulbs (*Allium cepa* L.) under Two Storage Methods. *Greener Journal of Biological Sciences*, 15(1): 18–22. doi: 10.15580/gjbs.2025.1.121624195
- Matumoto AdO, Gonçalves-Segundo PR. 2023. A Social-Semiotic Approach to Color Analysis in Digital Media: Theory and Practice. *Revista de Estudos Da Linguagem*, 31(4): 2009–2065. doi: 10.17851/2237-2083.31.4.2009-2065
- Mohammadi V, Ansari K, Gouton P, Attig H. 2024. Developing a New Method of Transformation for Obtaining XYZ Color Values from RGB Images for Agricultural Applications. *Sensors*, 24(23). doi: 10.3390/s24237728
- Mutia AK. 2019. Pengaruh Kadar Air Awal pada Bawang Merah (*Allium ascalonicum* L.) terhadap Susut Bobot dan Tingkat Kekerasan Selama Penyimpanan pada Suhu Rendah. *Gorontalo Agriculture Technology Journal*, 2: 30–37.
- Pathare PB, Opara UL, Al-Said FAJ. 2013. Colour Measurement and Analysis in Fresh and Processed Foods: A Review. In *Food and Bioprocess Technology* (Vol. 6, Issue 1, pp.

- 36–60). Springer Science and Business Media, LLC. doi: 10.1007/s11947-012-0867-9
- Sandoval JRM, Sandoval EM, Rosas MEM, Velasco MMM. 2018. Color Analysis and Image Processing Applied in Agriculture. In Colorimetry and Image Processing. InTech. doi: 10.5772/intechopen.71539
- Sharma K, Asnin L, Ko EY, Lee ET, Park SW. 2015. Phytochemical composition of onion during long-term storage. *Acta Agriculturae Scandinavica Section B: Soil and Plant Science*, 65(2): 150–160. doi: 10.1080/09064710.2014.983151
- Sukorini H. 2024. Shallot extension shelf life with fogging and the storage temperature. *BIO Web of Conferences*, 143, 01010. doi: 10.1051/bioconf/202414301010
- Vojnović Đ, Ilin Ž, Kostić A, Adamović B, Kilibarda S, Pezo L, Popović-Đorđević J. 2025. Phytochemical composition, antioxidant potential, and bulb mass of differently colored onions under distinct sowing methods. *Quality Assurance and Safety of Crops and Foods*, 17(3): 108–126. doi: 10.15586/qas.v17i3.1549
- Wijoyo RB, Sulistyarningsih E, Wibowo A. 2020. Growth, Yield and Resistance Responses of Three Cultivars on True Seed Shallots to Twisted Disease with Salicylic Acid Application. *Caraka Tani: Journal of Sustainable Agriculture*, 35(1): 1–11. doi: 10.20961/carakatani.v35i1.30174
- Yoo WS, Kang K, Kim JG, Yoo Y. 2023. Image-Based Quantification of Color and Its Machine Vision and Offline Applications. *Technologies*, 11(2). doi: 10.3390/technologies11020049

Rahma SA · Budiarto R · Mubarok S

Analysis of source–sink organ dynamics in kaffir lime (*Citrus hystrix* DC.) at different plant ages

Abstract. Kaffir lime (*Citrus hystrix* DC.) is a leafy spice commodity widely used as a culinary ingredient, in bio-pharmaceuticals, and as a raw material for essential oil production. Despite its high demand, leaf productivity remains relatively low, highlighting the need for improved cultivation practices. A better understanding of source–sink dynamics is essential, as leaf production is strongly influenced by the balance between assimilate-producing and assimilate-consuming organs. Therefore, this study aimed to evaluate the source-sink capacity of kaffir lime plants of different ages as a preliminary step toward identifying cultivation strategies to enhance leaf production. The experiment was conducted at Bale Tatanen experimental field, Universitas Padjadjaran, Sumedang. This work used a randomized complete block design with five replications and three age treatments: A = 1 year, B = 2 years, and C = 3 years. The results showed the source-to-sink ratio in 1-, 2-, and 3-year-old plants were 53:47, 50:50, and 48:52, respectively. As kaffir lime plants grow older, their source–sink balance shifts because older plants allocate assimilates more efficiently to developing organs. In this study, increasing plant age was associated with a greater capacity to develop sink organs, which in turn supports leaf expansion and overall leaf productivity. This study offers baseline information on the source–sink proportions of kaffir lime plants at different ages, providing a foundation for improving cultivation practices. The findings support better harvest timing and more accurate yield estimation to enhance planning efficiency.

Keywords: Citrus · Roots · Shoots · Source-capacity · Sink-capacity

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Introduction

Kaffir lime (*Citrus hystrix* DC.) is a high-value horticultural commodity, primarily due to its leaves, which serve as a key ingredient in culinary preparations and as a raw material in the food, beverage, and cosmetics industries (Budiarto et al., 2024). The increasing demand for both fresh and dried kaffir lime leaves, driven by the growth of the culinary and herbal industries (Abidin et al., 2020), underscores the need for cultivation strategies that sustainably optimize leaf production.

As a perennial species, the growth and leaf yield of kaffir lime are strongly influenced by the balance between the source system (photosynthate-producing organs such as mature leaves) and the sink system (photosynthate-utilizing organs such as shoots and young leaves) (Goldschmidt & Koch, 2017; Li et al., 2024). Variations in plant age and size can lead to differences in source capacity and sink strength (Sunmonu & Kudo, 2014), thereby shaping vegetative growth patterns (Smith et al., 2018). A comprehensive understanding of source-sink dynamics is therefore essential as a foundation for developing targeted cultivation strategies, particularly those aimed at manipulating plant physiological processes to maximize leaf production.

The source-sink relationship plays a crucial role in determining plant growth and productivity, as it governs the allocation of photosynthates between producing organs (sources, e.g., mature leaves) and utilizing organs (sinks, e.g., shoots, young leaves, stems, and roots) (Amarullah, 2021). Managing this allocation effectively is key to sustaining vegetative growth and optimizing productivity (White et al., 2016; Yu et al., 2015). In perennial crops such as kaffir lime (*Citrus hystrix* DC.), insights into these physiological processes provide a foundation for designing cultivation practices that enhance leaf production (Li et al., 2024; Budiarto et al., 2023).

Plant age strongly influences source and sink capacities. Younger plants typically exhibit higher sink demand due to rapid vegetative development, whereas older plants tend to have greater source capacity from mature leaves, often exceeding sink requirements (Chen et al., 2021). In leaf-oriented cultivation systems, strategies such as close planting spacing can suppress reproductive development and promote

continuous leaf production (Haque & Sakimin, 2022).

This study investigates source and sink capacities in 1-, 2-, and 3-year-old kaffir lime plants under a close-planting system designed to maintain vegetative growth. The analysis focuses on vegetative sinks (young leaves, young stems, and roots) while excluding reproductive organs, aiming to clarify age-related differences in photosynthate allocation patterns and their implications for sustainable leaf yield optimization.

Materials and Methods

This research was conducted at the open field of Bale Tatanen garden, Universitas Padjadjaran, Jatiningor District, Sumedang Regency, West Java Province. Present experiment used kaffir lime (*Citrus hystrix* DC. cv. Puri Agrihorti) seedling as plant materials. The plant materials were produced through vegetative method, i.e., grafting, combining kaffir lime scion into Rangpur lime (*Citrus limonia* Osbeck) rootstock, produced by local seedling producer in Majalengka. There were three tested-plant age in present experiment, i.e., 1, 2, and 3 years after grafting. All plant were raised in Inceptisol soil growing bed supplemented with goat manure.

The relationship between source and sink in kaffir lime plants of different ages was analyzed through destructive sampling. Classification of plant organs into source or sink categories was conducted using a morphometric approach. The experimental design employed was a Randomized Block Design (RBD) consisting of 3 treatments with 5 replications. The treatments were based on plant age, 1-, 2-, and 3-years-old kaffir lime plants. Observations were carried out by measuring and weighing plant components according to the defined parameters. All measurements were performed on the same day as the destructive sampling to ensure that the plant materials remained fresh.

The observation parameters consisted of plant height (cm), leaf length (cm), number of young and mature leaves, fresh weight of young and mature leaves (g), fresh weight of young and mature stems (g), fresh weight of shoots and roots (g), dry weight of shoots and roots (g), and source-sink capacity. Young leaves—characterized by pale light-green color, thin and soft texture, glossy surface, and small size

(Budiarto et al., 2022)—were classified as sink organs, whereas mature leaves—dark-green, thicker and firmer in texture, with a rough or dull surface and fully expanded size (Budiarto et al., 2022)—were classified as source organs. Young stems—identified by their bright green color, smooth surface, soft and flexible texture, and small diameter—were classified as sink organs, whereas mature stems—brownish green to dark brown in color indicating lignification, with a rough, hard, and rigid texture and larger diameter—were classified as source organs.

Data Analysis. Data were analyzed using analysis of variance (ANOVA) based on a randomized complete block design (RCBD) at a 5% significance level, followed by Tukey's Honestly Significant Difference (HSD) test (5%) for post hoc comparisons.

Result and Discussion

Plant height and leaf length. According to the results (Table 1), plant height exhibited a gradual increase with advancing plant age. This increment in height indicates the cumulative effect of progressive and continuous vegetative growth, which is strongly associated with the dynamic balance between source capacity and sink demand within the plant. As plants age, photosynthetic organs such as leaves expand in size and increase in number, thereby enhancing the source capacity of the plant to produce assimilates through photosynthesis (Leng et al., 2025). On the other hand, young organs such as apical meristems, roots, and lateral branches that function as sinks also develop more actively in older plants (Cheng et al., 2022), thus increasing the demand for assimilates and supporting overall plant growth (Doidy et al., 2024).

A balanced relationship between source capacity and sink demand is crucial to ensure efficient carbon distribution, particularly to meristematic tissues that determine plant height (Amarullah, 2021). In addition, older plants tend to have a more developed vascular system (xylem and phloem) (Swidrak et al., 2014; Yang & Wang, 2016). This allows for greater efficiency in the transport of water, nutrients, and photosynthates, thereby supporting a higher growth rate (Vadera et al., 2025).

Leaf length serves as a key indicator of morphological growth and biomass accumulation in kaffir lime. Longer leaves are

closely associated with greater surface area, which enhances light absorption and photosynthetic capacity (Telaumbanua et al., 2024). As plants age, leaf development becomes more optimal in both number and size, thereby increasing carbon productivity and assimilate supply (Poorter et al., 2015; Weraduwege et al., 2015). This improvement is supported by a more developed vascular system that facilitates efficient distribution of water and nutrients, promoting cell expansion and leaf elongation (Yang & Wang, 2016).

Table 1. Plant height and leaf length of kaffir lime at different ages

Treatment	Plant height (cm)	Leaf length (cm)
A: 1 year old	68.00 a	4.58 a
B: 2 years old	93.80 b	6.70 b
C: 3 years old	187.80 c	8.64 c

Note: Mean values followed by the same letter in the same column are not significantly different according to Tukey's HSD test at the 0.05 significance level.

Within the source–sink framework, longer leaves contribute significantly to source capacity due to their higher photosynthetic potential (Nuranisa et al., 2020). In three-year-old kaffir lime plants, the presence of longer leaves suggests enhanced assimilate production to sustain the growth of sink organs such as young leaves, stems, roots, and shoots (Lambers et al., 2019). This reflects a transition toward greater physiological stability, allowing more efficient assimilate production and allocation (Chang & Zu, 2017).

Number and weight of young and mature leaves. An increase in leaf number directly influences the source–sink relationship in kaffir lime. Mature leaves serve as the primary source by producing assimilates through photosynthesis, while young leaves act as sinks due to their active growth and limited photosynthetic ability (Zhang et al., 2023). With advancing plant age, the rise in mature leaf number significantly enhances source capacity (Table 2), thereby providing sufficient energy and carbon to sustain the growth of sink organs such as young leaves, shoots, and meristematic tissues (Bielczynski et al., 2017; Durand et al., 2018). The coexistence of more mature and young leaves in older plants indicates a more efficient and balanced source–sink system (White et al., 2016).

A higher sink capacity, reflected in the increased number of young leaves, creates strong demand for assimilates, which physiologically stimulates metabolic activity in source organs (Fernie et al., 2020). The abundance of mature leaves also contributes to an elevated overall photosynthetic rate, ensuring a continuous assimilate supply to support vegetative growth and leaf regeneration (Wu et al., 2018). As a result, older kaffir lime plants tend to accumulate greater biomass and exhibit faster canopy development (Sunmonu & Kudo, 2014).

The increase in leaf biomass not only reflects the quantitative rise in leaf number but also indicates an enhanced physiological capacity of the plant to produce, store, and distribute photosynthates more efficiently (Weraduwege et al., 2015). This is closely related to the concept of source-sink dynamics, in which mature leaves, functioning as the primary photosynthetic organs, act as sources, while young leaves undergoing cell expansion and tissue development act as active sinks (Yu et al., 2015; Jansson et al., 2015; Koley et al., 2019).

The increase in mature leaf biomass signifies an expansion of active surface area for photosynthesis, which directly enhances the plant's capacity to produce soluble carbohydrates such as sucrose and glucose through carbon fixation (Liu et al., 2022). In older plants, particularly at three years of age, the accumulation of biomass in mature leaves indicates that the plant has reached a vegetative phase with a more developed phloem transport system, enabling efficient translocation of photosynthates from sources to sink tissues (Milne et al., 2015; Nam et al., 2022). This activity is critical, as young leaves, functioning as sinks, not only require energy for cell expansion but also for the construction of internal structures such as chloroplasts and incompletely developed vascular tissues (Van Campen, 2016).

In young plants, source limitation leads to higher competition among sink organs, thereby slowing leaf growth (White et al., 2016). Conversely, older plants exhibit a more stable source-sink ratio, allowing a more proportional allocation of photosynthates to support extensive sink growth (Burnett et al., 2016), such as the sharp increase in the number and weight of young leaves. This condition demonstrates that enhanced source capacity accelerates sink expansion, while stronger sink growth also stimulates greater source activity (Fatichi et al., 2014). Moreover, the increase in young leaf biomass in three-year-old plants indicates greater potential for continued growth (Table 2). Active sinks such as young leaves will remain a primary target for carbon allocation, facilitating the formation of a larger canopy (Fatichi et al., 2019). This is particularly important in kaffir lime, where canopy development is directly correlated with the productivity of vegetative organs (Feng et al., 2016).

Young and mature stems weight. A greater biomass of young stems in older plants indicates that meristematic growth activity remains high in the stem, which functions as part of the active sink system (Rademacher et al., 2022). Young stems continue to grow through cell division and elongation, thus requiring large amounts of carbon and energy supplied by source tissues, particularly mature leaves (Czedik-Eysenberg et al., 2016; Kocurek et al., 2020). Meanwhile, mature stems that have undergone lignification and secondary tissue formation serve as supporting and more efficient conducting organs (Asaoka et al., 2024). The significant increase in mature stem biomass in three-year-old plants (Table 3) demonstrates that cambial thickening is actively taking place, contributing substantially to the accumulation of vegetative organ mass (Guada et al., 2018).

Table 2. Number and weight of young and mature leaves of kaffir lime at different ages

Treatment	Number of young leaves	Number of mature leaves	Young leaves weight (g)	Mature leaves weight (g)
A: 1 year old	49.00 a	86.00 a	17.00 a	33.00 a
B: 2 years old	64.00 a	180.40 a	21.00 a	87.00 a
C: 3 years old	319.60 b	574.00 b	144.00 b	263.00 b

Note: Mean values followed by the same letter in the same column are not significantly different according to Tukey's HSD test at the 0.05 significance level.

Table 3. Young and mature stems weight of kaffir lime at different ages

Treatment	Young stems weight (g)	Mature stems weight (g)
A: 1 year old	38.14 a	57.21 a
B: 2 years old	86.40 a	129.60 a
C: 3 years old	306.00 b	459.00 b

Note: Mean values followed by the same letter in the same column are not significantly different according to Tukey's HSD test at the 0.05 significance level.

Table 4. Fresh and dry weight of shoots and roots of kaffir lime at different ages

Treatment	Shoots fresh weight (g)	Roots fresh weight (g)	Shoots dry weight (g)	Roots dry weight (g)
A: 1 year old	149.36 a	24.64 a	44.81 a	7.39 a
B: 2 years old	320.00 a	109.00 a	96.00 a	32.70 a
C: 3 years old	1,172.00 b	332.00 b	351.60 b	99.60 b

Note: Mean values followed by the same letter in the same column are not significantly different according to Tukey's HSD test at the 0.05 significance level.

The relationship between stem biomass and the source-sink system can also be understood from the perspective of long-term carbon allocation. In older plants, carbon distribution is not only directed toward young organs but also allocated to permanent tissues such as mature stems (Hartmann et al., 2018). Mature stems play a role in storing energy reserves in the form of starch and in maintaining the integrity of the vascular system (Plavcova & Jansen, 2015). Therefore, greater biomass of mature stems can be considered an indicator of long-term carbon allocation efficiency (Hartmann et al., 2018), whereas young stem biomass reflects the ongoing potential for vertical and lateral growth (Speck & Burgert, 2011). The coexistence of both young and mature stems with significant biomass in three-year-old plants illustrates a balance between active growth and the differentiation of functionally mature tissues.

Fresh and dry weight of shoots and roots.

The shoot system, comprising leaves, stems, and branches, is the center of source activity where photosynthesis and assimilate production occur. The greater the shoot biomass, the larger the photosynthetic surface area of the plant (Chen et al., 2020; Zhang et al., 2020), thereby increasing carbon production capacity through CO₂ fixation (Dhami & Cazzonelli, 2021). Table 4 showed that three-year-old plants have the highest shoot dry weight (Table 4), implying that the tissues formed are more structural and durable, such as lignin and sclerenchyma, which are essential for supporting continued growth and facilitating assimilate transport to sink organs (Putra et al., 2023).

Conversely, roots function as active sinks that require carbon supply from sources for growth, elongation, and lateral root formation (Waheed et al., 2025). The sharp increase in root biomass at three years of age suggests extensive root system development to support greater water and nutrient demands (Carvalho & Foulkes, 2019). The balance between shoot and root biomass reflects that the plant has achieved an optimal shoot-root ratio (Punyasu et al., 2024). This ratio is important because roots are not only passive sinks but also long-term carbon storage sites in the form of starch (Irving, 2015), and they influence stability and efficiency of nutrient uptake from the soil (Lopez et al., 2023).

The increase in both shoot and root biomass indicates synchronization between production (source) activity and the distribution and utilization of photosynthetic products (sink). In young plants, source capacity remains limited, leading to lower root dry weight as sinks. However, in three-year-old plants, the more developed vascular and hormonal systems enable more efficient communication between organs (Savage et al., 2016), thus allowing assimilate allocation to be more effective in generating large and balanced biomass growth (Wang & Ruan, 2016).

In addition, the increase in dry weight compared to fresh weight reflects greater tissue density and structural compounds (cellulose and lignin) accumulation (Afzal et al., 2022). This indicates that the plant has reached an advanced vegetative phase and physiological stability. Plants in this condition have stronger adaptability to environmental stresses,

supported by a well-developed root system and a broad canopy that sustain photosynthesis and transpiration (Koevoets et al., 2016).

Source-sink capacity. The results indicated that plant age influenced the distribution of source and sink capacities. Mature leaves and mature stems were classified as source organs, whereas young leaves, young stems, and roots represented sink organs. The relative contribution of each organ changed progressively with increasing plant age. The 1-year-old plant display a total source capacity for about 53%, while the sink capacity was 47% (Figure 1). The dominance of source capacity indicates that the plants were still in the early growth phase, focusing on the development of photosynthetic tissues (Körner, 2015). The proportion of source and sink capacities became balanced in 2-year-old plants, each accounting for 50% (Figure 2). This indicates that the plant's metabolic system

began to stabilize, with an increased distribution of photosynthates toward actively growing sink organs, particularly the roots, which showed a significant increase from 15% to 25%. The decrease in the proportion of young leaves from 10% to 5% suggests that most leaves had matured and functionally served as source organs. Later on, sink capacity increased to 52% in 3-year-old plants, while source capacity decreased to 48% (Figure 3). This indicates that the plants had entered an active vegetative growth phase with a higher demand for photosynthates to support the development of new tissues such as young stems and roots (Burnett, 2019). Roots remained the dominant sink organs, followed by young stems and young leaves. The relative proportion of mature leaves declined, not because their number decreased, but due to the increasing activity and demand of sink organs (Brant & Chen, 2015).

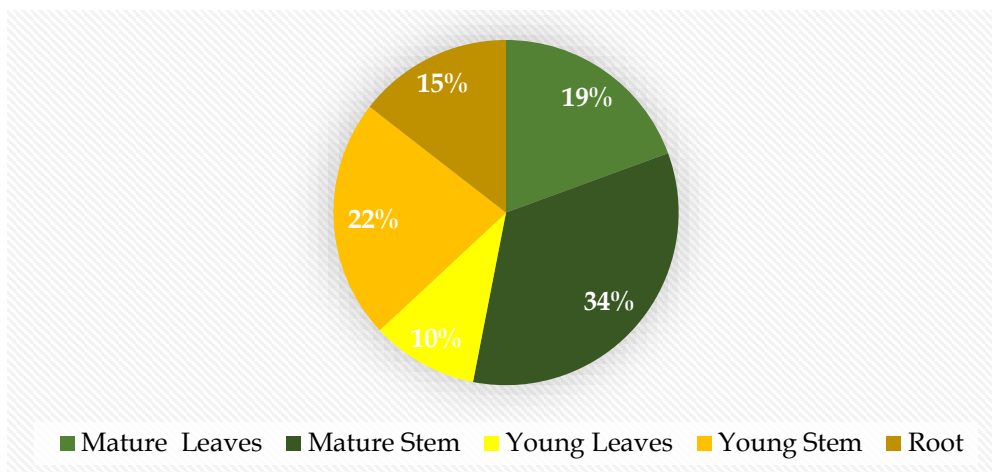


Figure 1. Source and sink capacity diagram for 1-year-old kaffir lime plants

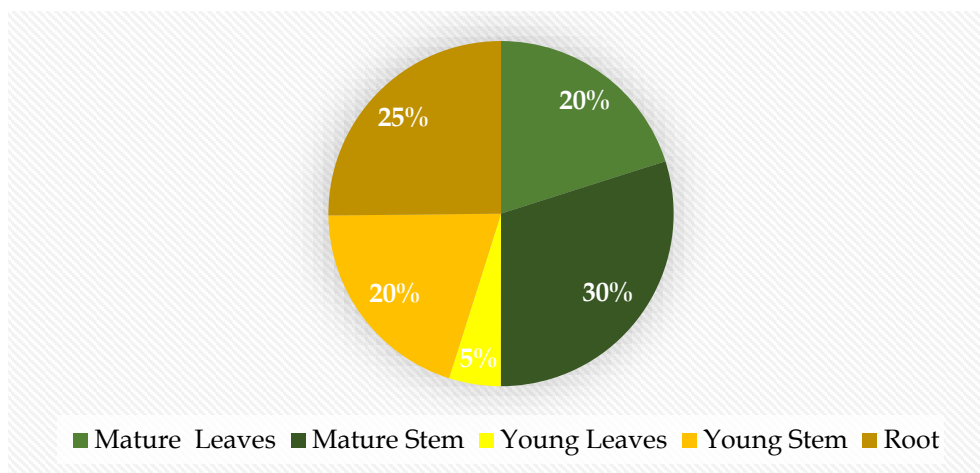


Figure 2. Source and sink capacity diagram for 2-year-old kaffir lime plants

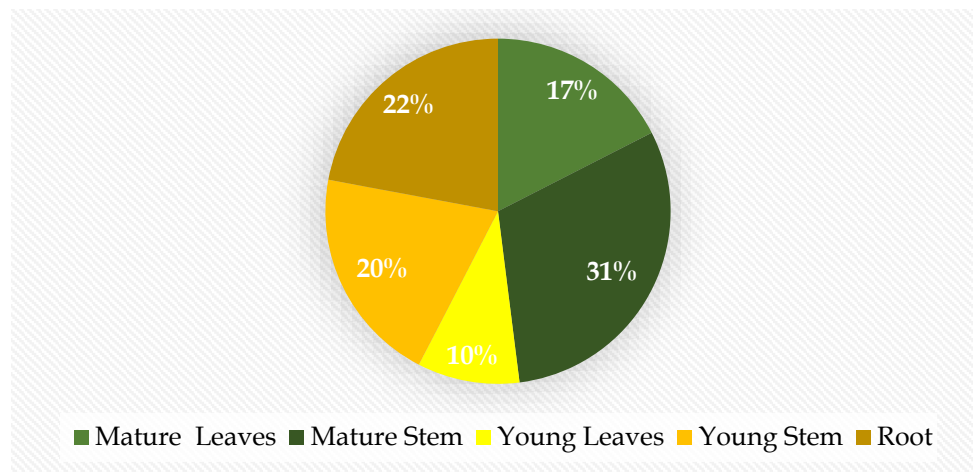


Figure 3. Source and sink capacity diagram for 3-year-old kaffir lime plants

In young plants, growth is constrained by source capacity because developing shoots have relatively low photosynthetic ability compared to the energy required for growth (Trugman & Anderegg, 2024). Conversely, in older plants, growth is more often limited by sink capacity, as growth rates slow while photosynthetic activity remains high (Burnett et al., 2018; Company et al., 2017). The transition from source-limited to sink-limited growth typically occurs as plants begin entering the reproductive phase, when metabolism shifts toward seed or fruit production (Lv et al., 2020; White et al., 2016). This shift aligns with plant physiology theory, which states that with increasing age, sink capacity rises due to the enlargement, increased number, and complexity of source organs (Burnett, 2019). Young organs and roots continue to grow as major consumers of photosynthates, thereby influencing assimilate distribution patterns within the plant (Anuradha et al., 2017).

Plant age showed a positive correlation with the increasing number of mature leaves, which serve as the primary organs of photosynthesis. Mature leaves have greater photosynthetic capacity compared to young leaves (Budiarto et al., 2022) because their anatomical structure and chlorophyll content are more fully developed (Wu et al., 2018). As the number of mature leaves increases, overall photosynthetic capacity also rises, producing more assimilates available for distribution to various plant organs. This enhanced photosynthetic capacity directly contributes to increased sink capacity, as sink organs such as shoots, roots, and buds gain

greater access to photosynthates (Pawar & Rana, 2019; Singh et al., 2017). Thus, plant aging not only strengthens the source system through a higher number of mature leaves but also enhances the efficiency and capacity of sink organs, which are critical for supporting plant growth and productivity (Sonnewald & Fernie, 2018).

Older plants also exhibit a greater ability to store carbon in permanent structures such as stems, branches, and roots (Plavcová et al., 2016). This activity can be categorized as carbon trading, a process of capturing and storing carbon from the atmosphere into plant biomass (Nath et al., 2015; Shen et al., 2020). This process holds important ecological value as it helps reduce atmospheric carbon dioxide (CO₂) concentrations (Kukah et al., 2024) and highlights the role of aging plants in climate change mitigation through enhanced carbon storage capacity (Nunes et al., 2020).

Overall, the combined increase in mature leaf biomass, stem thickening, and root development with plant age reflects a coordinated shift in source-sink balance. Older plants accumulate greater source capacity through expanded mature leaf area, while simultaneously supporting stronger sink activity in developing shoots and roots. This integrated response demonstrates that the progression of plant age does not enhance individual organs in isolation but reshapes the entire assimilate allocation system, ultimately strengthening both the source and sink components of kaffir lime's growth physiology.

Conclusion

The study demonstrates that plant age significantly influences the source-sink dynamics in kaffir lime. As the age of the kaffir lime plant increases, the plant's ability to form sink organs also increases. Younger plants tend to be source-dominated, relying on limited photosynthetic tissues to support growth, while older plants show a gradual increase in sink capacity through the development of young leaves, stems, and roots. By the third year, sink demand exceeds source supply, reflecting active vegetative growth and greater assimilate allocation to developing organs. These findings highlight the importance of managing age-related source-sink balance as a foundation for optimizing cultivation practices aimed at maximizing leaf production in kaffir lime.

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References

- Abidin MRZ, Ishak FAC, Ismail IA, Juhari NH. 2020. Issues and Challenges of Malaysian Local Herbs toward the Development of Local Herbs-Based Culinary Products. *Food Studies*, 10(3): 13-30. <https://doi.org/10.18848/2160-1933/CGP/v10i03/13-30>
- Afzal RA, Pennells J, Yamauchi Y, Annamalai PK, Nanjundan AK, Martin DJ. 2022. Lignocellulosic plant cell wall variation influences the structure and properties of hard carbon derived from sorghum biomass. *Carbon Trends*, 7(1): 1-12.
- Amarullah. 2021. Sink source relationship dalam tanaman. Banda Aceh: Syiah Kuala University Press.
- Anuradha, Goyal R, Bishnoi C. 2017. Assimilate partitioning and distribution in fruit crops: A review. *Journal of Pharmacognosy and Phytochemistry*, 6(3): 479-484.
- Asaoka M, Badel E, Ferjani A, Nishitani K, Hamant O. 2024. Contributions of lignification, tissue arrangement patterns, and cross-sectional area to whole-stem mechanical properties in *Arabidopsis thaliana*. *Journal of Plant Research*, 137(5): 773-783.
- Bielczynski LW, Łącki MK, Hoefnagels I, Gambin A, Croce R. 2017. Leaf and plant age affects photosynthetic performance and photoprotective capacity. *Plant Physiology*, 175(4): 1634-1648.
- Brant AN, Chen HYH. 2015. Patterns and mechanisms of nutrient resorption in plants. *Critical Reviews in Plant Sciences*, 34(5): 471-486.
- Budiarto R, Mubarak S, Nanda MA, Nabiiyu M, Haris M, Jaya IS. 2023. The increase in kaffir lime leaf production due to gibberellin is diminished by pruning. *Horticulturae*, 9(9):1018.
- Budiarto R, Poerwanto R, Santosa E, Efendi D, Agusta A. 2022. Comparative and correlation analysis of young and mature kaffir lime (*Citrus hystrix* DC) leaf characteristics. *International Journal of Plant Biology*, 13(3): 270-280.
- Budiarto R, Wathoni N, Mubarak S, Sauman J. (2024). Recent overview of kaffir lime (*Citrus hystrix* DC) dual functionality in food and nutraceuticals. *Journal of Agriculture and Food Research*, 18(1): 101384.
- Burnett AC. 2019. Source-sink relationships. *eLS*, 1(1): 1-27.
- Burnett AC, Rogers A, Rees M, Osborne CP. 2018. Nutrient sink limitation constrains growth in two barley species with contrasting growth strategies. *Plant Direct*, 2(1): 1-13.
- Burnett AC, Rogers A, Rees M, Osborne CP. 2016. Carbon source-sink limitations differ between two species with contrasting growth strategies. *Plant Cell and Environment*, 39(11): 2460-2472.
- Campany CE, Medlyn BE, Duursma RA. 2017. Reduced growth due to belowground sink limitation is not fully explained by reduced photosynthesis. *Tree Physiology*, 37(1): 1042-1054.
- Carvalho P, Foulkes MJ. 2019. Roots and uptake of water and nutrients. In: *Crop Science*. Springer Science+Business Media New York. p. 107-130.
- Chang, T. G., & Zhu, X. G. (2017). Source-sink interaction: a century old concept under the

- light of modern molecular systems biology. *Journal of Experimental Botany*, 68(16) : 4417-4431.
- Chen J, Liu L, Wang Z, Zhang Y, Sun H, Song S, Li C. 2020. Nitrogen fertilization increases root growth and coordinates the root-shoot relationship in cotton. *Frontiers in Plant Science*, 11: 880.
- Chen Q, Lu X, Guo X, Xu M, Tang Z. 2021. A source-sink model explains the difference in the metabolic mechanism of mechanical damage to young and senescing leaves in *Catharanthus roseus*. *BMC Plant Biology*, 21: 154.
- Cheng S, Wang Q, Manghwar H, Liu F. 2022. Autophagy-mediated regulation of different meristems in plants. *International Journal of Molecular Sciences*, 23(11): 1-15.
- Czedik-Eysenberg A, Arrivault S, Lohse MA, Feil R, Krohn N, Encke B, Stitt M. 2016. The interplay between carbon availability and growth in different zones of the growing maize leaf. *Plant Physiology*, 172(2): 943-967.
- Dhami N, Cazzonelli CI. 2021. Short photoperiod attenuates CO₂ fertilization effect on shoot biomass in *Arabidopsis thaliana*. *Physiology and Molecular Biology of Plants*, 27(4): 825-834.
- Doidy J, Wang Y, Gouaille L, Goma-Louamba I, Jiang Z, Pourtau N, Le Gourrierec J, Sakr S. 2024. Sugar transport and signaling in shoot branching. *International Journal of Molecular Sciences*, 25(23).
- Durand M, Mainson D, Porcheron B, Maurousset L, Lemoine R, Pourtau N. 2018. Carbon source-sink relationship in *Arabidopsis thaliana*: the role of sucrose transporters. *Planta*, 247(3): 587-611.
- Fatichi S, Leuzinger S, Körner C. 2014. Moving beyond photosynthesis: From carbon source to sink-driven vegetation modeling. *New Phytologist*, 201(4): 1086-1095.
- Fatichi S, Pappas C, Zscheischler J, Leuzinger S. 2019. Modelling carbon sources and sinks in terrestrial vegetation. *New Phytologist*, 221(2): 652-668.
- Feng G, Luo H, Zhang Y, Gou L, Yao Y, Lin Y, Zhang W. 2016. Relationship between plant canopy characteristics and photosynthetic productivity in diverse cultivars of cotton (*Gossypium hirsutum* L.). *Crop Journal*, 4(6): 499-508.
- Fernie AR, Bachem CW, Helariutta Y, Neuhaus HE, Prat S, Ruan YL, Sonnewald U. 2020. Synchronization of developmental, molecular and metabolic aspects of source-sink interactions. *Nature Plants*, 6(2): 55-66.
- Goldschmidt EE, Koch KE. 2017. Citrus. In: Photoassimilate distribution in plants and crops: source-sink relationships. Routledge. p. 797-824.
- Guada G, García-González I, Pérez-de-Lis G, Vázquez-Ruiz RA, Montserrat-Martí G. 2018. Dry matter content during extension of twigs, buds and leaves reflects hydraulic status related to earlywood vessel development in *Quercus pyrenaica* Willd. *European Journal of Forest Research*, 137(1): 307-319.
- Haque MA, Sakimin SZ. 2022. Planting arrangement and effects of planting density on tropical fruit crops – A review. *Horticulturae*, 8(6): 1-17.
- Hartmann H, Adams HD, Hammond WM, Hoch G, Landhäusser SM, Wiley E, Zaehle S. 2018. Identifying differences in carbohydrate dynamics of seedlings and mature trees to improve carbon allocation in models for trees and forests. *Environmental and Experimental Botany*, 152(1): 7-18.
- Irving LJ. 2015. Carbon assimilation, biomass partitioning and productivity in grasses. *Agriculture*, 5(4): 1116-1134.
- Jansson C, Vogel J, Hazen S, Brutnell T, Mockler T. 2018. Climate-smart crops with enhanced photosynthesis. *Journal of Experimental Botany*, 69(16): 3801-3809.
- Kocurek M, Kornas A, Wierzchnicki R, Lüttge U, Miszalski Z. 2020. Importance of stem photosynthesis in plant carbon allocation of *Clusia minor*. *Trees*, 34(4): 1009-1020.
- Koevoets IT, Venema JH, Elzenga JTM, Testerink C. 2016. Roots withstanding their environment: Exploiting root system architecture responses to abiotic stress to improve crop tolerance. *Frontiers in Plant Science*, 7(1): 1-19.
- Koley S, Raorane ML, Junker BH. 2019. Shoot tip culture: a step towards ¹³C metabolite flux analysis of sink leaf metabolism. *Plant Methods*, 15(1): 48.
- Körner C. 2015. Paradigm shift in plant growth control. *Current Opinion in Plant Biology*, 25: 107-114.
- Kukah ASK, Xiaohua J, Osei-Kyei R, Perera S. 2024. How carbon trading contributes to

- reduction in emission of greenhouse gases: a narrative literature review. *Journal of Facilities Management*, 23(3): 463–479.
- Lambers H, Oliveira RS, Pons TL. 2019. Growth and allocation. In *Plant Physiological Ecology*. Cham: Springer International Publishing, p. 385–449.
- Leng H, Zhou L, Yan W. 2025. Variations in leaf photosynthesis and its limitations at different canopy positions in mature camphor trees. *Forests*, 16(4).
- Li SY, Hussain SB, Vincent C. 2024. Response of carbon fixation, allocation, and growth to source–sink manipulation by defoliation in vegetative citrus trees. *Physiologia Plantarum*, 176(3): e14304.
- Liu L, Hao L, Zhang Y, Zhou H, Ma B, Cheng Y, Tian Y, Chang Z, Zheng Y. 2022. The CO₂ fertilization effect on leaf photosynthesis of maize (*Zea mays* L.) depends on growth temperatures with changes in leaf anatomy and soluble sugars. *Frontiers in Plant Science*, 13: 1–15.
- Lopez G, Ahmadi SH, Amelung W, Athmann M, Ewert F, Gaiser T, Seidel SJ. 2023. Nutrient deficiency effects on root architecture and root-to-shoot ratio in arable crops. *Frontiers in Plant Science*, 13: 1067498.
- Lv X, Zhang Y, Zhang Y, Fan S, Kong L. 2020. Source-sink modifications affect leaf senescence and grain mass in wheat as revealed by proteomic analysis. *BMC Plant Biology*, 20(257): 1–17.
- Milne RJ, Offler CE, Patrick JW, Grof CP. 2015. Cellular pathways of source leaf phloem loading and phloem unloading in developing stems of *Sorghum bicolor* in relation to stem sucrose storage. *Functional Plant Biology*, 42(10): 957–970.
- Nam H, Gupta A, Nam H, Lee S, Cho HS, Park C, Hwang I. 2022. JULGI-mediated increment in phloem transport capacity relates to fruit yield in tomato. *Plant Biotechnology Journal*, 20(8): 1533–1545.
- Nath AJ, Lal R, Das AK. 2015. Managing woody bamboos for carbon farming and carbon trading. *Global Ecology and Conservation*, 3: 654–663.
- Nunes LJR, Meireles CIR, Gomes CJP, Ribeiro NMCA. 2020. Forest contribution to climate change mitigation: Management oriented to carbon capture and storage. *Climate*, 8(21): 1–20.
- Nuranisa S, Sudiana E, Yani E. 2020. Hubungan umur dengan stok karbon pohon duku (*Lansium parasiticum*) di Desa Kalijajar Kecamatan Kaligondang Kabupaten Purbalingga. *BioEksakta: Jurnal Ilmiah Biologi Unsoed*, 2(1): 146–151.
- Pawar R, Rana VS. 2019. Manipulation of source-sink relationship in pertinence to better fruit quality and yield in fruit crops: A review. *Agricultural Reviews*, 40(3): 200–207.
- Plavcová L, Jansen S. 2015. The role of xylem parenchyma in the storage and utilization of nonstructural carbohydrates. In *Functional and Ecological Xylem Anatomy*. Springer, p. 209–234.
- Plavcová L, Hoch G, Morris H, Ghiasi S, Jansen S. 2016. The amount of parenchyma and living fibers affects storage of nonstructural carbohydrates in young stems and roots of temperate trees. *American Journal of Botany*, 103(4): 603–612.
- Poorter H, Jagodzinski AM, Ruiz-Peinado R, Kuyah S, Luo Y, Oleksyn J, Sack L. 2015. How does biomass distribution change with size and differ among species? An analysis for 1200 plant species from five continents. *New Phytologist*, 208(3): 736–749.
- Punyasu N, Thaiprasit J, Kalapanulak S, Saithong T, Postma JA. 2024. Modeling cassava root system architecture and the underlying dynamics in shoot-root carbon allocation during the early storage root bulking stage. *Plant and Soil*, 507(1): 863–880.
- Putra SP, Santosa S, Salsinha YCF. 2023. Waterlogging and salinity stress affecting growth and morphological character changes of *Limnocharis flava*. *Biodiversitas Journal of Biological Diversity*, 24(1).
- Rademacher T, Fonti P, LeMoine JM, Fonti MV, Bowles F, Chen Y, Richardson AD. 2022. Insights into source/sink controls on wood formation and photosynthesis from a stem chilling experiment in mature red maple. *New Phytologist*, 236(4): 1296–1309.
- Savage JA, Clearwater MJ, Haines DF, Klein T, Mencuccini M, Sevanto S, Turgeon R, Zhang C. 2016. Allocation, stress tolerance and carbon transport in plants: How does phloem physiology affect plant ecology? *Plant Cell and Environment*, 39(4): 709–725.
- Shen N, Zhao Y, Deng R. 2020. A review of carbon trading based on an evolutionary perspective. *International Journal of*

- Climate Change Strategies and Management, 12(5): 739–756.
- Singh SK, Nath V, Swati Sharma ESM. 2017. Source-sink relationship in litchi versus mango: A concept. *International Journal of Current Microbiology and Applied Sciences*, 6(3): 500–509.
- Smith MR, Rao IM, Merchant A. (2018). Source-Sink Relationships in Crop Plants and Their Influence on Yield Development and Nutritional Quality. *Frontiers in Plant Science*, 9(1):1889.
- Sonnewald U, Fernie AR. 2018. Next-generation strategies for understanding and influencing source-sink relations in crop plants. *Current Opinion in Plant Biology*, 43: 63–70.
- Speck T, Burgert I. 2011. Plant stems: Functional design and mechanics. *Annual Review of Materials Research*, 41(1): 169–193.
- Sunmonu N, Kudo G. 2014. How do sink and source activities influence the reproduction and vegetative growth of spring ephemeral herbs under different light conditions? *Journal of Plant Research*, 127(4): 503–511.
- Swidrak I, Gruber A, Oberhuber W. 2014. Xylem and phloem phenology in co-occurring conifers exposed to drought. *Trees - Structure and Function*, 28(4): 1161–1171.
- Telaumbanua PH, Nazara RV, Zebua HP, Samudin S, Monde A, Purba JH, Sulistiyowati R, Oksifa A, Harti R, Telaumbanua ED, Kartini NL, Khide P, Mendrofa T. 2024. *Dasar-Dasar Agronomi. Azzia Karya Bersama*.
- Trugman AT, Anderegg LDL. 2024. Source vs sink limitations on tree growth: from physiological mechanisms to evolutionary constraints and terrestrial carbon cycle implications. *New Phytologist*, 245(1): 966–981.
- Vadera H, Pandya J, Mehta S. 2025. A review: The Elucidation of Source-Sink Relationship. *Life Sciences Leaflets*, 179(1): 12-35.
- Van Campen JC. 2016. Early leaf development in rice (*Oryza sativa* L.): structure, physiology, and gene expression (Doctoral dissertation, University of Sheffield).
- Waheed A, Qiao X, Muhammad M, Yiremaikebayi Y, Yingying X, Xu H, Wahab A. 2025. Plant root-mediated carbon sequestration and nutrient cycling in grassland ecosystems under land use and climate change. *Agriculture, Ecosystems & Environment*, 393: 109865.
- Wang L, Ruan YL. 2016. Shoot-root carbon allocation, sugar signalling and their coupling with nitrogen uptake and assimilation. *Functional Plant Biology*, 43(2): 105–113.
- Weraduwage SM, Chen J, Anozie FC, Morales A, Weise SE, Sharkey TD. 2015. The relationship between leaf area growth and biomass accumulation in *Arabidopsis thaliana*. *Frontiers in Plant Science*, 6: 167.
- White AC, Rogers A, Rees M, Osborne CP. 2016. How can we make plants grow faster? A source-sink perspective on growth rate. *Journal of Experimental Botany*, 67(1): 31–45.
- Wu Y, Gong W, Wang Y, Yong T, Yang F, Liu W, Wu X, Du J, Shu K, Liu J, Liu C, Yang W. 2018. Leaf area and photosynthesis of newly emerged trifoliolate leaves are regulated by mature leaves in soybean. *Journal of Plant Research*, 131(4): 671–680.
- Yang JH, Wang H. 2016. Molecular mechanisms for vascular development and secondary cell wall formation. *Frontiers in Plant Science*, 7(356): 1–8.
- Yu SM, Lo SF, Ho THD. 2015. Source-sink communication: regulated by hormone, nutrient, and stress cross-signaling. *Trends in Plant Science*, 20(12): 844–857.
- Zhang J, Zhang L, Wang Q, Liu J, Sun Y. 2023. Diurnal regulation of leaf photosynthesis is related to leaf-age-dependent changes in assimilate accumulation in *Camellia oleifera*. *Plants*, 12(11): 1–15.
- Zhang N, Van Westreenen A, Evers JB, Anten NP, Marcelis LF. 2020. Quantifying the contribution of bent shoots to plant photosynthesis and biomass production of flower shoots in rose (*Rosa hybrida*) using a functional-structural plant model. *Annals of Botany*, 126(4): 587–599.

