# Planta Tropika

0216 499X
E-ISSN: 2528-7079
Vol. 13 No. 1
February 2025

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# Planta Tropika

Planta Tropika focuses related to various themes, topics and aspects including (but not limited) to the following topics Agro-Biotechnology, Plant Breeding, Agriculture Waste Management, Plant Protection, Soil Science, Post Harvest Science and Technology, Horticulture. Planta Tropika published two times a year (February and August) by Universitas Muhammadiyah Yogyakarta in collaboration with Indonesian Association of Agrotechnology / Agroecotechnology (PAGI) and Perhimpunan Agronomi Indonesia (PERAGI). The subscriptions for one year : IDR 350.000.

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# Editorial

Journal of Planta Tropika ISSN 0216-499X published by Study Program of Agrotechnology, Faculty of Agriculture, Universitas Muhammadiyah Yogyakarta, is journal presenting scientific articles of agricultural science (Journal of Agro Science). With full sense of gratitude to the Almighty Allah, Volume 13 Number 1 for the year of 2025 has been published.

In this edition, Journal of Planta Tropika presents ten research articles in the field of Agro sciences comprising soil and plant nutrition, plant protection, agrobiotechnology, post-harvest technology. The scientific articles discuss about:

(1) Thidiazuron-Induced Somatic Embryogenesis in *Cymbidium bicolor* Orchid In Vitro, (2) Growth and Yield of Mint (*Mentha spicata* L.) as Affected by Composition of Charcoal Husk and Organic Fertilizer, (3) Effects of Cricket and Fruit Fly Flour in Growth Media on *Beauveria bassiana* (Bals.) Vuill Pathogenicity Against *Zeugodacus cucurbitae* (Coquillet) Prepupae, (4) Antagonistic activity of *Trichoderma harzianum* against *Aspergillus parasiticus* and *Mucor circinelloides* in corn plant (*Zea mays* L.), (5) Formulation and Market Acceptability of Dragon Fruit (*Selenicerius undatus*) Flavored Mead, (6) Effects of Biocontrol Product Bio P60 and Liquid Organic Fertilizer on The Development of Fusarium Wilt and Yield of Shallot, (7) Antagonistic Effect of Nitrogen Fertilizer and Rhizobium on Growth, Nodulation and Yield of Peanut (*Arachis hypogaea* L.) in Acidic Soil, (8) Assessing Soil Nutrient and Biomass Contributions to Peatland Formation, (9) Effects of Arbuscular Mycorrhiza-Enriched Bio-compost and Organic Fertilizer on Reducing Heavy Metal Absorption in Shallots, (10) Effects of Soil Ameliorants on Growth And Yield of Elephant Grass (*Pennisetum Pupureum*) in Post-Tin Mining Land.

The editors would like to thank the authors, reviewers, executive editors, leaders and LRI UMY for their participation and cooperation. Our hope, this journal can be useful for readers or be a reference for other researchers and useful for the advancement of the agriculture.

Editors

# GUIDE FOR AUTHORS

**TYPE OF PAPERS**

PLANTA TROPIKA receives manuscripts in the form of research papers in Bahasa Indonesia or English. The manuscript submitted is a research paper that has never been published in a journal or other publication.

**SUBMISSION**

The submission of the manuscript is done through our journal website http://journal.umy.ac.id/index.php/pt/index. If you need information regarding the process and procedure for sending the manuscript, you can send it via email at plantatropika@umy.ac.id. Editor's address: Program Studi Agroteknologi, Fakultas Pertanian, Universitas Muhammadiyah Yogyakarta, Jl. Brawijaya, Tamantirto, Kasihan, Bantul, Telp (0274) 387646 psw 224, ISSN: 2528-7079.

**ARTICLE STRUCTURE**

The submitted manuscripts should consist of 15-20 pages of A4 size paper with 12-point Times New Roman fonts, 1.5 spacing with left-right margin and top-bottom of the paper is 2.5 cm each. All manuscript pages including images, tables and references should be page-numbered. Each table or picture should be numbered and titled.

The systematic of the manuscript writing is as follows:

**TITLE** : The title should be written in a concise but informative manner describing the content of the research. Avoid abbreviations or formulas. Font style is Tahoma 18pt.

**AUTHOR NAMES** : The author names should be written in lowercase letters (only the first letter of the words is written in uppercase) and should be written from the first author and followed by the others along with the marker of each author's affiliation.

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**EMAIL** : Please list one of authors' email address used for paper's correspondence.

**ABSTRACT** : Abstract written in English. Abstract appears after the Title in the manuscript. Abstract must be solid consisting of an introduction and objectives, research methods, results, and conclusions. Abstract must be written in one paragraph. Libraries are not allowed to be written in the abstract section. Writing abbreviations should be avoided. Abstract consists of 150-200 words followed by keywords. Abstract is written in justify.

**INTRODUCTION** : This section describes: (i) the general background of the research (concise and clear), (ii) a review of the results of previous research that is relevant and up to date, (iii) clearly provides a statement of novelty (gap analysis) which contains the urgency and novelty of the research, as well as the objectives of the study. The introduction is written without numbering and / or pointers. The introduction is written in 750-1000 words.

**MATERIALS AND METHOD** : This section should state the time and place for conducting the research. All materials and methods used such as chemicals for analysis, treatment and experimental design must be clearly and concisely stated. The use of methods should be based on previous research. Materials and methods should be written in 400 - 600 words. Subsection heading might be applied if there are many sections appeared.

**RESULT AND DISCUSSION** : In this section the results and discussion must be written in one section. Both must be written in a continuous manner starting from the main results to supporting results and completed with discussion. The unit of measurement used must follow international standards. Equations are written in Times New Roman or Symbol fonts. If there are several equations, give each equation a number in sequence and place it on the far right, namely (1), (2), and so on. The use of signs is allowed to make equations more concise. Italic fonts are used for variables, bold for vectors. Example:

$$\alpha + \beta = \chi \qquad (1)$$

Results and discussion should be written between 2500 - 3000 words. It can be grouped in several sub-section heading.

**CONCLUSION** : In this section, conclusions must be written clearly and concisely referring to the research results to answer the research objectives or hypotheses, and not repeat the discussion. Conclusions are written critically, logically and honestly based on existing facts, and be careful if there are generalizations. This section is written in paragraph form, not using numbering or bullets. Results and discussion should be written between 40-80 words.

**ACKNOWLEDGEMENT** : This section is used if needed. This section is provided for authors to express their gratitude to either the party who funded the research, facilities, or suggestions; also for a statement if the article is part of a thesis / dissertation.

**REFERENCES** : All libraries used should be noted in this section. The use of bibliography writing style follows the style of the American Psychological Association (APA) 6th Edition and is arranged in alphabetical order (https://apastyle.apa.org/style-grammar-guidelines/references/examples). Manuscripts must use a minimum of 30 references and 80% come from journals (**for the new submission starting January 2024**). The majority of the literature used consists of primary literature (published in the last 5 years). Unpublished data and personal communications are not allowed to be cited. Between one library and the next one separated by one space. It is mandatory to include the DOI of cited references, if available. **Author are suggested to use reference tools, such as Mendeley, Zotero and Endnote.**

## EXAMPLES ON HOW TO WRITE REFERENCES

References are written in alphabetical order according to the rules below:

### REFERENCE TO A BOOK

Pessarakli, M. (2001). *Handbook of Plant and Crop Physiology* (4th Ed.). CRC Press.