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The effects of nanobubble fertigation and soil conditioners to improve nutrient dynamics, soil microbial activity, and chili productivity

Abstract. Drip fertigation is a fertilization method that integrates nutrient delivery with an irrigation system, which is capable of optimizing the direct delivery of nutrients to the root zone. This experiment aims to investigate the effect of drip fertigation with nanobubble technology, and soil conditioners on the population of Phosphate-Solubilizing Bacteria (PSB) and *Azotobacter* spp., available phosphorus, total nitrogen, growth, and yield of red chili. The study used a Strip Plot Design with two factors and three replications. The first factor was nutrient application (solid NPK fertilizing as control; drip fertigation; and drip fertigation with nanobubble technology), and the second factor was soil conditioner application (cow manure fertilizer; bioameliorant; and ameliorant). The measured parameters included PSB and *Azotobacter* spp. population, available phosphorus, total nitrogen, growth components (plant height, number of leaves, and stem diameter), and yield components (number of fruits per plant, fruit weight per plant, and yield quality). The results showed that the interaction between drip fertigation with nanobubble technology and soil conditioners significantly influenced the increase in PSB population and available phosphorus. Drip fertigation treatment with nanobubble and ameliorant application gave the best effect on the PSB population and P available. Nutrient application significantly affected plant height, stem diameter, number of fruits per plant, and fruit weight per plant. Soil conditioner application significantly affected total nitrogen, plant height, number of leaves, stem diameter, number of fruits per plant, and fruit weight per plant.

Keywords: Ameliorant · *Azotobacter* · Beneficial microbes · Nutrient · P-solubilizer

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Introduction

Red chili (*Capsicum annuum* L.) is an important vegetable commodity that plays a vital role in both domestic and international markets. Total chili production (red chilies and cayenne peppers) in Indonesia in 2023 was 3.11 million tons. With this high production, Indonesia has good market potential in world trade. According to the Statistics Indonesia (2023), red chili consumption in Indonesia in 2023 was 675 thousand tons. This figure increased from 636.56 thousand tons in 2022 to 596.14 thousand tons in 2021. This indicates that the level of consumption and demand for red chili continues to increase annually. The increase in red chili consumption each year indicates that red chili production needs to be increased to meet consumption needs. Despite continued increases in production, the continued growth in total consumption suggests that domestic red chili supply could still be further increased to reduce dependence on imports.

Meeting the increasing need for red chili consumption can be achieved through the utilization of suboptimal agricultural land, such as Inceptisols soil. Modifying the growing medium through soil conditioners, such as cow manure fertilizer, bioameliorant, and ameliorant, is known to improve the physical and chemical properties of the soil, increase pH, and support soil microbial activity (Avifah et al., 2022; Antonius et al., 2018). The application of liquid nutrients through drip fertigation can support nutrient availability in the root zone. Nutrient solutions are liquid inorganic fertilizers containing essential macro and micro nutrients for plants. Fertigation is a method of nutrient delivery performed simultaneously with irrigation (Kafkafi & Tarchizky, 2011; Yuan et al., 2014). The effectiveness of this system can be enhanced through the integration of nanobubble technology, which involves nano-sized gas bubbles (100–200 nm) that exhibit high stability in water.

Nanobubble technology is known to enhance the solubility of dissolved oxygen and accelerate the release of nutrients in the soil, thereby supporting optimal plant growth (Alqaramah et al., 2025). Phosphorus (P) and nitrogen (N) are two essential macronutrients in plant tissue formation. In addition, soil microbial activity such as phosphate-solubilizing bacteria play an important role in solubilizing P from an

unavailable form into a form available to plants (Campos et al., 2018) and *Azotobacter* spp. plays an important role in N fixation (Kholida & Zulaika, 2015). Studies on drip fertigation combined with nanobubble technology and soil conditioners remain limited; therefore, this study aims to determine their effects on the population of phosphate-solubilizing bacteria, available phosphorus, soil nitrogen, and the growth and yield of chili.

Materials and Methods

The experiment was conducted from August to December 2024 at the Bale Tatanen Experimental Field, Faculty of Agriculture, Universitas Padjadjaran, located at an altitude of \pm 750 meters above sea level (m asl). The materials used in this study included Inceptisol soil from Jatininggor; red chili seeds of the Baja F1 variety; single inorganic fertilizers comprising urea, SP-36 and KCl; AB mix solution, consisted of stock solution A (LI-A) containing $\text{Ca}(\text{NO}_3)_2$, KNO_3 , and Fe-EDTA, and stock solution B (LI-B) containing KH_2PO_4 , $(\text{NH}_4)\text{SO}_4$, K_2SO_4 (ZK), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, CuSO_4 , ZnSO_4 , H_3BO_3 , MnSO_4 , and Mo-NH_4 ; cow manure fertilizer; ameliorant, consisted of a mixture of coconut shell biochar, sugarcane bagasse compost, guano, and dolomite; bioameliorant, consisted of a mixture of ameliorant and biofertilizers (phosphate-solubilizing bacteria, nitrogen-fixing bacteria, and *Trichoderma* sp.).

The research conducted pot experiment, used a strip-plot design with two factors and repeated three times. The first factor was nutrient application, consisted of three levels, namely solid NPK fertilizing (as control); drip fertigation; and drip fertigation with nanobubble technology. The second factor was soil conditioner type, consisted of three levels, namely cow manure (20 tons ha^{-1} as control); bioameliorant (4 ton ha^{-1} ameliorant + 200 kg ha^{-1} biofertilizers) and ameliorant (4 ton ha^{-1}).

NPK fertilizer application is side placement by dose 300 kg ha^{-1} urea, 300 kg ha^{-1} SP-36, and 300 kg ha^{-1} KCl given at 3, 6, and 9 weeks after planting (WAP). The volume flowed from drip fertigation with nanobubble technology or without nanobubble technology is 200 ml AB mix solution/polybag. The solution is dripped by concentration 800 ppm (early vegetative stage, from 1 to 2 WAP), 1200 ppm (late vegetative

stage, from 3 to 4 WAP), and 1600 ppm (generative stage). Soil conditioner is given by side placement, buried in the soil.

Watering is carried out based on the treatment design and its volume is determined based on field capacity. In the NPK treatment, watering is done manually. In the fertigation treatment, watering uses drip fertigation with nutrient solutions. Meanwhile, in the fertigation + nanobubble treatment, watering uses drip fertigation with nanobubble technology. Other plant maintenance were replanting, weeding, and control plant pests and diseases.

Soil samples were collected at the end of the vegetative stage. Rhizosphere soil samples were collected at approximately 100 g for biological analysis, and composite soil samples were collected at approximately 500 g for chemical analysis. The population of phosphate-solubilizing bacteria and *Azotobacter* was calculated using the Total Plate Count (TPC) method, Soil P-Availability measured using the Olsen and Bray I method, for measuring soil nitrogen by Kjeldahl Method.

Growth observations were conducted once a week, starting from 1 week after planting until the plants reached the end of vegetative phase. Measurements included plant height, number of leaves, and stem diameter. In addition, observations were made on the components of chili plant yields, including the number of fruits per plant, the weight of the fruits per plant, and the quality of the chili fruits. All collected data were tabulated in Microsoft Excel and then the data were analyzed using analysis of variance (ANOVA). When significant differences among treatment factors were detected, mean comparisons were performed using Duncan's Multiple Range Test (DMRT) at a 5% significance level.

Results and Discussion

PSB Population. Interactions occur between nutrient application techniques and soil conditioners on PSB populations. The application of drip fertigation using nanobubble technology and soil conditioners results in the best PSB populations (Table 1). Nanobubbles can increase oxygen levels in the root zone, thereby stimulating root growth, improving nutrient uptake, and creating more optimal environmental conditions for beneficial soil microbes (Zhou et al., 2019). This aligns with the statement by Chen et al. (2023), who noted that increased oxygenation due to nanobubbles can influence the activity and population of beneficial soil microbes such as PSB, which play a crucial role in maintaining soil fertility and plant health. Based on Table 1, the treatment of providing drip fertigation nutrients + nanobubbles and soil conditioners in the form of ameliorants gave the best results for the PSB population of 7.12×10^5 CFU mL⁻¹.

The application of ameliorant containing coconut shell biochar, sugarcane bagasse compost, dolomite, and guano can provide sufficient carbon sources for PSB. The high organic carbon content in coconut shell biochar (Situmorang et al., 2019), sugarcane bagasse compost (Salsavira, 2024), and guano (Yeri et al., 2024) can increase PSB population because organic carbon serves as an energy source for PSB. Previous research showed that ameliorant application of 4 t ha⁻¹ combined with nutrient solution can increase PSB population by up to 78.4% compared to the control (Fitriatin et al., 2024).

Table 1. Effect of nutrient applications and soil conditioners interaction on phosphate-solubilizing bacteria population at 6 WAP

Nutrient Application	PSB Population (10 ⁵ CFU mL ⁻¹)		
	Soil Conditioners		
	Cow Manure Fertilizer	Bioameliorant	Ameliorant
NPK Fertilizer	6.66 a A	6.58 a A	6.73 a A
Drip Fertigation	6.90 a A	6.66 a A	6.65 a A
Drip Fertigation + Nanobubble	6.74 a A	6.77 a A	7.12 b B

Note: Means followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 0.05 significance level. Capital letters are read horizontally, and small letters are read vertically.

***Azotobacter* spp. Population.** The results showed no interaction between nutrients and soil conditioner application on the *Azotobacter* spp. population (Table 2). High nitrogen availability from readily absorbable nutrient sources can inhibit the nitrogen-fixing activity of *Azotobacter* spp. These free-living, non-symbiotic bacteria typically reduce their nitrogen fixation processes when sufficient nitrogen is already present in the soil. This physiological response is consistent with their ecological role, as *Azotobacter* spp. preferentially activate nitrogen fixation under low-nitrogen conditions to compensate for the deficiency. Consequently, elevated nitrogen levels can suppress both their population density and biological activity (Sheikh Assadi et al., 2023).

Table 2. Effect of nutrient applications and soil conditioners on *Azotobacter* spp. population at 6 WAP

Treatments	<i>Azotobacter</i> spp. Population (10 ⁵ CFU mL ⁻¹)
Nutrient Applications	
NPK Fertilizer	7.02 a
Drip Fertigation	7.11 a
Drip Fertigation + Nanobubble	7.14 a
Soil Conditioners	
Cow Manure Fertilizer	7.05 a
Bioameliorant	7.09 a
Ameliorant	7.12 a

Note: Means followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 0.05 significance level.

Previous studies have demonstrated that the use of soil conditioners can markedly influence the population dynamics of *Azotobacter* spp. (Table 2). One of the key factors affecting this response is the relatively high carbon-to-nitrogen (C/N) ratio of the applied soil amendments and bio-ameliorants, which in this case was 27.93%. Such an elevated C/N ratio restricts the availability of nitrogen in plant-accessible forms, thereby limiting the proliferation and activity of nitrogen-fixing microorganisms such as *Azotobacter* spp. (Yihune & Addisu, 2024).

Soil P-Availability. Interactions occurred between the application of nutrients and soil conditioners on available phosphorus. The application of drip fertigation using nanobubble technology and ameliorant interacts to produce the best available phosphorus (Table 3). This is likely because ameliorant can support the release

of bound phosphorus, while drip fertigation with nanobubble technology enhances nutrient distribution efficiency due to its direct application to the plant root zone. According to Alqaramah et al. (2025), nanobubbles can improve nutrient absorption efficiency by plants and enhance microbial activity in the soil. Increased microbial activity promotes the decomposition and mineralization of organic compounds, and can release a certain amount of phosphorus ions, thereby increasing their availability in the soil (Pang et al., 2024).

This increase in available P is also closely related to the increase in the population and activity of PSB in the soil. This bacteria can produce the enzyme phosphatase, which dissolves organic phosphate into inorganic phosphate through a mineralization process, making P available to plants, especially in soils with low P content (Pan & Cai, 2023).

Table 3. Effect of Nutrient Applications and Soil Conditioners Interaction on Soil P-Availability

Nutrient Applications	Soil P-Availability		
	Soil Conditioners		
	Cow Manure Fertilizer	Bioameliorant	Ameliorant
NPK Fertilizer	64.92 c B	29.50 a A	20.19 a A
Drip Fertigation	51.21 b B	19.50 a A	42.28 b B
Drip Fertigation + Nanobubble	16.91 a A	44.41 b B	79.32 c C

Note: Means followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 0.05 significance level. Capital letters are read horizontally, and small letters are read vertically

The ameliorant used in this study contained sugarcane bagasse compost and guano. According to Saldaña et al. (2014), the application of bagasse compost can increase the available phosphorus content in the soil due to the release of CO₂ from organic matter, which increases the CO₂ concentration in the soil and accelerates the decomposition process of phosphate minerals, thereby increasing the available phosphorus in the soil. The application of guano fertilizer enhances soil organic matter content and improves physical properties, particularly structure and porosity, thereby increasing the availability of essential nutrients such as phosphorus (Lubis et al., 2024).

Total Nitrogen. The results of the analysis showed that there was no interaction between the provision of nutrients and soil conditioners on total N. The analysis reveals that nutrient application exerts variable effects on soil total nitrogen (Total-N) levels, largely shaped by processes such as nitrogen volatilization, nitrate leaching, and plant competition for uptake. Numerous studies have underscored the complexity of nitrogen dynamics in soil systems. For example, Acharya and Shrestha (2015) reported that low soil nitrogen levels can result from rapid plant absorption, coupled with substantial losses through leaching and volatilization. These findings underscore a key characteristic of nitrogen as a highly mobile element, which often escapes the soil matrix rather than being efficiently retained.

Table 4. Effect of nutrient applications and soil conditioners on total Nitrogen

Treatments	Total N (%)
Nutrient Applications	
NPK Fertilizer	0.32 a
Drip Fertigation	0.36 a
Drip Fertigation + Nanobubble	0.39 a
Soil Conditioners	
Cow Manure Fertilizer	0.53 b
Bioameliorant	0.28 a
Ameliorant	0.26 a

Note: Means followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 0.05 significance level.

The application of cow manure fertilizer had a significant effect on total nitrogen. Based on Table 4, the soil conditioner treatment in the form of manure gave the best results for total N of 0.53%. Cow manure fertilizer contains organic nitrogen that decomposes into an available form (Moe et al., 2019). Increased microbial activity helps to maintain nitrogen in an available form and enriches the soil with total nitrogen (Khodaei Joghhan et al., 2010). This is supported by reports showing that the addition of organic matter, such as manure, increases the presence of nitrogen in soluble form in the soil, which in turn enhances nitrogen uptake efficiency by plants (Yang et al., 2018).

Plant Height. The application of drip irrigation, particularly when combined with nanobubble technology, significantly influenced

chili plant height at 6 weeks after planting (WAP) (Table 5). Drip fertigation facilitates precise nutrient delivery to the rhizosphere, thereby minimizing fertilizer losses and enhancing nutrient-use efficiency, which ultimately promotes plant growth (Zhao et al., 2023). Moreover, the enrichment of soil oxygen concentration through nanobubble technology has been reported to improve photosynthetic activity, stimulate gibberellin production, regulate microbial dynamics, and further enhance nutrient uptake efficiency (He et al., 2022). The subsequent section presents the documentation of chili plant growth following the application of nutrient inputs and soil amendments at 6 WAP (Figure 1).



Figure 1. Comparison of chili height in response to different nutrient applications and soil conditioners: N1 (NPK Fertilizer), N2 (Drip Fertigation), N3 (Drip Fertigation + Nanobubble), P1 (Cow Manure Fertilizer), P2 (Bioameliorant), and P3 (Ameliorant)

As highlighted by Howe et al. (2024), cow manure is a valuable source of essential nutrients required for plant development, including nitrogen (N), phosphorus (P), and potassium (K), along with various macro- and micronutrients. These nutrients occur in both organic and inorganic fractions, with the organic component undergoing microbial mineralization that gradually releases them into plant-available forms. The efficiency and extent of this mineralization process are largely determined by microbial activity and are strongly influenced by environmental factors such as soil temperature, moisture levels, and the physicochemical characteristics of both the soil and the manure. Beyond the primary nutrients, cow manure also provides secondary nutrients such as calcium (Ca) and magnesium (Mg), as well as trace elements including zinc (Zn), iron (Fe), manganese (Mn), copper (Cu), sulfur (S), and

boron (B). These nutrients may be available directly in bioavailable forms or as compounds that can be rapidly converted into accessible forms. The integration of cow manure into agricultural practices has consistently been linked with improved soil fertility and enhanced crop productivity (Dhiman et al., 2019).

Number of Leaves. The analysis results show that nutrient application has no significant effect on the number of leaves (Table 6). The nutrient formulations used in this approach are designed to supply both macro- and micronutrients in water-soluble forms, ensuring immediate availability for plant uptake. Among these, nitrogen is the most prominent and plays a vital role in promoting leaf development, as it is integral to photosynthesis and overall vegetative growth (Santana et al., 2021).

When combined with nanobubble technology, drip fertigation not only enhances oxygen availability in the rhizosphere but also stimulates root development and supports more robust vegetative growth (Zahra et al., 2025).

The use of cow manure in conjunction with soil ameliorants has been demonstrated to significantly enhance plant growth, notably reflected by an increase in number leaves count at 5–6 WAP. This effect is primarily attributed to the organic matter in cow manure and ameliorants, which improves soil physical properties by enhancing porosity and aeration. Such improvements optimize nutrient availability and uptake – particularly nitrogen – thereby promoting healthier and more vigorous vegetative development (Artacho et al., 2009).

Table 5. Effect of nutrient applications and soil conditioner on chili plant height at 1-6 WAP

Treatments	Plant Height (cm)					
	1 WAP	2 WAP	3 WAP	4 WAP	5 WAP	6 WAP
Nutrient Applications						
NPK fertilizers	25.84	34.14	47.73	67.52	79.46	91.35 a
Drip fertigation	25.32	35.31	49.49	70.29	84.64	98.25 b
Drip fertigation with nanobubble	24.84	36.16	49.97	71.77	85.38	101.77 b
Soil Conditioners						
Cow manure fertilizer	26.25	37.07 b	52.39 b	73.76 b	87.29 b	101.80 c
Bioameliorant	24.84	33.20 a	46.18 a	63.65 a	76.75 a	92.24 a
Ameliorant	24.90	35.35 ab	48.63 ab	72.17 b	85.44 b	97.32 b

Note: Means followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 0.05 significance level.

Table 6. Effect of nutrient applications and soil conditioner on the number of chili plant leaves at 1-6 WAP

Treatments	Number of Leaves (blades)					
	1 WAP	2 WAP	3 WAP	4 WAP	5 WAP	6 WAP
Nutrient Applications						
NPK fertilizers	11.55	17.70	33.88	73.14	115.18	173.63
Drip fertigation	12.11	17.66	35.11	78.11	115.33	162.55
Drip fertigation with nanobubble	12.14	18.25	38.88	76.40	128.07	181.40
Soil Conditioners						
Cow manure fertilizer	12.37 a	18.77 b	40.81 b	87.59 b	138.07 b	190.74 b
Bioameliorant	11.51 a	16.70 a	30.70 a	65.48 a	94.14 a	143.85 a
Ameliorant	11.92 a	18.15 ab	36.36 ab	74.59 ab	126.37 b	183.00 b

Note: Means followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 0.05 significance level.

Stem Diameter. The application of drip fertigation and drip fertigation with nanobubble technology had a significant effect on stem diameter (Table 7). This technique optimizes the supply of both macro- and micronutrients, thereby supporting essential physiological processes required for vigorous plant growth. Among these nutrients, nitrogen is particularly crucial, as it stimulates the development of vegetative structures, including stems, which play a fundamental role in sustaining overall plant performance (Dianawati et al., 2023). The application of drip irrigation with nanobubble technology enhances gas transport efficiency to cells, improves membrane structure and protein activity, thereby supporting stem growth (Liu et al., 2013; Seddon et al., 2012).

The incorporation of cow manure together with soil ameliorants has been shown to substantially promote plant growth, as indicated by a significant increase in stem diameter at 3–4 weeks after planting (WAP). This effect is primarily associated with the high organic matter

content of cow manure and ameliorants, which improve soil physical properties by enhancing porosity and aeration. Such improvements facilitate more efficient nutrient dynamics, particularly nitrogen uptake, thereby stimulating vigorous vegetative growth (Artacho et al., 2009).

Number of Fruits per Plant. The combination of drip fertigation and nanobubble technology has been shown to markedly improve fruit production in crop systems (Table 8). Drip fertigation ensures the precise and efficient delivery of macro- and micronutrients in soluble forms, facilitating rapid uptake by plants. When coupled with nanobubbles, this system further enhances nutrient utilization and physiological efficiency, particularly during the generative phase of development. In fruiting crops such as chili peppers, these synergistic effects translate into a significant increase in yield (Giri et al., 2024). Nanobubble formulations enhance nutrient absorption efficiency through controlled-release mechanisms (Zhou et al., 2019).

Table 7. Effect of Nutrient Applications and Soil Conditioners on Chili Plant Stem Diameter at 1-6 WAP

Treatments	Stem Diameter (mm)					
	1 WAP	2 WAP	3 WAP	4 WAP	5 WAP	6 WAP
Nutrient Applications						
NPK fertilizers	3.67	4.40	5.31	6.60	7.46	8.57 a
Drip fertigation	3.68	4.36	5.52	7.00	7.74	9.44 b
Drip fertigation with nanobubble	3.75	4.59	5.55	7.03	7.57	9.68 b
Soil Conditioners						
Cow manure fertilizer	3.67	4.34	5.80 b	7.27 b	8.00	9.58
Bioameliorant	3.71	4.37	5.21 a	6.57 a	7.25	8.95
Ameliorant	3.72	4.63	5.38 ab	6.80 ab	7.52	9.16

Note: Means followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 0.05 significance level.

Table 8. Effect of Nutrient Applications and Soil Conditioner on the Number of Fruits per Plant

Treatments	Number of Fruits per Plant
Nutrient Applications	
NPK Fertilizer	37.07 a
Drip Fertigation	31.59 a
Drip Fertigation + Nanobubble	48.44 b
Soil Conditioners	
Cow Manure Fertilizer	47.59 b
Bioameliorant	28.77 a
Ameliorant	40.74 b

Note: Means followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 0.05 significance level.

The application of cow manure combined with soil ameliorants has been demonstrated to significantly increase the number of fruits per plant. This improvement is associated with the abundant supply of key nutrients, particularly nitrogen (N), phosphorus (P), potassium (K), and organic carbon, which collectively enhance plant vigor and productivity. These elements are essential for cellular development and carbohydrate metabolism (Ogundare et al., 2015). Among them, phosphorus is especially critical, as it is absorbed predominantly in the form of hydrogen phosphate (HPO_4^{2-}) and dihydrogen phosphate (H_2PO_4^-) ions. These soluble species represent the most plant-available forms of phosphorus in the soil solution and are primarily taken up through diffusion (Wang et al., 2024). The efficiency of phosphorus absorption is strongly regulated by soil pH, with the optimal range for uptake typically between 4.5 and 5.0. Within this range, plants preferentially utilize H_2PO_4^- , emphasizing the importance of maintaining suitable soil pH conditions for effective nutrient management (García et al., 2025).

Fruit Weight per Plant. The application of drip fertigation with nanobubble technology has a significant effect on fruit weight per plant (Table 9). The application of drip fertigation integrated with nanobubble technology has a pronounced effect on fruit weight per plant. This system enhances the precision and efficiency of nutrient delivery, thereby supporting the biosynthesis of proteins, carbohydrates, and lipids that are fundamental to fruit development and weight accumulation (Camarero et al., 2023). The improved nutrient availability promotes optimal metabolic functioning within the plant, ensuring that assimilates are efficiently allocated to developing fruits. Potassium (K), in particular, plays a pivotal role as a macronutrient influencing plant growth, physiology, and yield. Acting as a key enzyme activator, potassium regulates numerous biochemical processes, including photosynthesis, osmotic balance, and assimilate transport. Given its high uptake demand—second only to nitrogen—potassium is indispensable for sustaining physiological efficiency and maximizing fruit production (Al-Raddadi et al., 2024). Nanobubbles enhance oxygen availability in the root zone, support root respiration, and improve water and nutrient

absorption efficiency, thereby supporting fruit filling and weight increase (Wu et al., 2019).

Table 9. Effect of nutrient applications and soil conditioners on fruit weight per plant

Treatments	Fruit Weight per Plant (g)
Nutrient Applications	
NPK Fertilizer	177.90 a
Drip Fertigation	174.47 a
Drip Fertigation + Nanobubble	261.91 b
Soil Conditioners	
Cow Manure Fertilizer	250.07 b
Bioameliorant	160.14 a
Ameliorant	204.14 ab

Note: Means followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 0.05 significance level.

The use of cow manure has been shown to significantly increase fruit weight per plant, mainly due to its rich composition of macro- and micronutrients, particularly N, P, and K, which are essential for protein synthesis, assimilate production, and fruit tissue development. In addition to nutrient supply, cow manure contributes substantial organic matter that enhances soil fertility, structural stability, and water retention capacity. These improvements foster better root growth and nutrient uptake, ultimately resulting in higher crop productivity (Ikeh et al., 2023). Furthermore, its ability to improve soil water absorption enhances nutrient uptake efficiency, thereby supporting optimal fruit growth.

Quality of Chili Yields. Irrigation with nanobubble-enriched water significantly improves the quality of chili fruits, with grade I peppers showing up to a 14.45% increase in fruit quality compared to conventional fertilization using urea, SP-36, and KCl. The macro- and micronutrients delivered through drip irrigation play a critical role in meeting plant nutritional demands for producing high-quality fruit. Potassium, in particular, is an essential macronutrient that supports growth and enhances fruit quality (Meylia & Koesriharti, 2018). Moreover, nanobubbles improve irrigation efficiency by increasing nutrient uptake and water-use efficiency, thereby boosting crop yield and fruit quality without requiring additional water or fertilizer inputs (Liu et al., 2019).

Table 10. Effect of nutrient applications and soil conditioners on chili quality

Treatments	Yield Quality (%)					
	I	II	Good Quality (I+II)	Improvement in Quality (I+II) (%)	III	BS
Nutrient Applications						
NPK fertilizers	5.63 a	59.98	65.61	-	30.20	4.19
Drip fertigation	8.30 a	65.36	73.66	12.27	20.85	5.49
Drip fertigation with nanobubble	14.96 b	60.13	75.09	14.45	20.26	4.65
Soil Conditioners						
Cow manure fertilizer	6.36	64.71	71.07	-	24.57	4.36
Bioameliorant	13.56	60.81	74.37	4.64	20.51	5.12
Ameliorant	8.98	59.94	68.92	-3.03	26.23	4.85

Note: Means followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 0.05 significance level.

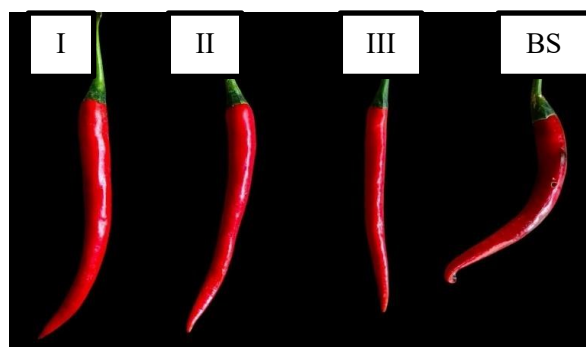


Figure 2. Comparison of Chili Quality in Response to Different Nutrition and Soil Conditioners: I (Premium), II (Medium), III (Standard), BS (Below Standard)

The chili classification system categorizes chili fruits into four quality grades, ranging from Grade I to Below Standard (Figure 2). This grading is primarily based on external attributes such as color uniformity, shape and size consistency, cleanliness, and the presence of physical damage or decay. Higher grades are characterized by uniform red coloration, typical fruit morphology, and minimal defects or contamination. In contrast, lower grades display reduced uniformity, irregular or undersized fruits, and a greater incidence of visible dirt, damage, or deterioration.

Table 10 shows soil conditioner with bioameliorants indicates an 4.64% improvement in fruit quality (I+II) compared to the application of cow manure. This is because the bioameliorants used in this study contain guano and biological agents. The high phosphorus

content in guano fertilizer plays an important role in fruit quality formation and increases plant resistance to disease (Taofik et al., 2018). Biological agents can improve soil structure and increase nutrient absorption, thereby supporting optimal plant growth and producing fruit of better quality (Nicolson et al., 2021). This finding is consistent with the results of Widiyanti et al. (2022), who reported that biological agents such as *Trichoderma* sp. and *Pseudomonas fluorescens* significantly improved dragon fruit quality and increased yield by up to 30%.

Conclusion

The interaction between drip fertigation with nanobubble technology and soil conditioners had a significant effect on increasing the population of PSB and available P. Nutrient treatment has a significant effect on plant height at 6 WAP, stem diameter at 6 WAP, and the quality of chili peppers of grade I. Soil conditioner treatment has a significant effect on plant height at 2–6 WAP, number of leaves at 3–6 WAP, stem diameter at 3–4 WAP, number of fruits per plant, and fruit weight per plant.

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References

- Acharya R, Shrestha BB. 2015. Physiochemical characteristics of soil of a mixed *Shorea robusta* forest in Rupandehi District, Nepal. *Journal of Natural History Museum*, 26: 155-162.
<https://doi.org/10.3126/jnhm.v26i0.14139>
- Alqaramah R, Fevria R, Vauzia, Razak A. 2025. Influence of Tithonia POC and nano bubble technology on hydroponic pakcoy (*Brassica rapa* L.) quality. *Jurnal Biologi Tropis*, 25: 1384-1391.
<https://doi.org/10.29303/jbt.v25i2.8782>
- Al-Raddadi TM, Al-Khateeb LA, Sadaka MWM, Bahaffi SO. 2024. Trace element speciation and nutrient distribution in *Boerhavia elegans*: evaluation and toxic metal concentration across plant tissues. *Toxics*, 13: 1-14.
<https://doi.org/10.3390/toxics13010014>
- Antonius S, Sahputra RD, Nuraini Y, Dewi TK. 2018. Manfaat pupuk organik hayati, kompos dan *biochar* pada pertumbuhan bawang merah dan pengaruhnya terhadap biokimia tanah pada percobaan pot menggunakan tanah ultisol. *Jurnal Biologi Indonesia*, 14: 234-250.
<https://doi.org/10.47349/jbi/14022018/243>
- Artacho P, Bonomelli C, Meza F. 2009. nitrogen application in irrigated rice grown in mediterranean conditions: effects on grain yield, dry matter production, nitrogen uptake, and nitrogen use efficiency. *Journal of Plant Nutrition*, 32: 1574-1593.
- Avifah N, Zainabun, Jufri Y. 2022. Pemberian Beberapa Macam Amelioran untuk Memperbaiki Sifat-Sifat Kimia Tanah Sawah. *Jurnal Ilmiah Mahasiswa Pertanian*, 7: 604-614.
- Camarero MC, Briegas B, Corbacho J, Labrador J, Gallardo M, Gomez-Jimenez MC. 2023. characterization of transcriptome dynamics during early fruit development in olive (*Olea europaea* L.). *International Journal of Molecular Sciences*, 24: 961.
- Campos P, Borie F, Cornejo P, López-Ráez JA, López-García Á, Seguel A. 2018. Phosphorus acquisition efficiency related to root traits: is mycorrhizal symbiosis a key factor to wheat and barley cropping?. *Frontiers in Plant Science*, 9: 752.
- Chen W, Bastida F, Liu Y, Zhou Y, He J, Song P, Kuang N, Li Y. 2023. Nanobubble oxygenated increases crop production via soil structure improvement: the perspective of microbially mediated effects. *Agricultural Water Management*, 282: 108263.
- Dhiman D, Sharma R, Sankhyan NK, Sepehya S, Sharma SK, Kumar R. 2019. Effect of regular application of fertilizers, manure and lime on soil health and productivity of wheat in an acid alfisol. *Journal of Plant Nutrition*, 42: 2507-2521.
<https://doi.org/10.1080/01904167.2019.1659317>
- Dianawati M, Cahyaningrum H, Suwitono B. 2023. The determination of nitrogen concentration in the g2 potato seed production on fertigation system. *e3s web of conferences*, 425.
<https://doi.org/10.1051/e3sconf/202342501005>
- Fitriatin BN, Dupa PSE, Fauzian NO, Wong MY, Simarmata T. 2024. The Influence of vegetative, Nutrient Solution and Biofertilizer on Soil P, Plant P Uptake, and Yield of Red Chili. *Jurnal Agro*, 11(1): 107-117.
- García BA, Torre JAF, Santos-Villalobos S, Espinoza-Canales A, Echavarría-Cháirez FG, Bañuelos HG. 2025. Phosphorus dynamics and sustainable agriculture: the role of microbial solubilization and innovations in nutrient management. *Current Research in Microbial Sciences*, 8: 100326.
<https://doi.org/10.1016/j.crmicr.2024.100326>
- Giri J, Ghawade S, Panchbhai D, Nagre P, Kadu P, Deshmukh M. 2024. Influence of drip fertigation and foliar spray of boron on yield and nutrient uptake by *Cucumber* cv. Himangi. *Asian Journal of Soil Science and Plant Nutrition*, 10: 146-154.
<https://doi.org/10.9734/ajssp/2024/v10i1220>
- He J, Liu Y, Wang T, Chen W, Liu B, Zhou Y, Li Y. 2022. Effects of nanobubble in subsurface drip irrigation on the yield, quality, irrigation water use efficiency and nitrogen partial productivity of watermelon and muskmelon. *International Agrophysics*, 36(3): 163-171.
<https://doi.org/10.31545/intagr/150413>
- Fitriatin BN, Rokayah EH, Elmyra EN, Simarmata T, Fauziah NO, Fakhurroja H. 2025. The effects of nanobubble fertigation and soil conditioners to improve nutrient dynamics, soil microbial activity, and chili productivity. *Jurnal Kultivasi*, 24(3): 309-320.