### REFERENCE TO A JOURNAL PUBLICATION

Aiman, U., Tantriati, T., & Sriwijaya, B. (2017). Pemberian Macam Konsorsium Bakteri Hasil Isolasi Tumbuhan Pantai pada Kangkung (*Ipomoea reptans* Poirs.). *Planta Tropika*, *5*(1), 1–6. https://doi.org/10.18196/pt.2017.065.1-6

### REFERENCE TO A THESIS/DISSERTATION

Miranda, C. (2019). *Exploring the lived experiences of foster youth who obtained graduate level degrees: Self-efficacy, resilience, and the impact on identity development* (Publication No. 27542827) [Doctoral dissertation, Pepperdine University]. PQDT Open. https://pqdtopen.proquest.com/doc/2309521814.html?FMT=AI

### REFERENCE TO AN ARTICLE IN PROCEEDING

Sarjiyah, Setiawan, D. A., & Rineksane, I. A. (2021). Shallot extract enhance root growth in crystal guava (*Psidium guajava*) stem cuttings. *IOP Conference Series: Earth and Environmental Science*, *752*(1), 012050. https://doi.org/10.1088/1755-1315/752/1/012050

### REFERENCE TO A REPORT GOVERNMENT

Ministry of Agriculture. (2019). *Taking time: Support for improve agriculture product* (MOA Publication No. 18-2059). Indonesia. Department of Crop Production, Ministry of Agriculture. https://psp.pertanian.go.id/

**TABLE AND FIGURE FORMATTING :** Table labels are placed on top of the table, while image labels are at the bottom of the image. When referring to a table, write down a specific table, for example Table 1. Examples of writing tables and figures are as follows.

**Table 1.** Table format (Verdana 10pt, align left, space 1, spacing before 0 pt, after 0 pt, without a period)

| Head of table | Head of table column<br>Sub-column header | Sub-column header |
|---|---|---|
| Contents | Contents | Contents |

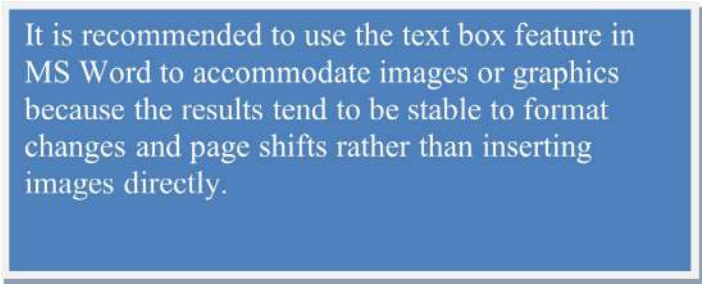


**Figure 1.** Examples of image captions (Verdana 10pt, center, space 1, spacing before 0 pt, after 0 pt, without a period)

# Thidiazuron-Induced Somatic Embryogenesis in *Cymbidium bicolor* Orchid In Vitro

10.18196/pt.v13i1.15306

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**ABSTRACT**

*Cymbidium bicolor* is a highly hunted and traded orchid, leading to a decline in its wild population. Orchid conservation can be achieved through tissue culture, particularly via somatic embryogenesis. Thidiazuron (TDZ) is a growth regulator used to induce somatic embryogenesis. This study aimed to determine the optimal TDZ concentration for somatic embryo formation. Stem explants of C. bicolor were cultured on *Murashige Skoog* (MS) medium with TDZ concentrations of 0, 1, 2, and 3 ppm. Observations were conducted weekly for two months using a stereo microscope and OptiLab. Variables observed included the percentage of green explants, somatic embryo formation time, the number of explants forming somatic embryos, and the number and morphology of somatic embryos. The study was arranged in a Completely Randomized Design (CRD) with 14 replications. Results showed that TDZ addition influenced somatic embryo formation and maintained the green color of explants. Media with TDZ promoted faster growth and larger embryo size compared to media without TDZ. The optimal concentration was 1 ppm TDZ, which produced the highest number of embryos (172) and the fastest formation time compared to other concentrations (TDZ 0: 27, TDZ 2 ppm: 60, TDZ 3 ppm: 39).

**Keywords**: Clone; Differentiation; Plant propagation; Somatic embryo

## INTRODUCTION

Indonesia is well-known to have a high diversity of orchids (Dewi et al., 2024). One of the orchids utterly popular in Indonesia is *Cymbidium bicolor,* whose flower characteristic resembles a boat (Pratama et al., 2021). The beauty of the *C. bicolor* flower causes this flower to be hunted from the forest so it can be traded, and its number in nature has begun to decrease. Yudaputra et al. (2024) state that the existence of orchids in the wild continues to decline, caused by habitat destruction and overexploitation.

Practical propagation efforts are needed to preserve *C. bicolor* orchid (Pratama et al., 2021). Conventional orchid propagation methods take a long time and a large area (Syamsiah et al., 2020). In addition, the natural propagation of orchids requires a suitable pollinator, even with the help of humans. Therefore, plant tissue culture was chosen as an effective method of propagation. Plant propagation through tissue culture can be done in three ways: adventitious shoot formation, lateral shoot proliferation, and somatic embryogenesis (Pardede et al., 2021). Propagation through somatic embryogenesis aims to form embryos from genetically identical somatic tissue (Kong et al., 2020).

Article History
Received : 24 January 2024
Revised : 04 July 2024
Accepted : 03 September 2024

The success of regeneration through somatic embryogenesis is influenced by various factors, including the composition of the media and growth regulators (Ardiyani et al., 2020). The medium used in this study was Murashige Skoog (MS). This medium is often used because most plant cultures that use this medium produce an optimal response. Nutrients contained in MS media are thought to be able to optimize explants that have the potential for the formation of cell competence to form somatic embryos that occur when plant tissue is injured (Lopez et al., 2022).

PGR types and their concentrations in tissue culture depend on the desired goal or direction of plant growth (Murgayanti et al., 2020). One type of PGR widely used for somatic embryo induction is Thidiazuron (TDZ). This hormone is not structurally similar to other natural cytokinins, especially those based on purines. Furthermore, the action of TDZ to induce somatic embryogenesis sets it apart from other purine-based cytokinins (Pardede et al., 2021). The chemical structure of TDZ is stable, its degradation is slower than BAP, and its biological activity is higher to stimulate better embryogenesis growth (Rostiana, 2020). Pardede et al. (2021) stated that TDZ alone had been found to replace the role of auxin and other cytokinins in influencing the somatic embryogenesis process because TDZ can fulfill substantial prerequisites for inducing somatic embryos.

Thidiazuron controls many gene expressions, chloroplast development, secondary metabolite synthesis, cell specification, dedifferentiation, and differentiation. Growth in somatic embryos is triggered by TDZ activity during cell differentiation (Restanto et al., 2023). Pyati (2022) research on *Dendrobium ovatum* showed that 1 ppm TDZ combined with 0.5 ppm NAA resulted in the optimum somatic embryos. Thidiazuron with a concentration of 3 ppm combined with 1 ppm NAA showed the highest number of somatic embryos in *Phalaenopsis amabilis* cultured (Mose et al., 2020). Ghahremani et al. (2021) research on *Phalaenopsis amabilis* cv. Jihan cultured on medium supplemented with 3 ppm TDZ produced the highest number of somatic embryos. Mahendran & Bai (2012) conducted a study on the induction of somatic embryos in *C. bicolor* using explant sources in the form of Protocorm Like Bodies (PLB), where the best response was obtained at 1 ppm BAP and 2,4-D 2 ppm, which formed somatic embryos to the globular phase.

Thidiazuron diffuses into plants, and cellularly, it will act as a signal to increase the number of purines and convert adenine to adenosine. Adenosine will be converted into ribonucleotides (Pardede et al., 2021). Ribonucleotide activity will affect the synthesis of proteins that will act as enzymes isopentenyl transferase (IPT), nucleoside 5-monophosphate phosphoribohydrolase (LOG), and dehydrogenase (CKX), which are involved in the synthesis of cytokinins (Zhao et al., 2024). Nucleoside 5-monophosphate phosphoribohydrolase (LOG) is a regulator and second messenger that will affect cell division (Chen et al., 2022). In cell division, cytokinins (TDZ) signal to restructure the development towards the embryogenic pathway. This signal will trigger the embryogenic developmental path that leads to the formation of somatic embryos, where cells that initially do not have competence become embryonic competence due to the restructured pathway (Zhao et al., 2024).

In addition to adding TDZ to the culture media, it can also be supported by vitamins such as peptone, which is rich in nitrogen and amino acids. According to Carnelos et al. (2022), the presence of nitrogen is positively correlated with the concentration of cytokinins. Peptone is an additional supplement in tissue culture media (Krisdianto et al., 2020). Adding peptone can affect the composition of

organic nutrients in the media. Therefore, the induction of somatic embryos of *C. bicolor* orchids on MS media was carried out with various concentrations of TDZ to accelerate the induction response of somatic embryos to obtain a higher somatic embryo phase compared to somatic embryo studies with *C. bicolor* (Mahendran & Bai, 2012).

In this study, results were obtained in the form of intact plants, in contrast to Mahendran & Bai (2012) research, which had just reached the globular phase, which means that TDZ could spur the formation of Somatic Embryo to the coleoptile phase, exceeding the ability of BAP in Mahendran & Bai (2012) research, which only reached the globular phase.

## MATERIALS AND METHODS

### Research Design

The research was conducted in January - May 2021 at the Tissue Culture Laboratory, Diponegoro University, Semarang. The research was arranged in a single-factor, completely randomized design (CRD), consisting of four treatments with fourteen replications, totaling 56 experimental units. The treatments were Thidiazuron with concentrations of 0 ppm (T0), 1 ppm (T1), 2 ppm (T2), and 3 ppm (T3). Murasige Skoog, with the addition of 1 g/L peptone, was used as basal media for all treatments.

### Sterilization

Culture glassware, pipette, and scalpel were immersed in 5% natrium hypochlorite solution for ± 15 – 30 minutes, then sterilized in an autoclave at 121°C with a pressure of 15 Psi or 2 atm for 15 minutes.

### Media Preparation

A 100 ppm TDZ stock solution was prepared by weighing 10 mg of TDZ and adding 3-4 drops of HCl. Then, it was shaken and dissolved with 100 ml of distilled water until evenly distributed. Ready-to-use MS media, sucrose, agar, and peptone were weighted according to their respective dosages on the analytical balance. 50 mL of distilled water was put into a beaker and heated on a hot plate. MS media of 4.43 g/L was put into a beaker and homogenized. 1 g/L peptone was put into a beaker and homogenized on a hot plate using a magnetic stirrer at 200-300 rpm. Sucrose 30 g/L was added, and the solution was left until homogeneous. 8 g/L agar powder was added. The concentrations of growth regulators used were TDZ 0 ppm, 1 ppm, 2 ppm, and 3 ppm. Thidiazuron hormone with various concentrations was added by taking several ml using a syringe, and the solution was allowed to boil. The distilled water was added until the volume reached 100 mL. Then, the media were poured into the Erlenmeyer, covered with aluminum foil, and the middle was pressed and sealed using plastic wrap. The media were sterilized by autoclaving for 15 minutes at 121°C with a pressure of 15 Psi or 2 atm. After sterilization, sterile media were poured into Petri dishes in LAF, then covered with plastic wrap and stored on a culture rack.

### Somatic Embryo Induction

The explants used in this research were stems from the *C. bicolor* orchid, which grew from plantlets. The stems were cut into ±2 cm; in the middle of the stems, they were given a shallow wound

using a scalpel and placed horizontally on a petri dish containing MS+1 g/L peptone media with 4 TDZ treatments. Explants were planted under sterile conditions in the LAF.

## Somatic Embryo Observation

Visual observations of the somatic embryo of *C. bicolor* were made weekly for two months using OptiLab and a stereo microscope. First, the petri dish containing the explant was put in the base of the stage plate of a stereo microscope and was observed with 4x10 magnification. The variables observed were the percentage of green explants, the formation time of somatic embryos, the number of explants that formed somatic embryos, somatic embryos, and somatic embryo morphologies.

## Statical Analysis

All data were analyzed using IBM SPSS Statistics 25 software and Microsoft Excel. Variance (ANOVA) was analyzed on the somatic embryo percentage and continued with the Duncan Multiple Range Test (DMRT) at the 5% level.

# RESULTS AND DISCUSSION

## Explant's Response

This study showed that adding growth regulators (PGR) in the form of thidiazuron affected the explants to remain green. Browning often occurs in the tissue culture process, inhibiting vitro regeneration. Browning reduces growth rate, shoot, and root differentiation (Bariyyah & Putri, 2021) and can even cause death (Permadi et al., 2023).

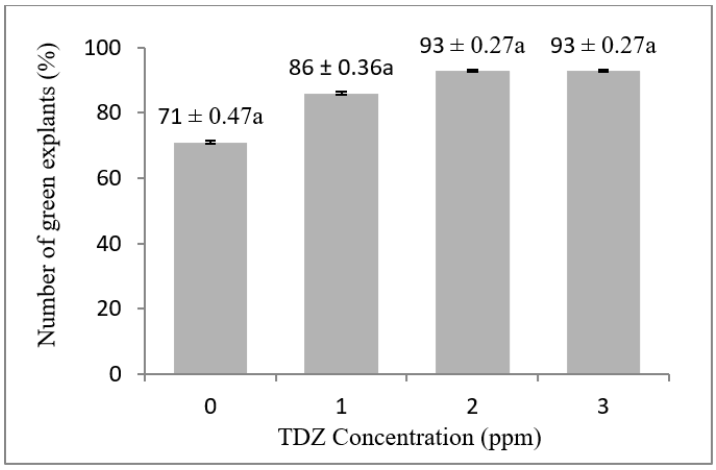


**Figure 1**. Percentage of green explants of *Cymbidium bicolor* orchid stems planted with TDZ treatment (0, 1, 2, 3 ppm) 8 weeks after planting (WAP) with fourteen replications. Values represent the percentage ± standard deviation. Values followed by the same letters are not significantly different according to the Duncan Test at a 0.05 significance level.

Based on the percentage of green explants (Figure 1), the addition of thidiazuron (TDZ) affected the greenness of the explants. The percentage of green explants indicated a tendency that with the addition of TDZ concentration, the browning of *C. bicolor* explants decreased (Figure 1). The percentage of green explants showed that the administration of TDZ increased the chlorophyll content produced by *C. bicolor* explants. Yuniati & Isda (2024) stated that TDZ could affect the formation/

synthesis of chlorophyll, increasing the amount of chlorophyll in explants. This statement is supported by Mihovilovic et al. (2020), showing that administering TDZ with the right concentration could increase the chlorophyll content in *Amelanchier alnifolia*.

According to Sumihar et al. (2021), explant color is one of the live indicators of explants in tissue culture propagation. In addition, the color of the explants also indicates maximum growth. The green color of the explants indicates that the plant cells are actively photosynthesizing and dividing, but if the explants are brown, it indicates that the explants are starting to become inactive and the cells are less viable or dying (Figure 2; A, C, E, G). Many factors affect brownings, including plant type and genotype, explant damage, media composition, and culture conditions (Amente & Chimdessa, 2021).