- Howe JA, McDonald MD, Burke J, Robertson I, Coker H, Gentry TJ, Lewis KL. 2024. Influence of fertilizer and manure inputs on soil health: A review. *Soil Security*, 16: 100155. <https://doi.org/10.1016/j.soisec.2024.100155>
- Ikeh AO, Ndaeyo NU, Ikeh CE. 2023. Effects of integrated fertilization on soil sustainability and cassava (*Manihot esculenta* Crantz) yield in an ultisol. *Journal of Current Opinion in Crop Science*, 4: 89-102. <https://doi.org/10.62773/jcocs.v4i2.197>
- Kafkafi U, Tarchitzky J. 2011. *Fertigation: A Tool for Efficient Fertilizer and Water Management* (pp. 141- 141). Paris: International Fertilizer Industry Association (IFA) International Potash Institute (IPI).
- Khodaei Joghana A, Ghalavand A, Aghaalikhani M, Gholamhoseini M, Dolatabadian A. 2010. Comparison among different integrated nutrition management for soil micro and macro elements after winter wheat harvesting and yield. *Notulae Scientia Biologicae*, 2: 107-111.
- Kholida FT, Zulaika E. 2015. Potensi *Azotobacter* sebagai penghasil hormon IAA (*Indole 3-Acetic Acid*). *Jurnal Sains dan Seni ITS*, 4: 75-77.
- Liu S, Kawagoe Y, Makino Y, Oshita S. 2013. Effects of nanobubbles on the physicochemical properties of water: the basis for peculiar properties of water containing nanobubbles. *Chemical Engineering Science*, 93: 250-256. <https://doi.org/10.1016/j.ces.2013.02.004>
- Liu Y, Zhou Y, Wang T, Pan J, Zhou B, Muhammad T, Li Y. 2019. Micro-nano bubble water oxygation: synergistically improving irrigation water use efficiency, crop yield and quality. *Journal of Cleaner Production*, 222: 835-843.
- Lubis FA, Kusbiantoro D, Asbur Y, Purwaningrum Y, Kurniawan IS. 2024. Analysis study of soil phosphorus availability on the growth of oil palm seedlings (*Elaeis guineensis* Jacq.) with the application of liquid fertilizer and guano fertilizer (bat manure) in main-nursery. *Central Research Institute for Agriculture*, 18: 58-66.
- Meylia RD, Koesriharti. 2018. Pengaruh pemberian pupuk fosfor dan sumber kalium yang berbeda terhadap pertumbuhan dan hasil tanaman tomat (*Lycopersicon esculentum* Mill.). *Jurnal Produksi Tanaman*, 6(8): 1934-1941.
- Moe K, Htwe AZ, Thu TTP, Kajihara Y, Yamakawa T. 2019. Effects on npk status, growth, dry matter and yield of rice (*Oryza sativa*) by organic fertilizers applied in field condition. *Agriculture*, 9: 1-15. <https://doi.org/10.3390/agriculture9050109>
- Nicolson M, Smith J, Patel R. 2021. Enhancing soil health and plant growth through the use of biological control agents. *Soil Biology & Biochemistry*, 58: 98-109.
- Ogundare S, Mohammed S, Owolabi J. 2015. Soil application of cow dung with foliar application of boost extra, effect on growth and yield of okra in an ultisol, Nigeria. *Journal of Agricultural Studies*, 4: 73. <https://doi.org/10.5296/jas.v4i1.8579>
- Pan L, Cai B. 2023. Phosphate-Solubilizing Bacteria: Advances in Their Physiology, Molecular Mechanisms and Microbial Community Effects. *Microorganisms*. 11(12):2904. doi: 10.3390/microorganisms11122904. PMID: 38138048; PMCID: PMC10745930.
- Pang F, Qing L, Solanki MK, Wang Z, Xing YX, Dong DF. 2024. Soil phosphorus transformation and plant uptake driven by phosphate-solubilizing microorganisms. *Frontiers in Microbiology*, 15: 1-13.
- Saldaña MI, Álvarez RG, Cruz MDCR, Solís JDÁ, Fernández JMP, García CFO. 2014. The Influence of organic fertilizers on the chemical properties of soil and the production of *Alpinia purpurata*. *Ciencia e Investigacion Agraria*, 41: 215-224. <https://doi.org/10.4067/s0718-16202014000200008>
- Salsavira K. 2024. analisa kandungan c-organik tanah dan total populasi mikroorganisme tanah sebelum dan setelah aplikasi pupuk organik blotong pada lahan tebu PTPN XI di Kebun Mrawan dan Kebun RVO Tapen. *Jagad Tani; Jurnal Ilmu Pertanian*, 1: 1-11.
- Santana DC, Flores MS, Cotrim MF, Rodrigues EV, Santos A, Teodoro LPR, Teodoro PE. 2021. Genotype × trait biplot and canonical correlations for spectral and agronomic traits in corn. *Agronomy Journal*, 113: 1197-1204. <https://doi.org/10.1002/agj2.20581>

- Seddon JRT, Lohse D, Ducker WA, Craig VSJ. 2012. A deliberation on nanobubbles at surfaces and in bulk. *ChemPhysChem*, 13: 2179–2187. <https://doi.org/10.1002/cphc.201100900>
- Sheikh-Assadi M, Khandan-Mirkohi A, Taheri M, Babalar M, Sheikhi H, Nicola S. 2023. arbuscular mycorrhizae contribute to growth, nutrient uptake, and ornamental characteristics of static (*Limonium sinuatum* [L.] mill.) subject to appropriate inoculum and optimal phosphorus. *Horticulturae*, 9: 564. <https://doi.org/10.3390/horticulturae9050564>
- Situmorang Y, Nurbaity A, Simarmata T. 2019. Efek komposisi dan dosis amelioran terhadap sifat tanah dan hasil tanaman cabai (*Capsicum annum* L.) pada inceptisols. *Jurnal Agrotek Indonesia*, 4: 26-29.
- Statistics Indonesia. 2023. *Produksi Tanaman Sayuran*. Jakarta: Badan Pusat Statistik
- Taofik A, Setiati Y, Purnama L. 2019. Kombinasi guano kelelawar dengan pupuk urea dalam budidaya buncis, *Phaseolus vulgaris*. In *Seminar Nasional Pembangunan Pertanian Berkelanjutan Berbasis Sumber Daya Lokal* (pp. 156-168).
- Wang X, Guo H, Wang J, He P, Kuzyakov Y, Ma M, Ling N. 2024. Microbial phosphorus-cycling genes in soil under global change. *Global Change Biology*, 30. <https://doi.org/10.1111/gcb.17281>
- Widiantini E, Prabowo M, Rizki F. 2022. Field evaluation of biological control agents for dragon fruit cultivation. *Journal of Sustainable Agriculture*, 24: 375-386.
- Wu Y, Lyu T, Yue B, Tonoli E, Verderio EAM, Ma Y, Pan, G. 2019. Enhancement of tomato plant growth and productivity in organic farming by agri-nanotechnology using nanobubble oxygation. *Journal of Agricultural and Food Chemistry*, 67: 10823–10831. <https://doi.org/10.1021/acs.jafc.9b04117>
- Yang L, Bai J, Liu J, Zeng N, Cao W. 2018. Green manuring effect on changes of soil nitrogen fractions, maize growth, and nutrient uptake. *Agronomy*, 8. <https://doi.org/10.3390/agronomy8110261>
- Yeri N, Fikrinda W, Hamzah A. 2024. Pemberian mikotricho dan guano terhadap pertumbuhan dan hasil tanaman kacang tanah (*Arachis hypogaea* L.). *Jurnal Penelitian Pertanian Terapan*, 24: 8-16.
- Yihune E, Addisu S. 2024. Assessment of physicochemical properties and heavy metal content of floriculture soil in amhara region of northwest ethiopia. *The Scientific World Journal*, 2024. <https://doi.org/10.1155/2024/9945257>
- Yuan H, Cheng M, Pang S, Li L, Wang H, NA S. 2014. Construction and performance experiment of integrated water and fertilization irrigation recycling system. *Transactions of the Chinese Society of Agricultural Engineering*, 30: 72-78.
- Zahra A, Fauziah NO, Ambarita DDM, Fakhurroja H, Turmuktini T, Fitriatin BN, Simarmata, T. 2025. Deep insights into drip-based nanobubble fertigation technology for enhancing nutrient availability and boosting cash crop vegetable productivity and quality. *Journal of Ecological Engineering*, 26: 459–471. <https://doi.org/10.12911/22998993/205227>
- Zhao L, Teng M, Zhou L, Li Y, Sun J, Zhang Z, Wu F. 2023. Hydrogen nanobubble water: a good assistant for improving the water environment and agricultural production. *Journal of Agricultural and Food Chemistry*, 71: 12369–12371. <https://doi.org/10.1021/acs.jafc.3c04582>
- Zhou Y, Zhou B, Xu F, Muhammad T, Li Y. 2019. Appropriate dissolved oxygen concentration and application stage of micro-nano bubble water oxygation in greenhouse crop plantation. *Agricultural Water Management*, 223: 105713. <https://doi.org/10.1016/j.agwat.2019.10>

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Production analysis of seed-derived tea plants (*Camellia sinensis* (L.) O. Kuntze) across two different seasons

Abstract. Tea (*Camellia sinensis* (L.) O. Kuntze) is a major economic crop in Indonesia, yet its productivity is strongly influenced by environmental variability, particularly rainfall seasonality. Fluctuating yields under dry and wet conditions pose challenges for sustainable tea production. This study aimed to assess the effects of contrasting seasonal rainfall on seed-derived tea plants' yield and yield components. The research was conducted at a seed-derived tea plantation block in IRITC, West Java, across dry and wet seasons using field observations on tea yield, shoot proliferation, plucking quality, and environmental parameters. Statistical analyses included independent t-tests to compare seasonal differences and Pearson correlation to evaluate the relationships between environmental variables and production outcomes. The findings revealed that tea yield and shoot growth were higher in the wet season than in the dry season, which was statistically significant. Correlation analysis showed that temperature negatively influenced yield and shoot development, while rainfall, soil moisture, and relative humidity were strongly and positively associated with production performance. Conversely, plucking quality (fine and coarse leaves) remained stable across both seasons, indicating resilience to climatic variability and the role of consistent management practices. In short, the wet season provides more favorable conditions for seed-derived tea cultivation, especially in enhancing shoot growth and yield components. At the same time, adaptive management is required to mitigate temperature stress during the dry season.

Keywords: Dry seasons · Seed-derived · Wet seasons · Tea yields · Yield components

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Introduction

Tea (*Camellia sinensis* L. (O.) Kuntze) is one of the most widely cultivated and economically important crops globally, primarily valued for its leaves used in producing various tea products. Tea production supports millions of livelihoods and represents a significant source of export income for several countries. Indonesia is the seventh-largest tea-producing country in the world with a total production of 129.259 tons in 2021 (Cahyaningsih et al., 2025). Tea production is one of the important contributors to Indonesia's foreign exchange earnings. However, despite the increasing global demand for tea, the productivity of tea in Indonesia has shown a fluctuating trend (Sita & Rohdiana, 2021). In 2022, the export volume decreased to 44.92 thousand tons, compared with 49.03 thousand tons in 2018. This unstable trend highlights the challenge of maintaining sustainable tea production in Indonesia, particularly under varying environmental conditions.

The productivity of tea is significantly influenced by both genetic and environmental factors, among which rainfall is a critical factor influencing plant performance. Rainfall regulates vegetative growth and modulates the secondary metabolite accumulation (Langát, et al., 2018; Boehm et al., 2016). Yield components such as pekoe shoots, dormant shoots, young, mature, and damaged leaves are crucial indicators of tea plant growth and productivity. These components exhibit considerable sensitivity to fluctuations in soil moisture availability, reflecting the plants' physiological adaptation to varying rainfall regimes (Ochieng et al., 2016; Langát, 2022). Therefore, understanding rainfall influence is important for agronomic performance and ensuring tea quality and market competitiveness.

Tea plants derived from seeds and those established through clonal cuttings differ primarily in their genetic diversity and uniformity. Seed-grown plants arise from sexual productivity, resulting in genetic variability among individuals, which enhances adaptability and resilience to environmental stresses genetically identical to the parent genotype, offering uniformity in growth, flavor, and quality, which is advantageous for commercial production but limits genetic adaptability (Xia et al., 2020). The genetic base of seed-derived plants may provide greater drought tolerance through enhanced water-use efficiency and survival

under moisture-deficient conditions. Conversely, in high rainfall conditions, genetic variability may enable better adjustment to excessive soil moisture and associated risks such as root diseases, thereby supporting physiological resilience to waterlogging (Ding et al., 2021). Thus, seed-derived plants offer genetic diversity that supports adaptation to varying rainfall conditions, whereas cloned plants favor uniformity but may be more vulnerable to environmental extremes.

Low and high rainfall conditions differentially affect tea plants' tea yields and yield components. Limited water availability can cause a reduction in the number and growth of pekoe buds and young leaves. It also prolongs shoot dormancy, lowers the biomass and photosynthetic activity of mature leaves, and increases the occurrence of diminished overall productivity and quality, with the severity depending on the intensity and duration of drought as well as the tea plant's tolerance mechanisms (Hasan et al., 2023). Mature and damaged leaves tend to increase under high rainfall and humid conditions, where fungal infections and related stresses are more prevalent, negatively affecting leaf quality (Nyabundi et al., 2016). These contrasting responses highlight that rainfall impacts yield quantitatively and alters the balance of yield components, directly influencing the final harvested product.

Despite the recognized impact of seasonal factors on tea cultivation, limited research has specifically addressed the effect of different seasonal conditions on tea yields and yield components in seed-derived tea plants. Most existing studies have focused primarily on clonal varieties or have examined tea yields and yield components traits independently, without integrating their interactions within seed-derived populations that exhibit greater genetic and physiological diversity. Investigating yield traits such as pekoe shoots, dormant shoots, young leaves, mature leaves, and damaged leaves, alongside phenolic and flavonoid contents under contrasting rainfall regimes, could provide valuable insights into adaptive responses and help optimize cultivation practices across diverse agroclimatic conditions (Abdullah et al., 2024). This knowledge gap is critical in Indonesia, where seed-derived tea fields are still widely found, but little is known about their performance under different seasonal conditions.

Rainfall is well acknowledged for its influence on the trade-off between growth and

quality in tea production. This complexity is particularly relevant in seed-derived tea populations, which contain a broader genetic base than clonal cultivars, making them potentially more resilient but also more variable in response to environmental factors (Abdullah et al., 2024). Understanding these dynamics is critical for sustaining yield stability and components under increasingly variable rainfall patterns caused by climate change. Therefore, this study aims to evaluate the effects of contrasting rainfall regimes on the yield components of seed-derived tea plants in order to identify seasonal advantages and provide practical recommendations for sustainable tea cultivation. By assessing growth performance, leaf quality, and production output, the research intends to provide empirical data on how seasonal dynamics influence seed-derived tea plants and to identify which season provides optimal conditions for tea production. Such insights will help determine the extent to which environmental factors affect agronomic traits and economic yield during each season, thereby supporting the development of more effective management and harvesting strategies.

Materials and Methods

The experiment was conducted at the Experimental Field in Indonesia Research Institute for Tea and Cinchona (IRITC), Gambung, Ciwidey, at an altitude of ± 1.350 m asl. The research was conducted from October 2023 to August 2024. The plant material comprised seed-derived tea (A2 block) established since 1907, planted by R.E. Kerhoven, cultivated at a spacing of 120×110 cm. Samples were collected from a seed-derived tea field covering an area of 1 ha and consisting of approximately 8,000 plants. Harvesting was carried out three times each season, and each of these harvests was considered a replication. This study used existing field blocks with plants of similar age and management, so samples were taken using a blocking plot design, meaning the same plot areas were consistently observed each season. To obtain representative values for each replicate, all observations from each harvest were averaged. The primary parameters assessed included tea yields and yield components: shoot analysis and

plucking analysis (fine plucking and coarse plucking). Supportive environmental and biotic parameters such as rainfall, temperature, relative humidity, soil moisture, and tea looper caterpillar incidence were also monitored. Seasonal differences in this research were evaluated based on the rainfall data recorded at the research site, which were classified according to the Oldeman climate classification, where a wet month is defined as having rainfall exceeding 200 mm, and a dry month as having less than 100 mm (Maru et al., 2016). Statistical analyses included the Shapiro-Wilk test to assess normality and Levene's test for homogeneity of variance. Differences between treatments (dry and wet seasons) were evaluated using independent samples t-tests at the 5% significance level. Additionally, Pearson correlation analysis was employed to determine relationships between tea plant production, yield components, and environmental factors.

Results and Discussion

Environmental factors. Environmental factors showed distinct seasonal patterns significantly affecting tea growth, productivity, and pest dynamics. The temperature was higher during the dry season (23.08 °C) than during the wet season (22.19 °C), which was significant at the 5% level. Elevated temperatures during the dry season can influence physiological processes in tea plants, including photosynthesis, evapotranspiration, and leaf development. Previous studies have reported that temperatures between 23 °C and 26 °C optimize metabolic activity and new shoot growth, whereas temperatures exceeding 28 °C may reduce yield and leaf quality (Benti et al., 2023). Li et al. (2025) stated that high temperatures can accelerate biochemical pathways, causing drought stress in plants, which lowers chlorophyll content and antioxidant capacity if soil moisture is insufficient. Moreover, drought stress driven by temperature can reduce catechin synthesis, a critical component of tea quality, particularly in subtropical regions (Omer et al., 2025). These findings highlight the need for adaptive management strategies to mitigate heat stress during hotter dry seasons to optimize tea yields (Yan et al., 2021).

Table 1. Environmental conditions between the two seasons in tea plantation

Seasons	Temperature (°C)	Relative humidity (%)	Soil moisture (%)	Pest (<i>Hyposidra talaca</i>) incidence (%)
Dry seasons	23.08 ± 0.11	83.08 ± 2.88	29.37 ± 7.59	9.27 ± 1.04
Wet seasons	22.19 ± 0.43	89.93 ± 1.01	37.46 ± 0.75	8.73 ± 1.54
T-test	*	*	ns	ns
C.V. (%)	3.32	2.26	7.67	16.36

Note: Values are expressed as mean ± standard deviation (SD). ns indicates non-significant differences ($p > 0.05$) according to the independent t-test. C.V. = coefficient of variation.

Relative humidity was significantly higher in the wet season (89.93%) compared to the dry season (83.08%), reflecting increased atmospheric moisture associated with rainfall. Elevated humidity improves stomatal conductance and reduces transpiration stress, supporting leaf turgor and promoting shoot growth. High atmospheric humidity creates a microclimate conducive to optimal physiological function, enhancing photosynthetic efficiency (Urban et al., 2017). However, increased humidity also influences pest and disease dynamics, as fungal pathogens and some insect pests often proliferate under wet conditions (Tapia et al., 2025). Thus, seasonal relative humidity fluctuations affect tea growth and require integrated pest and disease management strategies to maintain plant health and productivity.

In contrast, soil moisture showed higher average values in the wet season (37.46) compared to the dry season (29.37), but the difference was not statistically significant. This lack of significance may result from variability in soil properties such as texture, drainage, or agronomic practices like irrigation, mulching, or soil management, which moderate water availability (Hou et al., 2025). Stable soil moisture even during drier periods can sustain root water uptake, maintaining physiological functions and supporting consistent production (Li et al., 2023). Long-term organic and integrated soil management practices have improved water retention, promoting sustainable tea yields across fluctuating moisture conditions (Topa et al., 2025).

From a biotic perspective, the tea looper caterpillar (*Hyposidra talaca*) showed relatively stable population levels across seasons (9.27 in the dry season vs. 8.73 in the wet season), with no significant difference. Although the analysis did not show significant results in the percentage of *H. talaca* attack in two type difference seasons, this pest is an important threat to tea plants. Attacks caused by *H. talaca* can result in leaf perforation and the loss of young shoots on tea plants, which may lead to high yield losses

(Kusumah et al., 2023). Larval development and population dynamics of this pest are influenced by temperature and humidity, where warmer temperatures accelerate larval development and increase the potential number of annual generations, and adequate moisture enhances growth while lowering mortality (Roy et al., 2017). Although seasonal differences in temperature and humidity were observed, the relatively stable population suggests that other factors, including microclimate variability and management practices, may moderate pest abundance. Nonetheless, the potential for outbreaks remains higher during warm, humid periods, emphasizing the importance of continuous environmental monitoring and timely pest management interventions.

Overall, the findings highlight that temperature and atmospheric humidity are the primary environmental factors strongly affected by seasonal shifts, whereas local conditions and management practices moderate soil moisture and pest population levels. Understanding these interactions is essential for optimizing tea production, as it identifies periods when plants are exposed to environmental stress and when pest pressures may intensify. The interplay between abiotic factors (temperature, humidity, soil moisture) and biotic factors (tea looper caterpillar) underscores the complexity of seasonal effects in tea agroecosystems and the need for integrated management strategies to sustain productivity and crop quality.

Tea yields and yield components. The comparative analysis of tea yields between the dry and wet seasons revealed a significant increase in production during the wet season. Tea yields rose from 281.23kg per ha in the dry season to 635.33kg per ha in the wet season, and the difference was statistically significant at the 5% level. This increase reflects the positive role of rainfall and moisture in supporting growth and development. The observed significant increase

in tea production during the wet season aligns with agronomic principles, where optimal moisture conditions during the growth phase stimulate leaf expansion and bud development, which are critical for plucking quantity (Chen et al., 2025a). The enhanced water availability during the wet season likely improved leaf hydration, turgor, and photosynthetic efficiency, promoting shoot elongation and overall biomass accumulation (Ndagijimana et al., 2024).

Similar findings have been reported in Rwanda, where seasonal rainfall correlates strongly with tea production, and adequate soil moisture was critical in supporting leaf expansion and yield (Dushimimana & Nahayo, 2022). Moreover, climatic stability, including moderate temperature and consistent precipitation, amplifies seasonal yield differences by enhancing physiological processes essential for tea productivity (Ndagijimana et al., 2024). Therefore, the observed significant increase in tea yields during the wet season highlights the crucial role of seasonal rainfall and water availability in maximizing tea production, while also illustrating the importance of local microclimate and agronomic practices in modulating these effects.

Shoot analysis exhibited a significant seasonal effect, increasing from 38.66 in the dry season to 41.67 in the wet season, with the difference at 5%. Water availability, temperature, and relative humidity strongly influence shoot growth in tea plants. Higher moisture and moderate temperatures during the wet season promote bud break, leaf emergence, and shoot elongation, leading to greater biomass accumulation (Jayasinghe et al., 2018; Wulansari et al., 2022). Increased water availability enhances dry matter partitioning and new leaf production, while elevated CO₂ and stable climatic conditions further boost photosynthesis and shoot proliferation in tropical environments (Benti et al., 2023; Zhang et al., 2020). Recent studies

confirm that rainfall and temperature interplay significantly regulate shoot growth and related metabolites, underscoring the importance of favorable seasonal conditions for tea development (Tukhvatshin et al., 2025).

Plucking analysis revealed that fine leaf proportion increased slightly from 75.66% in the dry season to 81.33% in the wet season. In contrast, coarse plucking decreased from 62.16% to 59.33%, but neither change was statistically significant. This indicates that seasonal variation affects shoot quantity rather than leaf class distribution. Leaf quality stability is supported by intrinsic plant regulation and agronomic practices such as consistent plucking frequency (Jayasinghe et al., 2018; Benti et al., 2023). Seasonal studies further show that chemical quality parameters, including catechins, methylxanthines, and flavor-related metabolites, remain relatively stable across harvests despite environmental fluctuations, ensuring consistent functional quality of fine plucking leaves used for high-grade tea (Tang et al., 2020; Ye et al., 2022; Chen et al., 2025b).

Overall, the findings demonstrate that tea yields and shoot growth respond strongly to seasonal climatic variation, with wet-season conditions significantly enhancing production through improved rainfall and moisture availability. In contrast, leaf plucking quality remains relatively stable across seasons, reflecting intrinsic plant regulation and the buffering role of consistent agronomic practices. These results highlight that rainfall-driven variability is the key determinant of yield performance, while leaf quality is maintained through physiological resilience and management strategies. Therefore, adaptive practices such as soil water conservation, irrigation management, and integrated pest control are essential to sustain productivity and ensure tea quality under changing climatic conditions.

Table 2. Yields and yield components of tea between two seasons at the tea plantation

Seasons	Tea yields (kg ha ⁻¹ per plucking round)	Shoot Analysis (%)	Plucking Analysis (%)	
			Fine Plucking	Coarse Plucking
Dry seasons	281.23 ± 170.13	38.66 ± 2.51	75.66 ± 8.73	62.16 ± 4.46
Wet seasons	635.33 ± 85.45	41.67 ± 0.57	81.33 ± 3.05	59.33 ± 1.52
T-test	*	*	ns	ns
C.V. (%)	3.29	3.78	3.27	2.11

Note: Values are expressed as mean ± standard deviation (SD). ns indicates non-significant differences (p > 0.05) according to the independent t-test. C.V. = coefficient of variation.

Correlation between tea yields, yield components (kg ha⁻¹ per plucking round), pest incidence (%), and environmental conditions such as rainfall, temperature, and humidity.

Pearson correlation analysis evaluated the relationships between environmental factors (air temperature, relative humidity, soil moisture, rainfall, and tea looper infestation) and tea production parameters, including overall yield, shoot quality, and leaf plucking characteristics. Correlation coefficients (r) ranged from -1 to +1, with values close to +1 indicating strong positive relationships, values close to -1 indicating strong negative relationships, and values near 0 indicating weak or no association. Statistical significance was denoted by an asterisk (*).

Tea yields exhibited a strong negative correlation with air temperature ($r = -0.84^*$), indicating that higher temperatures adversely affect production by disrupting physiological and biochemical processes essential for growth (Omer et al., 2025). Conversely, relative humidity ($r = 0.97^*$) and rainfall ($r = 0.95^*$) showed strong positive correlations with production, suggesting that higher moisture availability supports shoot growth, leaf hydration, and metabolic functions, thereby enhancing yield (Omer et al., 2025; Benti et al., 2023). Soil moisture ($r = 0.52$) and tea looper infestation ($r = -0.58$) showed moderate, non-significant correlations, indicating that their impact on yield is more variable and likely moderated by local management practices and microclimatic conditions (Dushimimana & Nahayo, 2022; Siahaan et al., 2024). Overall, these results confirm that tea production is highly sensitive to temperature extremes and moisture availability, consistent with previous studies highlighting the critical role of stable environmental conditions in maintaining physiological processes necessary for optimal yield.

Shoot proliferation and quality were similarly influenced by environmental factors. Air temperature was negatively correlated with shoot growth ($r = -0.66^*$), reflecting the inhibitory effect of elevated temperatures on bud break and shoot elongation (Jayasinghe et al., 2018). In contrast, relative humidity ($r = 0.78^*$), soil moisture ($r = 0.94^*$), and rainfall ($r = 0.71^*$) exhibited strong positive correlations with shoot development, supporting active cell division and elongation in tea shoots under favorable moisture conditions (Benti et al.,

2023). Tea looper infestation showed a moderate negative correlation with shoot proliferation ($r = -0.52$), although this was not statistically significant, suggesting that while insect herbivory can reduce shoot vigor, environmental conditions primarily govern shoot growth (Jayasinghe et al., 2018; Benti et al., 2023). These findings emphasize that shoot development is particularly responsive to water availability and climatic stability, underscoring the importance of integrated environmental and pest management strategies to sustain high-quality shoot production.

The fine and coarse leaf plucking analysis revealed weak and non-significant correlations with all environmental factors, indicating that leaf quality remains relatively stable across seasonal and climatic variations. Fine leaf proportion increased slightly from the dry to wet season, while coarse leaf proportion decreased slightly, but neither change was statistically significant. This stability is attributed to intrinsic physiological regulation within the plant and consistent farm management practices, such as selective plucking and maintaining plucking frequency (Deka et al., 2021; Siahaan et al., 2024). Leaf quality parameters, including catechin and methylxanthine concentrations, are largely unaffected by moderate fluctuations in environmental conditions, suggesting that both biological and agronomic factors buffer plucking outcomes.

The correlation analysis demonstrates that tea yields and shoot quality are strongly influenced by environmental variables, with temperature exerting a negative effect and relative humidity, soil moisture, and rainfall exerting positive effects. Fine and coarse leaf plucking remain largely unaffected by these factors, reflecting intrinsic plant stability and the role of consistent management practices. These findings are consistent with the broader literature, highlighting the importance of moderate temperatures, sufficient moisture, and rainfall for optimal tea growth and production, while leaf quality remains resilient to seasonal variability (Omer et al., 2025; Jayasinghe et al., 2018; Benti et al., 2023; Deka et al., 2021; Siahaan et al., 2024). Understanding these correlations provides critical insights for designing adaptive agronomic strategies to optimize yield, shoot quality, and leaf characteristics in the face of climatic variability.

Table 3. Correlation between tea yields, yield components, and pest incidence with environmental conditions such as rainfall, temperature, and humidity.

	Rainfall	Temperature	Relative Humidity	Soil Moisture	Pest (<i>Hyposidra talaca</i>) incidence
Tea yields	0.95 *	-0.84 *	0.97 *	0.52	-0.58
Shoot analysis	0.71 *	-0.66*	0.78 *	0.94*	-0.52
Fine plucking	0.51	-0.4	0.52	0.88	0.45
Coarse plucking	-0.51	0.4	-0.52	-0.88	-0.45

Conclusion

This study demonstrates that seasonal variation plays a critical role in the productivity of seed-derived tea plants, with yields and shoot proliferation generally higher during the wet season than the dry season. The wet season provided more favorable conditions, particularly through increased rainfall and relative humidity, significantly enhancing shoot growth and contributing to better yield components. Shoot growth as a primarily yield component in tea plants increases significantly during the wet seasons, indicating the importance of water availability in supporting active vegetative growth. In contrast, the quality of leaf plucking (fine and coarse plucking) were not show significant differences between two type seasons, indicating that plucking quality is more influenced by plant physiology and the method of plucking. For practical applications, tea farmers are encouraged to optimize harvesting during the wet season to take advantage of improved shoot growth, while implementing adaptive measures such as soil moisture conservation and shading during the dry season to mitigate heat stress. Future research should extend to long-term seasonal monitoring and consider additional environmental factors, including solar radiation and soil nutrient dynamics, to develop more comprehensive strategies for sustaining tea production under increasingly variable climatic conditions.

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References

- Abdullah ATM, Sayka MI, Rahman MM, Sharif M, Khan TA, Jahan S, Mazumdar MM, Hoque MM. 2024. Tea (*Camellia sinensis*) cultivated in three agro-ecological regions of Bangladesh: Unveiling the variability of methylxanthine, bioactive phenolic compound, and antioxidant activity. *Heliyon*, 10
- Benti T, Debela A, Bekele Y, Suleman S. 2023. Effect of seasonal variation on yield and leaf quality of tea clone (*Camellia sinensis* (L.) O. Kuntze) in South West Ethiopia. *Heliyon*, 9
- Boehm R, Cash SB, Anderson BT, Ahmed S, Griffin TS, Robbat Jr A, Stepp JR, Han W, Hazel M, Orians CM. 2016. Association between empirically estimated monsoon dynamics and other weather factors and historical tea yields in China: results from a yield response model. *Climate*, 8;4(2):20
- Cahyaningsih S, Wulandari SA, Kemala N. 2025. Analisis Daya Saing Ekspor Teh Indonesia di Pasar Internasional. *Jurnal Media Agribisnis*
- Chen Z, Sui Y, Wisniewski M. 2025a. Current and future perspectives on tea production. *Industrial Crops & Products*, 235
- Chen L, Zhang S, Feng Y, Jiang Y, Yuan H, Shan X, Zhang Q, Niu L, Wang S, Zhou Q, Li J. 2025b. Seasonal variation in non-volatile flavor substances of fresh tea leaves (*Camellia sinensis*) by integrated lipidomics and metabolomics using UHPLC-Q-Exactive mass spectrometry. *Food Chemistry*, 462:140986 (b)
- Deka H, Sarmah PP, Devi A, Tamuly P, Karak T. 2021. Changes in major catechins, caffeine, and antioxidant activity during CTC processing of black tea from North East India. *RSC advances*, 11(19), 11457-11467
- Ding ZJ, Shi YZ, Li GX, Harberd NP, Zheng SJ. 2021. Tease out the future: How tea research

- might enable crop breeding for acid soil tolerance. *Plant Communications*, 2(3)
- Dushimimana FC & Nahayo L. 2022. Impact of climate variability on tea production in Rwanda. *Global Scientific Journal*, 10: 1286-1297
- Hasan R, Islam AFMS, Maleque, MA, Islam MS, Rahman MM. 2023. Effect of drought stress on leaf productivity and liquor quality of tea: A Review. *Asian J. Soil Sci. Plant Nutr*, 9, 1-10
- Hou X, Hu W, Li Q, Fan J, Zhang F. 2025. Evaluating of four irrigation depths on soil moisture and temperature, and seed cotton yield under film-mulched drip irrigation in Northwest China. *Agronomy*, 15(7), 1674
- Jayasinghe HASL, Suriyagoda LDB, Karunarathne AS, Wijeratna MA. 2018. Modelling shoot growth and yield of Ceylon cultivar TRI-2025 (*Camellia sinensis* (L.) O. Kuntze). *The Journal of Agricultural Science* 156, 200-214
- Kusumah RYM, Kurniawati F, Kristanto ED, Parasian F, Christian M. 2023. Analisis filogenik *Hyposidra talaca nucleopolyherovirus* (HytaNPV) yang diisolasi dari perkebunan teh Gunung Mas, Bogor, Jawa Barat dan virulensinya terhadap *Hyposidra talaca Walker*. *Jurnal Entomologi Indonesia*, 20(2).
- Langát J K. 2018. Effect of total solar radiation and rainfall on yield of different tea (*Camellia sinensis* (L.) O. Kuntze) clones at two sites in Kenya. *Journal of Agricultural Science*, 10(6)
- Langát J. 2022. Response of Tea (*Camellia sinensis*) to rainfall and temperature patterns in Kenya. *Ajausud African Journal of Agriculture and Utilisation of Natural Resources for Sustainable Development*, 1(1)
- Li G, Long H, Zhang R, Drohan PJ, Xu A, Niu L. 2023. Stable soil moisture alleviates water stress and improves morphogenesis of tomato seedlings. *Horticulturae*, 9(3), 391
- Li C, Zhang L, Wang X. 2025. Field performance of heat-tolerant traits in tea and cultivation factors affecting summer leaf functional stability. *Plant Gene and Trait*, 16
- Maru R, Leo M, Rahim S, Basram NF. 2016. Oldeman climate zoning for the agricultural area. *Proceedings of International Conference on Mathematics, Science, Technology, Education, and their Applications*, 26-27
- Ndagijimana J, Hafashimana A. 2024. Tea production response to seasonal rainfall variability: Evidence from Rwanda. *Agricultural Sciences*, 15: 909-938
- Nyabundi KW, Owuor PO, Netondo GW, Bore JK. 2016. Genotype and environment interactions of yields and yield components of tea (*Camellia sinensis*) cultivars in Kenya. *American Journal of Plant Science*, 7: 855-869
- Ochieng J, Kirimi L, Mathenge, M. 2016. Effects of climate variability and change on agricultural production: The case of small scale farmers in Kenya. *NJAS-Wageningen journal of life sciences*, 77: 71-78
- Omer, AAA, Zhang CH, Liu J, Shan ZG. 2025. Comprehensive review of mapping climate change impacts on tea cultivation: bibliometric and content analysis of trends, influences, adaptation strategies, and future directions. *Frontiers in Plant Science*, 15
- Roy S, Das S, Handique G, Mukhopadhyay A, Muraleedharan N. 2017. Ecology and management of the black inch worm, *Hyposidra talaca Walker* (Geometridae: Lepidoptera) infesting *Camellia sinensis* (Theaceae): A review. *Journal of integrative agriculture*, 16(10): 2115-2127
- Siahaan AJ. 2024. the influence of climate elements to tea plant productivity (*Camellia Sinensis* L.) at Bah Butong Plantation PTPN IV 2005-2009. *Jurnal Agroteknologi*, 12(2): 1-7
- Sita K, Rohdiana D. 2021. Analisis kinerja dan prospek komoditas teh. *Radar Opini Dan Analisis Perkebunan*, 2(1): 1-7
- Tang S, Liu Y, Zheng N, Li Y, Ma Q, Xiao H, Zhou X, Xu X, Jiang T, He P, Wu L. 2020. Temporal variation in nutrient requirements of tea (*Camellia sinensis*) in China based on QUEFTS analysis. *Scientific Reports*, 10(1): 1745.
- Tapia L, Castillo-Novales D, Riquelme N, Valencia AL, Larach A, Cautín R, Besoain X. 2025. Rainfall and high humidity influence the seasonal dynamics of spores of *Glomerellaceae* and *Botryosphaeriaceae* genera in avocado orchards and their fruit rot association. *Agronomy*, 15(6): 1453.
- Țopa DC, Căpșună S, Calistru AE, Ailincăi C. 2025. Sustainable practices for enhancing soil health and crop quality in modern agriculture: A review. *Agriculture*, 15(9)

- Tukhvatshin M, Peng Q, Zhao X, Liu J, Xiang P, Lin J. 2025. Identifying meteorological factors influencing catechin biosynthesis and optimizing cultivation conditions of tea plant (*Camellia sinensis*). *Frontiers in Plant Science*, 16:1532880
- Urban J, Ingwers M, McGuire MA, Teskey RO. 2017. Stomatal conductance increases with rising temperature. *Plant signaling & behavior*, 12(8)
- Wulansari R, Athallah FNF, Pramudita AA. 2022. Effect of slope and year of pruning of tea plants on soil water content in Indonesian tea plantations. *Soil Science Annual*, 73(4)
- Xia EH, Tong W, Wu Q, Wei S, Zhao J, Zhang ZZ, Wei CL, Wan XC. 2020. Tea plant genomics: achievements, challenges and perspectives. *Horticulture research*, 7
- Yan Y, Jeong S, Park CE, Mueller ND, Piao S, Park H, Joo J, Chen X, Wang X, Liu J, Zheng C. 2021. Effects of extreme temperature on China's tea production. *Environmental Research Letters*, 16(4)
- Ye F, Guo X, Li B, Chen H, Qiao X. 2022. Characterization of effects of different tea harvesting seasons on quality components, color and sensory quality of "Yinghong 9" and "Huangyu" large-leaf-variety black tea. *Molecules*, 27(24):8720
- Zhang Q, Li T, Wang Q, LeCompte J, Harkess RL, Bi G. 2020. Screening tea cultivars for novel climates: Plant growth and leaf quality of *Camellia sinensis* cultivars grown in Mississippi, United States. *Frontiers in Plant Science*, 11:280

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Effects of integrated biological and chemical fertilization on pecco and banji shoot development in tea plants

Abstract. Tea productivity and quality are largely determined by shoot structure, particularly the balance between pecco shoots (tender buds and young leaves) and banji shoots (older, less productive shoots). Conventional reliance on inorganic fertilizers often enhances overall growth but does not necessarily improve shoot structure, while biofertilizers alone provide limited nutrient availability. This study aimed to evaluate the effects of combining microbial-based liquid biofertilizers with inorganic fertilizers on the structure of tea shoots. The experiment was carried out on seed-derived tea plants using a randomized block design with several fertilization treatments, including sole biofertilizer, sole inorganic fertilizer, and their combinations. The parameters observed were the number and weight of pecco and banji shoots. The results revealed that the combined application of microbial-based biofertilizers and inorganic fertilizers significantly increased both the number and weight of pecco shoots compared with single applications. Conversely, the proportion of banji shoots decreased under integrated fertilization treatments, indicating an improvement in shoot structure toward more productive harvests. The synergistic effects of microbial activity and balanced nutrient supply enhanced nutrient uptake, regulated shoot differentiation, and promoted a higher ratio of productive shoots. In conclusion, the application of biological and chemical fertilizers affected the growth of pecco shoots and banji shoots of tea plants. Treatment 40 L/ha biological + 100% chemical fertilizer produced the highest weight and number of pecco, while treatment 30 L/ha biological + 75% chemical fertilizer produced equivalent results, allowing for a reduction in chemical fertilizer dosage by up to 25%.

Keywords : Banji shoots · Biofertilizer · Integrated fertilization · Pecco shoots · Tea plant

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Introduction

The quality of tea is determined by the composition of primary and secondary metabolites, including amino acids, flavonoids, theanine, and alkaloids such as caffeine, which collectively influence flavor, aroma, and functional properties (Wu et al, 2025). In tea production systems, harvestable young shoots consisting of the apical bud (pecco) and adjacent leaves play a central role in determining both yield and quality. Beyond total shoot biomass, the structural composition of tea shoots—particularly the proportion and weight of pecco and banji shoots—has a direct influence on harvesting efficiency and the consistency of raw material quality. Therefore, optimizing tea shoot structure represents a key objective in sustainable tea plantation management.

Nutrient management is one of the most critical agronomic practices for regulating tea shoot growth and development. In many tea plantations, nitrogen, phosphorus, and potassium required for continuous shoot flushing are primarily supplied through chemical fertilizers (Manzoor et al., 2024). Although chemical fertilizers can rapidly enhance shoot growth, excessive or prolonged use often leads to nutrient imbalance, soil degradation, and reduced nutrient use efficiency. Moreover, inappropriate fertilization practices may negatively affect metabolite composition, thereby compromising tea quality (Zhang et al., 2025).

Previous studies have demonstrated the positive effects of chemical fertilizers and biofertilizers on tea growth and yield. However, most existing research focuses on general growth performance or total yield, while limited attention has been given to how fertilization strategies influence tea shoot structural characteristics. In particular, the combined application of chemical fertilizers and microbial-based biofertilizers remains underexplored with respect to their role in shaping shoot structure, despite their importance for both productivity and quality. Based on available literature, there is a lack of integrated studies that explicitly evaluate how such combined fertilization strategies affect tea shoot structural composition, especially pecco development.

Microbial-based biofertilizers containing plant growth-promoting rhizobacteria (PGPR) offer a promising approach to improving fertilizer efficiency and sustainability in tea

plantations. PGPR enhances nutrient availability through mechanisms such as nitrogen fixation, nutrient solubilization, and phytohormone production, which may regulate shoot initiation and development (Yorlady et al., 2024). The novelty of this study lies in evaluating how the combination of biofertilizers with reduced chemical fertilizer doses affects tea shoot structure, rather than focusing solely on yield or nutrient uptake. The findings are expected to provide scientific evidence for optimizing fertilization strategies that support sustainable tea production while maintaining high-quality shoot characteristics.

Materials And Methods

The experiment was conducted from July to November 2024 at the Tea and Cinchona Research Center experimental Plantation in Gambung, Bandung Regency. The elevation ranged from $\pm 1,000$ m to 1,800 m above sea level, rainfall ranged from 2,000 to 3,000 mm/year, and the average air temperature was 14 to 25°C. The soil order was mostly Andisol.

The materials used in this experiment included seed-derived tea plants aged 75–85 years and Fertomax PPKS liquid biofertilizer. Based on laboratory analysis, the biofertilizer contained a consortium of functional microorganisms with populations $\geq 10^7$ cfu mL⁻¹, including nitrogen-fixing bacteria (*Azospirillum* sp. and *Azotobacter* sp.), phosphate-solubilizing bacteria (*Bacillus* sp. and *Pseudomonas* sp.), and indole-3-acetic acid (IAA)-producing bacteria. In addition, the biofertilizer contained plant growth hormones, namely auxin (≥ 65 ppm), gibberellin (≥ 82 ppm), and cytokinin (≥ 34 ppm). Chemically, it contained organic carbon ($\geq 0.17\%$), total nitrogen ($\geq 0.12\%$), P₂O₅ ($\geq 0.006\%$), K₂O ($\geq 0.022\%$), and micronutrients including Fe, Cu, Zn, and B.

Inorganic fertilization was conducted using single-nutrient fertilizers rather than compound NPK, consisting of urea (46% N), SP-36 (36% P₂O₅), KCl (60% K₂O), and kieserite (27% MgO). Application rates were calculated based on the standard fertilizer recommendation for mature tea plants grown on Andisol soils issued by the Indonesian Tea and Quinine Research Institute Gambung with annual rates of 558 kg N ha⁻¹, 75 kg P₂O₅ ha⁻¹, 214 kg K₂O ha⁻¹, and 119 kg MgO ha⁻¹. During the experimental period, 30%

of the annual recommendation was applied at three fertilization levels (50%, 75%, and 100%). For a 100 m² plot, the 100% rate corresponded to 1.67 kg urea, 0.23 kg SP-36, 0.64 kg KCl, and 0.36 kg kieserite, with proportional adjustments for the lower levels.

The equipment used in this experiment included a knapsack sprayer for applying liquid organic and inorganic fertilizers; measuring cups for preparing treatment solutions; pruning shears for harvesting shoots; plastic bags for sample collection and storage; an analytical balance for weighing shoot samples; and an oven for drying the samples.

The experiment employed a Randomized Block Design (RBD) comprising ten treatments with three replications, yielding a total of 30 experimental plots. Each plot measured 10 × 10 m, giving a total experimental area of 3,000 m². The treatment combinations of liquid biofertilizer and chemical fertilizer, ranging from the sole application of 100% recommended chemical fertilizer to reduced levels of chemical fertilizer supplemented with 20–40 L/ha biofertilizer. The treatments included: (A) 100% recommended chemical fertilizer (control), (B) 20 L/ha biofertilizer + 50% recommended chemical fertilizer, (C) 30 L/ha biofertilizer + 50% recommended chemical fertilizer, (D) 40 L/ha biofertilizer + 50% recommended chemical fertilizer, (E) 20 L/ha liquid biofertilizer + 75% recommended chemical fertilizer, (F) 30 L/ha biofertilizer + 75% recommended chemical fertilizer, (G) 40 L/ha biofertilizer + 75% recommended chemical fertilizer, (H) 20 L/ha biofertilizer + 100% recommended chemical fertilizer, (I) 30 L/ha biofertilizer + 100% recommended chemical fertilizer, and (J) 40 L/ha biofertilizer + 100% recommended chemical fertilizer. The research method used was a completely randomized design with 10 treatment combinations. Each treatment was repeated three times.

All data collected were subjected to variance analysis and followed by Duncan's multiple range test in SASMAgri software to test the differences between treatments. Chemical fertilizer is applied once after the initial harvest by scattering it on the tea rows, while liquid biofertilizer is applied twice at two-month intervals, namely after the initial harvest and

after the second harvest. Plucking is carried out every 30 days using plucking shears with a medium plucking (p+2m, p+3m, b+2, b+3) (Santoso et al., 2020). A total of 100 g of shoot samples from each plot were collected for the analysis of pecco and dormant shoot numbers, with sampling conducted routinely after each production plucking at a one-month plucking cycle. Plant maintenance is carried out by mechanical weed control and routine pest control.

Results and Discussions

Weight of pecco shoots. Measuring the fresh weight of pecco shoots is important because this parameter reflects the production capacity of tea buds, the physiological condition of the plant, nutrient absorption efficiency, and the plant's response to the cultivation practices applied. Pecco shoots play a crucial role because they are the youngest part of the plant and are rich in secondary metabolites, such as polyphenols, caffeine, and amino acids, which significantly impact the quality of the tea brew. The fresh weight of tea buds can be used as an indicator of the vegetative growth of tea shoots and is closely related to the potential yield and quality of the tea produced (Turkmen et al., 2009).

The analysis results show that the combination of chemical fertilizer and biofertilizer has a significant effect on the fresh weight of pecco shoots. Fresh weight of pecco shoots differed significantly among treatments (Table 1), indicating that shoot productivity responded to the interaction between biofertilizer rate and chemical fertilizer level. The highest fresh weight was observed in treatment J (40 L ha⁻¹ biofertilizer + 100% chemical fertilizer), confirming that sufficient nutrient supply combined with microbial activity can maximize shoot biomass. This condition suggests that adequate nutrient availability from chemical fertilizers, combined with the role of microbes in biofertilizers, can promote optimal shoot growth. Microbes effective in biological fertilizers are known to increase nutrient availability through nitrogen fixation, phosphate solubilization, and the production of growth regulators that stimulate shoot growth (Miao et al., 2024).

Table 1. Combined application of biofertilizers and chemical fertilizers on the weight of fresh pecco shoots

Treatment	Weight of fresh shoot (g)
A 100% recommended chemical fertilizer (control)	62 bc
B 20 L/ha biofertilizer + 50% recommended chemical fertilizer	93 ab
C 30 L/ha biofertilizer + 50% recommended chemical fertilizer	77 abc
D 40 L/ha biofertilizer + 50% recommended chemical fertilizer	51 c
E 20 L/ha biofertilizer + 75% recommended chemical fertilizer	84 ab
F 30 L/ha biofertilizer + 75% recommended chemical fertilizer	95 ab
G 40 L/ha biofertilizer + 75% recommended chemical fertilizer	48 c
H 20 L/ha biofertilizer + 100% recommended chemical fertilizer	90 ab
I 30 L/ha biofertilizer + 100% recommended chemical fertilizer	71 bc
J 40 L/ha biofertilizer + 100% recommended chemical fertilizer	112 a
CV	11.89%

Note: Means followed by different letters in the same column indicate significant differences based on Duncan's Multiple Range Test. CV – coefficient of variance.

Interestingly, treatments with lower doses of chemical fertilizer, namely B (20 L/ha biofertilizer + 50% chemical fertilizer), C (30 L/ha biofertilizer + 50% chemical fertilizer), E (20 L/ha biofertilizer + 75% chemical fertilizer), F (30 L/ha biofertilizer + 75% chemical fertilizer), and H (20 L/ha biofertilizer + 100% chemical fertilizer) treatments were not significantly different from treatment J. This shows that the combination of biofertilizer with a 25–50% reduction in chemical fertilizer is still capable of producing equivalent fresh shoot weight. The use of biofertilizers allows for a reduction in chemical fertilizer inputs without reducing productivity. Thus, the integration of biofertilizers is a potential strategy for improving fertilizer efficiency and reducing the negative environmental impacts of excessive chemical fertilizer use (Kumar et al., 2022).

Physiologically, the increase in fresh weight of pecco shoots is closely related to the supply of essential nutrients, especially nitrogen, phosphorus, and potassium. Nitrogen is needed for the formation of chlorophyll and proteins, phosphorus plays a role in energy metabolism and meristematic tissue growth, while potassium regulates osmotic balance and the translocation of photosynthates to shoot tissues (Shambhavi et al., 2016). Optimal nutrient availability will increase cell division and enlargement in young shoots, resulting in higher fresh weight of pecco (Ruan et al., 2010). The role of biofertilizer in increasing nutrient availability and stimulating physiological activity supports more vigorous shoot growth (Nepolean et al., 2012).

Thus, the use of biofertilizers in tea fertilization systems can be a sustainable

approach. In addition to increasing the fresh weight of pecco shoots, the integration of biofertilizers enables the efficient use of chemical fertilizers without compromising productivity. These implications are very important in the management of modern tea plantations, which are required to maintain a balance between productivity, cost efficiency, and environmental sustainability. According to Miao et al. (2024) The rational application of fertilizers in tea plantation improves the soil environment of tea plantations, enhances the quality of tea leaves, and achieves sustainable tea production.

Weight of banji shoots. In addition to pecco shoots, which are the main target in tea harvesting, tea plants also produce banji shoots, which are shoots that do not have young leaves because their apical shoots are dead or undeveloped. Banji shoots are unproductive because they cannot be harvested to produce young leaves, so their contribution to tea yield is very limited. The presence of banji shoots can even reduce the quality of the harvested buds, because buds that contain too many banji shoots will reduce the yield and quality of the tea brew. Measuring the weight of tea shoots is important for evaluating the effectiveness of cultivation practices. This parameter describes the extent to which a practice can reduce the formation of unproductive shoots and increase the proportion of economically valuable shoots. Thus, analyzing the weight of tea shoots can provide a more comprehensive picture of tea shoot growth, not only in terms of productivity but also in terms of yield quality.

The results of statistical analysis in Table 2 show that the application of various combinations of chemical and organic fertilizers

did not have a significant effect on the weight of banji shoots. This indicates that the formation of banji shoots is more influenced by plant physiological factors and environmental conditions, while fertilization factors also indirectly affect the growth of banji shoots.

Banji shoots formation is caused by the cessation of apical meristem activity, preventing the terminal bud from developing into a productive shoot. This phenomenon can be caused by internal factors such as apical dominance and growth hormone balance, especially cytokinin and auxin, as well as external factors such as light availability, temperature, and environmental stress (Chen et al., 2014). Thus, although fertilization can increase nutrient supply, it does not necessarily reduce the formation of banji shoots.

The presence of banji shoots in the composition of harvested shoots is undesirable because banji shoots are classified as unproductive shoots that cannot be harvested further. The dominance of banji shoots will reduce the quality of the shoots produced and have implications for a decline in the quality of the tea brew, because this part tends to produce coarse biomass with lower secondary metabolite content (Wang et al., 2022). Supporting this interpretation, Wulansari and Rezamela (2017) reported that a high proportion of dormant or bird shoots (40–30%) reflects plant stress arising from nutritional imbalance and unfavorable environmental conditions, which ultimately

induce shoot dormancy and banji shoot formation.

From a management perspective, the absence of significant differences in banji shoot weight among fertilization treatments suggests that improving tea shoot quality cannot rely on fertilization manipulation alone. Instead, effective suppression of dormant shoots requires integrated crop management practices, including appropriate pruning regimes, shade management, and proper plucking techniques that regulate shoot turnover and hormonal balance. The integration of these practices is therefore expected to play a more decisive role in reducing banji shoot dominance and increasing the proportion of productive pecco shoots.

Number of pecco shoots. The number of pecco shoots is an important parameter in determining the potential production of high-quality tea. Not only the fresh weight, but also the number of pecco that appear will directly affect the productivity of the plant because pecco are the main ingredient in plucking (Anjarsari et al., 2024). The higher the number of pecco formed, the greater the chance of increasing the yield and quality of tea buds that can be harvested. Variations in the number of pecco between treatments reflect the physiological response of plants to environmental conditions and cultivation treatments. Therefore, analyzing the number of pecco is an important step in understanding the dynamics of tea shoots' growth more comprehensively.

Table 2. Combined application of biofertilizers and chemical fertilizers on the weight of banji shoots

Treatment	Weight of banji shoot (g)
A 100% recommended chemical fertilizer (control)	249 a
B 20 L/ha biofertilizer + 50% recommended chemical fertilizer	250 a
C 30 L/ha biofertilizer + 50% recommended chemical fertilizer	242 a
D 40 L/ha biofertilizer + 50% recommended chemical fertilizer	247 a
E 20 L/ha biofertilizer + 75% recommended chemical fertilizer	245 a
F 30 L/ha biofertilizer + 75% recommended chemical fertilizer	248 a
G 40 L/ha biofertilizer + 75% recommended chemical fertilizer	265 a
H 20 L/ha biofertilizer + 100% recommended chemical fertilizer	268 a
I 30 L/ha biofertilizer + 100% recommended chemical fertilizer	270 a
J 40 L/ha biofertilizer + 100% recommended chemical fertilizer	238 a
CV	10.68%

Note: Means followed by different letters in the same column indicate significant differences based on Duncan's Multiple Range Test. CV – coefficient of variance.

Table 3. Combined application of biofertilizers and chemical fertilizers on the number of pecco shoots

Treatment	Number of Pecco Shoots
A 100% recommended chemical fertilizer (control)	80 b
B 20 L/ha biofertilizer + 50% recommended chemical fertilizer	108 ab
C 30 L/ha biofertilizer + 50% recommended chemical fertilizer	100 ab
D 40 L/ha biofertilizer + 50% recommended chemical fertilizer	95 b
E 20 L/ha biofertilizer + 75% recommended chemical fertilizer	105 ab
F 30 L/ha biofertilizer + 75% recommended chemical fertilizer	125 a
G 40 L/ha biofertilizer + 75% recommended chemical fertilizer	102 ab
H 20 L/ha biofertilizer + 100% recommended chemical fertilizer	100 ab
I 30 L/ha biofertilizer + 100% recommended chemical fertilizer	94 b
J 40 L/ha biofertilizer + 100% recommended chemical fertilizer	129 a
CV	14.91%

Note: Means followed by different letters in the same column indicate significant differences based on Duncan's Multiple Range Test. CV – coefficient of variance.

Statistical analysis shows that the combination of biofertilizer and chemical fertilizer affects the number of pecco shoots (Table 3). The number of pecco shoots was the most responsive parameter to fertilization treatments. Treatment J produced the highest number of pecco shoots, followed closely by Treatment F (30 L ha⁻¹ biofertilizer + 75% chemical fertilizer). Notably, Treatment F achieved pecco numbers statistically equivalent to the full chemical fertilizer treatment, demonstrating that a 25% reduction in chemical fertilizer input did not reduce productivity.

This result clearly indicates that Treatment F represents the best compromise between productivity and input efficiency. The enhanced pecco formation is likely associated with improved nutrient availability and phytohormone production by biofertilizer-associated microbes, which stimulate bud initiation and meristem activity. These findings strongly support the role of biofertilizers in improving fertilizer efficiency and sustaining high-quality tea production. This is in line with the concept of sustainable agriculture, which emphasizes input efficiency and reducing environmental impact (Le et al., 2021). A high number of pecco is an important indicator in tea production, as pecco are the main raw material for high-quality tea (Owuor & Obanda, 2007). Younger tea plant leaves contain higher concentrations of phenolic compounds than the stems and roots, contributing both significantly to flavor and pharmacological health benefits (Techane et al., 2025). Thus, these results show that the integration of biological and chemical fertilizers not only

maintains productivity but also has the potential to increase fertilization efficiency.

The increase in the number of shoots in the combination of biological fertilizer and chemical fertilizer treatments is thought to be related to the role of microbes in biological fertilizers, which can increase nutrient availability and stimulate shoot growth through the production of phytohormones such as auxin and cytokinin (Vejan et al., 2016). The presence of chemical fertilizers as a source of macro nutrients directly supports the formation of new tissue, so that the synergy between the two can accelerate the growth of productive shoots.

Number of banji shoots. In tea cultivation, in addition to pecco shoots which have high economic value, there are also banji shoots or inactive buds. Dormant buds generally do not develop into productive buds and are therefore considered to be less profitable vegetative parts. A high proportion of dormant buds in a bud population can reduce plucking efficiency and result in a decline in the quality of the harvested buds (Cheruiyot et al., 2007). Therefore, agronomic management that can suppress the formation of banji shoots is an important factor in efforts to improve tea quality and productivity.

Statistical analysis shows that the number of banji shoots significantly varies between treatments (Table 4). Treatment A produced more banji shoots than the other treatments, but was not significantly different from treatments C, D, G, H, and I. A high number of banji shoots is undesirable in tea production because these buds are inactive and do not develop into productive buds.

Table 4. Combined application of biofertilizers and chemical fertilizers on the number of banji shoots

Treatment	Number of Banji Shoots
A 100% recommended chemical fertilizer (control)	371 a
B 20 L/ha biofertilizer + 50% recommended chemical fertilizer	296 c
C 30 L/ha biofertilizer + 50% recommended chemical fertilizer	311 abc
D 40 L/ha biofertilizer + 50% recommended chemical fertilizer	333 abc
E 20 L/ha biofertilizer + 75% recommended chemical fertilizer	288 c
F 30 L/ha biofertilizer + 75% recommended chemical fertilizer	298 bc
G 40 L/ha biofertilizer + 75% recommended chemical fertilizer	361 ab
H 20 L/ha biofertilizer + 100% recommended chemical fertilizer	318 abc
I 30 L/ha biofertilizer + 100% recommended chemical fertilizer	317 abc
J 40 L/ha biofertilizer + 100% recommended chemical fertilizer	288 c
CV	10.54%

Note: Means followed by different letters in the same column indicate significant differences based on Duncan's Multiple Range Test. CV – coefficient of variance.

An increase in the number of banji shoots can reduce harvesting efficiency and affect the quality of the harvested shoots (Cheruiyot et al., 2009). The results show that the combination of certain biological and inorganic fertilizers, particularly in treatments B (20 L/ha biological fertilizer + 50% recommended inorganic fertilizer), E (20 L/ha biological fertilizer + 75% recommended inorganic fertilizer), and J (40 L/ha of organic fertilizer + 100% of the recommended inorganic fertilizer), were able to suppress the formation of bird beaks compared to treatment A without the optimal combination.

This phenomenon indicates that the application of organic fertilizer can play a role in improving the physiological balance of plants, so that photosynthetic energy is directed more towards the formation of productive shoots (pecco) rather than inactive shoots. Microbes in biofertilizers can increase nutrient availability and produce bioactive compounds that support cell division and differentiation (Yorlady et al., 2024). Thus, the right combination of fertilizers not only increases the number and weight of pecco buds but also reduces the proportion of banji shoots, resulting in optimal productivity and quality of tea buds. The sustainability implication (chemical fertilizer reduction) is well supported and could be emphasized more clearly as a key contribution.

Conclusion

The application of biological and chemical fertilizers significantly affected the growth of pecco shoots and banji shoots of tea plants.

Treatment J (40 L/ha biological + 100% chemical fertilizer) produced the highest weight and number of pecco shoots, while treatment F (30 L/ha biological + 75% chemical fertilizer) produced equivalent results, allowing for a reduction in chemical fertilizer dosage by up to 25%.

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References

- Anjarsari IRD, Wiharti NR, Maxiselly Y. 2024. Optimizing biofertilizer application and fertilizing technique for enhanced tea plant growth and flavonoid composition after stress. *Research on Crops*, 25: 724–730.
- Chen XH, Zhuang CG, He YF, Wang L, Han GQ, Chen C, He HQ. 2014. Photosynthesis, yield and chemical composition of Tieguanyin tea plants (*Camellia sinensis* (L.) O. Kuntze) in response to irrigation treatments. *Agricultural Water Management*, 97: 419–425.
- Cheruiyot EK, Mumera LM, Ng'etich WK, Hassanali A, Wachira F. 2007. Polyphenols as potential indicators for drought tolerance in tea (*Camellia sinensis* L.). *Bioscience*

- Biotechnology and Biochemistry, 71: 2190–2197.
- Cheruiyot EK, Mumera LM, Ng'etich WK, Hassanali A, Wachira FN. 2009. High fertilizer rates increase susceptibility of tea to water stress. *Journal of Plant Nutrition*, 33: 115–129.
- Kumar S, Diksha, Sindhu SS, Kumar R. 2022. Biofertilizers: An ecofriendly technology for nutrient recycling and environmental sustainability. *Current Research in Microbial Sciences*, 3: 100094.
- Le VS, Lesueur D, Herrmann L, Hudek L, Ngoc L, Lambert Q. 2021. Sustainable tea production through agroecological management practices in Vietnam: A review. *Environmental Sustainability*, 4: 589–604.
- Manzoor, Ma L, Ni K, Ruan J. 2024. Influence of organic and inorganic fertilizers on tea growth and quality and soil properties of tea orchards. *Plants*, 13: 1–22.
- Miao P, Pang X, Zhang M, Cheng W, Zhou Z, Li Y, Wang H, Jia X, Ye J, Zhang Q. 2024. Enhancing soil health and tea plant quality through integrated organic and chemical fertilization strategies. *Horticulturae*, 10: 1–14.
- Nepolean P, Jayanthi R, Pallavi RV, Balamurugan A, Kuberan T, Beulah T, Premkumar R. 2012. Role of biofertilizers in increasing tea productivity. *Asian Pacific Journal of Tropical Biomedicine*, 2: 1443–1445.
- Owuor PO, Obanda M. 2007. The use of green tea (*Camellia sinensis*) leaf flavan-3-ol composition in predicting plain black tea quality potential. *Food Chemistry*, 100: 873–884.
- Ruan J, Haerdter R, Gerendás J. 2010. Impact of nitrogen supply on carbon/nitrogen allocation: A case study on amino acids and catechins in green tea (*Camellia sinensis* (L.) O. Kuntze) plants. *Plant Biology*, 12: 724–734.
- Santoso J, Suprihatini R, Widayat W, Johan E, Rayati DJ, Dharmadi A. 2020. Petunjuk Kultur Teknis Tanaman Teh. Edisi Ketiga. Gambung. Pusat Penelitian Teh dan Kina.
- Shambhavi S, Kumar R, Sharma SP, Verma G, Sharma RP, Sharma SK. 2016. Long-term effect of inorganic fertilizers and amendments on productivity and root dynamics under maize-wheat intensive cropping in an acid Alfisol. *Journal of Applied and Natural Science*, 9: 2004–2012.
- Techane T, Muhammed A, Abdullahi A, Abamecha N, Hamza M. 2025. Contribution of nutritional and bioactive components of tea leaves in management of non-communicable chronic diseases: A comprehensive review. *Discover Food*, 5: 1–25.
- Turkmen N, Sari F, Velioglu S. 2009. Factors affecting polyphenol content and composition of fresh and processed tea leaves. *Akademik Gıda*, 7: 29–40.
- Vejan P, Abdullah R, Khadiran T, Ismail S. 2016. Role of plant growth-promoting rhizobacteria in agricultural sustainability: A review. *Molecules*, 21: 1–17.
- Wang C, Han J, Pu Y, Wang X. 2022. Tea (*Camellia sinensis*): A review of nutritional composition, potential applications, and omics. *Applied Sciences*, 12: 1–20.
- Wu T, He J, Deng X, Wang X, Yuan W, Wang Q, Chen X, Zou M, An H, Wang B, Che R. 2025. Influencing Factors and Regulatory Mechanisms of Fresh Tea Leaf Quality: A Review. *Foods*, 14:1–27.
- Wulansari R, Rezamela E. 2017. Pengaruh aplikasi pupuk daun mineral dan organik cair terhadap peningkatan pertumbuhan bibit teh siap salur. *Creative Research Journal*, 2: 135–146.
- Yorlady M, Zuluaga A, Fattorini R. 2024. Plant-microbe interactions in the rhizosphere for smarter and more sustainable crop fertilization: The case of PGPR-based biofertilizers. *Frontiers in Microbiology*, 15: 1–17.
- Zhang S, Zhang H, Zhang L, Li Z, Mao Y, Zhang L, Ge S, Hu Q, Chen C, Han W, Yan P, Li X. 2025. Effects of organic fertilizer substitution for chemical fertilizer on tea yield and quality: A meta-analysis focusing on alkali-hydrolyzable nitrogen dynamics. *Soil and Tillage Research*, 254.

Fariroh I · Harsanti RS · Nisa A · Restanto DP · Putra RA

Growth of porang (*Amorphopallus muelleri*) seedlings as influenced by seed maturity and the presence of sarcotesta

Abstract. Porang seed maturity based its position on spadix position significantly impacts germination. The presence of the seed coat (sarcotesta) also influenced germination and initial seedling growth. This study aimed to explore the effect of seed maturity and the presence of sarcotesta to the seedling growth. The experiment was conducted from January to June 2023. A two factor Randomized Complete Block Design was employed, examining three maturity levels based on spadix position (physiological maturity seed from the tip spadix (red colour), half physiological maturity seed from the middle spadix (orange colour), and immature seed from the base spadix (green colour)), and the presence of sarcotesta (seed with sarcotesta, and seed without sarcotesta). Every treatment replicated six times, each experimental unit consisted of five seeds, resulting in a total of 180 seeds used in the experiment. The observation parameters were seed germination capacity, seedling height, leaf canopy, seedling fresh weight, seedling dry weight, number of seedling roots, and tuber diameter. The result showed that physiologically mature seeds produce the highest germination capacity and seedling dry weight compared to others. No significant correlation was found between the level of seed maturity including the presence of sarcotesta on all observed parameters. Seeds obtained from the top of the spadix (red fruit) showed higher germination capacity compared to others. The removal of sarcotesta significantly enhanced all growth parameters, resulting in the highest seedling height, leaf canopy area, fresh and dry seedling weight, number of seedling roots, and tuber diameter in porang.

Keywords: Base spadix · Middle spadix · Morphophysiological dormancy · Tip spadix

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Introduction

Porang was found in tropical areas, from Africa expanded to Asia, including Indonesia (Turhadi & Indriyani, 2015). Porang plants are characterized by their elevated glucomannan content found in their tubers. Glucomannan is a hemicellulose-type polysaccharide that is water-soluble, operates as a hydrocolloid, and is low in calories. Additionally, it is gluten-free, which renders it highly suitable for application as both an industrial raw material and a food ingredient (Yanuriati et al., 2017). The higher glucomannan content in porang than other tubers make the exports of porang as food and industrial raw materials have increased in recent years (Hidayah et al., 2022)

Porang is commonly propagated through the use of small tubers, bulbils (referred to as frogs), and seeds. However, the propagation by small tubers or bulbils tends to be less efficient and may incur higher costs (Palupi et al., 2024). Among the three propagation materials, seeds are particularly advantageous because each plant generally produce approximately 300 seeds (Harijati & Widoretno, 2018). The limited availability of rare porang seeds poses a significant constraint on the propagation of porang through generative seeds, as it takes approximately four to five years for porang to produce seeds (Gusmalawati et al., 2023).

Numerous factors significantly influence seed quality, including seed genotype, habitat of origin, local variety, seed purity, morphology, dimensions, and maturity (Yan & Chen, 2020). The successful cultivation of porang relies heavily on the availability of seeds that exhibit the necessary physiological characteristics and are present in adequate quantities (Riptanti et al., 2023). One notable challenge in porang cultivation is the issue of immature seeds, as the maturity level of these seeds directly impacts their germination rates. The maturity of seeds influences the activity of the endophytic microbiome in *A. muelleri* seeds. During the mid to late stages of seed development, microbial functional genes that contribute to the production of resistant compounds like phenols, flavonoids, and alkaloids become significantly enriched, thereby boosting the resistance and environmental adaptability of *A. muelleri* seeds (Yang et al., 2022). Endophytic bacteria can enhance plant adaptability by improving nutrient mobilization, nitrogen fixation, and phosphate

solubilization, as well as by providing resistance against pathogens (Wang et al., 2021).

Furthermore, the presence of the seed coat is also an essential factor in seed development. Porang seeds are characterized by a thick testa, a thin tegmen, and a rafid sac (Dewi et al., 2015). The seed coat comprises an outer layer known as the testa and an inner layer referred to as the tegmen, both of which play crucial roles in supporting seed development. *Amorphophallus muelleri* tubers and seeds are rich in glucomannan, which is known for its high viscosity, solubility, and swelling properties, as well as its excellent film-forming and gel characteristics in water-based solutions. The substantial glucomannan content in the seeds prevents germination. Raising the germination temperatures can decrease the elasticity and strength of glucomannan, thereby facilitating germination (Herranz et al., 2013). According to Fadhilah et al. (2025), subjecting seeds to dry preheating at 35°C for four weeks can disrupt seed dormancy by causing the seed coat to fracture and tear, thus speeding up the germination of *A. muelleri* seeds. This research aims to investigate how seed maturity and the presence of the sarcotesta, along with their interaction, affect germination results.

Materials and Methods

This experiment was conducted from January to June 2023 at the Seed Technology Laboratory and Greenhouse of Agronomy Study Program, Faculty of Agriculture, University of Jember. The seed material used 3-4 months old porang seed with the differences in maturity and physical condition. This research used Randomized Complete Block Design with two factors. The first factor was seed maturity in three level based on Figure 1A, the second factor is the presence of sarcotesta (Figure 1B). Every treatment replicated six times, each experimental unit consisted of five seeds, resulting in a total of 180 seeds used in the experiment.

Seed selection. Porang seeds were harvested from healthy, well-developed plants that possessed seed-bearing spadixes. The seeds were obtained from various maturity stages, ranging from green to orange and red (Figure 2). Red seeds were collected from the spadix tip, indicating that the seeds had reached physiological maturity. Orange seeds were

collected from the middle of the spadix, representing the half-physiological maturity phase. Meanwhile green seeds were obtained from the basal of the spadix, signifying an immature stage. Subsequently, the collected seeds were peeled and selected based on health criteria, ensuring they were free from diseases like mold and decay.

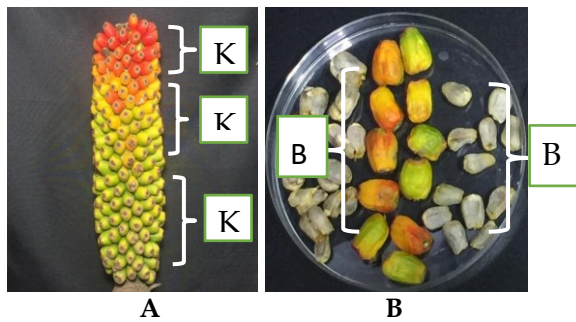


Figure 1. A) Maturity level of porang seeds; K1= Physiological maturity seed from the tip spadix (red); K2= Half physiological maturity seed from the middle spadix (orange); K3= Immature seed from the base spadix (green). B) The presence of sarcotesta; B1= Seed with sarcotesta; B2= Seed without Sarcotesta



Figure 2. Maturity stages from green, orange, and red (left to the right)

Seed cleaning. The selected porang seeds were subsequently washed and soaked in distilled water for 24 hours in an Erlenmeyer flask. Seed separation using common separation methods include to remove lightweight and foreign material to separate seeds by weight. Clearing sarcotesta was done for the treatment only.

Germinating Seed. The germination media consisted of a 1:1 mixture of soil and rice husk ash, which was previously sterilized using an autoclave. There are 6 treatments, every treatment replicated 6 times, so total of the treatment was 36 unit. Each experimental unit

consisted of 5 seeds, resulting in a total of 180 seeds used in the experiment. Seeds were then sown in germination trays (72 holes), measuring 4.5 cm x 4.5 cm x 4.2 cm, received one seed.

Seedling maintenance. The germination trays were sprayed daily with distilled water to maintain adequate moisture and prevent desiccation during the germination period. Throughout this process, non-viable seeds, those that were moldy and soft were eliminated. Seeds that were moldy but remained firm were rinsed with distilled water before being returned to the tray.

Observation Variable. Seed Germination (%). Germination data was carried out to determine the initial quality of the seeds before used. Germination percentage (GP) was assessed 35 days after planting (DAP) or 5 weeks after planting. Gusmalawati et al. (2023) reported that the porang seed dormancy lasted for 4-8 weeks (1-2 months). Since the International Seed Testing Association (ISTA) has not established specific criteria for normal porang seedlings, Hamdi et al. (2022) defined seed as a normal seedling if its plumule emergence reached 1 cm (Figure 3).



Figure 3. Normal seedling in porang

The germination rate was subsequently calculated using the following formula:

$$GP (\%) = \frac{\text{number of normal seedling}}{\text{number of seeds tested}} \times 100\%$$

Seedling height (cm). Seedling height was measured using a ruler, from the base of the stem to the tip of the coleoptile. This measurement was taken once at 50 DAP (Figure 4A), based on the experiment of Fadhillah et al. (2025) which observed the final count of germination at 57 DAP.

Leaf canopy (cm²). Leaf canopy diameter, which reflects the spread of the crown, was measured using a ruler. The diameter was assessed by measuring the widest horizontal spread of the leaves at 50 DAP (Figure 4B).

Seedling fresh weight (g). Seedling fresh weight was measured at 50 DAP immediately after harvest. This measurement, which reflects the plant's metabolic activity, was recorded using a digital scale (Figure 4C).

Seedling dry weight (g). Seedling dry weight, reflecting the accumulation of biomass and net CO₂ assimilation, was determined at 50 DAP. Samples were dried in an oven until a constant weight was achieved, and the final weight was the recorded using a digital scale.

Number of seedling roots. The number of seedling roots indicates the growth of the seedling, measured mechanically by counting the number of roots that have formed on the 50 DAP (Figure 4D).

Tuber diameter (cm). The diameter of the porang tuber was early harvested from the seedling using a ruler on the 50 DAP (Figure 4E).

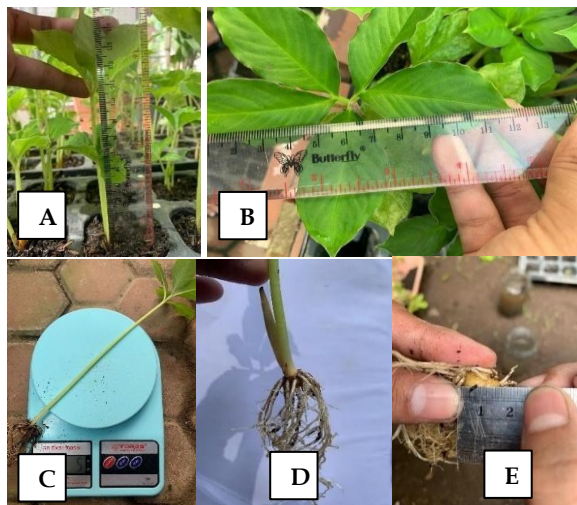


Figure 4. The measurement of A) plant height, B) leaf area, C) seedling fresh weight, D) root number, E) tuber diameter

Data Analysis. Data were analyzed using Analysis of Variance (ANOVA). When significant differences were detected, further comparisons among treatment means were performed using Duncan's Multiple Range Test (DMRT) at α 5% significance level ($\alpha = 0.05$).

Results and Discussion

The ANOVA analysis indicated no significant interaction between seed maturity and the presence of sarcotesta for any of the observed parameters, which included seedling height, leaf canopy, seedling fresh and dry weight, number of seedling roots and tuber diameter of porang. Only the single factors demonstrated a significant influence of these parameters.

Seed Germination (%). Seeds collected from the spadix tip (red) resulted in the highest total number of normal seedlings (17.5 seedlings) (Figure 5A) and germination of 70% (Figure 5B). This suggests that seeds at the spadix tip (red) reached physiological maturity earlier than those in the middle (orange) and base sections (green) of the spadix. Physiologically mature seeds tend to have a higher dry weight resulting from embryo ripening. However, this germination capacity is considered low because it is still below the standard for good seed quality, which is 80%. The existence of sarcotesta is thought to inhibit germination, resulting in an initial germination below 80%. These findings are consistent with the results of (Gusmalawati et al., 2023), which also reported that seeds harvested from the spadix tip at 4 weeks after planting exhibited a faster germination rate compared with orange and green spadix. This attributed to the sequential maturation of seeds along the porang spadix, where the seeds located at the tip attain physiological maturity earlier, thus resulting in faster germination. The viability of porang seeds originating from the spadix base was the lowest (31.0±19.92%) relative to seeds from the middle (81.7±32.5%) and tip of the porang spadix (77.7±25.5%).

Wardani et al. (2019) reported that seed germination was directly correlated with fruit maturity. Seeds derived from red fruit showed the highest germination rate, measuring 92±7.71%. This was followed by seeds from yellow fruit (64.5±5.86%) and lastly the seeds from the green fruit (37.77±8.2%) at 130 DAP. The lower germination observed in yellow and green seeds is due to they had not yet reached physiological maturity at the time of harvest. Consequently, these immature seeds resulted in lower germination uniformity and required longer time for first shoot emergence and 60% germination. This difference is attributed to the incomplete formation of food reserves stored

within the embryo of seeds harvested before full physiological maturity.

Yang et al. (2022) speculate that fully mature *Amorphopallus muelleri* seeds and their coats contained an abundance of plant growth promoting microorganisms, which may enhance resistance and facilitate the robust seedling establishment. There were strong correlation between seed maturity, indicated by fruit color and germination rate. Red seeds exhibited the highest germination to 100%, significantly outperforming yellow seeds (8.33%) and green seeds (0%). The superior performance of red seeds is linked to the microbial community within the seed coat. The relative abundance of genus *Streptomyces*, *Bradyrhizobium*, *Pseudomonas*, *Bacillus* and *Burkholderia* were markedly higher in the red seed coats. Since the seed coat acts as the primary protective layer, this microbial community enhanced the higher seed resistance against plant pathogens.

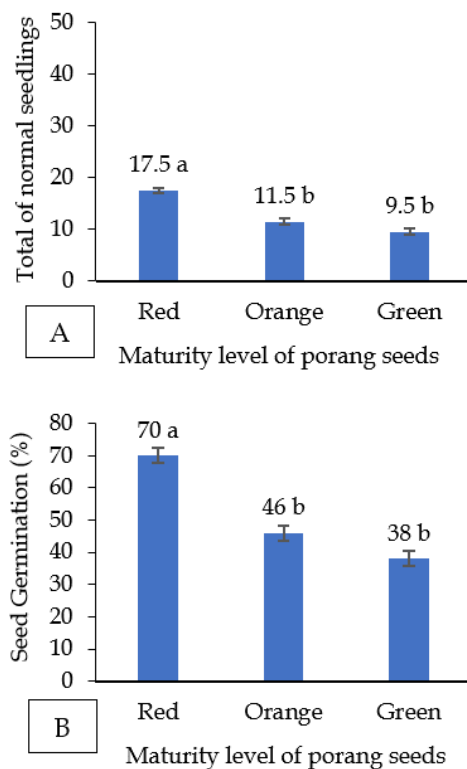


Figure 5. A) Total of normal seedlings and B) germination percentage of porang seeds in different maturity levels

Seedling Height (cm) and Leaf Canopy (cm²).

The presence of sarcotesta significantly inhibited early porang seedling growth. At 50 DAP, seedlings from seeds with the sarcotesta removed exhibited a

significantly greater height (10.48 cm) and leaf canopy (9.25 cm²) compared to intact seeds (9.00 and 6.93 cm², respectively) (Figure 6A, Figure 6B). This inhibition by the thick sarcotesta layer resulted in dwarfed seedling and reduced leaf size. This result aligned with the seedling height range (10.97 – 16.47 cm) reported by Gusmalawati et al. (2023) for extracted porang seeds. Meanwhile the seedling height observed for intact seeds at 50 DAP (9.00 cm) was also considerably higher than the 6.43 cm reported by Wardani et al. (2019) for intact seeds at 130 DAP. Hamdi et al. (2022) reported that porang seedling growth from seed without sarcotesta was considerably slower than that from bulbil. Seedlings from seed without sarcotesta reached only 4.8 cm at 50 DAP and 8.0 cm at 70 DAP.

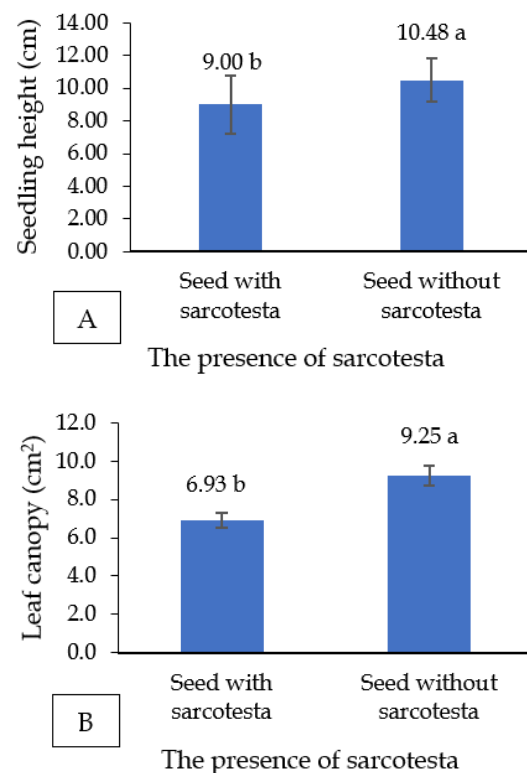
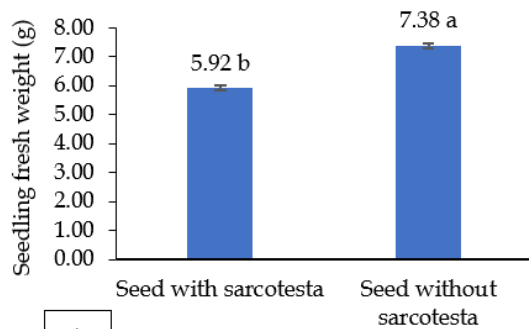


Figure 6. A) Seedling height and B) leaf canopy of porang seedlings in the presence of sarcotesta treatments

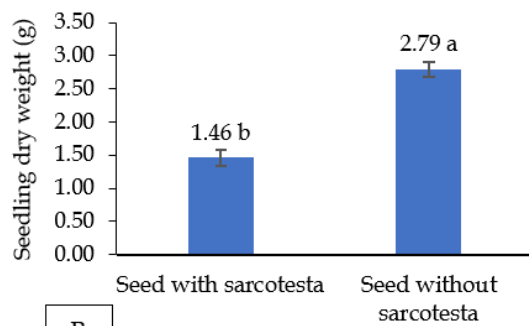
The sarcotesta acts as a physical barrier, impeding the uptake of water and oxygen essential for germination, consequently delaying the emergence of vegetative organs. Porang seed is hypothesized to exhibit morphophysiological dormancy, where its embryo requires a period post-harvest development, followed by a physical dormancy phase imposed by the thick sarcotesta. Indriyani & Widoretno (2016) further

explained that physiological dormancy in porang prevents the formation of new shoots, requiring tubers, bulbils, or seeds to pass a specific seasonal cycle before growth resumes. The duration of this seed dormancy was reported by Gusmalawati et al. (2023) to last for 4-8 weeks post-harvest. To overcome this, Fadhilah et al. (2025) successfully demonstrated that dry heat treatment (25 or 30 °C) applied for four weeks significantly reduced the time of physical dormancy, accelerating germination from 57 DAP to 37 DAP.

Seedling Fresh and Dry Weight (g). Porang seedlings derived from seed without sarcotesta exhibited significantly more fresh and heavier than seed with sarcotesta. The absence of the sarcotesta likely removed barrier to growth, which at 50 DAP resulted in a greater mean seedling fresh weight of 7.38 g (Figure 7A) and a higher mean seedling dry weight of 2.79 g (Figure 7B). Seedling weight is a key indicator of seed potential in developing biomass, where high-quality seed yields greater seedling dry weight. The increasing in seedling weight is inline with enhanced seedling height and leaf canopy, indicating that sarcotesta elimination promoted more vigorous and rapid growth.