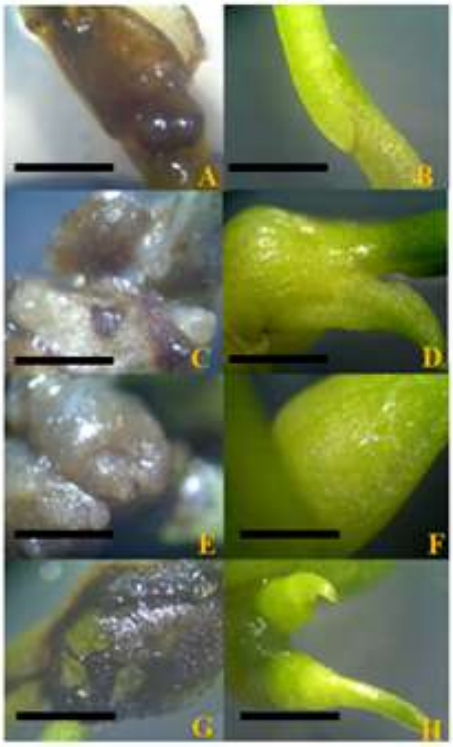


**Figure 2.** Explants of *Cymbidium bicolor* orchids that remained green (B, D, F, H) and those that experienced browning (A, C, E, G). A-B=TDZ 0, C-D=TDZ 1 ppm, F-E=TDZ 2 ppm, G-H=TDZ 3 ppm

The explants turned brown (Figure 2; A, C, E, G) due to the inhibition of nutrient absorption in the media, so it could be seen that the growth of the explants was disturbed (Figure 2; A, C, E, G). Growth and morphogenesis were not inhibited in explants that did not experience browning (Figure 2; B, D, F, H). According to Jaiswal et al. (2021), the browning condition of explants may also result from nutrient depletion, which halts their growth and necessitates periodic transfers to a fresh growing media. Browning can also occur due to stress caused by cutting and the release of phenol, which is then oxidized. Zhao et al. (2021) stated that the wound caused by cutting encouraged the release of phenol that will be oxidized to quinone by the Polyphenol Oxidase (PPO) enzyme. This irreversible growth inhibition occurs when the phenol is oxidized to the enzyme quinone, polymerizing and oxidizing the protein to an increasing amount of melanic compounds. Phenol that causes explants to brown and dry out can eventually lead to the death of the explants (Punja et al., 2019).

The growth and morphogenesis of explants in vitro are controlled by the balance and interaction of endogenous and exogenous PGRs. Genetic or endogenous traits originating from within the plant include the ability of cells to absorb nutrients available in the media. Meanwhile, exogenous properties can be in the form of technical influences on the implementation of culturing and the addition of PGR to the media.

The presence of TDZ in the media can stimulate the development of chloroplasts in explants (Rineksane et al., 2021). Thidiazuron can affect the color of explants to remain green. The use of TDZ at the right concentration can inhibit leaf discoloration (Bariyyah & Putri, 2021), maintain leaf functionality (Erland et al., 2020), reduce post-harvest browning of lychee fruit pericarp (Fahima et al., 2019), and inhibit leaf senescence (Wang et al., 2019b).

## Somatic Embryo Formation

The induction of somatic embryogenesis in this study is direct embryogenesis, which is superior in terms of time reduction because it does not go through the callus stage (Adri, 2019). There is no dedifferentiation stage in direct embryogenesis, where there is little genetic reprogramming, and embryonic cells are formed directly from the explant surface (Xu et al., 2019). Direct embryogenesis minimizes the occurrence of genetic changes induced by tissue culture processes (Jayusman, 2021).

This study used Thidiazuron (TDZ) treatment because TDZ can induce somatic embryos (Restanto, 2023). Thidiazuron is an effective growth regulator in inducing somatic embryos, and it must be used at proper concentrations (Lizawati et al., 2023). The method used in this study was an induction of somatic embryogenesis with various concentrations of TDZ (0, 1, 2, 3 ppm) for eight weeks.

Administration of TDZ at low concentrations induces somatic embryogenesis faster than at high concentrations (Lizawati et al., 2023). Statistical analysis results on the formation of somatic embryos showed that the TDZ concentration of 1 ppm significantly differed from the treatment with TDZ 0, 2, and 3 ppm in *C. bicolor* explants, where 1 ppm TDZ was the treatment with the most optimal results. The results were obtained from the analysis of the number and percentage of somatic embryos formed from the four TDZ treatments, as shown in Table 1, where the values are presented in different letters (a/b) and have different significance at $p < 0.05$.

**Table 1.** Number and percentage of somatic embryos of *Cymbidium bicolor* Orchid explants with Thidiazuron treatment at concentrations of 0-3 ppm in 0-8 weeks after planting (WAP) with fourteen replications

| TDZ Concentration (ppm) | Explants Forming Somatic Embryos (%) | Total Somatic Embryos | Average Somatic Embryos per Explant |
|---|---|---|---|
| 0 | 93 | 27 | 1.93±1.27[a] |
| 1 | 100 | 172 | 12.29±7.55[b] |
| 2 | 100 | 60 | 4.29±6.32[a] |
| 3 | 93 | 39 | 2.79±2.55[a] |

Remarks: Values represent means ± standard deviations. Values followed by different letters show a significant difference based on the 95% Duncan test.

The findings of this investigation showed that 93% of the explants grown in a medium containing TDZ 0 and 3 ppm generated somatic embryos. All explants produced 100% somatic embryos when

cultivated on media treated with TDZ 1 and 2 ppm. The findings of this investigation showed that 93% of the explants grown in a medium containing TDZ 0 and 3 ppm generated somatic embryos. All explants produced 100% somatic embryos when cultivated on media treated with TDZ 1 and 2 ppm. A total of 172 somatic embryos were created at 1 ppm TDZ, 60 somatic embryos at 2 ppm TDZ, 39 somatic embryos at 3 ppm TDZ, and only 27 somatic embryos at 0 ppm TDZ (Table 1). The treatments between TDZ 0, 2, and 3 ppm were not significantly different (Table 1), so it was found that a concentration of 1 ppm TDZ induced the most optimal somatic embryos compared to other treatments. This follows previous studies on the induction of somatic embryos of *Dendrobium ovatum* by Pyati (2022), which found that 1 ppm TDZ was the most effective in increasing somatic embryo induction.

Based on the observations that have been made, the administration of TDZ in tissue culture media greatly affected the response of *C. bicolor* orchid stem explants, as indicated by an increase in the mean somatic embryos formed (Figure 3). At a concentration of 0 ppm, somatic embryos were formed because the explants of *C. bicolor* had endogenous hormones capable of inducing somatic embryos. The added thidiazuron can activate metabolic processes, such as carbohydrate metabolism, ROS (Reactive Oxygen Species) metabolism, photosynthesis, and protein synthesis, which are needed for the transformation process. This process causes somatic cells to dedifferentiate and then become meristems that trigger the induction of somatic embryos (Erland et al., 2020; Ali et al., 2022; Feher, 2019).

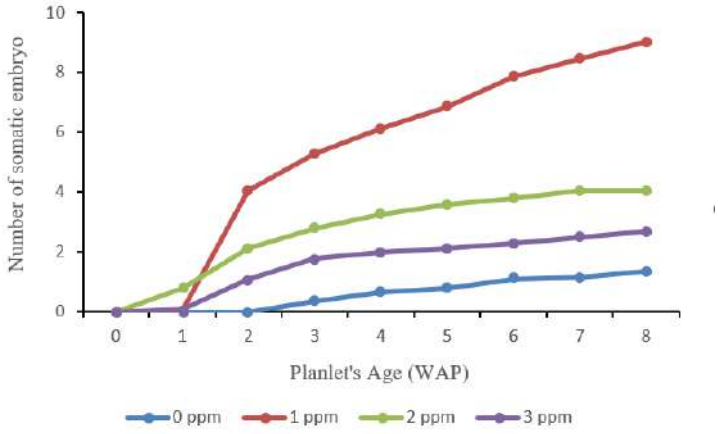


**Figure 3.** Average number of somatic embryos of *Cymbidium bicolor* formed after TDZ treatment (0,1,2,3 ppm) from 0-8 weeks after planting (WAP).