A
The presence of sarcotesta



B
The presence of sarcotesta

Figure 7. A) Seedling fresh weight and B) seedling dry weight of porang in the presence of sarcotesta treatments

Many seed coats including sarcotesta contain chemical compounds, such as germination inhibitors, which prevent premature germination. The elimination of the sarcotesta effectively removes this inhibitor. This facilitates the optimal action of endogenous growth hormones like gibberellins, consequently accelerating the rate of seedling growth and resulting in significantly higher biomass at 50 DAP. Chenyin et al. (2023) reported that several common germination inhibitors such as phenols, flavonoids, aldehydes, aromatic oils, alkaloids, and amides are frequently detected in diverse plant organs, including the seed, fruit, leaf, stem, and root. Isnaini et al. (2025) detailed the presence of secondary metabolites specifically alkaloids, flavonoids, saponins, tannins, and steroids in porang tuber and leaves, though the study did not directly address the role of these compounds as germination inhibitors within the sarcotesta. As the porang seed matures, the sarcotesta undergoes a chromatic transition from green through orange to red. This shift strongly suggests the accumulation of anthocyanins, which are flavonoid-group pigments known to impart red, pink, purple, and blue. Zhao et al. (2025) reported that porang seeds also contained *p*-coumaric acid, a compound known to inhibit germination and classified as a germination destructor by Chenyin et al. (2023).

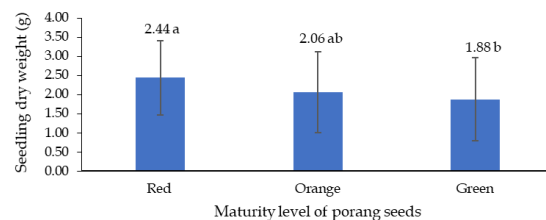


Figure 8. Seedling dry weight of porang seeds in different maturity levels

The seedling dry weight for seedlings derived from red and orange fruits was not significantly different, measuring 2.44 and 2.06 g, respectively (Figure 8). Seeds collected from the tip and middle portions of the spadix were confirmed to have already attained the physiological maturity phase at three to four months after planting. However, subsequent analysis showed that seeds harvested from red fruits generally resulted in a higher seedling dry weight also total number of normal seedlings and germination percentage. This observation suggests that if the harvest period is prolonged,

red seeds likely achieve a more advanced stage of maturity compared to seeds from orange fruits, thereby enhancing their overall vigor. Seed harvesting is not performed at the phase of physiological maturity (the agronomic yield harvest), but rather several weeks after, when viability and vigor had reached their optimum. During physiological maturity, the seed are still undergoing the seed-filling process, resulting in an increasing dry weight. In contrast, at harvest maturity, the grain-filling process had reached its maximum, the seed protection mechanism is fully developed, and the seed desiccation is complete. Sari et al. (2019) explained that seed harvesting should be performed gradually when the fruit turns red, specifically at 10-11 months after plating or 8-9 months after anthesis. Wardani et al. (2019) added that seed from red fruit planted in soil and cocopeat media resulted in the higher seedlings (8 cm), larger petiole diameter (8 cm), and wider canopy diameter (3 mm). This is likely to the sufficient availability of food reserves within the seed, which are utilized to accelerate shoot and root growth.

Number of Seedling Roots and Tuber diameter (cm). Seeds without the sarcotesta resulted in a higher number of seedling roots (13.79) (Figure 9A) and a greater tuber diameter (1.70 cm) (Figure 9B). The elimination of sarcotesta removes both of physical and chemical dormancy, which allows for faster absorption of water and oxygen also frees the seed from inhibiting agents. This optimal condition facilitates radicle protrusion and the efficient allocation of energy from food reserves to develop a stronger seedling structure, as evidenced by the higher number of roots formed at 50 DAP or 7 weeks after sowing. Consistent with these findings, Sari et al. (2019) reported that seeds with eliminated sarcotesta showed an increase in the number of seedling roots, from 3.0 to 12.0 roots, between 3 and 6 weeks after sowing. Furthermore, Hamdi et al. (2022) found that seedlings grown from seeds produced a root length of 12.08 cm, which was not significantly different from seedlings grown from bulbil (16.36 cm). This suggests that both seeds and bulbils possess a comparable total amount of food reserves capable of supporting vegetative growth, particularly root elongation and maturation. With sufficient energy derived from these food reserves, the roots are able to undergo maturation and form lateral roots, which ultimately maximizes the absorption of water and nutrients essential for overall plant health and tuber development.

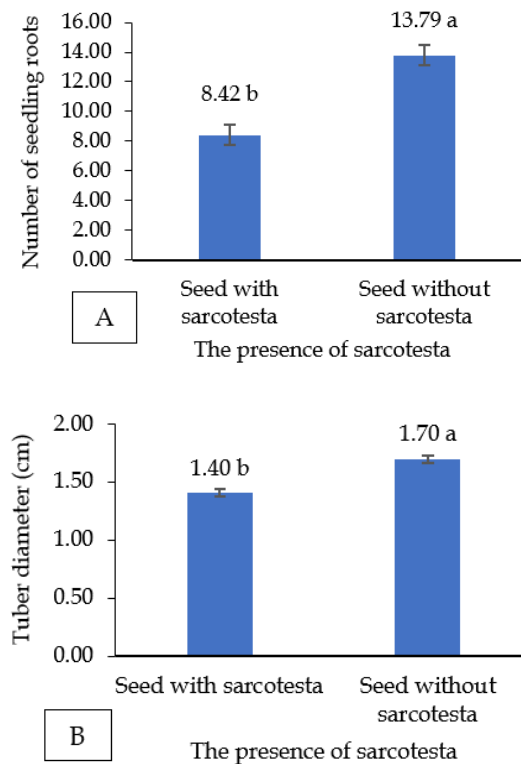


Figure 9. A) Number of seedling roots and B) tuber diameter of porang in the presence of sarcotesta treatments

The average tuber diameter harvested at 27 months after planting from spadixes developed from seeds without sarcotesta was 1.53 cm (Gusmalawati et al., 2023), whereas this study found a slightly higher average of 1.7 cm (Figure 9B) at 1.6 months after planting (50 DAP). Sarcotesta removed from seeds also yielded greater fresh and dry seedling weights, indicating higher seed vigor. This suggests an enhanced capacity for efficient and effective transport of food reserves to growth organs, such as the roots and leaves. Furthermore, the resulting larger leaf canopy area allows for greater light interception, leading to maximum carbohydrate accumulation in the porang tuber. Previous research reported that porang seeds exhibit physiological dormancy primarily due to the sarcotesta barrier (Harijati & Widoretno, 2018). Removing the seed coat or fruit flesh significantly increased the average germination percentage to over 90%. The removal of sarcotesta enhances seed permeability to water and oxygen, consequently accelerating seedling growth and promoting higher accumulation of food reserves in the developing tuber.

Conclusion

There is no correlation between the level of seed maturity and the presence of sarcotesta on all observed parameters. Seeds obtained from the top of the spadix (red fruit) showed higher germination capacity compared to others. The removal of sarcotesta eliminate the causes of dormancy and germination inhibitors, thereby facilitating optimal growth in seedling height (10.48 vs 9.00 cm), leaf canopy area (9.25 vs 6.93 cm²), fresh and dry seedling weight (7.38 and 2.79 g, respectively), number of seedling roots (13.79 vs 8.42), and tuber diameter (1.7 vs 1.4 cm). Future research on causes of seed dormancy and chemical compounds contained in sarcotesta is needed to identify growth inhibitors in porang.

References

- Chenyin P, Yu W, Fenghou S, Yongbao S. 2023. Review of the current research progress of seed germination inhibitors. *Horticulturae*, 9(4): 1-12. <https://doi.org/10.3390/horticulturae9040462>
- Dewi DFK, Azrianingsih R, Indriyani S. 2015. Struktur embrio porang (*Amorphophallus muelleri* Blume) dari berbagai variasi ukuran biji. *Jurnal Biotropika*, 3(3): 146-150.
- Fadhilah S, Widajati E, Ilyas S, Palupi ER, Qadir A. 2025. Protocol development for assessing seed moisture content and germination testing in *Amorphophallus muelleri* Blume. *Journal of Tropical Crop Science*, 12(1): 132-144. <https://doi.org/10.29244/jtcs.12.01.132-144>
- Gusmalawati D, Indriyani S, Azrianingsih R, Abidin Z. 2023. Seed viability and growth test of porang (*Amorphophallus muelleri* Blume) from several variation of spadix parts. *G-Tech: Jurnal Teknologi Terapan*, 7(4): 1315-1324. <https://doi.org/10.33379/gtech.v7i4.3042>
- Hamdi MFFA, Diyanti AR, Mutia YD. 2022. Germination study of three types porang seeds originated from Pacitan Regency. *Jurnal Folium*, 6(1): 23-36. <https://doi.org/10.33474/folium.v6i1.14275>
- Harijati N, Widoretno W. 2018. The effects of scarification on seed germination of porang (*Amorphophallus muelleri*). AIP Conference Proceedings, 2019(November 2017). <https://doi.org/10.1063/1.5061841>
- Herranz B, Tovar CA, de Zaldivar BS, Borderias AJ. 2013. Influence of alkali and temperature on glucomannan gels at high concentration. *LWT - Food Science and Technology*, 51: 500-506. <https://doi.org/10.1016/j.lwt.2012.11.023>
- Hidayah SN, Hidayat R, Triani N. 2022. Study of paclobutrazol dosage and seed size on growth and yield of porang (*Amorphophallus onchophyllus* P.). *Jurnal Teknik Pertanian Lampung (Journal of Agricultural Engineering)*, 11(4): 574. <https://doi.org/10.23960/jtep-l.v11i4.574-588>
- Indriyani S, Widoretno W. 2016. The effect of photoperiod to break dormancy of porang's (*Amorphophallus muelleri* Blume) tuber and growth. *Research Journal of Life Science*, 3(3): 166-171. <https://doi.org/10.21776/ub.rjls.2016.003.03.5>
- Isnaini H, Yuhana TM, Sholehah W, Jannah A. 2025. pharmacological benefits of porang (*Amorphophallus muelleri* Blume): A Review. *Biota: Jurnal Ilmiah Ilmu-Ilmu Hayati*, 10(2): 169-180. <https://doi.org/10.24002/biota.v10i2.10308>
- Palupi T, Darussalam, Aprizkiyandari S. 2024. Seed invigoration to improve viability, seed vigour and growth of porang seedlings. *IOP Conference Series: Earth and Environmental Science*, 1356(1). <https://doi.org/10.1088/1755-1315/1356/1/012027>
- Riptanti EW, Irianto H, Mujiyo. 2023. Challenges in the development of porang tuber processing industry with the fishbone diagram approach. *Universal Journal of Agricultural Research*, 11(5): 768-776. <https://doi.org/10.13189/ujar.2023.110503>
- Sari M, Santosa E, Lontoh AP, Kurniawati A. 2019. Seed quality and seedling growth of iles-iles (*Amorphophallus muelleri* Blume) from different growing media. *Jurnal Ilmu Pertanian Indonesia*, 24(2): 144-150. <https://doi.org/10.18343/jipi.24.2.144>
- Turhadi, Indriyani S. 2015. Uji daya tumbuh porang (*Amorphophallus muelleri* Blume) dari berbagai variasi potongan biji. *Jurnal Biotropika*, 3(1), 1-6.
- Wang H, Narsing Rao MP, Gao Y, Li X, Gao R, Xie

- Y, et al. 2021. Insights into the endophytic bacterial community comparison and their potential role in the dimorphic seeds of halophyte *Suaeda glauca*. *BMC Microbiol*, 21:143. <https://doi.org/10.1186/s12866-021-02206-1>
- Wardani IB, Harijati N, Mastuti R. 2019. the study of growth and its polyembryonic properties of porang seeds (*Amorphophallus muelleri* Blume) from various fruit colors in different planting media. *The Journal of Experimental Life Sciences*, 9(2): 122-127. <https://doi.org/10.21776/ub.jels.2019.009.02.10>
- Yan A, Chen Z. 2020. The control of seed dormancy and germination by temperature, light and nitrate. *The Botanical Review*, 86(1): 39-75. <https://doi.org/10.1007/s12229-020-09220-4>
- Yang M, Qi Y, Liu J, Wu Z, Gao P, Chen Z, Huang F, Yu L. 2022. Dynamic changes in the endophytic bacterial community during maturation of *Amorphophallus muelleri* seeds. *Frontiers in Microbiology*, 13(September): 1-12. <https://doi.org/10.3389/fmicb.2022.996854>
- Yanuriati A, Marseno DW, Rochmadi, Harmayani E. 2017. Characteristics of glucomannan isolated from fresh tuber of porang (*Amorphophallus muelleri* Blume). *Carbohydrate Polymers*, 156: 56-63. <https://doi.org/10.1016/j.carbpol.2016.08.080>
- Zhao Y, Yang M, Wang Q, Li L, Gao P, Qi Y, Guo JW, Wei H, Liu J, Zhao J, Huang F, Yu L. 2025. Multi-omics profiling of *Amorphophallus muelleri* seeds: Insights into germination and ABA regulation. *Scientia Horticulturae*, 348(April). <https://doi.org/10.1016/j.scienta.2025.114217>

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Fruit characteristics of F1 NIL *Sletr1-2* vary with parental background

Abstract. The *Sletr1-2* allele, which reduces ethylene sensitivity in tomatoes, offers a promising approach to extending fruit shelf life by slowing ripening-related metabolism. However, the trait's success depends on compatibility with the recipient cultivar's genetic background. This study evaluated fruit characteristics of F1 near-isogenic line (NIL) hybrids carrying *Sletr1-2* in the commercial backgrounds 'Costoluto', 'Oxheart', and 'Sart Roloise'. The field trial was carried out between May and August 2024 at the Experimental Station of Universitas Padjadjaran, Sumedang, Indonesia, following a randomized complete block design with eight biological replicates for each treatment. The results revealed that fruit characteristics of F1 NIL *Sletr1-2* hybrids depend on parental background: NIL *Sletr1-2* x 'Oxheart' produced small fruit size, thin pericarp, and poor visual quality, while NIL *Sletr1-2* x 'Costoluto' and NIL *Sletr1-2* x 'Sart Roloise' showed more commercial performance, with bigger fruit size. These findings highlight the critical role of parental background in optimizing *Sletr1-2*-linked traits and inform breeding strategies that balance postharvest resilience with commercial quality.

Keywords: Ethylene · Fruit · Postharvest · Tomato

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Introduction

The increasing frequency of extreme weather events associated with global climate change has posed significant challenges to agricultural productivity, particularly in horticultural crops such as tomatoes (*Solanum lycopersicum*) (Singh et al., 2022). As climacteric fruits with a high water content (~94% of their total weight), tomatoes are highly susceptible to postharvest deterioration due to their elevated respiration rates and accelerated ripening, especially under heat-stress conditions. (Alenazi et al., 2020; Mubarok, Okabe, et al., 2015). In response to these challenges, recent research has focused on breeding strategies to develop tomato cultivars with extended fruit shelf life.

Hybrid tomato breeding has shown considerable promise in improving both yield potential and fruit quality. However, a critical limitation remains in integrating desirable traits, particularly abiotic and biotic stress tolerance, along with postharvest longevity, without compromising agronomic performance. (Patanè et al., 2021). Advances in tomato genetics have identified the *SlETR1* gene, which encodes an ethylene receptor, as a key regulator of ethylene-mediated responses. (Saadah et al., 2023). A specific allele, *Sletr1-2*, has been associated with reduced ethylene sensitivity, delayed ripening, and extended shelf life while maintaining essential agronomic traits. (Mubarok et al., 2023).

The *Sletr1-2* mutation, located within the second transmembrane domain of the ethylene receptor, disrupts ethylene perception, resulting in firmer fruit and approximately 4–5 days of prolonged shelf life compared to wild-type lines (Saito et al., 2011). Despite its potential for postharvest improvement, mutant lines carrying *Sletr1-2* often exhibit agronomic drawbacks, including smaller fruit size and higher acidity, which limit their commercial appeal (Mubarok et al., 2016).

To overcome these limitations, the *Sletr1-2* allele can be introgressed into elite cultivars through the development of Near-Isogenic Lines (NILs). This approach employs repeated backcrossing between a donor parent carrying the target gene and a recipient parent with superior agronomic traits, resulting in NILs that retain the genetic background of the elite cultivar while incorporating the beneficial mutation (Di Giacomo et al., 2020). Hybrid F1 NILs carrying *Sletr1-2* are thus expected to combine stress

tolerance and shelf-life extension with desirable fruit quality attributes from the elite parental lines.

Nonetheless, the successful application of this breeding strategy depends on the genetic compatibility between *Sletr1-2* mutants and the commercial parental backgrounds. Incompatibilities can lead to undesirable physiological interactions, such as impaired heat tolerance or reduced fruit quality traits, namely size, flavour, and nutritional content. (Nguyen et al., 2019). These potential trade-offs underscore the necessity for a comprehensive evaluation of fruit quality parameters and secondary metabolite profiles in F1 NIL *Sletr1-2* hybrids derived from diverse genetic backgrounds. Therefore, the present study aims to evaluate and compare the fruit characteristics of F1 NIL *Sletr1-2* hybrids derived from different parental lines.

Materials and Methods

Plant Materials and Cultivation. Near-isogenic lines (NILs) carrying the *Sletr1-2* allele were crossed with three homozygous commercial tomato cultivars, 'Costoluto', 'Oxheart', and 'Sart Roloise', to produce F1 hybrid progenies. The field experiment was conducted from May to August 2024 at the Universitas Padjadjaran Experimental Station, Sumedang, Indonesia, using a randomized complete block design with eight biological replicates per treatment.

Seeds were germinated in seedling trays containing a 1:1 (v/v) mixture of cocopeat and rice husk charcoal. At the 5–6 true leaf stage, seedlings were transplanted into field plots and fertilized with NPK 15:15:15 at a total rate of 600 kg ha⁻¹, applied in three split doses: 50% at transplanting, 25% at three weeks after planting (WAP), and the remaining 25% at six WAP. All plants were maintained under uniform agronomic practices throughout the growing season.

Pericarp Thickness. To evaluate internal fruit morphology, three fruits per plant at the breaker plus six days (Br+6) stage were cut transversely, and the number of locules was recorded and averaged. Pericarp thickness was measured at the equatorial region of each fruit using a digital caliper and expressed in millimeters (mm). The fruit-set percentage was calculated as the ratio of the total number of fruits to the total number of flowers per plant and served as an indicator of reproductive success.

Titrateable Acidity (TA). Titrateable acidity was determined following the procedure by Mauro et al. (2020). Five g of the homogenized tomato sample were diluted with 50 mL of distilled water, and the solution was titrated with 0.1 N NaOH until the pH reached 8.1. TA (%) was calculated using the formula:

$$TA = \frac{mL\ NaOH \times N\ (NaOH) \times 0.0064 \times 100}{g\ sampel}$$

Total Soluble Solids (TSS). TSS content was measured using a handheld refractometer (Euromex, Arnhem, Netherlands). A 5 g fruit sample was blended, centrifuged at 1,000 rpm, and 1000 µL of the supernatant was placed onto the refractometer lens. Measurements were performed in triplicate for each sample.

Statistical Analysis. All quantitative data were subjected to one-way analysis of variance (ANOVA), and treatment means were compared using Duncan's Multiple Range Test (DMRT) at a 5% significance level. Statistical analyses were conducted using SPSS software version 25.0 (IBM Corp., Armonk, NY, USA).

Results and Discussion

Number of Fruits per Truss and Fruit Set. The F1 NIL *Sletr1-2* hybrids showed variation in fruit number per truss, with statistically significant

differences among parental backgrounds. The average number ranged between 4.47 and 4.87 fruits per truss (Figure 1). The hybrid derived from 'Oxheart' produced the highest fruit number (4.87), although not significantly different from that of the 'Costoluto' (4.75). The cross with 'Sart Roloise' resulted in the lowest fruit number (4.47). These differences may reflect variations in inflorescence architecture and flower fertility influenced by parental genetic contribution. The number of fruits per truss influenced the plant yield. Mubarok et al. (2023) reported that fruit weight was affected by the number of fruits on the plant.

Fruit set is defined as the ratio of the number to the number of flowers. The reduction in fruit set had a consequential impact on plant yield (Mubarok et al., 2023). The statistical data analysis showed that the fruit ratio among the F1 NIL *Sletr1-2* was comparable. The values ranged from 74% ('Sart Roloise') to 75% ('Costoluto') (Figure 1). The consistent fruit set among all parental backgrounds suggests that the introgression of *Sletr1-2* did not adversely affect pollination efficiency or fertilization success under field conditions, and that all hybrid lines had comparable reproductive performance. Our previous study reported that environmental factors, such as temperature, significantly affect fruit formation (Mubarok et al., 2023). Ezura et al. (2019) reported that heat stress increased pollen sterility, leading to pollen development failure and pollination failure.

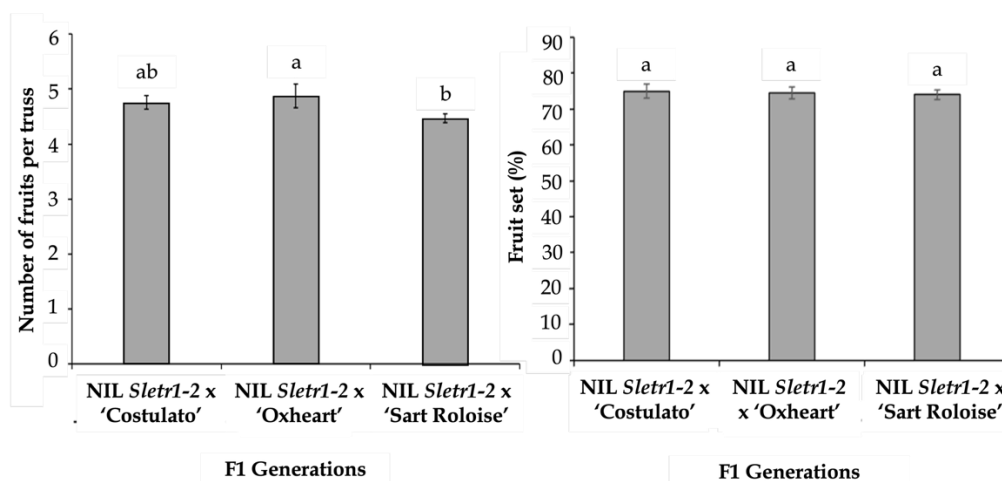


Figure 1. Number of fruits per truss and fruit set in various F1 NIL *Sletr1-2* tomato plants from three parental backgrounds ('Costoluto', 'Oxheart', and 'Sart Roloise'). The average values followed by the same letter are not significantly different based on Duncan's Multiple Range Test at the 5% level.

Locule Number and Pericarp Thickness.

Locule number, which reflects the internal complexity and potential seed-bearing capacity of the fruit, varied significantly between hybrids (Figure 2). The F1 NIL *Sletr1-2* from 'Sart Roloise' parent produced the most locules, averaging 4.98 per fruit, indicating a more segmented internal fruit architecture. This was followed by the NIL *Sletr1-2* x 'Costoluto' fruit at 4.58, and finally, the NIL *Sletr1-2* x 'Oxheart' resulted in the lowest locule number at 4.28. Although all fruits fell within the typical multilocular category, the degree of locule formation differed in a way that highlights the influence of each parent line's inherent fruit morphology on this trait.

Pericarp thickness is a structural trait associated with fruit firmness. Among the hybrids, significant differences were observed (Figure 2). The F1 NIL *Sletr1-2* from 'Costoluto' parental background displayed the thickest pericarp at 3.98 mm, providing a potential advantage for mechanical strength and water retention. The F1 NIL *Sletr1-2* from 'Sart Roloise' parental background followed closely with a pericarp thickness of 3.73 mm, while the F1 NIL *Sletr1-2* from 'Oxheart' parental background had the thinnest pericarp, averaging only 3.11 mm. These results indicate that pericarp thickness is significantly influenced by the genetic contribution of the commercial parent and that selecting the parental line is crucial for determining this attribute.

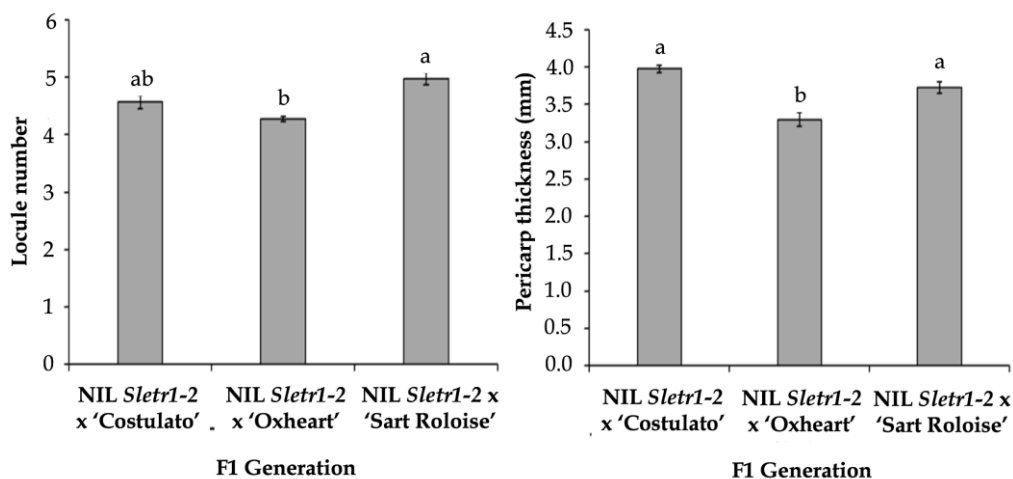


Figure 2. Locule number and pericarp thickness in various F1 NIL *Sletr1-2* tomato plants from three parental backgrounds ('Costoluto', 'Oxheart', and 'Sart Roloise'). The average values followed by the same letter are not significantly different based on Duncan's Multiple Range Test at the 5% level.

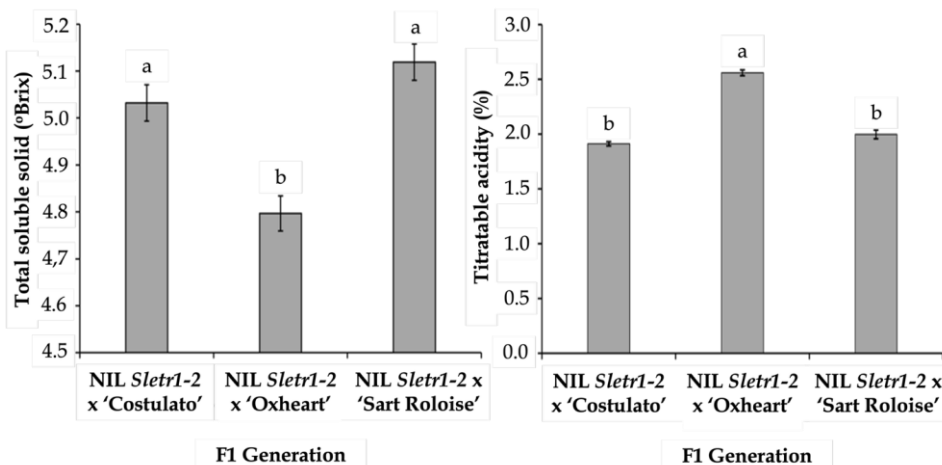


Figure 3. TSS and TA in various F1 NIL *Sletr1-2* tomato plants from three parental backgrounds ('Costoluto', 'Oxheart', and 'Sart Roloise'). The average values followed by the same letter are not significantly different based on Duncan's Multiple Range Test at the 5% level.

Total Soluble Solids (TSS) and Titratable Acidity (TA). TSS values, indicative of sugar content and flavor, ranged from 4.80 to 5.12 °Brix across hybrids (Figure 3). The NIL *Sletr1-2* × ‘Sart Roloise’ had the highest TSS (5.12 °Brix), which was significantly greater than the NIL *Sletr1-2* × ‘Oxheart’ hybrid (4.80 °Brix), but not different from the NIL *Sletr1-2* × ‘Costoluto’ (5.02 °Brix). These results highlight that the parental background influences fruit sweetness and that the *Sletr1-2* introgression does not suppress soluble sugar accumulation.

Titrate acidity is an indicator of sourness. Statistical analysis showed a significant difference among the F1 NIL *Sletr1-2* hybrid lines (Figure 3). The NIL *Sletr1-2* × ‘Oxheart’ had the highest TA value at 2.56%, indicating a more acidic flavor profile. In comparison, the NIL *Sletr1-2* × ‘Costoluto’ and × ‘Sart Roloise’ recorded significantly lower acidity levels at 1.91% and 2.00%, respectively. These differences reflect how each parent's genetic contribution affects organic acid biosynthesis and accumulation, thereby influencing overall flavor perception. The fruit flavor is influenced by the combination of sweet and acidic. TSS and TA are mostly used to estimate sugar and organic acids, respectively (Mubarok et al., 2015). In our study, we reported that the TSS and TA of F1 NIL *Sletr1-2* hybrid lines are dependent on the parental background. The parental line that crosses with NIL *Sletr1-2* will affect the fruit taste quality and fruit characteristics. This finding was similar to the previous study by Mubarok et al. (2015), the effect of *Sletr1-2* mutation was dependent on the genetic background of the pure-line cultivar parent.

Introducing the *Sletr1-2* allele into commercial tomato lines offers a practical strategy to improve shelf life by reducing the plant's sensitivity to ethylene, the hormone responsible for triggering ripening. By slowing ethylene-mediated metabolic activity, this mutation extends the fruit's postharvest life (Rahmat et al., 2021). However, the success of this approach depends not only on the mutation itself but also on its interaction with the genetic background of the recipient cultivar (Mubarok, et al., 2015). In this study, F1 NIL *Sletr1-2* hybrids were developed using three different parental lines: Oxheart, Sart Roloise, and Costoluto. The results showed apparent differences in fruit quality, postharvest potential, and metabolite

content depending on the chosen parental background (Saadah et al., 2023).

Conclusion

This study demonstrates that parental background strongly influences the expression of fruit characteristics in F1 NIL *Sletr1-2*. The ‘Oxheart’ background tended to amplify less desirable attributes, including lower pericarp thickness, lower TSS, higher acidity (TA), and smaller fruit size, which collectively reduce firmness, sweetness perception, and postharvest durability. These features make ‘Oxheart’ a less suitable candidate for commercial improvement when combined with the *Sletr1-2* allele. In contrast, ‘Costoluto’ and ‘Sart Roloise’ contributed to improved fruit size. Integrating ethylene-insensitive traits into elite tomato backgrounds requires strategic parental selection to balance storability, fruit size, flavour, and consumer-preferred qualities. These results demonstrate that integrating ethylene-insensitive traits such as *Sletr1-2* into elite tomato backgrounds requires careful parental selection to balance extended storability with desirable consumer-oriented attributes, including sweetness, firmness, and optimal fruit size.

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References

- Alenazi MM, Shafiq M, Alsadon AA, Alhelal IM, Alhamdan AM, Solieman THI, Ibrahim AA, Shady MR, Al-Selwey WA. 2020. Improved functional and nutritional properties of tomato fruit during cold storage. *Saudi Journal of Biological Sciences*, 27:1467–1474.
- Di Giacomo M, Luciani MD, Cambiaso V, Zorzoli R, Rodríguez GR, Pereira da Costa JH. 2020. Tomato near isogenic lines to unravel the genetic diversity of *S. pimpinellifolium* LA0722 for fruit quality and shelf life

- breeding. *Euphytica*, 216(8): 1-15.
- Mauro RP, Rizzo V, Leonardi C, Mazzaglia A, Muratore G, Distefano M, Sabatino L, Giuffrida F. 2020. Influence of harvest stage and rootstock genotype on compositional and sensory profile of the elongated tomato cv. "Sir Elyan." In *Agriculture* 10:82.
- Mubarok S, Ezura H, Anas, Kusumiyati, Rostini N, Suminar E, Wiguna G. 2015. Impacts of *Sletr1-1* and *Sletr1-2* mutations on the hybrid seed quality of tomatoes. *Journal of Integrative Agriculture*, 18:1170-1176.
- Mubarok S, Okabe Y, Fukuda N, Ariizumi T, Ezura H. 2015. Potential use of a weak ethylene receptor mutant, *Sletr1-2*, as breeding material to extend fruit shelf life of tomato. *Journal of Agricultural and Food Chemistry*, 63:7995-8007.
- Mubarok S, Okabe Y, Fukuda N, Ariizumi T, Ezura H. 2016. Favorable effects of the weak ethylene receptor mutation *Sletr1-2* on postharvest fruit quality changes in tomatoes. *Postharvest Biology and Technology*, 120:1-9.
- Mubarok S, Qonit MAH, Rahmat BPN, Budiarto R, Suminar E, Nuraini A. 2023. An overview of ethylene-insensitive tomato mutants: Advantages and disadvantages for postharvest fruit shelf-life and future perspective. *Frontiers in Plant Science*, 14, 1079052.
- Mubarok S, Jadid N, Widiastuti A, Derajat Matra D, Budiarto R, Lestari FW, Nuraini A, Suminar E, Pradana Nur Rahmat B, Ezura H. 2023. Parthenocarpic tomato mutants, *iaa9-3* and *iaa9-5*, show plant adaptability and fruiting ability under heat-stress conditions. *Frontiers in Plant Science*, 14:1090774.
- Nguyen C, Verdeprado H, Demeter Z, Sanada-Morimura S, Matsumura M, Virk P, Brar D, Horgan F, Yasui H, Fujita D. 2019. The Development and characterization of near-isogenic and pyramided lines carrying resistance genes to brown planthopper with the genetic background of japonica rice (*Oryza sativa* L.). *Plants*, 8:498.
- Patanè C, Siah S, Pellegrino A, Cosentino SL, Siracusa L. 2021. Fruit yield, polyphenols, and carotenoids in long shelf-life tomatoes in response to drought stress and rewatering. *Agronomy*, 11.
- Rahmat BPN, Wicaksana N, Mubarok S, Ramadhan MJ, Putri MZ, Ezura H. (2021). Respons dua generasi tomat mutan insensitif etilen *Sletr1-2* terhadap cekaman kekeringan. *Kultivasi*, 20:1.
- Rosales M, Ruiz J, Hernandez J, Soriano M, Castilla N, Romero L. 2006. Antioxidant content and ascorbate metabolism in the exocarp of cherry tomatoes in relation to temperature and solar radiation. *Plant Cell. Environ.*, 27:423-435.
- Saadah IR, Wiguna G, Anas, Mubarok S, Hamdani KK, Soedomo RP. 2023. Variability and selection of tomato *Sletr1-2* mutants backcross population to improve yield and fruit quality. *IOP Conference Series: Earth and Environmental Science*, 1255:1.
- Saito T, Ariizumi T, Okabe Y, Asamizu E, Hiwasa-Tanase K, Fukuda N, Mizoguchi T, Yamazaki Y, Aoki K, Ezura H. 2011. TOMATOMA: a novel tomato mutant database distributing Micro-Tom mutant collections. *Plant & Cell Physiology*, 52:283-296.
- Singh AK, Mishra P, Kashyap SP, Karkute SG, Singh PM, Rai N, Bahadur A, Behera TK. 2022. Molecular insights into mechanisms underlying thermo-tolerance in tomato. *Frontiers in Plant Science*, 13:1-15.

Hindersah R · Fitriyani F · Endo K · Nakayama M · Mubarok S · Nugraha GN

Effects of supplemental LED lighting and biofertilizer on yield and fruit quality of Japanese strawberry cultivars in Indonesia

Abstract. Growing strawberries in soilless substrates in the greenhouse relies on fertilizers and supplemental light to boost yield and fruit quality. The research evaluated the properties of the biofertilizer and its effectiveness on the yield of Japanese strawberry cultivars Nanatsuboshi and KS-75 under controlled greenhouse conditions, with and without light-emitting diodes (LED) as supplemental light. The cell viability and plant-growth-promoting properties of a liquid consortium biofertilizer consisting of nitrogen-fixing bacteria and phosphate-solubilizing microbes, were characterized. Laboratory analysis confirmed the biofertilizer's composition, and a greenhouse experiment using a completely randomized design tested treatments of biofertilizer, LED, and their combination. The biofertilizer contained specific phytohormones and organic acids, with bacterial and fungal populations exceeding 10^7 and 10^5 CFU/mL, respectively. The 3-month greenhouse experiment showed that the biofertilizer, LED, and their combination reduced fruit number and weight in the strawberry cultivar Nanatsuboshi but increased them in KS-75. However, the total fruit weight of Nanatsuboshi was higher than that of KS-75 due to its larger size. The treatments did not increase the fruit total soluble solids (TSS). These findings indicate that KS-75 exhibited a stronger response to biofertilizer and LED treatments than Nanatsuboshi.

Keywords: Biofertilizer properties · Fruit number · Fruit weight · Light-emitting diodes · Total soluble solids

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Introduction

Strawberries (*Fragaria × ananassa* Duch.) are temperate crops that have adapted to high-altitude tropical regions, such as those found in Indonesia. Their economic importance drives increasing Indonesian demand for sweet, large, and appealing fruit. Most cultivars in Indonesia originated from seeds imported from the United States and later evolved into local varieties, whereas only a limited number of Japanese strawberries are grown there.

The cultivar, climate, and cultivation methods strongly influence the fruit's TSS. Local strawberries typically have a sweetness level of 5-8 °Brix (Hindersah et al., 2023; Romiyadi & Putri, 2024), which is considered sour, whereas Japanese strawberries range from 10-14 °Brix (Ikegaya, 2024). In general, optimal temperatures for strawberry growth are 18-25 °C, and optimal relative humidity is 60-69%. A temperature difference of 10-12 °C between day and night enhances sugar accumulation (Wu et al., 2021). High temperatures and humidity impede flower development, hinder pollination and fruit set, and reduce yield and quality (Muneer et al., 2017).

Greenhouse cultivation offers a promising method for controlling microclimates and enabling year-round production. Because natural sunlight varies throughout the day, supplemental artificial lighting is essential. LED technology provides high light intensity, adjustable wavelengths, and low heat emission. LEDs emit specific wavelengths, including red and blue, that meet plants' photosynthetic needs and influence photosynthesis, morphogenesis, and flowering. In subtropical regions, cultivating strawberries in greenhouses under LED lighting increased leaf chlorophyll levels, vegetative growth, above-ground dry matter, and fruit production compared with those without LED (Choi et al., 2015; Nakayama, 2025; Hwang et al., 2025). The beneficial effects of red and blue LED on the yield and total soluble solids of American strawberries grown in conventional greenhouses in Indonesia have been documented (Adrian et al., 2024). However, similar research on Japanese strawberries is lacking.

In greenhouses, strawberries are often grown in soilless substrates, simplifying management and reducing pest and disease incidence. Most growers in Indonesia still depend on chemical fertilizers for soilless-based cultivation. Biofertilizers provide an eco-friendly

and cost-effective source of nutrients. They typically contain nitrogen-fixing bacteria (NFB) and phosphorus-solubilizing microbes (PSM). The NFBs convert atmospheric N₂ into ammonia, NH₃ (Pajares & Bohannan, 2016), which is further transformed into available ammonium and then nitrate (Longepierre et al., 2022). Meanwhile, PSMs release organic acid to convert inert inorganic phosphorus to available one and hence increase its bioavailability (Yadav et al., 2015).

Beneficial bacteria *Azotobacter*, *Azospirillum*, and *Acinetobacter* (endophytic N-fixer), and *Pseudomonas*, have been reported to promote plant growth, enhance plant health, and increase strawberry yield (Hosseini et al., 2022; Morais et al., 2019). The positive impact of *Penicillium* and *Trichoderma* on strawberry growth was reported (Fitriatin et al., 2025). Many of these microbes also synthesize phytohormones that are involved in strawberry plant development (de Andrade et al., 2019; Rueda et al., 2016). Biofertilizer application in strawberries enhanced juice content, total soluble solids, and total sugar (Kumar et al., 2019). However, data on the response of Japanese strawberry cultivars to beneficial bacterial inoculation in tropical regions are limited.

Integrating lighting and biofertilizers provides a synergistic solution to optimize yields and fruit sweetness in Japanese strawberry production in tropical regions. Nonetheless, the microbial composition and metabolite content of biofertilizers determine their effectiveness in boosting plant yield. This study aimed to characterize the plant-growth-related properties of the biofertilizer consortia and evaluate their effectiveness on the cv. Nanatsuboshi and KS-75 strawberry yields and sweetness under controlled greenhouse conditions, with and without LED lighting. This research presents the first report on the application of biofertilizers for growing Japanese strawberries in an environmentally controlled greenhouse in Indonesia.

Materials and Methods

The laboratory analysis was carried out in June 2024. A pot experiment was conducted in the environmentally-controlled greenhouse at the Faculty of Agriculture, Universitas Padjadjaran, from August to November 2024.

Biofertilizer Characterization. The consortia biofertilizer consisted of heterotrophic-aerobic *Azotobacter chroococcum*, *A. vinelandii*,

Acinetobacter sp., *Azospirillum* sp., *Pseudomonas cepacia*, and *Penicillium* sp. Microbes were scaled up separately in 30 L of molasses-based growth media within a 45 L bioreactor. The initial inoculum concentration in the media was 5%, and the culture was incubated for 3 days at room temperature with agitation at 130 rpm. All microbial cultures were mixed in a balanced ratio before bottling. PT Pupuk Kujang provided the biofertilizer in collaboration with the Faculty of Agriculture, Universitas Padjadjaran.

Population counts and metabolite analyses in the biofertilizer were performed one month after bottling. The population of each microbial strain was quantified in specific media using the serial dilution and plate count method (Ben-David & Davidson, 2014). A total of 0.1 mL of the microbial suspension was taken from the diluted BF, poured onto the sterilized plates, and mixed with 20 mL of the media. The plates were incubated at 30 °C for 3 days for bacteria and 5 days for *Penicillium* fungi. Each colony with a diameter of 1 mm or more was counted. The microbial counts were replicated three times.

The opportunistic pathogen *Escherichia coli* and *Salmonella* spp. were enumerated using the Most Probable Number method (Weaver et al., 1994). Indole-3-acetic acid (IAA) was measured by spectrophotometer after extraction with Salkowski's reagent (Patel et al., 2018), while gibberellins (GA3, GA4 and GA5) and cytokinins (zeatin and kinetin) were determined through HPLC with mobile phase of acetonitrile/water (50/50; v/v) and a flow 1.0 ml/min; the detection was performed by a UV detector set at 205 nm (Macías et al., 2014). Specific organic acids, including lactic, oxalic, citric, and acetic, were measured by high-performance liquid chromatography (Risanti et al., 2025).

The strawberry cultivars used in this study were Nanatsuboshi and KS-75, which had been grown in beds for one year. The growth medium in the trays on the raised beds consisted of a 9:9:1 volume ratio of coco peat, rice husk, and peat moss. Both strawberry cultivars were imported from Okinawa prefecture, Japan, and provided by the Japan Premium Vegetable Project in collaboration with the Faculty of Agriculture, Universitas Padjadjaran.

Greenhouse Experiment. The greenhouse temperature was maintained at 20 °C at night and 25-30 °C during the day, with a relative humidity

of 60%. LEDs with a wavelength of 400-800 nm were installed 60 cm above the cultivation beds (Figure 1), and automatically turned on daily from 4:00-6:00 pm and 6:00-8:00 am, and when outside sunlight was less than 300 W/m².

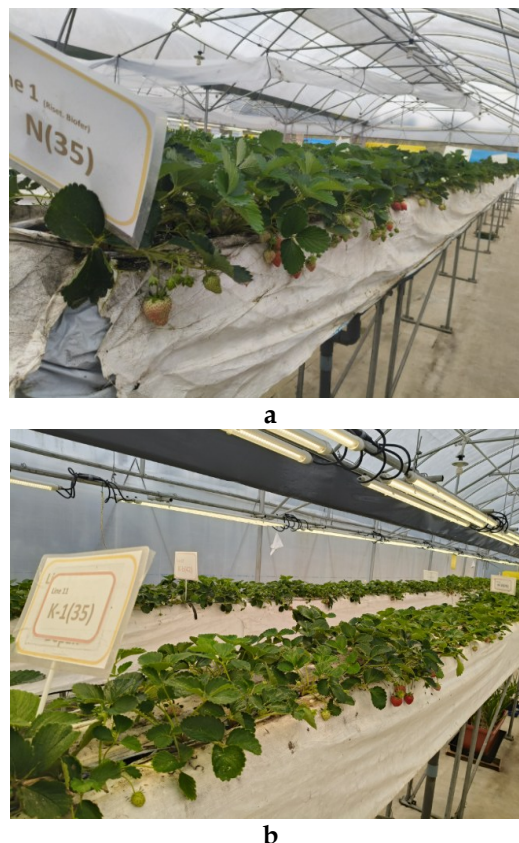


Figure 1. The strawberries cv Nanatsuboshi grown with natural light (a) and KS-75 cultivars grown in the raised bed under LED (b)

Photosynthetic photon flux density (PPFD) was recorded every 5 minutes using quantum photosynthetically active radiation sensors. Data were stored in a Decagon EM50 data logger. During the experiment, the average PPFD under natural and LED light were 179.62 and 166.10 mol/m²/s (Table 1), while the average daylight integral (DLI) values were 15.52 and 14.35 mol/m²/day (Table 2).

Table 1. The photosynthetic photon flux density during the experiment

PPFD (mol/m ² /s)	Non-LED	LED
Sum	16345.60	15115.46
Minimum	94.01	115.79
Maximum	222.19	207.14
Mean	179.62	166.10

Table 2. Daylight integral during the experiment

DLI (mol/m ² /day)	Non-LED	LED
Sum	1412.26	1305.98
Minimum	10.00	8.12
Maximum	17.90	19.20
Mean	15.52	14.35

Monthly PPFD and DLI under LED and non-LED areas were shown in Figure 2.

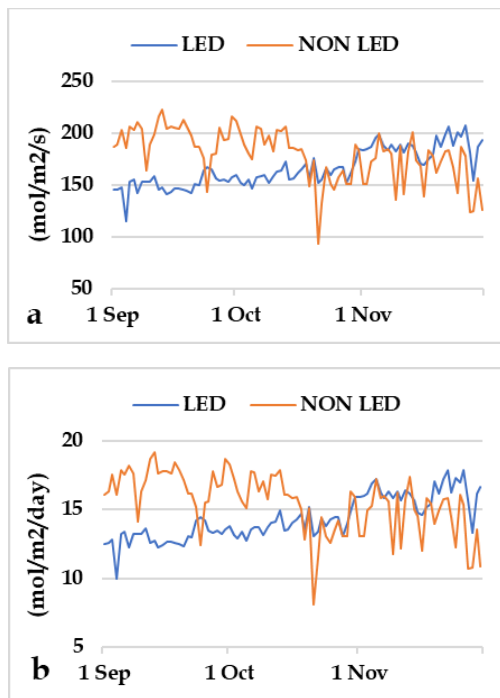


Figure 2. The dynamic of photosynthetic photon flux density (a) and daylight integral (b) from September to November 2024 under LED and natural (non-LED) lighting

Experimental Design and Implementation.

The experiment was conducted using a randomized complete block design to test one independent factor with four treatments and five replications. The treatment factors included A) natural lighting without biofertilizer, B) LED lighting without biofertilizer, C) natural lighting with biofertilizer, and D) LED lighting with biofertilizer. Each treatment was placed in 5 trays, each containing seven plants.

The strawberry cultivars used in this study were Nanatsuboshi and KS-75, which had been grown in beds for one year. The growth medium on the raised beds consisted of coco peat, rice husk, and peat moss (9:9:1; v:v:v). Both cultivars were imported from Okinawa prefecture, Japan,

and provided by the Japan Premium Vegetable Project in collaboration with the Faculty of Agriculture, Universitas Padjadjaran.

Plant nutrients were supplied through fertigation. The nutrient solution was prepared by mixing the A and B nutrient solutions with 3% citric acid in the specified composition. The A solution contained 1,200 ppm CaNO₃, 350 ppm KNO₃, and 40 ppm Fe; the B solution contained 800 ppm MgSO₄, 300 ppm KH₂PO₄, 50 ppm K₂SO₄, 10 ppm MnSO₄, 10 ppm boric acid, 5 ppm CuSO₄, 2 ppm (NH₄)₂MoO₄, and 5 ppm ZnSO₄. All nutrient solutions were placed in separate containers. The nutrient solution was applied at an average EC of 0.5-0.7 dS/m and a pH of 5.7. The daily fertigation was adjusted to deliver 50 mL over 10 minutes per hour from 6 am to 4 pm.

Biofertilizer at a 5% (v:v) concentration and 20 mL volume was applied to the leaves every two weeks for two months. Plants without fertilizer were sprayed with the same volume of water. Before biofertilizer application, the plants were in the fruiting stage, and no fruits or flowers were removed. Natural pesticides were applied regularly, 2-3 times a week, to control aphids and mites. The caterpillars that sometimes attack the leaves were manually removed and squashed. The experiment maintained three crowns per plant and eight leaves per crown. All runners and side roots were removed. The mature fruits were harvested three times a week.

Parameters and statistical analysis. The number of fruits, their total weight, and the total soluble solids (TSS) of both the fruit tip and the intact fruit were measured. The fruit tip is the low part of the fruit; one-third of the height of the fruit is cut to get the fruit tip. The fruits were weighed using a digital scale (1000 HPS2, Sojikyoo, Japan), and TSS was measured with a refractometer (PAL-1, Atago, Ltd., Tokyo, Japan). All data were analysed using one-way analysis of variance (ANOVA) at $p < 0.05$. The means were compared using the Tukey Honestly Significant Difference (HSD) test at $p < 0.05$. Statistical analyses were performed using SPSS version 25.

Results and Discussion

Biofertilizer Properties. All microbes inoculated into molasse-based liquid biofertilizer were recovered using specific growth media (Table 3). The biofertilizer contained a low population of opportunistic pathogens, *E. coli* and *Salmonella*

spp., each at <3 MPN/mL. The phytohormones, including IAA, gibberellins, and cytokinins, were present in biofertilizer (Table 3). Lactic, oxalic, and acetic acids were found in the biofertilizer, but citric acid was not detected.

The number of microbial populations in a biofertilizer is the primary factor determining its quality and effectiveness. The populations of bacteria and fungi were more than 10⁷ and 10⁵ CFU/mL, respectively, which complies with the Indonesian Ministry of Agriculture Regulation No. 261 of 2019 about biofertilizer. The liquid media support heterotrophic microbial growth, as molasses provides 29-40% sucrose, 4-14% glucose, 0.5-4.5% protein, 0.3-1.5% amino acids, phosphorus, potassium, and other nutrients (Jamir et al., 2021). Heterotrophic microbes in biofertilizer metabolize saccharides for energy and carbon, as well as proteins and amino acids for nitrogen sources.

Rhizobacteria can produce IAA through both tryptophan-dependent and tryptophan-independent pathways. Notable examples of rhizobacteria that produce IAA without external tryptophan include *Azotobacter*, *Pseudomonas*

(Ahmad et al., 2005), and *Acinetobacter* (Lin et al., 2018). In contrast, most *Azospirillum* species rely on exogenous Trp for IAA biosynthesis (Carreno-Lopez et al., 2000). In this study, *Azotobacter*, *Pseudomonas*, and *Acinetobacter* mainly contributed to IAA production in the culture, as no external Trp was added.

The presence of GA and CK in biofertilizer reconfirmed that *Azotobacter*, *Azospirillum*, and *Pseudomonas* can synthesize both phytohormones, as discussed elsewhere. Molasses provides disaccharides and other nutrients for bacterial growth and the production of phytohormones, which are then released into the surrounding medium (Hindersah et al., 2020). In this study, the reactor headspace and continuous agitation maintain an aerobic environment during biofertilizer production. All microbes in the biofertilizer exhibit heterotrophic and aerobic metabolism rather than fermentation, resulting in a low organic acid content.

Strawberries Yield. Biofertilizer, LED, and their combination significantly reduced the number of fruits per plant in Nanatsubohsi but increased it in KS-75 (Table 5).

Table 3. Microbial composition of the liquid consortia biofertilizer

Microbes	Methods	Growth Media	Population	Unit
<i>A. vinelandii</i>	TPC ¹	Vermani Agar	2.16 × 10 ⁷	CFU/mL
<i>A. chroococcum</i>	TPC	Ashby Agar	1.37 × 10 ⁷	CFU/mL
<i>P. cepacia</i>	TPC	Pikovskaya Agar	9.74 × 10 ⁷	CFU/mL
<i>Acinetobacter</i>	TPC	Tryptic Soy Agar	1.55 × 10 ⁹	CFU/mL
<i>Azospirillum</i> sp.	TPC	Okon Agar	3.85 × 10 ⁷	CFU/mL
<i>Pencillium</i> sp.	TPC	Pikovskaya Agar	1.21 × 10 ⁵	CFU/mL
<i>Escherichia coli</i>	MPN ²	EMBA ⁴	< 3	MPN/mL
<i>Salmonella</i> sp.	MPN	SSA ⁵	< 3	MPN/mL
Patogenicity	HS Test ³	Tobacco plants	Negative	-

¹Total Plate Count, ²Most Probable Number, ³in-planta hypersensitive test, ⁴Eosin Methylene Blue Agar, ⁵Salmonella Shigella Agar.

Table 4. Phytohormones and organic acid composition of liquid consortia biofertilizer

Metabolites	Concentration (mg/L)
IAA	34.01
GA3	167.09
GA4	38.07
GA5	43.90
Zeatin	198.23
Kinetin	21.86
Lactic acid	2.73
Oxalic acid	4.97
Citric acid	nd ¹
Acetic acid	32.51

¹Not detected

Table 5. Fruit number of 'Nanatsuboshi' and 'KS-75' strawberry in response to different supplemental LED lighting and biofertilizer treatments over three months of observation

Treatments	Fruit number per plant in each month					
	Nanatsuboshi			KS-75		
	1 st	2 nd	3 rd	1 st	2 nd	3 rd
Control (without BF ¹ , natural light)	4.0 b	2.8 a	4.8 c	1.0 a	1.0 a	1.5 a
BF, natural light	2.7 a	2.5 a	3.5 ab	1.6 ab	1.3 a	1.6 a
Without BF, LED	2.3 a	2.0 a	3.0 a	1.5 a	2.2 b	2.7 b
BF, LED	2.7 a	3.5 a	4.0 b	2.3 b	2.7 b	2.3 b

Mean values (n=6) followed by different letters in the same column were significantly different according to the Tukey HSD test at $p < 0.05$. ¹Biofertilizer

Table 6. Fruit weight per plant of 'Nanatsuboshi' and 'KS-75' strawberry in response to different supplemental LED lighting and biofertilizer treatments over three months of observation

Treatments	Fruit weight (g/plant) in each month					
	Nanatsuboshi			KS-75		
	1 st	2 nd	3 rd	1 st	2 nd	3 rd
Control (without BF ¹ , natural light)	39.5 a	26.6 ab	43.7 b	8.6 a	7.4 a	9.6 a
BF, natural light	37.9 a	24.3 a	31.6 a	13.7 b	12.5 b	14.3 ab
Without BF, LED	23.6 a	21.5 a	31.3 a	13.1 b	15.9 b	14.8 ab
BF, LED	26.7 a	32.5 b	39.7 b	19.6 c	19.7 c	17.5 b

Mean values (n=6) followed by different letters in the same column were significantly different according to the Tukey HSD test at $p < 0.05$. ¹Biofertilizer

Table 7. Total fruit number and fruit weight of 'Nanatsuboshi' and 'KS-75' strawberry in response to different supplemental LED lighting and biofertilizer treatments over three months of observation

Treatments	Fruit number		Fruit weight (g/plant)	
	Nanatsuboshi	KS-75	Nanatsuboshi	KS-75
	Control (without BF ¹ , natural light)	11.67 c	3.50 a	109.93 b
BF, natural light	8.67 a	4.67 a	93.74 ab	40.48 b
Without BF, LED	7.67 a	6.33 b	76.43 a	43.85 b
BF, LED	10.17 b	7.33 b	98.87 ab	56.79 c

Mean values (n=6) followed by different letters in the same column were significantly different according to the Tukey HSD test at $p < 0.05$. ¹Biofertilizer

Regardless of statistical analysis, the number of fruits in Nanatsuboshi fluctuated over the three-month observation period. In the first and third months, all treatments reduced fruit number, whereas in the second month, biofertilizer with natural light and LED without biofertilizer did not affect fruit number. The fruit number of treated plants in the first and third months was 35.8% and 27% lower than the control, respectively. The pattern of fruit number increase in KS-75 was consistent across all months of observation, as evidenced by the positive effects of biofertilizer and LED treatments. The increase in fruit number under LED with biofertilizer was about 130%, 170%, and 53% from the first to the third month, respectively.

The Biofertilizer with natural light and LED without biofertilizer reduced fruit weight in Nanatsuboshi, but biofertilizer application in the LED area maintained the total fruit compared to the control (Table 6). In contrast, all treatments increased the fruit weight in KS-75. The KS-75 grown with biofertilizer under LED lighting during the first, second, and third months had 127%, 166%, and 82% more fruit weight, respectively, compared to the control. The reduced yield in the second month might be due to the lowest PPFD and DLI levels, which are 94.02 mol/m²/s and 8.12 mol/m²/day, respectively. Changes in light that directly affect photosynthesis and carbohydrate allocation to the fruit.

The trend in fruit yield over three months (Table 7) was comparable to the monthly data; a decrease in the number of Nanatsuboshi fruits after treatments was observed. Meanwhile, the KS-75 yield was enhanced by the treatments.

The monthly fruit numbers and weights varied widely because flowers and fruits were not thinned. In general, the Nanatsuboshi was dominated by medium-sized fruit (16-26 g), but the small-sized KS-75 (6-15 g) was the dominant size. The fruit size of Nanatsuboshi is genetically larger than that of KS-75.

Anthesis and fruit set also varied among plants within each treatment, possibly due to differences in plant physiological status and uneven nutrient supply. Moreover, the experiment was conducted on reproductive plants without removing flowers or fruits beforehand. Because strawberry plants naturally vary in their flowering and fruiting stages, they cannot all be harvested at the same time.

Beneficial microbes likely enhance yield by stimulating photosynthesis. The bacteria in the biofertilizer fix nitrogen and produce phytohormones (Hindersah et al., 2021). Spraying beneficial bacteria onto plant shoots growing in soil can improve N status and hence chlorophyll content, photosynthetic rate, and biomass (Efthimiadou et al., 2020). In the phyllosphere, nitrogen and phytohormones are absorbed by leaves through stomata or the epidermis of leaves.

Biofertilizer is applied by foliar spraying, which may alter the phyllosphere microbial composition in Nanatsuboshi, thereby repressing indigenous beneficial microbes. The phyllosphere of Nanatsuboshi may lack essential nutrients needed for fruit development. Some of the sprayed biofertilizer inevitably reaches the growth media. Exogenous bacteria modify the community structure of the rhizosphere and growth media, resulting in a different composition of root exudates that provide nutrients. In the case of Nanatsuboshi, this change may not be suitable for both bacterial and plant growth. However, further research is required to identify the key factors underlying the yield reduction.

A biofertilizer can decrease the yield of a specific strawberry cultivar due to incompatibility with the cultivar, climate, or an unbalanced nutrient profile in the substrate. Microbes often face numerous challenges when introduced into the shoots. Different strawberry varieties respond uniquely to biofertilizers

because of their distinct genetics and physiology, and how these interact with the introduced microbes and their activities in providing nutrients. A commercial biofertilizer composed of nitrogen-fixing and phosphorus-solubilizing bacteria slightly decreased the yield of the Dely cultivar but reduced that of Joly (Tomic et al., 2015).

LED lighting significantly impacts yield, both fruit number and yield. Photoperiod, genotype (cultivar), and temperature interact to influence growth and, subsequently, yield, which determines whether growth is vegetative or generative (Bradford et al., 2010). The LED usually increases strawberry yield (Pérez-Romero et al., 2024; Nakayama & Nakazawa, 2023). The results of this study for KS-75 are consistent with the 3.1-fold and 2.5-fold increase in yield of the Nanatsuboshi and Benihoppe cultivars, respectively, under LEDs compared to a conventional subtropical greenhouse (Nakayama & Nakazawa, 2023).

Lower yields might result from unsuitable light intensity, spectrum, or duration for the Nanatsuboshi cultivar, leading to plant stress or nutrient imbalance. Under white LED, strawberry cv Jewel had more severe *Colletotrichum gloeosporoides* than plants grown under ambient light, and all strawberry cultivars showed higher injury rating (Smith et al., 2022). Bacteria in the phyllosphere face alternating environmental conditions, including direct effects of specific LED wavelengths; for example, white LED alters the capacity of *Pseudomonas* sp. DR 5-09 for substrate utilization (Gharaie et al., 2017).

Light influences plant-microbe interactions by modulating root exudation patterns, which in turn shape the rhizosphere microbiome. Light intensity affects root exudation in the rhizosphere (Martin et al., 2018), and consequently, microbial composition, thereby regulating the nutrient cycle. Increased yield may result from improved nutrient composition in the substrate. Furthermore, light affects phyllosphere microbes and leaf nutrients by altering gene dominance (Kong et al., 2024), thereby altering the availability of nutrients, such as sugars and proteins, on the leaf surface (Larsen et al., 2020).

The strawberry cv. KS-75 responded positively to the LED light and its interaction with Biofertilizer. Nanatsuboshi fruit yield decreased significantly under LED light, possibly because changes in photoperiod and temperature reduced positive plant responses, including microbial

proliferation and activity in the rhizosphere and phyllosphere. Physiological parameters, including leaf area, nutrient content, and leaf chlorophyll, should be monitored in the following experiment, as all traits directly relate to photosynthesis and carbohydrate allocation to flowers and fruits.

The total soluble solids. Neither biofertilizer in natural light, LED without biofertilizer, nor LED and biofertilizer treatments affected TSS in intact fruit or fruit tips in either Nanatsuboshi or KS-75 (Tables 8 and 9). Over three months, TSS remained constant for both varieties. Without statistical analysis, the TSS of intact fruit was likely lower than that of the fruit tip, since sugar in strawberries usually accumulates in the fruit tip. The TSS of Nanatsuboshi was lower than that of KS-75, even though the yield of Nanatsuboshi was higher than that of KS-75 (Table 7). In general, the TSS of intact fruit of both cultivars, mainly KS-75, was higher than that of the local strawberry (Romiyadi & Putri, 2024). However, the TSS of both cultivars agrees with that of local strawberries grown with

humic acid and biofertilizer-coated fertilizer (Hindersah et al., 2023).

The fruit tip of strawberries is usually sweeter than the rest of the fruit because maturity begins at the tip. The TSS of KS-75 is higher than that of Nanatsuboshi. The TSS increases in intact fruit were more pronounced in KS-75 following biofertilizer inoculation. Fruit sweetness is directly determined by leaf sugars, which are generated during photosynthesis and serve as the primary source of sugars accumulated in the fruit. This preliminary experiment did not reveal a direct effect of LED, biofertilizer, or their combination on photosynthesis.

Sugar transport and accumulation directly require potassium, boron (B), and P. Biofertilizers did not affect sweetness, possibly because phosphate-solubilizing bacteria did not supply sufficient P for sugar transport. Furthermore, B might precipitate in the medium due to phosphate binding. The white LEDs used in this study may be less effective than blue LEDs, which increase sugar (sucrose and fructose) production (Hwang et al., 2025).

Table 8. Total soluble solids of 'Nanatsuboshi' and 'KS-75' strawberry intact fruit in response to different supplemental LED lighting and biofertilizer treatments over three months of observation

Treatment	TSS of intact fruit (°Brix) in each month					
	Nanatsuboshi			KS-75		
	1 st	2 nd	3 rd	1 st	2 nd	3 rd
Control (without BF ¹ , natural light)	8.7 a	8.5 a	8.5 a	8.7 a	9.3 a	9.2 a
BF, natural light	8.5 a	8.9 a	8.9 a	9.2 a	9.8 a	9.9 a
Without BF, LED	8.3 a	8.9 a	8.7 a	9.5 a	9.3 a	9.6 a
BF, LED	8.4 a	8.7 a	9.1 a	9.5 a	9.6 a	9.6 a

Mean values (n=6) ± standard deviation followed by different letters in the same column were significantly different according to Tukey HSD test at p < 0.05. ¹Biofertilizer

Table 9. Total soluble solids of 'Nanatsuboshi' and 'KS-75' strawberry tip fruit in response to different supplemental LED lighting and biofertilizer treatments over three months of observation

Treatment	TSS of Tip Fruit (°Brix) in each month					
	Nanatsuboshi			KS-75		
	1 st	2 nd	3 rd	1 st	2 nd	3 rd
Control (without BF ¹ , natural light)	9.2 a	9.0 a	9.1 a	9.1 a	9.7 a	9.6 a
BF, natural light	9.0 a	9.8 a	9.5 a	9.6 a	10.4 a	10.3 a
Without BF, LED	9.5 a	9.4 a	9.3 a	10.0 a	9.6 a	10.1 a
BF, LED	9.0 a	9.4 a	9.80 a	10.0 a	9.5 a	10.1 a

Mean values (n=6) ± standard deviation followed by different letters in the same column were significantly different according to the Tukey HSD test at p < 0.05. ¹Biofertilizer

The research limitations were that the flowers and fruit were not removed beforehand, resulting in inconsistent fruiting status among plants and non-simultaneous harvest times. Further experiments should also include physiological parameters such as chlorophyll content and the uptake of essential macronutrients and specific micronutrients that regulate photosynthesis. Despite the results of this experiment, strawberry cultivation in greenhouses in Indonesia using biofertilizers remains promising. However, the limitations of the experiment described above need to be addressed.

Conclusion

This study successfully characterized the microbial and biochemical properties of the liquid consortia biofertilizer, which met the Indonesian biofertilizer quality standards and contained high populations of N-fixing and P-solubilizing microbes, as well as plant-growth-related phytohormones such as IAA, gibberellins, and cytokinins. The evaluation of its effectiveness under controlled greenhouse conditions demonstrated that the two Japanese strawberry cultivars responded differently to the biofertilizer and LED treatments. Biofertilizer and LED lighting consistently enhanced fruit number, total fruit weight, and TSS in cv. KS-75, indicating strong compatibility with both inputs. In contrast, Nanatsuboshi exhibited a reduced number of fruits under biofertilizer and LED treatments, although its fruit-tip TSS remained unchanged. However, biofertilizer application in the LED area maintained the fruit weight compared to the control. These findings highlight a significant interaction between cultivar, biofertilizer, and supplemental lighting, suggesting that KS-75 is better suited for integrated biofertilizer-LED cultivation systems in tropical greenhouses.

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References

- Adrian M, Fauzan Y, Fathoni MAN, Poerwanto R, Inoue E, Matra DD. 2024. Optimizing light conditions for enhanced strawberry growth in tropical lowlands. *Acta Agrobotanica*, 77(7): 1-17. DOI: 10.5586/aa/194248
- Ahmad F, Ahmad I, Khan MS. 2005. Indole acetic acid production by the indigenous isolates of *Azotobacter* and fluorescent *Pseudomonas* in the presence and absence of tryptophan. *Turkish Journal of Biology*, 29(1): 29-34. <https://journals.tubitak.gov.tr/biology/vol29/iss1/5%0AThis>
- Choi HG, Moon BY, Kang NJ. 2015. Effects of LED light on the production of strawberry during cultivation in a plastic greenhouse and in a growth chamber. *Scientia Horticulturae*, 189: 22-31, <https://doi.org/10.1016/j.scienta.2015.03.022>
- Ben-David A, Davidson CE. 2014. Estimation method for serial dilution experiments. *Journal of Microbiological Methods*, 107: 214-221. <https://doi.org/10.1016/j.mimet.2014.08.023>
- Bradford E, Hancock JF, Warner RM. 2010. Interactions of temperature and photoperiod determine expression of repeat flowering in strawberry. *Journal of the American Society for Horticultural Science*, 135(2): 102-107. <https://doi.org/10.21273/JASHS.135.2.102>
- Carreno-Lopez R, Campos-Reales N, Elmerich C, Baca BE. 2000. Physiological evidence for differently regulated tryptophan-dependent pathways for indole-3-acetic acid synthesis in *Azospirillum brasilense*. *Molecular and General Genetic*, 264: 521-530. <https://doi.org/10.1007/s004380000340>
- de Andrade FM, de Assis Pereira T, Souza TP, Guimarães PHS, Martins AD, Schwan RF, Pasqual M, Dória J. 2019. Beneficial effects of inoculation of growth-promoting bacteria in strawberry. *Microbiological Research*, 223-225: 120-128. <https://doi.org/10.1016/j.micres.2019.04.005>
- Efthimiadou A, Katsenios N, Chanioti S, Giannoglou M, Djordjevic N, Katsaros G. 2020. Effect of foliar and soil application of plant growth promoting bacteria on growth, physiology, yield and seed quality of maize under Mediterranean conditions. *Scientific Reports*, 10(1): 21060. <https://doi.org/10.1038/s41598-020-78034-6>
- Fitriatin BN, Nakayama M, Endo K, Hindersah R, Syifa N, Oktaviani M. 2025. Growth and yield

- of strawberry influenced by the application of phosphate solubilizing biofertilizer. *Soilrens*, 23, 22–31. <https://doi.org/10.24198/soilrens.v23i1.66114>
- Fitriyani F, Hindersah R, Devnita R, Mubarak S, Nakayama M. 2025. Characterization of nitrogen-fixing bacteria isolated from strawberry rhizosphere and their potency to increase seedling growth. *Biodiversitas*, 26(4): 1964–1974. <https://doi.org/10.13057/biodiv/d260445>
- Gharaie S, Vaas LAI, Rosberg AK, Windstam ST, Karlsson ME, Bergstrand KJ, Khalil S, Wohanka W, Alsanus BW. 2017. Light spectrum modifies the utilization pattern of energy sources in *Pseudomonas* sp. DR 5-09. *PLoS One*, 12: e0189862. [10.1371/journal.pone.0189862](https://doi.org/10.1371/journal.pone.0189862).
- Hindersah R, Setiawati MR, Asminran P, Fitriatin BN. 2020. Formulation of Bacillus and Azotobacter consortia in liquid cultures: Preliminary research on microbes-coated urea. *International Journal of Agriculture System*, 8(1), 1-10. DOI:10.20956/ijas.v8i1.2283
- Hindersah R, Ayu R, Aini P, Setiawati MR, Simarmata T, Indrawibawa D, Akutsu M. 2023. Boosting strawberry yield and fruit sweetness with humic substances and biofertilizer in soilless cocopeat-based culture. *Jurnal Ilmiah Pertanian*, 20(3): 1–11. <https://doi.org/10.31849/jip.v20i3.13242>
- Hindersah R, Kalay AM, Kesaulya H, Suherman C. 2021. The nutmeg seedlings growth under pot culture with biofertilizers inoculation. *Open Agriculture*, 6(1), 1-10. <https://doi.org/10.1515/opag-2021-0215>
- Hosseini A, Hosseini M, Schausberger P. 2022. Plant growth-promoting rhizobacteria enhance defense of strawberry plants against spider mites. *Frontiers in Plant Science*, 12, 1–12. <https://doi.org/10.3389/fpls.2021.783578>
- Hwang HS, Koo JK, Park EW, Yun JH, Hwang SY, Choi HE, Hwang JH, Yu J, Lim MY, Hwang SJ. 2025. Blue LED Supplemental Lighting Enhances Flowering and Fruit Yield of 'Kuemsil' Strawberries. *HorTechnology*, 35(4): 554-563. DOI: 10.21273/HORTTECH05686-25
- Ikegaya A. 2024. Composition of free sugars and organic acids in Japanese strawberry cultivars and their influence on the perception of sweetness and sourness. *Journal of Food Science*, 89(1): 614–624. <https://doi.org/10.1111/1750-3841.16842>
- Jamir L, Kumar V, Kaur J, Kumar S, Singh H. 2021. Composition, valorization and therapeutical potential of molasses: a critical review. *Environmental Technology Reviews*, 10(1): 131–142. <https://doi.org/10.1080/21622515.2021.1892203>
- Kong D, Ye Z, Dai M, Ma B, Tan X. 2024. Light Intensity modulates the functional composition of leaf metabolite groups and phyllosphere prokaryotic community in garden lettuce (*Lactuca sativa* L.) plants at the vegetative stage. *International Journal of Molecular Sciences*, 25(1451). <https://doi.org/10.3390/ijms25031451>
- Kumar S, Kundu M, Das A, Rakshit R, Siddiqui MW, Rani R. 2019. Substitution of mineral fertilizers with biofertilizer: An alternate to improve the growth, yield and functional biochemical properties of strawberry (*Fragaria × ananassa* Duch.) cv. Camarosa. *Journal of Plant Nutrition*, 42(15): 1818 – 1837. <https://doi.org/10.1080/01904167.2019.1643363>
- Kurokura T, Hiraide S, Shimamura Y, Yamane K. 2017. PGPR improves yield of strawberry species under less-fertilized conditions. *Environmental Control in Biology*, 55(3): 121–128. <https://doi.org/10.2525/ecb.55.121>
- Larsen DH, Woltering EJ, Nicole CCS, Marcelis LFM. 2020. Response of Basil Growth and Morphology to Light Intensity and Spectrum in a Vertical Farm. *Front. Plant Sci.*, 11:597906. doi: 10.3389/fpls.2020.597906
- Lin HR, Shu HY, Lin GH. 2018. Biological roles of indole-3-acetic acid in *Acinetobacter baumannii*. *Microbiological Research*, 216: 30–39. <https://doi.org/10.1016/j.micres.2018.08.004>
- Longepierre M, Feola Conz R, Barthel M, Bru D, Philippot L, Six J, Hartmann M. 2022. Mixed effects of soil compaction on the nitrogen cycle under pea and wheat. *Frontiers in Microbiology*, 12: 1–16. <https://doi.org/10.3389/fmicb.2021.822487>
- Martin BC, Gleeson D, Statton J, Siebers AR, Grierson P, Ryan MH, Kendrick GA, Martin BC. 2018. Low light availability alters root exudation and reduces putative beneficial microorganisms in seagrass roots. *Frontier in Microbiology*, 8: 2667. doi: 10.3389/fmicb.2017.026678
- Macías JM, Pournavab RF, Reyes-Valdés MH, Benavides-Mendoza A. 2014 Development of a rapid and efficient liquid chromatography method for determination of gibberellin A4 in plant tissue, with solid phase extraction for purification and quantification. *American*

- Journal of Plant Sciences, 5: 573-583.
<http://dx.doi.org/10.4236/ajps.2014.55072>
- Molina I, Weber K, Dos Santos DYAC, Ohlogge J. 2009. Transformation of a dwarf Arabidopsis mutant illustrates gibberellin hormone physiology and the function of a Green Revolution gene. *Biochemistry and Molecular Biology Education*, 37(3): 170-177.
<https://doi.org/10.1002/bmb.20263>
- Morais MC, Mucha Â, Ferreira H, Gonçalves B, Bacelar E, Marques G. 2019. Comparative study of plant growth-promoting bacteria on the physiology, growth and fruit quality of strawberry. *Journal of the Science of Food and Agriculture*, 99(12): 5341-5349.
<https://doi.org/10.1002/jsfa.9773>
- Muneer S, Gyeong Y, Soohoon P, Byoung K, Jeong R. 2017. Foliar or Subirrigation Silicon Supply Mitigates High Temperature Stress in Strawberry by Maintaining Photosynthetic and Stress-Responsive Proteins. *Journal of Plant Growth Regulation*, 36(4): 836-845.
<https://doi.org/10.1007/s00344-017-9687-5>
- Nakayama M, Nakazawa Y. 2023. Effects of environmental control and LED supplemental lighting on strawberry growth and yield in a subtropical climate. *Scientia Horticulturae*, 321: 112349.
<https://doi.org/10.1016/j.scienta.2023.112349>
- Nakayama M. 2025. Nighttime LED Supplemental Lighting Increases Production of Everbearing Strawberry in a Subtropical Climate. *The Horticulture Journal*, 94(3): 346-355. DOI
<https://doi.org/10.2503/hortj.SZD-019>
- Pajares S, Bohannan BJM. 2016. Ecology of nitrogen fixing, nitrifying, and denitrifying microorganisms in tropical forest soils. *Frontiers in Microbiology*, 7(JUL): 1-20.
<https://doi.org/10.3389/fmicb.2016.01045>
- Patel D, Patel A, Vora D, Menon S, Vadakan S, Acharya D, Goswami D. 2018. A resourceful methodology to profile indolic auxins produced by rhizo-fungi using spectrophotometry and HPTLC. *3 Biotech*, 8(10): 413. doi: 10.1007/s13205-018-
- Pérez-Romero LF, Stirling PJ, Hancock RD. 2024. Light-Emitting Diodes improve yield, quality and inhibitory effects on digestive enzymes of strawberry. *Scientia Horticulturae*, 332: 113192,
<https://doi.org/10.1016/j.scienta.2024.113192>
- Risanti RR, Hindersah R, Fitriatin BN, Suryatmana P, Maksum IP, Setiawati MR, Hanindipto FA, Nugraha GB. 2025. Exploring the Bacillus from vegetable rhizosphere for plant growth. *Journal of Ecological Engineering*, 26(1): 109-120.
<https://doi.org/10.12911/22998993/195286>
- Romiyadi, Putri NI. 2024. Uji Pertumbuhan dan Hasil Berbagai Varietas Tanaman Stroberi (*Fragaria sp.*) di Dataran Rendah. *Jurnal Greenation Pertanian Dan Perkebunan*, 2(2): 51-59. <https://doi.org/10.38035/jgpp.v2i2>
- Rueda D, Valencia G, Soria N, Rueda BB, Manjunatha B, Kundapur RR, Selvanayagam M. 2016. Effect of Azospirillum spp. and Azotobacter spp. on the growth and yield of strawberry (*Fragaria vesca*) in hydroponic system under different nitrogen levels. *Journal of Applied Pharmaceutical Science*, 6(1): 48-54.
<https://doi.org/10.7324/JAPS.2016.600108>
- Songsaeng A, Tittabutr P, Umnajkitikorn K, Boonkerd N. 2022. Application of Light-Emitting Diodes with Plant Growth-Promoting Rhizobacteria and Arbuscular Mycorrhiza. *Agronomy*, 2(2458). <https://doi.org/doi.org/10.3390/agronomy12102458>
- Smith BJ, Rezazadeh A, Stafne ET, Sakhanokho HF. 2022. Effect of light-emitting diodes, ultraviolet-b, and fluorescent supplemental greenhouse lights on strawberry plant growth and response to infection by the Anthracnose pathogen *Colletotrichum gloeosporioides*. *HortScience*, 57(8): 56 - 863. DOI: 10.21273/HORTSCI16591-22
- Tomic JM, Milivojevic JM, Pesakovic MI. 2015. The response to bacterial inoculation is cultivar-related in strawberries. *Turkish Journal of Agriculture and Forestry*, 39, 332-341. doi:10.3906/tar-1410-16
- Woomer PL. 1994. Most Probable Number Count. In Bottomley, S., Angle, C., & Weaver, R.W. (Eds.) *Methods of soil analysis Part 2. Soil Science Society of America*. pp 59-79
- Wu X, Han W, Yang Z, Zhang Y, Zheng Y. 2021. The difference in temperature between day and night affects the strawberry soluble sugar content by influencing the photosynthesis, respiration and sucrose phosphatase synthase. *Horticultural Science*, 48(4): 174-182.
<https://doi.org/10.17221/169/2020-HORTSCI>
- Yadav BK, Akhtar MS, Panwar J. 2015. Rhizospheric Plant-Microbe Interactions: Key Factors to Soil Fertility and Plant Nutrition. In: Arora, N. (eds) *Plant Microbes Symbiosis: Applied Facets*. Springer, New Delhi. https://doi.org/10.1007/978-81-322-2068-8_6. pp 127-145
- Hindersah R, Fitriyani F, Endo K, Nakayama M, Mubarak S, Nugraha GN. 2025. Effects of supplemental LED lighting and biofertilizer on yield and fruit quality of Japanese strawberry cultivars in Indonesia. *Jurnal Kultivasi*, 24(3): 353-363.

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The effect of phosphate fertilizer and organic matter on phosphorus status, pH, and cabbage yield in Andisols

Abstract Andisols are characterized by high phosphorus (P) retention, which often limits P availability for crops. This study aimed to analyze the independent and interactive effects of P fertilizer and organic matter (i.e. manure and rice straw compost) on P retention, total P, available P, P uptake, soil pH, and cabbage yield. This research was conducted in pot experiments in plastic house, applying P fertilizer (0, 90, 180, 270 kg ha⁻¹) and organic matter (20 kg ha⁻¹ of chicken, sheep, cow manures and rice straw compost) on Andisols planted with cabbage. The experiment used a factorial randomized design. The results showed no significant interaction between P fertilizer and organic matter for all measured parameters. However, individual applications of organic matter, particularly manure, significantly improved P uptake, soil pH₀, and cabbage yield, demonstrating its superior effectiveness compared to rice straw compost. Overall, manure application consistently outperformed rice straw compost in enhancing crop growth and P uptake. These findings underscore the importance of proper nutrient management strategies in Andisols to improve P availability and sustain optimal crop productivity.

Keywords: P uptake · Manure · Phosphate fertilizer · Andisols

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Introduction

Andisols, are distributed only approximately 0.8% of the earth's surface, yet they possess great potential for agricultural production (Anda et al., 2021; Hati et al., 2021). In Indonesia, for instance, many of most productive regions and densely populated area are located near active or dormant volcanoes. The unique physical, chemical, and mineralogical properties of these soils, such as their high porosity, dark color, and abundance of amorphous materials (including allophane, imogolite, and ferrihydrite), sesquioxides (oxides and hydroxides of aluminum and iron), and Al-humus complexes distinguish them from soils formed from other parent materials and largely account for their high productivity (Takahashi, 2020; Takahashi & Dahlgren, 2016). These soils are commonly situated in the agro-ecosystem zone and are commonly utilized for cultivating plantation crop and upland vegetable fields. However, the productivity of Andisols is often constrained by phosphorus deficiency despite their inherent fertility (Marpaung et al., 2023; Velásquez et al., 2016). Therefore, appropriate soil management strategies are essential to optimize their productivity and prevent degradation.

Phosphorus (P) in one of the macro nutrients required for plant growth, yet in Andisols the availability of P for plant is often low due to the strong sorption of P by non-crystalline aluminum (Al) and iron (Fe) that forms insoluble metal-phosphorus compounds (Gypser et al., 2018; Marpaung et al., 2022; Wang et al., 2019). The amount of P retention is also affected by pH of the system. Phosphate retention is generally highest in acidic to near-neutral conditions, pH 5.5-7, due to strong absorption of P onto iron and aluminum oxide. Under alkaline conditions (i.e. pH > 7) and very acidic conditions (i.e. pH < 5) P retention may decrease due to formation of Ca-phosphate and reduced adsorption by Fe and Al oxides, respectively (Gustafsson et al., 2012; Penn & Camberato, 2019).