Table 1 shows that TDZ 2 and 3 ppm concentrations were less than optimal due to a decrease in the yield of somatic embryos compared to TDZ 1 ppm because the concentrations were thought to be too high. Thidiazuron concentrations that are too high can cause abnormalities and inhibit somatic embryo growth. Thidiazuron is a herbicide that can kill tissue and cause deviations in plant tissue development (Pardede et al., 2021). This is related to the absorption and transfer of nutrients and the metabolism of TDZ. Suppose the concentration of TDZ given to plants is appropriate. In that case, it will increase the overall absorption of sugar from the culture media, increase primary metabolism, transfer terpene metabolism, and mediate stress metabolism through indoleamine and phenylpropanoid metabolism (Erland et al., 2020).

The average induction time in TDZ treatment of 0-3 ppm was 2 WAP (Figure 3). *C. bicolor* explants grown on TDZ-added media showed a faster induction time (1 WAP) (Figure 3). This is because the hormone spreads in the tissue quickly. Devireddy et al. (2020) stated that the spread of the hormone could be through the intercellular space/cytoplasm, not necessarily through the vascular system. Therefore, the induction of somatic embryos in *C. bicolor* orchids occurs rapidly.

Thidiazuron in tissue culture media at the right concentration will stimulate somatic embryogenesis (Budi, 2020). Figure 3 also shows that TDZ works at low concentrations; this is proved by the response to the emergence of the most optimal somatic embryos shown in the 1 ppm TDZ treatment, namely the emergence of somatic embryos in the first week after planting (WAP), and the mean somatic embryos formed were more significant than other treatments. These results significantly differed from the treatment of 0, 2, and 3 ppm, which indicated that against *C. bicolor* explants, TDZ worked at low concentrations.

Thidiazuron works at low concentrations, where the medium can quickly transfer and metabolize TDZ because the higher the concentration, the more complex the distribution and metabolism process. As a result, somatic embryo growth remains at an early, globular stage. In another sense, besides being able to induce somatic embryogenesis, the TDZ hormone can also inhibit the regeneration of somatic embryos, depending on its concentration and the type of plant being cultured (Pardede et al., 2021). Budi (2020) also reinforces this statement, stating that the hormone concentration will inhibit the plant's growth if it is too high.

Table 1 shows that the endogenous hormones in the explants have a role in forming somatic embryos. It is proven that somatic embryos were still formed even at 0 ppm TDZ treatment. Non-specific tissues, such as meristematic tissue, produce endogenous hormones that can be produced when stimulated (Hong et al., 2019). Stimuli that can affect hormone production are growth media and environmental conditions (Hong et al., 2019). When the hormone has reached a specific concentration, the previously inactive gene will begin to express (Fadón et al., 2020).

Apart from endogenous hormones, somatic embryos can be induced at 0 ppm media because *C. bicolor* already has cell competence to form somatic embryos. The media already contained nutrients such as nitrogen, amino acids, and vitamins and was also supported by peptone added to the media, which increased the nitrogen content (Krisdianto et al., 2020). MS media added with peptone and supported by endogenous hormones, as well as other environmental factors such as the appropriate temperature (±25°C) and sufficient light, worked synergistically in influencing and activating the formation of a cell's competence in forming somatic embryos.

The addition of exogenous PGR affects the activity of endogenous hormones. This activity is a factor that stimulates further growth and development (Burgel et al., 2020). The interaction between TDZ added to the media and endogenous hormones determines the direction of the development of an explant (Rahmah et al., 2020). This will trigger physiological changes and the formation of somatic embryos because cells that initially do not have embryonic capabilities become embryonic competencies. Narváez et al. (2019) stated that some cells acquired embryogenic abilities after adding TDZ hormone in culture media. This is proved by the study of Vallado et al. (2022), reporting the success of increasing the induction rate of somatic embryos in *Hippeastrum hybridum* using low

concentrations of TDZ (0, 0.5, 1, 1.5, and 2 ppm), which in that study, the TDZ 0 ppm also produced somatic embryos. However, with the addition of TDZ, even at low concentrations, the number of somatic embryos increased. Low TDZ concentrations have affected the formation of somatic embryos in *C. bicolor* orchids.

## Morphology of Somatic Embryo of *C. bicolor*

The results of the qualitative analysis of this study were obtained by observing the morphological structure formed on the explants of *C. bicolor* for eight weeks. The observation of the growth of somatic embryos in vitro showed a growth pattern of somatic embryos directly from explant cells without going through the callus phase (Figure 4). Somatic embryos were formed in the wounded area of the *C. bicolor* explant. However, if the area was browning, somatic embryos were formed near the wounded area (Figure 4). The incision wounds encourage the wounded explant cells to become meristematic and then actively divide, allowing the previously differentiated explants to generate totipotent somatic embryos (Wehbi et al., 2022). The wound causes TDZ to diffuse into plant tissues more easily so that TDZ will stimulate and induce somatic embryogenesis.

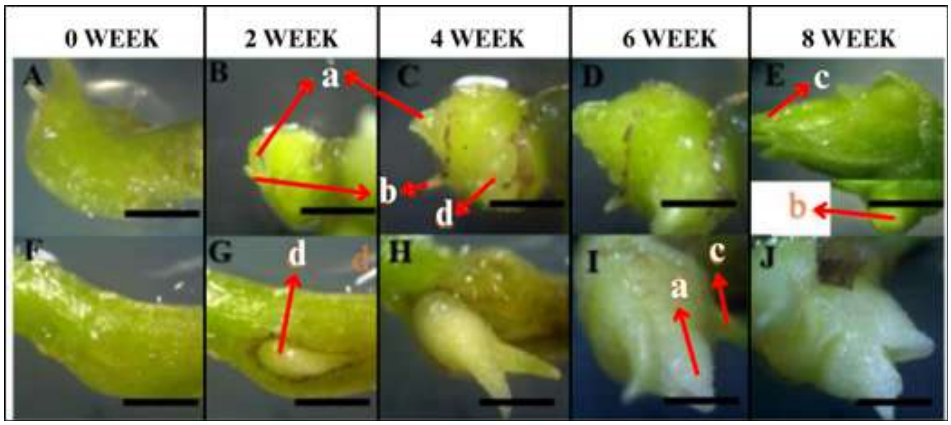


**Figure 4.** Development of *Cymbidium bicolor* Orchid Somatic Embryo with TDZ at concentrations of 1 ppm (A-E); 3 ppm (F-J). a: Shoot Apical Meristem (SAM), b: Root Apical Meristem (RAM), c: leaf, d: globular phase.

The development of somatic embryos of *C. bicolor* in vitro in the first week started with swelling of the embryo and some color changes to become brighter. In the second week (Figure 3 G), the embryo swelled and began to make a round shape, called the globular phase. The stages of the formation of somatic embryogenesis start from cell division into a collection of cells that form globular (Hernandez et al., 2019). TDZ treatment of 1 ppm at 2 WAP (Figure 4 B) formed a bipolar structure characterized by the appearance of SAM and RAM at the heart phase stage. In the 3 ppm TDZ treatment, SAM was formed first, then after 4 WAP, RAM began to appear. In Figure 4D, SAM and RAM continue to grow lengthwise and enlarge; this is the torpedo phase. SAM then developed into leaves at the coleoptile phase (Figure 4 E, J). Agustin et al. (2020) stated that SAM was formed before leaf primordia and would develop into mature leaves. The development of somatic embryos (Figure 4) corresponds to the development of *P. amabilis* orchid seeds that have been reported. In the first phase, the embryo is yellow; then, it will turn green, forming a bipolar structure, then leaf primordia, and the leaves will continue to form (Gulzar et al., 2020).

The explants used in this study were stems of the *C. bicolor* orchid plantlet. Orchid stems were chosen because they contain floral meristems (Wang et al., 2019a), which have high meristematic abilities. Loyola-Vargas et al. (2022) also stated that cells with high meristematic ability showed high regeneration potential and embryogenesis induction ability. Therefore, the induction of somatic embryogenesis is easier on stem explants. Somatic embryos of *C. bicolor* orchids growing on media containing TDZ experienced faster growth. Their size automatically became larger (Figure 5 B) compared to somatic embryos grown on media without the addition of TDZ (Figure 5 A, C, D); the number of somatic embryos produced was even higher (Figure 14 B, C, D). Thidiazuron can increase the growth rate of somatic embryos because TDZ is one of the most active cytokinins that induces more significant proliferation than other cytokinins. This hormone does more vigorous activity than other types of cytokinins.

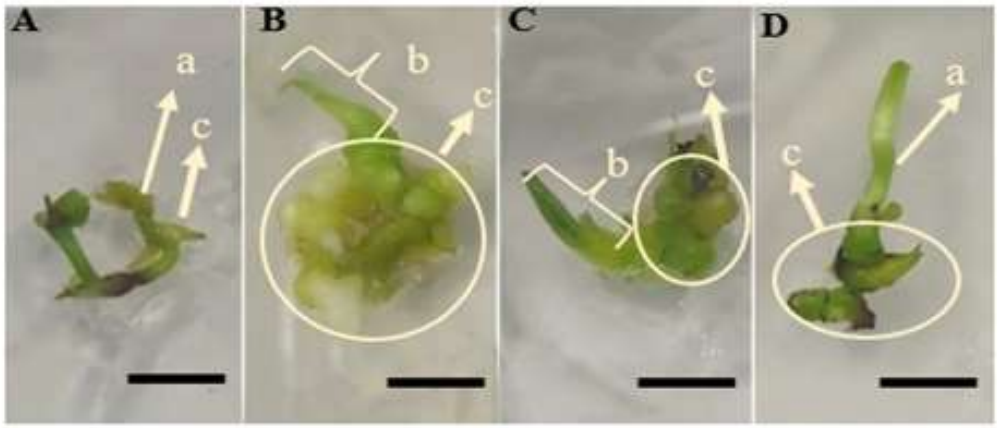


**Figure 5.** Morphology of Somatic Embryo of *Cymbidium bicolor* Orchid at 8 WAP with various concentrations of TDZ: 0 ppm (A), 1 ppm (B), 2 ppm (C), 3 ppm (D), showing shoot with leaf primordia (a), leaf primordia (b), and somatic embryo (c) (Scale bar = 5 mm)

Thidiazuron diffuses into plants and cellularly will act as a signal that will increase the number of purines and convert adenine to adenosine, then adenosine will be converted into ribonucleotides (Chandel, 2024; Erland et al., 2020). Ribonucleotide activity will affect the synthesis of proteins that will act as enzymes isopentenyltransferase (IPT), nucleoside 5-monophosphate phosphoribohydrolase (LOG), and dehydrogenase (CKX), which are involved in the synthesis of cytokinins (Chen et al., 2022). Nucleoside 5-monophosphate phosphoribohydrolase (LOG) is a regulator and second messenger that will affect cell division (Zhao et al., 2024). In the process of cell division, cytokinins (TDZ) signal to restructure development towards the embryogenic pathway. This will trigger the embryogenic developmental pathway that leads to the formation of somatic embryos, where cells that initially do not have competence become embryonic abilities due to the restructuring of the pathway (Zhao et al., 2024).

Therefore, this study stated that TDZ at a concentration of 1 ppm successfully induced somatic embryos in *C. bicolor*. The effectiveness of the research is highlighted by the ability to produce somatic embryos with low TDZ concentrations, eliminating the need for higher concentrations.

## CONCLUSION

Thidiazuron treatment stimulated the formation of somatic embryos. The optimal concentration of TDZ to stimulate the induction of somatic embryos in *C. bicolor* was 1 ppm, which induced somatic embryos the fastest (one week after implantation) and produced the highest number of somatic embryos.

## ACKNOWLEDGMENTS

The authors thank Diponegoro University for providing research on the *C. bicolor* somatic embryo induction culture. Gratitude is also conveyed to Dr. Endang Saptiningsih, M. Si, for the comments on the manuscript.

## AUTHORS CONTRIBUTIONS

NS and YN designed and conceived the experiments. AMI and NS experimented. AMI, NS, and YN contributed to the preparation of samples and interpretation of the results. The manuscript was primarily composed by AMI. All authors provided critical feedback and contributed to developing the research, analysis, and manuscript.

## COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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# Growth and Yield of Mint (*Mentha spicata* L.) as Affected by Composition of Charcoal Husk and Organic Fertilizer

10.18196/pt.v13i1.21758

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**ABSTRACT**

The high industrial demand for mint products, coupled with low domestic production, has led to a 63% import dependency. Enhancing mint production requires optimizing planting media and fertilization strategies. This study aimed to evaluate the effects of different soil-to-husk charcoal ratios and manure types on the growth and yield of mint (*Mentha spicata* L.). A factorial experiment was conducted from February to April 2022 at the Faculty of Agriculture, UNS, using a randomized complete block design (RCBD) with two factors: The first factor was soil-to-husk charcoal ratios consisting of 1 to 3, 1 to 1, and 3 to 1. The second factor was the manure type, which consisted of cow, goat, and chicken manure. Each treatment was replicated four times. The results showed that the P1 combined with the cow manure significantly increased the number of branches compared to P1 with goat manure. P1 also resulted in the highest number of leaves, leaf area, fresh weight, and dry weight. Cow manure yielded the highest values for leaf number, leaf area, and dry weight among manure treatments. These findings suggest that optimizing planting media composition and manure selection can enhance mint productivity, reducing reliance on imports.

**Keywords:** Biochar; Herbs; Husk; Manure; Mint

## INTRODUCTION

Mint (*Mentha spicata* L.) is a widely cultivated aromatic herb valued for its essential oils, medicinal properties, and applications in the food, pharmaceutical, and cosmetic industries. The genus *Mentha* is known for its high content of bioactive compounds, including menthol, carvone, and flavonoids, which contribute to its antimicrobial, antioxidant, and therapeutic properties (Hutsol et al., 2023) However, mint's chemical composition and nutritional value vary significantly depending on genetic, environmental, and agronomic factors (Lakušić et al., 2012).

The 17 global Sustainable Development Goals directly or indirectly influence soil and factors such as plant productivity, environmental sustainability, and human health (El-Ramady et al., 2022). One of the main goals of sustainable agriculture nowadays is to reduce the usage of chemical fertilizers to maintain sustainable crop productivity (Moradzadeh et al., 2021). Quality and production metrics are positively impacted by using organic and biofertilizers in cultivating crops of high economic value (El-Beltagi et al., 2023). Horticultural crops grown organically are of higher quality and yield a safe product that humans may use (Nada et al., 2022). Mint is a type of medicinal plant, which is a horticultural plant, so it is necessary to use planting media and apply organic fertilizer to support

Article History
Received : 29 February 2024
Revised : 31 July 2024
Accepted : 03 September 2024

growth and yield.

Despite its economic importance, domestic mint production in Indonesia remains insufficient to meet the demand for industrial needs. The country relies heavily on imports, with an average annual import volume of 76.10 tons, accounting for 63% of total industrial demand, and the import value reached 529.5 billion rupiah (Hasanah et al. 2019; BPS, 2016). The high dependency highlights the need for improved practices to enhance local production. Increasing mint productivity can be achieved through agronomic optimization, particularly by improving planting media composition and fertilization strategies, which play a critical role in plant growth and biomass accumulation (Song et al., 2019).