The chemistry of Andisols reflects the influence of high amounts of variable charge. The term 'variable charge' refers to characteristic of organic and inorganic soil constituents that possess varied charge on the reactive surface depending on the pH, ionic concentration, and composition of the soil solution (Van Ranst et al., 2017). In variable charge soils, P retention can be reduced by lowering the zero point of charge

(ZPC). ZPC arises from the properties of amphoteric oxides, e.g., aluminum (hydr-)oxide, allophane, or other amorphous substances in clay fractions which are the main characters of variable charged clay. According to Uehara & Gillman (1981), a decrease in point of zero net point charge (PZNPC) may increase the surface negative charge and increase cation exchange capacity. This surface charge characteristic is important in soil management since the physicochemical process that control nutrient availability occur at the soil particle surface (Uehara & Gillman, 1981). In tropical soil, Fe and Al oxides are the sources of variable charge and important agents in controlling P retention and its availability in topsoil (Barrow, 2017).

The incorporation of organic matter (OM) into the soil is one way to lower PZNPC (Van Ranst et al., 2017) and it also well known to soil fertility and productivity (Tong et al., 2020). OM has a low PZNPC, about pH 4, thereby playing a role in lowering PZNPC of the soils. The adsorption of organic anion secreted from plant roots or produced from the decomposition of plant residue can lower PZNPC of variable charge soils through increasing negative surface charge and masking positive charge. In addition, OM, particularly manure, can be the source of macro and micro-nutrients including phosphorus (Fang et al., 2021; Goldan et al., 2023). These studies also underlined that manure applications can reduce the use of phosphorus fertilizer. Recent research showed an appropriate fertilizer, combination NPK and organic fertilizer, can improve P uptake (Chen et al., 2023; Gao et al., 2023). This positive outcome underscores the potency of organic fertilizer in addressing challenges related to the use of P fertilizer, considering the rising of fertilizer price and its limited availability faced by farmers.

Andisols are characterized by high phosphorus fixation which often limits P availability. Although numerous studies have reported the beneficial effects of phosphate fertilizers and organic amendments on chemical soil properties and crop yield, results remain inconsistent in Andisols. Several cases showed that the application of phosphate fertilizer or/and organic matter did not lead to the expected improvement in P availability due to its interaction with amorphous Al and Fe compounds (Mabagala & Mng'ong'o, 2022; Velásquez et al., 2016). In addition, the low phosphorus zero point of charge (pH₀)

characteristic of Andisols plays a critical role in regulating nutrient dynamics, as soil pH relative to pH_0 strongly influences surface charge properties. Information on how different doses and types of organic matter influence P dynamics, either independently or in interaction with phosphate fertilizer remains limited. Understanding these effects is essential. Therefore, there is a need to evaluate both the individual and interactive effects of phosphate fertilizer and diverse organic matter types applied at different rates to identify nutrient management strategies that can effectively improve P availability and cabbage yield in Andisols.

Material and Method

Research areas. The research was conducted in plastic house at the experimental garden, Faculty of Agriculture, Universitas Padjadjaran, West Java (6°55' 0.728" S, 107°46' 18.4606" E). The research area has annual temperature and precipitation were ± 25 °C and ± 1500 -2000 mm per year, respectively. The soil used in this experiment was classified as Andisols originated from Pangalengan (Herdiansyah et al., 2024). We used the top layer (at 30 cm depth) of Andisols Pangalengan which was formerly planted by potato (*Solanum tuberosum*).

Materials and Tools. The treatments applied organic fertilizer such as cow manure, chicken manure, sheep manure, and rice straw compost, and inorganic fertilizer such as SP-36 (36% P_2O_5) as well as basic fertilizer such as urea, ZA, and KCl fertilizers. Cabbage (*Brassica oleraceae* var Grand 11) were used as plant indicator. Laboratory equipments and chemical reagents used in this research were analytical balance, pH meter, pipette, mechanical shaker, centrifuge, volumetric flask, H_2SO_4 , H_2O_2 , ammonium molybdate, $HClO_4$, Bray P, KH_2PO_4 , and KCl or $CaCl_2$.

Experimental Design and Treatments. The experiment used a Factorial Randomized Block Design (RBD) consisting of two factors (P fertilizer and organic fertilizer) with different types and doses (nine treatments). The treatments were $a_0 = 0$ kg ha^{-1} $P_2O_5 = 0$ g polybag⁻¹ SP-36; a_1 90 kg ha^{-1} $P_2O_5 = 1.6$ g polybag⁻¹ SP-36; a_2 180 kg ha^{-1} $P_2O_5 = 3.1$ g polybag⁻¹ SP-36; a_3 270 kg ha^{-1} $P_2O_5 = 4.6$ g polybag⁻¹ SP-36; $b_0 =$ without organic matter; $b_1 = 20$ ton ha^{-1} cow manure; $b_2 = 20$ ton ha^{-1}

$b_3 = 20$ ton ha^{-1} sheep manure; $b_4 = 20$ ton ha^{-1} rice straw compost, and five replicates for each treatment, thus total samples were 45 samples.

Research Procedures. The Andisol from Pangalengan were prepared as planting medium for pot experiment after cleaning the soils from the grass, filtered with 2 mm sieve, then weighed approximately 10 kg of field moist for each polybag with size 40 x 50 cm. Each polybag was filled with the soil and organic fertilizer was added in accordance with the treatments, then the mixed soils were stirred homogeneously. Watering was done every day until it reached the field capacity then incubated. The length of incubation was two weeks. After the incubation period, P fertilizer was applied in accordance with the treatment, as well as basic fertilizer N (Urea + ZA) of 0.61 g and 1.52 g polybag⁻¹, respectively. Urea and ZA were given first, followed by KCl fertilizer about 1.22 g polybag⁻¹. Cabbage seedlings were transplanted 21 days after sowing, with one seedling planted per polybag.

Laboratory Analysis. Selected chemical properties were conducted to analyze the effect of the dose of P fertilizer and the type of organic matter. Soil pH was measured in H_2O with a glass electrode pH meter at 1:5 soil:solution ratio. Soil pH_0 was conducted using pH-drift method in which we identified the point where changing the solution ionic strength does not produce a corresponding change in solution pH. P retention was determined by the method of Blakemore et al. (1981). Total P, available P, and P uptake were analyzed following the procedure by ISRIC (Van Reeuwijk, 2002). At harvest, the gross weight of cabbage was measured immediately after cutting, including the head and outer wrapper leaves. The wrapper leaves were then removed manually, and the fresh crop weight of the cabbage head was weighed. All weights were recorded in digital balance and expressed in grams per plant. Some observations that were not tested statistically were visual growth of plants, plastic house temperature, pest attack, and elemental deficiency symptoms. Chemical properties of organic materials (i.e. cow manure, chicken manure, sheep manure, and rice straw) were added in Supplementary 1. Fertilizer efficiency (g g^{-1}) was calculated following the formula:

$$P \text{ recovery efficiency} = \frac{(P \text{ uptake treatment} - P \text{ uptake control})}{\text{applied P}} \quad (\text{Eq.1})$$

Data Analysis. Statistical data analysis was done using IBM SPSS statistics for Windows version. Analysis of variance (ANOVA) was used to determine the effects of P fertilizer and organic matter independently and their interaction on measured variables, i.e. P-retention, P-total, P-available, P-uptake, pH, pH_0 , crop weight and gross weight. When ANOVA indicated significant effects, mean comparisons were performed using Duncan Multiple Range Test (DMRT) at 5% level, after checking assumptions of normality and homogeneity of variances (transformations applied where necessary).

Results and Discussion

Initial Soil Properties. The same soil type, namely Andisol originated from Pangalengan, as used in the study by Herdiansyah et al. (2024) was employed in this experiment. According to their morphological soil description, the soil was classified as Typic Hapludands with dominant silt loam and silty clay loam at the depth of 0-30 cm, soil P available 11.2-13.3 ppm, CEC 14.84-35.72 me. 100 g^{-1} , K_2O 0.14-0.22 mg. 100 g^{-1} , and organic carbon between 3.99-4.17%.

The effect of P fertilizer on measured variables. The application of various doses of P fertilizer did not have significant effect on all measured variables (i.e. P-retention, P-total, P-available, P-uptake, pH, pH_0 , crop weight, and gross weight) (Table 1 and 2). We observed that the P-total content increased with increasing the dose of P fertilizer although not significant. Increasing the P-total after being treated is expected due to the nature of P in the soil that always leads to the unabsorbed P form (immobile). On the other hand, the available P content tended to decrease with increasing doses of P fertilizer. It is suspected that some of the available P form were absorbed by plant and part of P was unavailable (i.e., total P or P retention). We observed there was a little gap between initial P available (section 3.1) and control P available. The difference may be attributed to soil re-equilibration processes during incubation and plant growth.

The increase doses of P fertilizer did not have significant effect on P-uptake, though the amount tended to increase. The lack of significant

influence of additional P fertilizer on the content of these P forms stands in contrast to results reported in earlier studies (Liu et al., 2024; Velásquez et al., 2016). The reason may be due to amount of initial P levels or strong P fixation through sorption by amorphous Fe or Al oxides so added P can be rapidly fixed into stable forms, preventing increases in available P, total P, or P uptake, which is common in Andisols (Gonzalez-Rodriguez & Fernandez-Marcos, 2018; Redel et al., 2016). We observed that the P-retention was between 55-64% (Table 1), which is lower than the typical Andisols ($\geq 85\%$). It may be attributed to a combination of methodological differences and intrinsic soil properties such as soil-solution ratio, short-range-order mineral content, organic matter-Al complexation, and legacy phosphorus saturation. Nevertheless, P retention values exceeding 60% remained within the high category, confirming the strong P-fixing nature of the Andisols studied. In addition, the limited response of P fertilizer on P form can be due to inadequate amount of P fertilizer (the highest treatment dose was $270\text{ kg ha}^{-1}\text{ P}_2\text{O}_5$) and narrow range of doses application. As an illustration, farmers in Pangalengan, based on their experience, applied P fertilizer with dose up to 360 kg ha^{-1} for cabbage plants.

The results indicated that P fertilization had no significant effect on soil pH which contrasts with findings reported in previous studies (Qaswar et al., 2020; von Tucher et al., 2018). It is possibly due to high buffering soil capacity, e.g. volcanic ash soils in Andisols or fertilizer application rate that was too low to influence pH. Regarding to pH_0 , the addition of P fertilizer to variable charged soils can increase negative surface charge which tends to decrease pH_0 . However, since Andisols have strong buffering of surface charge, addition of P fertilizer may not change the pH_0 . Furthermore, the absence of a significant effect of P fertilization on crop weight likely reflects adequate initial soil P availability, strong P fixation reducing plant uptake, and the possibility that other environmental or nutritional factors limited crop growth more than P supply. Previous studies found that additional P fertilizer with lime could strongly increase the crop weight (Dejene et al., 2023; Qaswar et al., 2020).

Table 1. Effect of phosphorus (P) fertilizer and organic material on average of P-retention, P-total, P-available, and P-Uptake

	P-Retention %	P-Total mg 100g ⁻¹	P-Available ppm	P-Uptake mg pot ⁻¹
P Fertilizer				
a ₀ (0 kg ha ⁻¹ P ₂ O ₅)	61.3 a	197.94 a	7.45 a	1387 a
a ₁ (90 kg ha ⁻¹ P ₂ O ₅)	63.0 a	197.53 a	8.84 a	1505 a
a ₂ (180 kg ha ⁻¹ P ₂ O ₅)	62.1 a	211.53 a	5.84 a	1433 a
a ₃ (270 kg ha ⁻¹ P ₂ O ₅)	61.7 a	202.02 a	6.81 a	1821 a
Organic Matter				
b ₀ (without organic matter)	62.9 a	206.14 a	7.06 a	1135 c
b ₁ (20 ton ha ⁻¹ cow manure)	62.4 a	195.30 a	6.02 a	1457 bc
b ₂ (20 ton ha ⁻¹ chicken manure)	64.8 a	208.31 a	6.36 a	1824 ab
b ₃ (20 ton ha ⁻¹ sheep manure)	55.8 a	201.48 a	7.32 a	2063 a
b ₄ (20 ton ha ⁻¹ rice straw compost)	64.3 a	200.06 a	9.42 a	1204 c

Note: Means followed by the same letter are not significantly different according to Duncan's multiple range test at the 5% significance level.

Table 2. Effect of phosphorus (P) fertilizer and organic material on average of pH, pH₀, crop weight, and gross weight

	pH	pH ₀	Crop weight g	Gross Weight g
P Fertilizer				
a ₀ (0 kg ha ⁻¹ P ₂ O ₅)	5.07 a	4.22 a	79.04 a	203.85 a
a ₁ (90 kg ha ⁻¹ P ₂ O ₅)	5.07 a	4.26 a	87.33 a	206.71 a
a ₂ (180 kg ha ⁻¹ P ₂ O ₅)	5.07 a	4.29 a	86.29 a	201.16 a
a ₃ (270 kg ha ⁻¹ P ₂ O ₅)	5.08 a	4.25 a	91.45 a	202.31 a
Organic matter				
b ₀ (without organic matter)	5.15 a	4.18 b	55.62 c	246.77 c
b ₁ (20 ton ha ⁻¹ cow manure)	5.15 a	4.30 a	77.14 b	287.86 b
b ₂ (20 ton ha ⁻¹ chicken manure)	5.16 a	4.27 ab	117.09 a	398.29 a
b ₃ (20 ton ha ⁻¹ sheep manure)	5.11 a	4.30 a	112.86 a	386.18 a
b ₄ (20 ton ha ⁻¹ rice straw compost)	5.17 a	4.23 ab	67.42 bc	281.94 b

Note: Means followed by the same letter are not significantly different according to Duncan's multiple range test at the 5% significance level.

The Effect of Organic Matter on Measured Variables. The results showed that the type of organic matter was seen to significantly affect P-uptake ($p < 0.05$), but not on P-retention, P-total, and P-available. The highest P-uptake was observed after applying sheep manure ($p < 0.05$), yet the carbon content on the sheep manure was 37.3%, lower than cow and rice straw compost (i.e. 65.2 and 47.7%, respectively) (Supplementary 1). Although sheep manure did not exhibit higher carbon content, P availability, or a lower C:P ratio compared with other organic amendments, its application resulted in the highest P uptake. This response suggests that P uptake in Andisols is governed not only by bulk soil P availability but also by rhizosphere processes and manure-soil interactions. In general, organic matter can

improve P-uptake efficiency by enhancing root growth, microbial activity, and P mobilization around the root zone (Poblete-Grant et al., 2025), even if the total measurable P in the bulk soil does not change. The increase of P-uptake can be due to the release of low molecular weight organic acids, e.g. citrate, oxalate, malate that bind strongly to Fe or Al oxides in Andisols releasing P from P-fixation. This result was in line with previous studies (Devaraja et al., 2021; Rahim et al., 2023). On the other hand, the lack of significant effect on P-available, P-total, or P-retention after organic matter application might be because plants immediately absorb P (i.e. real-time rhizosphere P), but not in bulk soil. Sheep and chicken manures was reported to increase phosphorus uptake because they contain more

phosphorus and release more rapid compared to cow manure (Mahmood et al., 2017). While beneficial for long-term soil organic matter, cow manure releases nutrients more slowly and is less effective for immediate crop P needs.

In general, the addition of organic fertilizer increased pH_0 (Table 2) than control b_0 , particularly cow and sheep manure ($p < 0.05$). This result was unexpected since organic matter generally tend to decrease pH_0 (Van Ranst et al., 2017). The rise of pH_0 after organic matter application is possibly due to the addition of basic cations (Ca^{2+} , Mg^{2+} , K^+) that increases soil positive charges and reduces the effective negative charge density of soil colloids. These processes shift the soil's point of zero charge to a higher pH. In variable-charge soils dominated by allophanic materials and Fe or Al oxides, this process can override the negative charge contribution from decomposed organic matter, thus an overall increase in pH_0 . Additionally, manure application induces the protonation of organic anions from the dissociation of weakly acidic functional groups on organic matter to form neutral molecules and this process can increase soil pH buffering capacity as some of exchangeable base cations release to soil solution, avoiding the soil from acidification (Shi et al., 2019). Furthermore, the crop weight was found to be significantly different after chicken and sheep manure applications than other treatments and control. This finding was in line with previous study which reported a moderate to high crop yield after chicken and sheep manure applications than cow manure (Mahmood et al., 2017; Mokgolo et al., 2019; Mukhtiar et al., 2018). Chicken and sheep manure significantly increase crop yield because they generally contain higher concentration of essential nutrients and decompose more rapidly. The application also improves soil chemical condition, i.e. P-uptake, as it was consistent with the increase of P-uptake in this research (Mahmood et al., 2017). In contrast, cow manure generally decomposes more slowly due to high fiber content and provides limited nutrient release, that possibly causes less crop yield than chicken manure.

The research also showed that manure fertilizers outperformed rice straw compost, as the rice straw compost application had less P content and crop yield (Table 1 and 2). Similar finding was reported by Dhaliwal et al. (2023). Manure fertilizer are generally more effective than straw compost,

especially in the short term, possibly due to faster nutrient mineralization (Zhao et al., 2024).

The Interaction Effect Between P Fertilizer and Organic Matter on Measured Variables. The interaction of P fertilizer and organic matter showed no significant effect with all the measured variables, i.e. P-retention, P-uptake, P-available, P-uptake, pH, pH_0 , crop yield, and gross weight. These results was in contrast to most studies that documented the improve soil pH, the availability of phosphorus, and the increase of crop yield after application of organic matter and P fertilizer (Jamal et al., 2023; Jin et al., 2023). The lack of significant effect after applying P fertilizer and organic matter indicating that both inputs may influence soil properties through independent mechanisms without producing synergistic or antagonistic effects. Nevertheless, each factor exhibited a significant individual effect (i.e. organic matter application increases P-uptake, crop yield, gross weight) which reflects their distinct pathways in modifying phosphorus dynamic in Andisols. This finding is consistent with Abbasi et al. (2015) who observed that the combined application of poultry manure, insoluble-rock phosphate, and phosphate-solubilizing bacteria resulted in growth, yield, and P uptake comparable to those obtained with individual treatments.

Fertilizer Phosphorus Efficiency. Chicken and sheep manures were shown to have the highest efficiency than other organic materials such as cow manure and rice straw compost (Fig.1). These types of manure contain P in a form that is more readily to plant, and this P is rapidly released (Mahmood et al., 2017). The chicken and sheep manures generally increase microbial biomass, stimulate phosphatase enzymes, and promote biological P cycling leading to higher P-uptake by the crop. We also found that the highest efficiency was with the dose of 90 kg ha^{-1} P in all different types of manure, and the efficiency decreased with higher doses. This result likely reflects the legacy effects of long-term phosphorus inputs. Repeated fertilization over time can lead to the accumulation of phosphorus in moderately labile and stable pools, thereby reducing crop responsiveness to additional P. Consequently, lower P applications may have better match to current crop demand, while higher rates predominantly contribute to non-productive P pools, leading to reduced fertilizer efficiency.

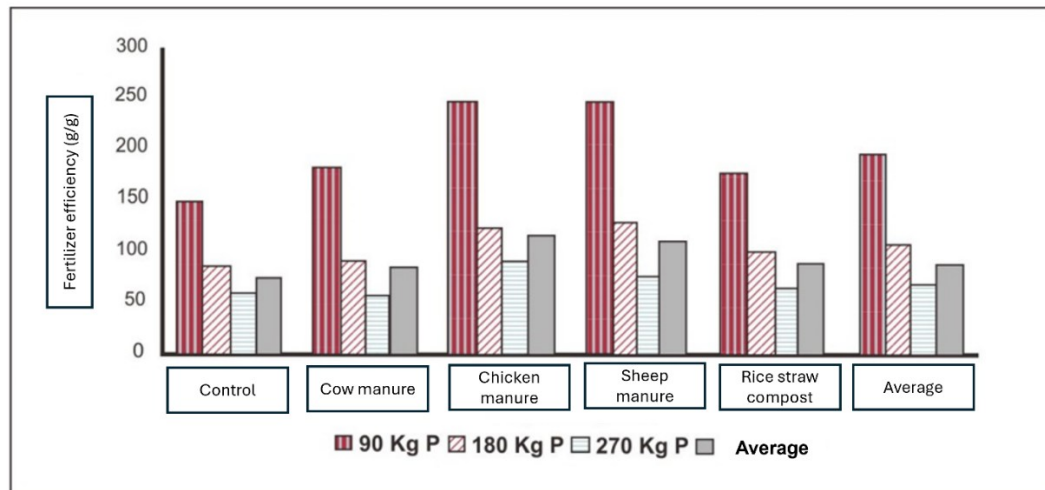


Figure 1. Phosphorus fertilizer efficiency (g g^{-1}) under different organic amendment and phosphorus application rates

Conclusions and Recommendation

Application of P fertilizer and organic matter on Andisols did not produce any significant interaction on P retention, total P, available P, P uptake, soil pH, and cabbage yield. However, each input, i.e. organic matter application, showed independent effect. Among the organic amendments tested, manure fertilizer significantly improved P uptake, increased soil pH₀, and enhanced cabbage yield. The highest efficiency of P fertilizer was at the dose of 90 kg ha⁻¹, underlining moderate P application rates over high inputs to maximize fertilizer efficiency. Overall, manure fertilizer performed better than rice straw compost in improving soil fertility and crop performance on Andisols. This highlights the critical importance of adopting effective nutrient management strategies in Andisols to optimize phosphorus availability and sustain high crop productivity.

References

- Abbasi MK, Musa N, Manzoor M. 2015. Mineralization of soluble P fertilizers and insoluble rock phosphate in response to phosphate-solubilizing bacteria and poultry manure and their effect on the growth and P utilization efficiency of chilli (*Capsicum annum* L.). *Biogeosciences*, 12: 4607–4619. <https://doi.org/10.5194/bg-12-4607-2015>
- Anda M, Kasno A, Ginting CB, Barus PA, Purwanto S. 2021. Response of Andisols to intensive agricultural land use: Implication on changes in P accumulation and colloidal surface charge. *IOP Conf. Ser. Earth Environ. Sci.*, 648. <https://doi.org/10.1088/1755-1315/648/1/012016>
- Barrow NJ, 2017. The effects of pH on phosphate uptake from the soil. *Plant Soil*, 410: 401–410. <https://doi.org/10.1007/s11104-016-3008-9>
- Blakemore LC, Searle PL, Daly BK. 1981. Methods for chemical analysis of soils. <https://doi.org/10.1111/aos.12317>
- Chen M, Zhang S, Liu L, Ding X. 2023. Influence of organic fertilization on clay mineral transformation and soil phosphorous retention: Evidence from an 8-year fertilization experiment. *Soil Tillage Res.*, 230: 105702. <https://doi.org/10.1016/j.still.2023.105702>
- Dejene M, Abera G, Desalegn T. 2023. The Effect of Phosphorus Fertilizer Sources and Lime on Acidic Soil Properties of Mollic Rhodic Nitisol in Welmera District, Central Ethiopia. *Appl. Environ. Soil Sci.*, 2023: 7002816. <https://doi.org/10.1155/2023/7002816>
- Devaraja M, Bavaraj K, Kumar M, Rajesh NL. 2021. Effect of phosphorus enriched organic manures on p-uptake and p-availability to onion crop in Vertisol. *Int. J. Chem. Stud.*, 9: 270–273. <https://doi.org/10.22271/chemi.2021.v9.i2d.11822>
- Dhaliwal SS, Sharma V, Verma V, Kaur M, Singh P, Gaber A, Laing AM, Hossain A, 2023. Impact

- of manures and fertilizers on yield and soil properties in a rice-wheat cropping system. *PLoS One*, 18: 1–21. <https://doi.org/10.1371/journal.pone.0292602>
- Fang P, Abler D, Lin G, Sher A, Quan Q. 2021. Substituting organic fertilizer for chemical fertilizer: Evidence from apple growers in china. *Land*, 10. <https://doi.org/10.3390/land10080858>
- Gao P, Zhang T, Lei X, Cui X, Lu Y, Fan P, Long S, Huang J, Gao J, Zhang Z, Zhang, H. 2023. Improvement of soil fertility and rice yield after long-term application of cow manure combined with inorganic fertilizers. *J. Integr. Agric.* <https://doi.org/10.1016/j.jia.2023.02.037>
- Goldan E, Nedef V, Barsan N, Culea M, Panainte-Lehadus M, Mosnegutu E, Tomozei C, Chitimus D, Irimia O. 2023. Assessment of Manure Compost Used as Soil Amendment – A Review. *Processes*, 11: 1–16. <https://doi.org/10.3390/pr11041167>
- Gonzalez-Rodriguez S, Fernandez-Marcos ML. 2018. Phosphate sorption and desorption by two contrasting volcanic soils of equatorial Africa. *PeerJ*, 2018: 1–14. <https://doi.org/10.7717/peerj.5820>
- Gustafsson JP, Mwamila LB, Kergoat K. 2012. The pH dependence of phosphate sorption and desorption in Swedish agricultural soils. *Geoderma*, 189–190: 304–311. <https://doi.org/10.1016/j.geoderma.2012.05.014>
- Gypser S, Hirsch F, Schleicher AM, Freese D. 2018. Impact of crystalline and amorphous iron- and aluminum hydroxides on mechanisms of phosphate adsorption and desorption. *J. Environ. Sci. (China)*, 70: 175–189. <https://doi.org/10.1016/j.jes.2017.12.001>
- Hati DP, Gani RA, Mulyani A, Husnain. 2021. Differences in Andisols properties as affected by horticulture land use and pine forest in Lembang Sub District, West Java. *IOP Conf. Ser. Earth Environ. Sci.*, 648. <https://doi.org/10.1088/1755-1315/648/1/012009>
- Herdiansyah G, Arifin M, Suriadikusumah A, Herawati A. 2024. Assessment of Andisol characteristics for the development of potato crops in Pangalengan. *J. Trop. Soils*, 30: 45–52. <https://doi.org/10.5400/jts.2025.v30i1.45-52>
- Jamal A, Saeed MF, Mihoub A, Hopkins BG, Ahmad I, Naeem A. 2023. Integrated use of phosphorus fertilizer and farmyard manure improves wheat productivity by improving soil quality and P availability in calcareous soil under subhumid conditions. *Front. Plant Sci.*, 14: 1–12. <https://doi.org/10.3389/fpls.2023.1034421>
- Jin Y, Zhang N, Chen Y, Wang Q, Qin Z, Sun Z, Zhang S. 2023. Quantitative Evaluation of the Crop Yield, Soil-Available Phosphorus, and Total Phosphorus Leaching Caused by Phosphorus Fertilization: A Meta-Analysis. *Agronomy*, 13. <https://doi.org/10.3390/agronomy13092436>
- Liu X, Zhang Y, Wang Z, Chen Z. 2024. The contribution of organic and chemical fertilizers on the pools and availability of phosphorus in agricultural soils based on a meta-analysis. *Eur. J. Agron.*, 156: 127144. <https://doi.org/10.1016/j.eja.2024.127144>
- Mabagala FS, Mng'ong'o ME, 2022. On the tropical soils; The influence of organic matter (OM) on phosphate bioavailability. *Saudi J. Biol. Sci.*, 29: 3635–3641. <https://doi.org/10.1016/j.sjbs.2022.02.056>
- Mahmood F, Khan I, Ashraf U, Shahzad T, Hussain S, Shahid M, Abid M, Ullah S. 2017. Effects of organic and inorganic manures on maize and their residual impact on soil physico-chemical properties. *J. soil Sci. plant Nutr.*, 17: 22–32.
- Marpaung AE, Karo B, Barus S, Hutabarat RC, Tarigan R. 2022. Application of organic fertilizer and phosphate solubilizing bacteria to increase the growth and yield of potatoes in Andisol. *Kultivasi* 21, 261–269. <https://doi.org/10.24198/kultivasi.v21i3.41485>
- Marpaung AE, Susilowati DN, Sopha GA, Siagian DR, Girsang SS, Tarigan R, Marpaung IS, Silitonga TF, Sabrina T, Rauf A, Karo B, Hutabarat RC, Barus S. 2023. The role of rhizosphere microbes as phosphate solubilizing bio fertilizers in shallot: a review. *IOP Conf. Ser. Earth Environ. Sci.*, 1255: 0–12. <https://doi.org/10.1088/1755-1315/1255/1/012003>
- Mokgolo MJ, Mzezewa J, Odhiambo JJO. 2019. Poultry and cattle manure effects on sunflower performance, grain yield and selected soil properties in Limpopo Province, South Africa. *S. Afr. J. Sci.*, 115: 1–7. <https://doi.org/10.17159/sajs.2019/6410>
- Mukhtiar A, Waqar A, Khalil M, Tariq M, Muhammad S, Hussain A, Kamal A. 2018. Evaluating the Potential Organic Manure for

- Improving Wheat Yield and Quality under Agro-Climatic Conditions of Pakistan. *Adv. Crop Sci. Technol.*, 06: 4–7. <https://doi.org/10.4172/2329-8863.1000349>
- Penn CJ, Camberato JJ. 2019. A critical review on soil chemical processes that control how soil pH affects phosphorus availability to plants. *Agric.* 9: 1–18. <https://doi.org/10.3390/agriculture9060120>
- Poblete-Grant P, Parra-Almuna L, Pontigo S, Rumpel C, Mora M de LL, Cartes P. 2025. Mechanisms Involved in Soil-Plant Interactions in Response to Poultry Manure and Phytase Enzyme Compared to Inorganic Phosphorus Fertilizers. *Agronomy*, 15. <https://doi.org/10.3390/agronomy15030660>
- Qaswar M, Dongchu L, Jing H, Tianfu H, Ahmed W, Abbas M, Lu Z, Jiangxue D, Khan ZH, Ullah S, Huimin Z, Boren W. 2020. Interaction of liming and long-term fertilization increased crop yield and phosphorus use efficiency (PUE) through mediating exchangeable cations in acidic soil under wheat-maize cropping system. *Sci. Rep.*, 10: 1–12. <https://doi.org/10.1038/s41598-020-76892-8>
- Rahim N, Yasin Z, Tahir MM, Majeed A, Yaqub A, Mahmood B. 2023. Rock Phosphate and Manures with Phosphorus Solubilizing Bacteria Increases the Growth, Yield and Phosphorus Uptake of Okra (*Abelmoschus esculentus*). *J. Appl. Res. Plant Sci.*, 5: 111–118. <https://doi.org/10.38211/joarps.2024.05.226>
- Redel Y, Cartes P, Velásquez G, Poblete-Grant P, Poblete-Grant P, Bol R, Mora ML. 2016. Assessment of phosphorus status influenced by Al and Fe compounds in volcanic grassland soils. *J. Soil Sci. Plant Nutr.*, 16: 490–506. <https://doi.org/10.4067/S0718-95162016005000041>
- Shi R-Y, Liu Z-D, Li Y, Jiang T, Xu M, Li J-Y, Xu, R-K. 2019. Mechanisms for increasing soil resistance to acidification by long-term manure application. *Soil Tillage Res.*, 185: 77–84. <https://doi.org/10.1016/j.still.2018.09.004>
- Takahashi T. 2020. The diversity of volcanic soils: focusing on the function of aluminum-humus complexes. *Soil Sci. Plant Nutr.*, 66: 666–672. <https://doi.org/10.1080/00380768.2020.1769453>
- Takahashi T, Dahlgren RA. 2016. Nature, properties and function of aluminum-humus complexes in volcanic soils. *Geoderma*. <https://doi.org/10.1016/j.geoderma.2015.08.032>
- Tong L, Zhu L, Lv Y, Zhu K, Liu X, Zhao R. 2020. Response of organic carbon fractions and microbial community composition of soil aggregates to long-term fertilizations in an intensive greenhouse system. *J. Soils Sediments*, 20: 641–652. <https://doi.org/10.1007/s11368-019-02436-x>
- Uehara G, Gillman G. 1981. The mineralogy, chemistry, and physics of tropical soils with variable charge clays. Westview Press, Inc., Colorado.
- Van Ranst E, Qafoku NP, Noble A, Xu R. 2017. Variable Charge Soils: Mineralogy and Chemistry, in: *Encyclopedia of Soil Science*, Third Edition. CRC Press, pp. 2432–2439. <https://doi.org/10.1081/E-ESS3-120053756>
- Van Reeuwijk L. 2002. Procedures for soil analysis, 4th ed, Technical paper. International Soil Reference and Information Centre (ISRIC), Wageningen. [https://doi.org/10.1016/0196-0709\(89\)90091-4](https://doi.org/10.1016/0196-0709(89)90091-4)
- Velásquez G, Calabi-Floody M, Poblete-Grant P, Rumpel C, Demanet R, Condrón L, Mora ML. 2016. Fertilizer effects on phosphorus fractions and organic matter in Andisols. *J. Soil Sci. Plant Nutr.*, 16: 294–304. <https://doi.org/10.4067/S0718-95162016005000024>
- von Tucher S, Hörndl D, Schmidhalter U. 2018. Interaction of soil pH and phosphorus efficacy: Long-term effects of P fertilizer and lime applications on wheat, barley, and sugar beet. *Ambio* 47: 41–49. <https://doi.org/10.1007/s13280-017-0970-2>
- Wang X, Phillips BL, Boily JF, Hu Y, Hu Z, Yang P, Feng X, Xu W, Zhu M. 2019. Phosphate sorption speciation and precipitation mechanisms on amorphous aluminum hydroxide. *Soil Syst.*, 3: 1–17. <https://doi.org/10.3390/soilsystems3010020>
- Zhao Z, Yang Y, Xie H, Zhang Y, He H, Zhang X, Sun S. 2024. Enhancing Sustainable Agriculture in China: A Meta-Analysis of the Impact of Straw and Manure on Crop Yield and Soil Fertility. *Agric.*, 14. <https://doi.org/10.3390/agriculture14030480> Supplementary 1. Results of organic fertilizers analysis

Amalia I · Qosim WA · Kusumiyati

Effect of nano edible coating, storage temperature, and packaging on water content, weight loss, and color on oyster mushrooms (*Pleurotus ostreatus*)

Abstract. Oyster mushrooms are one of the most commonly consumed mushrooms due to their high nutritional content, including protein, fiber, vitamins, and minerals, as well as their antioxidant properties. However, these mushrooms are easily damaged by microbes and poor post-harvest handling, which affects their quality. The combination of nano-edible coating, packaging, and storage temperature offers a solution to these issues. This study aims to evaluate the effects of nano-edible coating, packaging, and storage temperature on water content, weight loss, and the color (L^* , a^* , and b^*) of oyster mushrooms. The study was conducted at the Horticulture Laboratory, Faculty of Agriculture, Universitas Padjadjaran. The experimental design used was a Completely Randomized Block Design (CRBD) with 19 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 38 experimental units, each consisting of three mushrooms weighing 100 g, with fresh white color, no physical damage, and uniform shape and weight. This resulted a total of 114 mushroom samples. The results showed that the combination of nano edible coating treatment, packaging, and storage temperature significantly affected the water content, weight loss, and color parameters (L^* , a^* , b^*) of mushrooms at 3, 6, and 9 DAT. The combination of nano sodium alginate and nano aloe vera, when vacuum-packaged at 5 °C, produced the best results for water content, weight loss, and colour. These results indicated the potential of the treatment to maintain the quality of oyster mushrooms during storage.

Keywords: *Pleurotus ostreatus* · nano sodium alginate · nano aloe vera · packaging · yield quality

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Introduction

Oyster mushrooms (*Pleurotus ostreatus*) are a popular type of mushroom. Besides their delicious taste, oyster mushrooms serve as functional foods, significantly enhancing the nutritional value of foods (Effiong et al., 2024). Oyster mushrooms have a high carbohydrate content and are considered a functional food, containing various oligosaccharides, ergothioneine, and mono- and disaccharides that function as prebiotics and are very beneficial for maintaining a healthy gut microbiome (Li et al., 2015; Mishra et al., 2022).

Oyster mushrooms are high in protein, fiber, vitamins, minerals, and antioxidants. Oyster mushrooms are low in fat and calories, making them very suitable for a healthy eating pattern (Sassine et al., 2022). Oyster mushrooms are native to China and are now distributed worldwide, except in the Northwest Pacific region due to Arctic conditions. Oyster mushrooms cultivation methods developed rapidly in Germany during World War I and were later successfully implemented on a large scale (Piska et al., 2017). Despite their nutritional value and health benefits, oyster mushrooms also face significant challenges in production and distribution: their short shelf life due to their high-water content makes them susceptible to microbial spoilage and oxidation (Sassine et al., 2022).

Nano-edible coating technology has been developed as an innovative solution. Nano-edible coating is a thin layer based on natural ingredients applied to the surface of food using nano particles. This technology not only extends shelf life but also maintains the nutritional quality, flavor, and texture of oyster mushrooms (Sassine et al., 2022).

The main ingredients used in edible coating technology are sodium alginate and aloe vera. Sodium alginate has the potential to maintain the quality and extend the shelf life of horticultural products by inhibiting water loss and gas exchange (Zhu et al., 2019). Meanwhile, the gel (mucilage) obtained from aloe vera leaves is a polysaccharide-based coating material with anti-browning, antioxidant, and antifungal properties due to its anthraquinone and saponin content (Raghav et al., 2016). This mucilage has low barrier properties against moisture loss in some fruits (Mohammadi et al., 2021). Storing mushrooms at low temperatures is also an effort

to maintain mushroom quality and reduce physiological damage. For example, mushrooms stored at 3°C will maintain their appearance in good condition for up to 7 days. This is because low temperatures can slow the respiration rate and microbial activity in oyster mushrooms (Rahman et al., 2020).

One way to solve postharvest handling issues is through packaging. Proper packaging can protect fresh oyster mushrooms from environmental influences (sunlight and humidity). Many types of packaging are used to store fresh produce, such as mushrooms. Currently, most food products are packaged using plastic materials (Mohammadi et al., 2022).

Vacuum-packaged mushrooms in plastic have the advantage of extending their shelf life. Vacuum plastic packaging allows for the air circulation necessary for respiration, thus preventing the buildup of water vapor. Vacuum packaging can also inhibit the activity of microorganisms, which is the primary cause of softening (hardening) of oyster mushrooms (Aftukha & Purbasari, 2021). In addition to vacuum plastic packaging, biodegradable plastic packaging offers another alternative for short-term storage and helps reduce the environmental risks associated with conventional, non-degradable plastics (Shaikh et al., 2021).

The combination of nano-edible coating and packaging, along with storage temperature, is essential for maintaining postharvest oyster mushroom quality. Several studies, such as those showing that a nano-edible coating of sodium alginate is effective at forming a barrier against water loss (Chen et al. 2021) and those showing that aloe vera gel has strong antimicrobial properties (Nishant et al., 2023), have separately extended the shelf life of mushrooms. However, studies systematically combining both types of nano-edible coating (nano-sodium alginate and nano-aloe vera) with various packaging types (vacuum, wrap, and biodegradable) and storage temperatures (5 °C, 10 °C, and 25 °C) remain very limited. Postharvest handling of oyster mushrooms, along with proper temperature control, is crucial to maintaining their quality. Therefore, this study aims to evaluate the effects of combinations of nano-edible coating types, packaging, and storage temperature on the quality of oyster mushrooms. Identify the most effective treatments for the quality and shelf life of oyster mushrooms. It is hoped that this research will contribute to scientific

understanding of the synergistic mechanisms underlying the most effective combination treatments for maintaining the quality and extending the shelf life of post-harvest oyster mushrooms.

Materials and Methods

This research was conducted at the Horticulture Laboratory, Faculty of Agriculture, Universitas Padjadjaran, from August 2024 to May 2025. The materials used in this research included oyster 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% aluminium chloride, 2 M sodium acetate solution, distilled water, and quercetin. The tools used include an oven (Memmert, UM 300, Germany); analytical balance (Mettler Toledo, Switzerland); reflectance spectrophotometer (Konica Minolta, CM-600D, Japan); digital scales; thermo recorder; UV-Vis spectrophotometer (Shimadzu, Uv-1601, Japan); centrifuge; sonicator; pipette; measuring cup; 50 mL test tube; vacuum and sealer; knife; cutting board; grinder; water bath; and 10 mL glass bottle, cooler, refrigerator, and freezer.

The experiment design used a Completely Randomized Block Design (CRBD) with a combination of nano edible coating treatments consisting of nano sodium alginate (NSA) and nano aloe vera (NAV); packaging (biodegradable plastic, wrap plastic, and vacuum plastic), and storage temperatures (± 25 °C, 10 °C, and 5 °C). A total of 18 treatment combinations and one control without treatment (without coating and packaging, stored at room temperature) were each replicated twice, resulting 38 experimental units. Each unit consisting of 3 mushrooms (100 g) was observed at 0, 3, 6, and 9 days after treatment (DAT). The combination treatments are as follows:

- P0: Control (without coating treatment, packaging, storage 25 °C)
- P1: NSA + 25 °C + vacuum
- P2: NSA + 25 °C + wrap
- P3: NSA + 25 °C + biodegradable
- P4: NSA + 10 °C + vacuum
- P5: NSA + 10 °C + wrap
- P6: NSA + 10 °C + biodegradable
- P7: NSA + 5 °C + vacuum
- P8: NSA + 5 °C + wrap
- P9: NSA + 5 °C + biodegradable

- P10: NAV + 25 °C + vacuum
- P11: NAV + 25 °C + wrap
- P12: NAV + 25 °C + biodegradable
- P13: NAV + 10 °C + vacuum
- P14: NAV + 10 °C + wrap
- P15: NAV + 10 °C + biodegradable
- P16: NAV + 5 °C + vacuum
- P17: NAV + 5 °C + wrap
- P18: NAV + 5 °C + biodegradable

The data were analyzed using the F-test to assess the treatment effect, followed by the Scott-Knott test to compare treatments at the 5% level. The analysis was performed using Microsoft Excel software equipped with the Smart Stat XL add-in.