The selection of an optimal planting medium is crucial, as it influences root development, nutrient availability, water retention, and aeration. Previous studies suggest that well-balanced planting media should have high porosity, adequate moisture retention, and sufficient nutrient-holding capacity to support early root establishment, especially in vegetatively propagated plants such as mint (Dharaben Champaklal & Mansungbhai Chaudhari, 2023). Husk charcoal is widely recognized as a soil amendment that improves aeration and drainage while enhancing microbial activity and nutrient availability (Sodiq et al., 2019). Organic fertilizers, particularly animal manure, are essential for sustaining soil fertility and providing a slow-release nutrient source. Goat manure and husk charcoal have been reported to improve soil structure and enhance the supply of N, K, P, Mg, and Ca, which are critical for plant growth and development (Rayne & Aula, 2020). Moreover, organic fertilization is recommended for medicinal plants because it enhances secondary metabolite production, which is crucial for the pharmaceutical and essential oil industries (Kementrian Pertanian, 2013). The recommended application of organic manure for mint cultivation is 30 tons per hectare, yet the most effective combination of manure type and planting media composition remains unclear.

Despite the growing interest in optimizing mint cultivation, studies on the ideal combination of planting media and organic fertilizer to maximize growth and yield remain limited. While previous research has demonstrated the benefits of husk charcoal and manure application, their interaction in different proportions requires further investigation. Therefore, this study aimed to evaluate the effects of different soil-to-husk charcoal ratios and manure types on the growth and yields of *Mentha spicata* L. The findings are expected to contribute to sustained mind cultivation strategies, reducing import dependency while improving productivity and soil health.

## MATERIALS AND METHODS

The research was conducted in the greenhouse, Ecology and Crop Production Management, and Soil Chemistry Laboratory of the Faculty of Agriculture, Universitas Sebelas Maret, from February to April 2022. The experiment was arranged in a randomized complete block design. The first factor was the ratio weight of soil and husk charcoal, namely 1:3, 1:1, and 3:1. The second factor was the type of manure, namely cow, goat, and chicken, with a dose of 30 t ha or 577 g per polybag (Kementrian Pertanian, 2013). Polybags were prepared by mixing soil, husk charcoal, and manure according to the treatment. There were 12 polybags for each treatment. The organic fertilizer used was mature fertilizer, which is one of the most important organic fertilizer sources. Soil fertility increases

since manure provides nutrients such as nitrogen that soil microbes can take. Manure increases the water-holding capacity or soil structure (Ullah, 2023).

Plant management was carried out in accordance with the procedures for cultivating mint plants, including modifying the surrounding environment, watering, removing weeds, replanting, and controlling pests and diseases. Every afternoon, the plants were watered. Weeding was done to prevent weeds from competing for nutrients so that mint plants can absorb as many nutrients as possible. Disease-affected plant components were removed, and pests were eradicated manually.

The parameters observed included mint growth and yield. The growth parameters include plant height, number of leaves, branches, and leaf area. Plant height observations were carried out once a week. The observation of the number of leaves was done manually by counting the leaves. The calculation of leaf area was carried out using the paper gravimetric method using Formula (1). (Sitompul, 2005).

$$Leaf\ area = \frac{weight\ of\ sampels}{weight\ of\ paper} \times paper\ area \qquad (1)$$

The observation of the number of branch shoots was done by manually counting the branch shoots. Meanwhile, the variables of yield parameter include fresh weight and dry weight. The fresh weight of the plant was weighed after harvest by separating all parts of the plant from the roots. Weighing was carried out using digital scales. The dry weight of the plant was calculated after the plant parts separated from the roots were heated in the oven for 1 x 24 hours at a temperature of 60 degrees Celsius and then weighed until reaching constant weight.

The observation data were analyzed using the ANOVA test and SPSS software. The test was continued with Duncan's test with a 5% to determine a significant difference between treatments.

## RESULTS AND DISCUSSION

### Environment Conditions

Mint was planted in polybags in a greenhouse located at coordinates 7°33'38.54" South Latitude, 110°51'32.18" East Longitude, and an altitude of 106.2 meters above sea level. The air temperature at the research location ranges from 27° to 34°C, the average humidity is 78%, and the light intensity is 2146.3 lux. Higher air temperatures and lower relative humidity are ideal growing conditions for M. arvensis. Because the plants develop better in terms of plant height and leaf area index, which leads to a higher fresh yield, M. arvensis can produce valuable essential oil under these conditions. Syahirah et al. (2019) state that Indonesia's microclimate is suitable for mint plant growth. During the growth stages of plants, changes occur in the atmosphere. Environmental conditions need to be considered to support mint growth. Ideal temperature, humidity, and light intensity factors can increase the quantity and quality of mint. This indicates that the light intensity in the research environment is less than optimal for mint growth. Sunlight primarily affects photosynthetic and photostimulus processes, including pigment creation, chlorophyll synthesis, leaf growth, blooming, and plant bud development.

**Table 1.** Soil chemical properties before providing treatment with a composition of husk charcoal and organic fertilizer before experiment

| Properties | result | unit | VALUE |
|---|---|---|---|
| pH soil (actual method) | 6.67 | - | Neutral |
| Total N (Kjeldhal) | 0.12 | % | Low |
| Available P (Bray 1) | 4.14 | mg $kg^{-1}$ | Very low |
| Total K (Fotometer) | 0.37 | cmol(+)$kg^{-1}$ | Very low |
| Total organic C (Walkey & Black) | 3.99 | % | High |
| Ratio C/N | 33.25 | - | Very high |

Sources: Results of analysis from the Chemistry and Soil Conservation Laboratory of Faculty of Agriculture UNS (2022) * values were adjusted to Balittan in 2009.

The type of soil used is alfisol soil originating from Karangpandan, Karanganyar Regency. Alfisol soil itself is formed from the weathering of limestone and sedimentary rocks. Alfisol soil has a higher pH than that made from parent material or sandstone. The results of the soil chemical analysis conducted in the laboratory (Table 1) showed that the soil pH was neutral, but the available P and total K content was very low. The total N content and organic C were low, resulting in a very high C/N ratio.

## Plant Height

Various compositions of soil, husk charcoal, and various types of manure, as well as the interaction of both, did not affect the height of the mint plants. Based on Figure 1, mint plants treated with the composition of soil and husk charcoal with a ratio of 1:3 and chicken manure showed an average plant height of 56.58 cm. Meanwhile, those treated with a ratio of 1:3 and goat manure had a low average plant height of 40.1 cm. According to Tambunan et al. (2022), increasing the length of mint plants is called sympodial growth. Primary branches will form secondary branches, then tertiary branches continuously. This causes the main stem of the mint plant not to reach its maximum height because the branches grow even faster than the main stem.