The observation parameters included water content, weight loss, and color. The measurements were conducted on days 0, 3, 6, and 9 DAT. Water content determination is in accordance with SNI 01-2891-1992. Samples (4 g) were weighed to obtain the initial weight, dried in an oven at 105 °C for 3 hours, cooled in a desiccator, and reweighed until the weight was constant. Water content (%) was calculated using the equation:

$$\text{Water content (\%)} = \frac{(W_{\text{wet}} - W_{\text{dry}})}{W_{\text{dry}}} \times 100$$

W_{wet} = wet weight, W_{dry} = dry weight

Weight loss was observed by weighing mushrooms using an analytical balance to determine weight loss during storage. Mushroom weight loss was calculated using the equation:

$$W = \frac{(m_i - m_t)}{m_i} \times 100$$

W = weight loss, m_i = initial weight (g), m_t = final weight (g)

Color was measured on the top and bottom of oyster mushrooms (3 body mushroom/sample) using a spectrophotometer reflectance (Konica Minolta, CM-600D, Japan) to obtain the values of L^* , a^* , and b^* (Cavusoglu et al., 2021).

The oyster mushrooms, white, clean, and unpolluted, are damaged and then stored in a container with each weighing 100 g which has been coated 1% edible nano coating, made of Aloe vera and sodium alginate (300 nm),

provided by the Functional Nano University Centre of Excellence (FiNder U-CoE), Universitas Padjadjaran. The coating method is carried out by pouring nano sodium alginate and nano aloe vera powder evenly over the sieve onto the mushrooms that have been stored in the container, then brushing the powder over the surface of the mushroom fruit body evenly, let it sit for 5 - 10 minutes, then packing some of the mushrooms using plastic wrapping with a styrofoam *base* tightly, some mold packed inserted into biodegradable plastic, tied securely meeting and some mold again packed put in plastic vacuum. The oyster mushrooms have been packed in cans and stored at room temperature ($\pm 25^\circ\text{C}$), 5°C , and 10°C . Meanwhile, the oyster mushrooms treatment control, without coating and packaging, was stored at $\pm 25^\circ\text{C}$ on a bamboo-based tray lined with HVS paper.

Results and Discussion

Water content. Water content is a critical indicator of oyster mushroom quality. Based on Table 1, observations of water content in oyster mushrooms on days 0, 3, 6, and 9 DAT showed that water content decreased over time. Observations on day 0 DAT showed not significant differences between treatments based on anova (data not shown). The highest variation in water content was found in treatment P7 (NSA + 5°C + wrap) at 99.98%, while the lowest was

found in treatment P12 (NSA + 25°C + biodegradable) at 80.85 %. This indicated that oyster mushrooms naturally have a fairly high-water content. Observation on day 3 DAT showed different results across all treatments. Based on the Scott-Knot test at the 5% level, the treatments were grouped into two groups based on significant differences in water content. Treatment at room temperature (25°C) is marked with the notation 'a' (first group), namely P0, P1, P2, P3, P10, P11, P12, which have a lower water content value of 80.64 - 84.47% with faster water loss marked by the white color of the oyster mushrooms becoming less fresh and slightly brownish. Treatment at low temperatures of 5°C and 10°C is marked with the notation b (second group) with a water content of 86.30 - 93.72% with closed packaging (P4, P5, P6, P7, P8, P9, P13, P14, P15, P16, P17, P18) with the physical characteristics of the white color of the oyster mushrooms can still maintain their freshness. On day 6 DAT, the room-temperature treatment (25°C) generally showed lower water content (73.44 - 81.70%) than the low-temperature treatments (5°C and 10°C), with still high-water content (81.22 - 93.72%). Low storage temperatures (5°C and 10°C) can reduce water loss from respiration and transpiration. Observations on day 9 DAT showed rotting, dry, and damaged mushrooms, especially in the treatments stored at 25°C (room), which were not observed in treatments P1, P2, P3, P10, P11, and P12. An increase in respiration rate causes this.

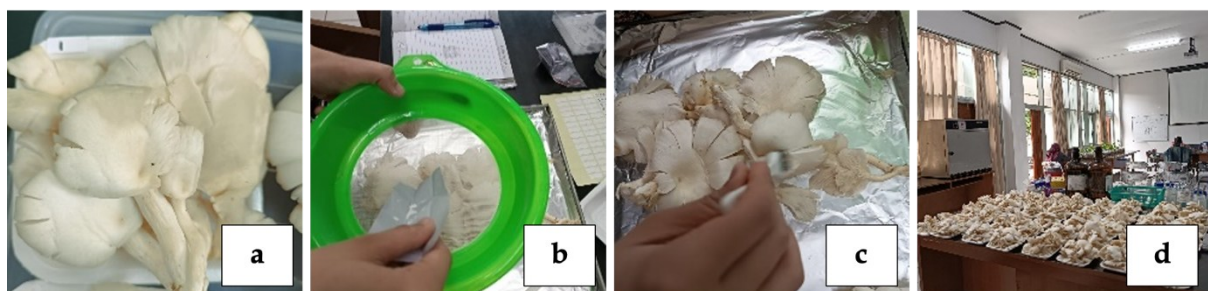


Figure 1. Mushroom coating process; (a) mushrooms weighed 100 g; (b) sprinkle coating powder over the mushrooms using a sieve; (c) coating process with a brush; (d) coated mushrooms are left to rest for 10 minutes, ready to be packaged and stored

Table 1. Effect of various combinations of nano coating, packaging, and storage temperature on the water content of the oyster mushrooms

Treatment	Water content (%)			
	0 DAT	3 DAT	6 DAT	9 DAT
P0 = Control (without treatment <i>coating</i> , packaging, storage 25 °C)	91.67 a	83.77 a	81.70 a	49.60 b
P1: NSA + 25 °C + vacuum	86.49 a	83.35 a	80.03 a	0.00 a
P2: NSA + 25 °C + wrap	84.87 a	82.82 a	80.80 a	0.00 a
P3: NSA + 25 °C + biodegradable	83.48 a	81.63 a	73.44 a	0.00 a
P4: NSA + 10 °C + vacuum	93.88 a	92.45 b	92.20 b	91.70 e
P5: NSA + 10 °C + wrap	94.40 a	92.27 b	91.50 b	90.40 e
P6: NSA + 10 °C + biodegradable	89.99 a	86.30 b	84.64 b	80.99 d
P7: NSA + 5 °C + vacuum	99.73 a	93.70 b	92.45 b	91.98 e
P8: NSA + 5 °C + wrap	99.98 a	92.76 b	91.75 b	91.09 e
P9: NSA + 5 °C + biodegradable	92.16 a	88.52 b	81.22 a	70.75 c
P10: NAV + 25 °C + vacuum	89.44 a	82.35 a	80.36 a	0.00 a
P11: NAV + 25 °C + wrap	84.51 a	84.47 a	81.21 a	0.00 a
P12: NAV + 25 °C + biodegradable	80.85 a	80.64 a	78.20 a	0.00 a
P13: NAV + 10 °C + vacuum	91.47 a	90.21 b	90.15 b	84.42 a
P14: NAV + 10 °C + wrap	93.72 a	93.70 b	91.79 b	83.81 a
P15: NAV + 10 °C + biodegradable	91.45 a	89.60 b	84.91 a	83.71 a
P16: NAV + 5 °C + vacuum	92.43 a	92.30 b	91.15 b	90.51 e
P17: NAV + 5 °C + wrap	92.90 b	90.36 b	90.06 b	89.28 e
P18: NAV + 5 °C + biodegradable	90.37 a	86.89 b	84.64 a	80.33 d

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. A value of 0.00 indicates that the samples had completely deteriorated and were unavailable for further analysis at 9 DAT

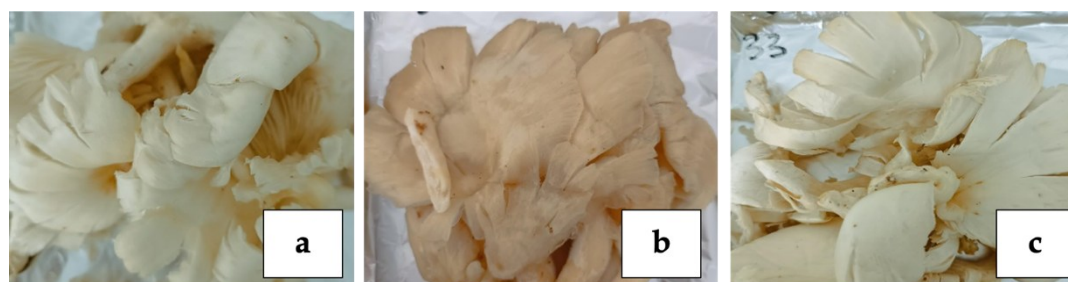


Figure 2. Visual comparison of the freshness of oyster mushrooms at 9 DAT at a temperature of 5 °C with nano sodium alginate and different packaging treatments: a. Vacuum plastic; b. Wrapping plastic, and c. Biodegradable plastic

Treatment control (P0) oyster mushrooms without packing will experience the process of respiration and transpiration, which is caused by the existence of interaction in a way constant to gas composition in the environment storage, so that metabolic activity happens normally, which shows degradation color going very fast browning (Yuniarto et al., 2024). Storage at 25 °C, open to air, results in severe damage with inferior texture and color. Under normal conditions, the water content of oyster mushrooms ranges from 85–95%. However, the surface structure of oyster mushrooms was less effective at protecting the fruit body, making it susceptible to water loss

through transpiration (Gong et al., 2025). This study showed that storage temperature played a more significant role in maintaining moisture content than packaging type. While packaging type had a slight effect on storage results, it wasn't as significant as temperature. For example, biodegradable plastic packaging has higher water vapor and oxygen permeability than vacuum packaging and plastic wrap (Kumari et al., 2023).

Visually, mushrooms packaged in biodegradable plastic appeared less fresh at the end of the observation period, with a rough and dry texture compared to those packaged in

vacuum packaging or plastic wrap. Low water content during storage is one of the main factors contributing to the decline in mushroom quality (Silva et al., 2025). The decrease in water content is often caused by cell damage and redistribution of internal water within the mushroom tissue (Zhang et al., 2018).

Vacuum and wrap packaging were more stable at maintaining oyster mushrooms water content on days 3, 6, and 9 DAT. This is because low oxygen in packaging can suppress oyster mushrooms respiration and prevent the production of water vapor, thereby increasing water content (Kusumiyati et al., 2025). Storage determines the stable water content if it is carried out using an edible layer, which can reduce breathing and the final products in the form of water vapor. The respiration rate can also be reduced by modifying natural atmospheric conditions as needed (Kandasamy, 2022). Vacuum packaging can prevent oxygen and water from entering. Evaporate, thus slowing down the respiration process (Othman et al., 2021).

Weight Loss. Based on Table 2, on the observation day 0 DAT, most treatments showed weight loss that was not significantly different (marked with the letter a), except for treatment P7 (NSA + 5 °C + vacuum) which showed the highest

weight loss (115.50 g), and P8 (NSA + 5 °C + biodegradable plastic) which showed lower weight loss (86.20 g). The value weight loss ranges from 86.20 to 103.30 g. However, from day 3, 6, and 9 DAT, there were significant differences among treatments, and the weight loss value of oyster mushrooms decreased over the 9 days of storage.

However, during storage, there was a decrease in different weights; real tend decreased in all treatments on observation days 3, 6, and 9 DAT. The most drastic weight shrinkage occurred in the treatment control (P0), namely oyster mushrooms without coating, without packaging, and stored at room temperature (25 °C). This showed that the transpiration and respiration processes are not under control during storage of oyster mushrooms without coating treatment and packaging at 25 °C. The is best, temporary treatments are, P4, P8, P13, P14, P16, and P17, with weight loss <20 g; influence temperature and packaging also affect storage. At low temperatures, it slows the respiration rate in fungi and oyster mushrooms with high water content. In contrast, vacuum packaging with plastic was known to reduce contact with oxygen, which can slow the oxidation and growth of microorganisms, compared to packaging with plastic wrap or biodegradable plastic.

Table 2. Effect of various combinations of nano coating, packaging, and storage temperature on the weight loss of the oyster mushrooms

Treatment	Weight loss (g)			
	0 DAT	3 DAT	6 DAT	9 DAT
P0 = Control (without treatment <i>coating</i> , packaging, storage 25 °C)	103.30 a	33.80 a	24.55 a	9.20 a
P1: NSA + 25 °C + vacuum	99.55 a	62.35 b	59.27 b	0.00 a
P2: NSA + 25 °C + wrap	99.90 a	87.00 c	56.00 b	0.00 a
P3: NSA + 25 °C + biodegradable	98.65 a	66.00 b	40.70 a	0.00 a
P4: NSA + 10 °C + vacuum	100.10 a	96.51 c	95.88 c	88.40 e
P5: NSA + 10 °C + wrap	99.90 a	97.70 c	91.91 c	87.65 e
P6: NSA + 10 °C + biodegradable	99.90 a	87.05 c	65.65 b	40.35 b
P7: NSA + 5 °C + vacuum	115.50 a	105.65 c	99.15 c	93.50 e
P8: NSA + 5 °C + wrap	94.55 a	94.25 c	93.45 c	89.75 e
P9: NSA + 5 °C + biodegradable	86.20 a	72.75 b	52.50 b	49.55 c
P10: NAV + 25 °C + vacuum	98.85 a	93.60 c	55.00 b	0.00 a
P11: NAV + 25 °C + wrap	99.65 a	87.70 c	61.65 b	0.00 a
P12: NAV + 25 °C + biodegradable	99.20 a	68.35 b	44.05 a	0.00 a
P13: NAV + 10 °C + vacuum	100.30 a	97.53 c	89.77 c	96.30 e
P14: NAV + 10 °C + wrap	99.80 a	92.36 c	90.20 c	90.36 e
P15: NAV + 10 °C + biodegradable	99.50 a	87.29 c	86.24 c	53.90 c
P16: NAV + 5 °C + vacuum	108.35 a	102.90 e	100.45 c	96.00 e
P17: NAV + 5 °C + wrap	112.25 a	101.70 c	93.90 c	75.00 d
P18: NAV + 5 °C + biodegradable	96.05 a	69.85 b	64.30 b	64.25 d

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

The application of a nano-edible coating containing sodium alginate and aloe vera showed significant effectiveness in reducing weight loss. Treatment with sodium alginate (P1-P9) and aloe vera (P10-P18) maintained weight loss better than the control. Like nano-edible coatings, aloe vera is a natural ingredient that contains a gel/hydrocolloid (Ababiel et al., 2023). Aloe vera gel, composed of polysaccharides and mucilage, acts as a deterrent to food spoilage by blocking the entry of O₂ and moisture. Furthermore, aloe vera gel contains various antibiotic and antifungal components, such as quinones and phenolic compounds (flavonoids), which play a role in delaying or inhibiting the growth of microorganisms that cause foodborne illness in humans or food spoilage (Ali & Abdul-Aziz, 2021).

Sodium alginate is known to help maintain oyster mushrooms during storage. This is caused by sodium alginate forming a potent gel that reacts with calcium ions. Hence, the sodium alginate film provides several benefits in preserving food, namely increasing the barrier to water, preventing microbial contamination, maintaining taste, and reducing weight (Jiang, 2013). Significant weight loss correlates with oyster mushroom mortality. The greater the weight loss, the greater the decline in oyster mushroom quality. This is indicated by damage to the fungus's cell membrane and cell wall, which results in the release and activation of the polyphenol oxidase (PPO) enzyme. The activated PPO enzyme catalyzes the oxidation of phenolic compounds to quinones, which then polymerize to form the brown pigment melanin, causing browning.

This suggests that weight loss is an important indicator in evaluating the quality decline of oyster mushroom products during storage (Liu et al., 2020). This shows that weight loss is an important indicator in evaluating the quality decline of oyster mushroom products during storage. The weight loss in oyster mushrooms is due to physiological changes, including water loss. Due to their high moisture content and respiratory activity, mushrooms rapidly lose moisture after harvest, leading to continuous weight loss, shrinkage, and turgor loss, accelerating their quality degradation (Castellanos-Reyes et al., 2021). Another factor causing weight loss is external factors related to storage, i.e., storage temperature, relative

humidity, and mechanical damage (Zhang et al., 2018). Unpackaged oyster mushrooms stored at high temperatures have a shelf life of only one day and may experience weight losses of up to 50%. This occurs because the oyster mushroom caps expand, leading to browning and a loss of turgor (Azevedo et al., 2017).

Brightness (L*). The surface color of oyster mushrooms was evaluated based on the L* value (brightness). Oyster mushrooms underwent significant changes over 9 DAT of storage. The L* value represents brightness, an important indicator of the visual quality of fresh horticultural products, including oyster mushrooms. Visually, the surface of oyster mushrooms appears white.

Based on Table 3. There was a drastic decrease in the L* value in the control treatment (P0) from 76.55 (0 DAT) to 52.80 (9 DAT). The occurrence of browning in oyster mushrooms indicates a decrease in brightness due to the enzymes polyphenol oxidase (PPO) and tyrosinase, which oxidize phenolic compounds into melanin. According to Jiang (2013), the high water content of oyster (85-95%) and their high respiratory activity after harvesting make them very susceptible to enzymatic browning. Respiratory activity is caused by a thin and porous layer on the surface epidermis of the mushroom (Sharma et al., 2024). This causes pigment degradation, altering the mushroom's surface color.

Treatment at 10°C showed the best results in maintaining brightness, such as P4 (NSA + 10°C + vacuum), which maintained the L* value up to 74.68 until the 9 DAT, while P6 (NSA + 10°C + biodegradable) reached 82.4. In addition, the low temperatures of 10 °C and 5 °C showed consistent brightness. The treatment of P9 (NSA + 5°C + biodegradable) produced an L* value of 82.41, an increase from the initial value of 62.96. Low temperatures can inhibit enzymatic activity, inhibit microorganism growth, and slow pigment degradation. This is in line with the research of Mohammadi et al. (2021), who reported that the combination of nano-coating and low-temperature storage, such as 4 °C, can maintain the L* value of button mushrooms (*Agaricus bisporus*). Nano-coating with cold storage (4 - 10 °C) has been shown to effectively slow pigment degradation and maintain mushroom visual quality for up to 12 days (Choi et al., 2016).

Table 3. Effect of various combinations of nano coating, packaging, and storage temperature on color L* value of the oyster mushrooms

Treatment	L*			
	0 DAT	3 DAT	6 DAT	9 DAT
P0 = Control (without treatment <i>coating</i> , packaging, storage 25 °C)	76.55 a	75.51 b	60.67 b	52.80 b
P1: NSA + 25 °C + vacuum	72.63 a	64.49 a	63.05 b	0.00 a
P2: NSA + 25 °C + wrap	81.68 a	59.84 a	55.15 a	0.00 a
P3: NSA + 25 °C + biodegradable	73.53 a	72.68 b	77.13 c	0.00 a
P4: NSA + 10 °C + vacuum	59.26 a	69.92 b	70.82 c	74.68 d
P5: NSA + 10 °C + wrap	71.12 a	66.91 a	64.30 b	71.21 c
P6: NSA + 10 °C + biodegradable	63.92 a	75.66 b	77.88 c	82.41 e
P7: NSA + 5 °C + vacuum	70.59 a	69.98 b	70.27 c	74.68 d
P8: NSA + 5 °C + wrap	67.48 a	71.36 b	70.55 c	71.21 c
P9: NSA + 5 °C + biodegradable	62.96 a	79.99 b	77.69 c	82.41 e
P10: NAV + 25 °C + vacuum	74.58 a	61.38 a	62.30 b	0.00 a
P11: NAV + 25 °C + wrap	74.77 a	56.24 a	52.05 a	0.00 a
P12: NAV + 25 °C + biodegradable	80.07 a	75.72 b	73.44 c	0.00 a
P13: NAV + 10 °C + vacuum	67.53 a	71.96 b	66.67 b	66.88 c
P14: NAV + 10 °C + wrap	68.17 a	71.65 b	68.88 c	68.41 c
P15: NAV + 10 °C + biodegradable	67.04 a	73.23 b	74.02 c	82.75 e
P16: NAV + 5 °C + vacuum	69.07 a	71.37 b	65.03 b	66.88 c
P17: NAV + 5 °C + wrap	61.95 a	62.99 a	67.25 b	68.41 c
P18: NAV + 5 °C + biodegradable	60.80 a	84.54 b	79.65 c	82.75 e

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

In contrast, high temperatures such as 25 °C in the NSA and NAV 25 °C treatments (P1-P3 and P10-P12) resulted in values of 0.00 because storage at high temperatures accelerates respiration and microbial activity, rendering the tissue unable to maintain viability and storing mushrooms at 20–25°C results in a decline in quality, including stem elongation, cap opening, texture softening, and color changes (Zhang et al., 2021). Oyster mushroom storage affects the effectiveness of edible coatings; high temperatures can cause pigment damage, accelerating the formation of brown pigments through non-enzymatic browning processes, including the Maillard reaction. In contrast, low temperatures, such as 4 °C, can delay undesirable changes in microbial counts (Kic, 2018). In the three comparisons of L* values at 5 °C, 10 °C, and 25 °C, it was reported that the combination of NSA + 5 °C or 10 °C + biodegradable packaging (P6, P9) is a fairly optimal method for maintaining the brightness of oyster mushrooms during storage. This is supported by the storage temperature and the packaging type. The packaging used by P6 and P9 is biodegradable plastic, which provides optimal gas exchange,

preventing the accumulation of CO₂ and ethylene. A high L* value (>80) indicates that the product remains visually attractive and marketable.

a* value. The a* value measures the level of redness-greenness. An increase in the a* value in oyster mushrooms indicates a decay process caused by enzymatic and non-enzymatic browning. This process, catalyzed by enzymes, results in brown pigmentation of the substrate (Cavusoglu et al., 2021). In Table 4, oyster mushrooms during storage showed that significant variations between treatments. At the beginning of storage (0 DAT), all treatments showed relatively low a* values, ranging from -4.48 to 0.83, with the control (P0) at 0.30. On day 3 DAT, a reddish color change was observed, indicated by an increase in the a* value in most treatments, including the control (P0), which showed a drastic increase to 2.95. Treatment P2 (NSA + 25 °C + wrap) showed the greatest increase, reaching 4.96, while treatments with vacuum plastic and biodegradable plastic at various temperatures tended to maintain lower a* values (0.42-0.84).

Table 4. Effect of various combinations of nano coating, packaging, and storage temperature on color a* value of the oyster mushrooms

Treatment	a*			
	0 DAT	3 DAT	6 DAT	9 DAT
P0 = Control (without treatment coating, packaging, storage 25 °C)	0.30 a	2.95 c	6.24 b	10.37 d
P1: NSA + 25 °C + vacuum	0.02 a	0.65 a	2.76 a	0.00 a
P2: NSA + 25 °C + wrap	-4.48 a	4.96 e	6.39 b	0.00 a
P3: NSA + 25 °C + biodegradable	0.01 a	0.72 a	0.60 a	0.00 a
P4: NSA + 10 °C + vacuum	0.22 a	0.48 a	-2.39 a	0.30 a
P5: NSA + 10 °C + wrap	0.20 a	2.06 b	2.04 a	1.65 b
P6: NSA + 10 °C + biodegradable	0.32 a	0.79 a	1.06 a	0.27 a
P7: NSA + 5 °C + vacuum	0.17 a	0.60 a	0.42 a	0.30 a
P8: NSA + 5 °C + wrap	0.12 a	1.18 a	2.29 a	1.65 b
P9: NSA + 5 °C + biodegradable	0.44 a	0.42 a	1.27 a	0.27 a
P10: NAV + 25 °C + vacuum	-0.04 a	0.13 a	3.28 a	0.00 a
P11: NAV + 25 °C + wrap	0.13 a	3.86 d	6.93 b	0.00 a
P12: NAV + 25 °C + biodegradable	-0.15 a	0.45 a	1.33 a	0.00 a
P13: NAV + 10 °C + vacuum	0.08 a	0.84 a	0.77 a	0.18 a
P14: NAV + 10 °C + wrap	0.57 a	1.63 b	2.24 a	2.37 c
P15: NAV + 10 °C + biodegradable	0.07 a	0.66 a	1.45 a	1.27 b
P16: NAV + 5 °C + vacuum	0.23 a	0.56 a	0.46 a	0.18 a
P17: NAV + 5 °C + wrap	0.53 a	2.04 b	2.05 a	2.37 c
P18: NAV + 5 °C + biodegradable	0.83 a	0.12 a	0.92 a	1.27 b

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

Table 5. Effect of various combinations of nano coating, packaging, and storage temperature on color b* of the oyster mushrooms

Treatment	b*			
	0 DAT	3 DAT	6 DAT	9 DAT
P0 = Control (without treatment coating, packaging, storage 25 °C)	14.96 a	27.40 c	29.17 c	0.00 a
P1: NSA + 25 °C + vacuum	12.04 a	18.63 a	25.92 c	4.00 b
P2: NSA + 25 °C + wrap	13.94 a	26.47 c	26.66 c	4.00 b
P3: NSA + 25 °C + biodegradable	14.06 a	14.68 a	17.57 a	4.00 b
P4: NSA + 10 °C + vacuum	13.00 a	16.61 a	17.35 a	0.00 a
P5: NSA + 10 °C + wrap	14.92 a	18.91 a	22.37 b	0.00 a
P6: NSA + 10 °C + biodegradable	13.83 a	16.99 a	18.89 a	0.00 a
P7: NSA + 5 °C + vacuum	15.05 a	18.35 a	17.85 a	0.00 a
P8: NSA + 5 °C + wrap	15.48 a	19.18 a	22.29 b	0.00 a
P9: NSA + 5 °C + biodegradable	25.19 b	16.46 a	19.83 a	0.00 a
P10: NAV + 25 °C + vacuum	12.57 a	18.82 a	26.11 c	4.00 b
P11: NAV + 25 °C + wrap	14.33 a	23.00 b	25.40 c	4.00 b
P12: NAV + 25 °C + biodegradable	13.48 a	14.94 a	19.31 a	4.00 b
P13: NAV + 10 °C + vacuum	13.84 a	18.38 a	17.67 a	0.00 a
P14: NAV + 10 °C + wrap	15.04 a	21.85 b	23.86 b	0.00 a
P15: NAV + 10 °C + biodegradable	13.82 a	16.96 a	18.64 a	0.00 a
P16: NAV + 5 °C + vacuum	15.99 a	18.23 a	18.45 a	0.00 a
P17: NAV + 5 °C + wrap	16.73 a	21.23 b	21.77 b	0.00 a
P18: NAV + 5 °C + biodegradable	17.18 a	15.27 a	17.49 a	0.00 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

A significant browning color change occurred on day 6 DAT, with the control (P0) reaching an a^* value of 6.24. Treatment P1 (NSA + 25 °C + vacuum) showed a value of 2.76, while P3 (NSA + 25 °C + biodegradable) was only 0.60. Treatment with low temperature (5 °C) showed a lower a^* value than high temperature. There was a color change indicating severe damage to oyster mushrooms on the day 9 DAT. The control (P0) experienced an increase in the a^* value, reaching 10.37, indicating severe color deterioration. Most treatments with nano coating showed a value of 0.00, indicating that the mushrooms had undergone severe color changes or damage. Treatments P5 (NSA + 10 °C + wrap), P8 (NSA + 5 °C + wrap), P14 (NAV + 10 °C + wrap), P15 (NAV + 10 °C + biodegradable), P17 (NAV + 5 °C + wrap), and P18 (NAV + 5 °C + biodegradable) still showed positive a^* value ranging from 1.27 - 2.37, indicating relatively better color quality.

A combination of nano-coating with low-temperature storage, such as 5 - 10 °C, and packaging under vacuum is highly recommended compared to packaging otherwise. The lower of oxygen created in the packaging vacuum. Unlike biodegradable packaging, high gas permeability allows oxygen to enter more readily, thereby accelerating decomposition. Uncontrolled packaging that allows oxygen to enter can accelerate oxidation, which causes browning/greening. The strategy to overcome this is to limit gas exchange, thereby reducing oxygen availability (Cavusoglu et al., 2021). Vacuum packaging, by minimizing oxygen exposure, is effective in inhibiting browning (Martha & Daniel, 2025).

The type of packaging, the combination of edible coatings, and storage temperature determine the color retention of oyster mushrooms. For example, a combination of nano aloe vera and nano sodium alginate at low temperatures is an effective option for maintaining color during storage of oyster mushrooms. This acts as an antimicrobial, an oxygen absorber for moisture, and a gas barrier (Rai et al. 2019).

Value b^* . The b^* value represents the colour coordinates of yellow (+) to blue (-) in the CIE $L a b^*$ color system. Browning is an indicator of quality decline, as evidenced by the b^* value of oyster mushrooms, which increases significantly during storage. The results of measuring the b^* value (yellowness) in white oyster mushrooms during storage with various nano coating

treatments, packaging, and storage temperatures are presented in Table 5.

The b^* value indicates the intensity of the yellow color in the sample, with higher values indicating a deeper yellow color. Values approaching zero indicate a loss of yellow color toward a neutral grayish color. On the initial observation day (0 DAP), b^* values ranged from 12.04 to 25.19. The control treatment (P0) showed that b^* value of 14.96, while the highest value was found in treatment P9 (NSA + 5 °C + biodegradable) with a value of 25.19. Treatment P9 had a high b^* value, indicating a more intense yellow color at the beginning of storage. In the initial stage, b^* values generally varied between treatments with nano sodium alginate (NSA) and various temperature and packaging combinations.

On 3 DAT, a significant increase in b^* values occurred in several treatments, particularly in the control (P0), which reached 27.40, as well as in P1 (18.63) and P2 (26.47). This increase in b^* values indicated an intensification of the yellow color in oyster mushrooms. However, it should be noted that the browning of oyster mushrooms is not only determined by an increase in b^* but also by a combination of a decrease in the L^* value (brightness) and an increase in the a^* value (redness). Interpretation of color changes requires consideration of all three color parameters (L , a^* , b^*). Treatments with low-temperature storage (5 °C and 10 °C) tend to show higher b^* values than storage at 25 °C, indicating greater inhibition of the degradation process and better color.

On day 6 (DAT), the control reached the highest b^* value of 29.17, indicating very significant color degradation. Treatment P3 (NSA + 25 °C + biodegradable) showed the lowest value, namely 17.57, indicating coating capability with biodegradable packaging in maintaining color. Treatment at low temperatures (5 °C and 10 °C) shows higher b^* values than 25 °C. On day 9 (DAT), some big samples show a b^* value of 0.00 or very low (0.00), indicating the sample has experienced total damage or is not worth analyzing further.

Edible coatings cover the entire exterior of the mushroom, acting as a barrier to oxygen, thus preventing gas exchange and moisture loss while maintaining quality (Dawadi et al., 2022). The use of nano-edible coatings, both nano sodium alginate (NSA) and nano aloe vera (NAV), has shown effectiveness in hindering improvements

in b^* value during storage. Smaller particle sizes effectively connect bulk materials and atomic or molecular structures. Smaller particles are known to have the potential to be effective catalysts for plant metabolism, providing better penetration into cells and enhancing protective activity on plant surfaces (Rosyada et al., 2019).

Conclusion

Based on the results of the research that has been done, some conclusions can be taken as follows:

1. The combination of nano-edible coating treatment, storage temperature, and packaging type had a significant effect on water content, weight loss, and color parameters (L^* , a^* , and b^* values) of oyster mushrooms on the 3, 6, and 9 DAT.
2. The combination of nano sodium alginate and nano aloe vera with vacuum packaging at 5 °C gave the best effect on water content, weight loss and colour value. These results indicated the potential of the treatment to maintain the quality of oyster mushrooms during storage.

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References

- Ababiel A, Aminah S, Suyanto A. 2023. Application of edible coating of *Aloe vera* on mushrooms oysters and their effect on chemical, physical, and sensory quality during Storage. *Journal of Food and Nutrition*, 13(2): 18-29.
- Aftukha, Aulia A, Purbasari A. 2021. Quality characteristics of oyster mushrooms (*Pleurotus ostreatus*) using various packaging methods in low temperature storage. 10(3): 327-37.
- Ali MR, Abdul Aziz ME. 2021. Application of edible film and coating based on aloe vera gel for preservation of physicochemical properties of *Physalis peruviana* L. fruits. *J. Microbiol Biotech Food* 11(3): 1-7
- Azevedo, Sílvia, Cunha LM, Oliveira JC, Mahajan PV, Fonseca SC. 2017. Modelling the influence of time, temperature and relative humidity conditions on the mass loss rate of fresh oyster mushrooms. *Journal of Food Engineering* 212: 108-12. <https://doi.org/10.1016/j.jfoodeng.2017.05.026>.
- Castellanos-Reyes K, Villalobos-Carvajal R, Beldarrain-Iznaga T. 2021. Fresh mushroom preservation techniques. *Foods*, 10(9), 2126. <https://doi.org/10.3390/foods10092126>
- Cavusoglu S, Uzun Y, Yilmaz N, Ercisli S, Eren E, Ekiert H, Elansary HO, Szopa A. 2021. Maintaining the quality and storage life of button mushrooms (*Agaricus bisporus*) with gum, agar, sodium alginate, egg white protein, and lecithin coating. *Journal of Fungi*, 7: 1-9.
- Chen, Jingxin, Ankang Wu, Mingliang Yang, Yonghong Ge, Penta Pristijono, Jianrong Li, Baocheng Xu, dan Hongbo Mi. 2021. Characterization of sodium alginate-based films incorporated with thymol for fresh-cut apple packaging. *Food Control* 126(March): 108063. [doi:10.1016/j.foodcont.2021.108063](https://doi.org/10.1016/j.foodcont.2021.108063).
- Choi MH, Kim GH, & Lee HS. 2016. Effects of ascorbic acid retention on juice color and pigment stability in blood orange (*Citrus sinensis*) juice during refrigerated storage. *Food Science and Biotechnology*, 25(5), 1273-1279. <https://doi.org/10.1007/s10068-016-0201-z>
- Dawadi E, Bahadur P, Bhandari S, Subedi S, Shrestha S. 2022. Heliyon Nutritional and post-harvest quality preservation of mushrooms: A review." *Heliyon* 8(12): e12093. <https://doi.org/10.1016/j.heliyon.2022.e12093>
- Effiong, Eno M, Afolabi IS, Chinedu SN. 2024. Assessing the nutritional quality of *Pleurotus ostreatus* (oyster mushroom). (January): 1-13. [doi:10.3389/fnut.2023.1279208](https://doi.org/10.3389/fnut.2023.1279208).
- Gong M, Zhang T, Wu Y, Shang J, Minggu E, Cao Y, Zhang J. 2025. Synergizing postharvest physiology and nanopackaging for edible mushroom preservation. *Food Chemistry*, 463: 1-12.
- Jiang T. 2013. Effect of alginate coating on physicochemical and sensory qualities of button mushrooms (*Agaricus bisporus*)
- Amalia I, Qosim WA, Kusumiyati. 2025. Effect of nano edible coating, storage temperature, and packaging on water content, weight loss, and color on oyster mushrooms (*Pleurotus ostreatus*). *Jurnal Kultivasi*, 24 (3): 373-385.

- under a high oxygen modified atmosphere. *Postharvest Biology and Technology* 76: 91–97. doi:10.1016/j.postharvbio.2012.09.005.
- Kandasamy P. 2022. Respiration rate of fruits and vegetables for modified atmosphere packaging: a mathematical approach. *Journal of Postharvest Technology*, 10: 88–102.
- Kic P. 2018. Mushroom drying characteristics and changes of colour. *Engineering for Rural Development*, 23(17): 432–38. doi:10.22616/ERDev2018.17.N009.
- Kumari S, Rao A, Kaur M, Dhanja G. 2023. Petroleum-based plastics versus bio-based plastics: A review. *Nature Environment and Pollution Technology*, 22: 1111–1124.
- Kusumiyati K, Soleh MA, Nurhadi B, Sutari W. 2025. Effects of different packaging treatments and storage durations on the quality of enoki mushrooms (*Flammulina velutipes*) with nano edible coating. *Australian Journal of Crop Science*, 19: 206–211.
- Li H, Zhang Z, Li M, Li X, Sun Z. 2015. Yield, size, nutritional value, and antioxidant activity of oyster mushrooms grown on perilla stalks. *Saudi Journal Of Biological Sciences*, 24(2):347-54. doi:10.1016/j.sjbs.2015.10.001.
- Liu Q, Kong W, Hu S, Kang Y, Zhang Y, Bun T. 2020. Postharvest Biology and Technology Effects of *Oudemansiella radicata* polysaccharide on postharvest quality of oyster mushroom (*Pleurotus ostreatus*) and its antifungal activity against *Penicillium digitatum*. *Postharvest Biology and Technology*, 166(March): 111207. doi:10.1016/j.postharvbio.2020.111207.
- Martha E, Daniel OE. 2025. Enzymatic browning in food and prevention. *International Journal of Innovative Biosciences Research*, 13: 44–53.
- Mishra V, Tomar S, Priyanka YP, Vishwakarma S, Singh MP. 2022. Elemental analysis, phytochemical screening and evaluation of antioxidant, antibacterial and anticancer activity of *Pleurotus ostreatus* through In vitro and In silico approaches. *Metabolites* 12, 821: 1–25. <https://doi.org/10.3390/metabo12090821>
- Mohammadi L, Khankahdani LH, Tanaka F, Tanaka F. 2021. Postharvest shelf-life extension of button mushroom (*Agaricus bisporus* L.) by aloe vera gel coating enriched with basil essential oil. *Environmental Control in Biology*, 59(2), 87–98. DOI: 10.2525/ecb.59.87
- Nishant, SA, Kumar K, Ashutosh R. 2023. Characterization of aloe vera gel - based edible coating with orange peel essential oil and its preservation effects on button mushroom (*Agaricus bisporus*). *Food and Bioprocess Technology*, 16(12): 2877–97. doi:10.1007/s11947-023-03107-z.
- Othman SH, Abdullah NA, Nordin N, Abdul Karim Shah NN, Mohd Nor MZ, Md Yunus KF. 2021. Shelf life extension of Saba banana: Effect of preparation, vacuum packaging, and storage temperature. *Food Packaging and Shelf Life*, 28: 1–7.
- Piska K, Sułkowska-Ziaja K, Muszyńska B. 2017. Oyster mushroom (*Pleurotus ostreatus*) – nutritional significance and biological activity. *Acta Scientiarum Polonorum Hortorum Cultus*, 16(1), 151–161.
- Raghav PK., Agarwal N, Saini M. 2016. Edible coating of fruits and vegetables: A Review. *International Journal of Scientific Research and Modern Education (IJSRME)*, 1(1): 188–204. ISSN (Online): 2455-5630.
- Rahman S, Hassan K, Talukder FU. 2020. Effect of low temperature on postharvest behaviors of oyster mushroom (*Pleurotus* spp.). *International Journal of Horticultural Science and Technology*, 7(3): 213–25. doi:10.22059/ijhst.2020.302076.365.
- Rai M, Ingle AP, Gupta I, Pandit R, Paralikar P, Gade A, Chaud MV, Dos Santos CA. 2019. Smart nano packaging for the enhancement of food shelf life. *Environmental Chemistry Letters*, 17: 277–290.
- Rosyada A, Sunarharum WB, Waziroh, E. 2019. Characterization of chitosan nano particles as an edible coating material. *IOP Conference Series: Earth and Environmental Science*, 230: 012043. <https://doi.org/10.1088/1755-1315/230/1/012043>
- Sassine YN, Shuleva N, El Sebaaly Z. (2022). Changes in *Pleurotus ostreatus* nutritional value and heavy metal profile as a result of supplementation with nano-additive. *Studies in Fungi*, 7(1):1-6. doi: 10.48130/SIF-2022-0012
- Shaikh S, Yaqoob M, Aggarwal P. 2021. An

- overview of biodegradable packaging in food industry. *Current Research in Food Science*, 4: 503-520.
- Sharma V, Singh P, Singh A. 2024. Shelf life extension of fresh mushrooms: From conventional practices to novel technologies – A comprehensive review. *Future Postharvest Food*, 1: 317-333
- Silva M, Vida M, Ramos AC, Lidon FJ, Reboredo FH, Goncalves EM. 2025. Effect of storage temperature on the quality and shelf life of *Hericium erinaceus* mushrooms. *Horticulture*, 11: 1-18.
- Yuniarto K, Aqidah F, Fuadi M. 2024. Engineering of storage methods to change sensory properties and respiratory activity of oyster Mushrooms. *Teknotan Journal*, 18(1): 7- 15 doi:10.24198/jt.vol18n1.2.
- Zhang K, Pu YY, Sun DW. 2018. Latest progress in post-harvest mushroom quality preservation (*Agaricus bisporus*): A review. *Trends in Food Science and Technology*, 78: 72-82.
- Zhang Y, Money D, Chen Y, Liu T, Zhang S, Fan H, Liu H, Li Y. 2021. Healthy function and tall valued utilization from edible mold. *Food Science and Man Health*, 10: 408-420.
- Zhu D, Guo R, Li W, Song J, Cheng F. 2019. Improved postharvest preservation effects of *Pholiota nameko* Mushroom by Sodium Alginate - Based Edible Composite Coating. *International Journal Food and Bioprocess Technology*, 12: 587-589.