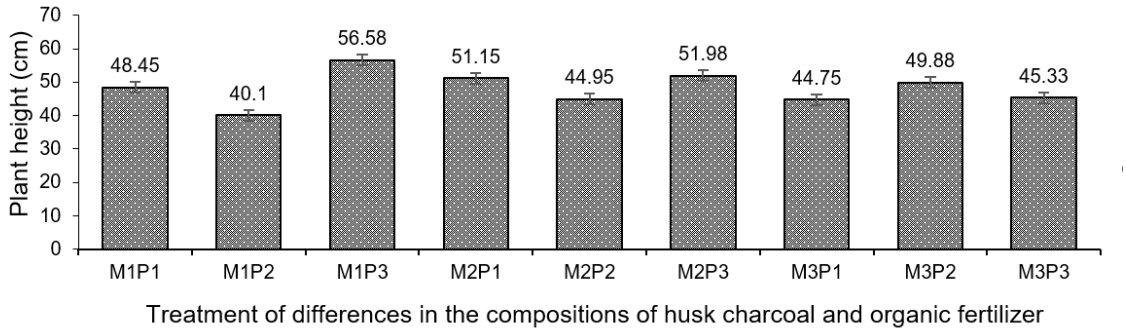


**Figure 1.** Effects of the composition of husk charcoal and the application of various types of manure on the height of mint plants. M1= soil: husk charcoal (1:3), M2 = soil: husk charcoal (1:1), M3: soil: husk charcoal (3:1), P1 = cow manure, P2= goat manure; P3 = chicken manure

## Number of Branches

Applying different kinds of manure and husk charcoal to the soil had an impact on the quantity of mint plant branches. Table 2 shows the number of mint branches with the highest value, which is

**Table 2.** Average number of branches as affected by differences composition of husk charcoal and organic fertilizer by treatments

| Treatment of composition soil and husk charcoal + type of manure | Number of branches (branch) | |
|---|---|---|
| (1:3) + cow | 47.00±7.37 | A |
| (1:3) + goat | 31.50±3.59 | C |
| (1:3) + chicken | 42.25±4.00 | A |
| (1:1) + cow | 40.25±2.59 | Ab |
| (1:1) + goat | 33.75±2.00 | bc |
| (1:1) + chicken | 33.00±2.53 | bc |
| (3:1) + cow | 32.50±2.89 | c |
| (3:1) + goat | 34.00±1.83 | bc |
| (3:1) + chicken | 35.00±1.12 | bc |
| Sig. | 0.01 | |
| CV% | 14.55% | |

Remarks: Values followed by the same letters indicate no significant difference based on Duncan's Multiple Range test (DMRT) at 5%.

47, produced by a 1:3 soil and husk charcoal composition with cow manure. The lowest number of branches, 31.5, was produced by a 1:3 soil and husk charcoal composition with goat manure.

The number of mint branches is one of the supporting factors that can influence the number of leaves. The number of branches in a plant will also affect the weight of the plant. According to Harahap et al. (2020), husk charcoal has an organic C content of 18.45% and a total soil N content of 1.07%, with a C: N ratio of 1 of 7.24. Furthermore, a good planting medium allows the roots to absorb water and nutrients optimally, thus influencing the formation of branches. When compared to a treatment without charcoal, applying biochar raises the amount of available soil nutrients, particularly available P and K (Zhang et al. 2024).

The availability of nitrogen in the growing medium affects the number of branches. Rosawanti (2019) states that the nitrogen content of organic fertilizer functions as a constituent of chlorophyll so that it can capture solar energy for photosynthesis. Besides, the amino acid (protein) content can influence growth in ways such as supporting branching, increasing leaf formation, and increasing the size and shape of plants. Furthermore, cow manure increased the yield of plant height, number of branches, fresh weight, and dry weight of mint plants.

## Number of Leaves

The soil and husk charcoal composition combined with the application of manure independently showed an effect on the number of mint plant leaves. Table 3 shows that ratio 1:1 produced the lowest number of mint leaves (167.17) compared to 1:3 and 3:1, with the respective values of 219.35 and 210.08. The use of cow manure showed the highest number of mint leaves (222.33) compared to goat manure and chicken manure, with values of 183.67 and 191.00 mint leaves, respectively (Table 4). Based on Table 3, the ratio 1:1 produced the lowest number of mint leaves compared to 1:3 and 3:1, with the respective values of 53 and 43. The use of cow manure showed the highest number of mint leaves compared to goat manure and chicken manure, with values of 39 and 32 mint leaves, respectively (Table 4).

In this research, the best organic fertilizer used was cow manure, resulting in an average number of leaves of 219.75. Providing organic fertilizer affects vegetative growth by increasing the number

**Table 3.** Average number of leaves, fresh weight of plants, dry weight of plants, and leaf area of the crop as affected by various compositions of soil and husk charcoal

| Composition of soil and husk charcoal | Number of leaves | Fresh weight of plant (g crop$^{-1}$) | Dry weight (g crop$^{-1}$) | Leaf area (cm$^2$ crop$^{-1}$) |
|---|---|---|---|---|
| 1:3 | 219.75±14.67a | 11.77±0.98a | 3.24±0.50a | 209.50±23.27a |
| 1:1 | 167.17±22.50b | 10.16±0.16ab | 2.55±0.01ab | 139.87±25.96b |
| 3:1 | 210.08±7.83a | 9.21±0.83b | 1.82±0.51b | 174.37±1.57b |
| Sig. | 0.13 | 0.06 | 0.16 | 0.38 |
| CV% | 17.73% | 20.52% | 37.78% | 27.70% |

Remarks: Values followed by the same letters indicate no significant difference based on Duncan's Multiple Range test (DMRT) at 5%.

**Table 4.** Average number of leaves, fresh weight of plants, dry weight of plants, and leaf area of the crop as affected by various types of manure

| Type of manure | Number of leaves | Fresh weight of plant (g crop$^{-1}$) | Dry weight (g crop$^{-1}$) | Leaf area (cm$^2$ crop$^{-1}$) |
|---|---|---|---|---|
| Cow manure | 222.33±16.50a | 11.49±0,78a | 2.96±0.30a | 211.21±24.48a |
| Goat manure | 183.67±10.84b | 8.71±1,18b | 1.84±0.49b | 149.38±19.24b |
| Chicken manure | 191.00±5.66ab | 10.94±0,40a | 2.80±0.19a | 164.26±8.72ab |
| Sig. | 0.13 | 0.06 | 0.16 | 0.38 |
| CV% | 17.73% | 20.52% | 37.78% | 27.70% |

Remarks: Values followed by the same letters indicate no significant difference based on Duncan's Multiple Range test (DMRT) at 5%.

of leaves and other plant growth parameters (Qulsum et al., 2021). Cow manure contains a number of nutrients and organic materials that can improve the fertility of the soil. Increases in N and P elements significantly affect plant physiological processes. Nitrogen plays a role in the formation of chlorophyll, so the photosynthesis process increases. Besides, it can stimulate the number of leaves. Meanwhile, phosphorus is a component of ADP and ATP, which are important in photosynthesis and ion absorption. The longer the presence of elements in the media will impact growth, namely the number of leaves that will form.

The highest average number of mint leaves was produced by a 1:3 soil and husk charcoal composition (M1). According to research by Gaso et al. (2022), applying a husk charcoal composition with a ratio of 4/5 produced the highest number of kale leaves compared to smaller ratios, namely 3/5, 2/5, and 1/5. This is also supported by research by Ahmad et al. (2021), which shows that using 300g of husk charcoal per plant, namely the highest dose, produces the highest average number of celery leaves. The aeration of organic planting media is comparable to that of rockwool planting media, and its high porosity can help plant nutrient solutions store more (Rahayu & Mulyani, 2022). The advantage of husk charcoal is that it can improve the physicochemical properties of the soil, namely porosity, root respiration, and soil moisture. Besides, husk charcoal can bind water, then release it into the micropores to be absorbed by plant roots, and can encourage the growth of microorganisms that are good for soil and plants.

## Fresh Weight of the Plant

Using various compositions of soil and husk charcoal and applying different types of manure independently showed an effect on the fresh weight of mint plants. Table 3 shows that the composition of soil and husk charcoal in a ratio of 3:1 resulted in the lowest fresh weight compared to a composition of soil and husk charcoal in a ratio of 1:3 and 1:1. The application of goat manure showed the

lowest fresh weight compared to cow manure and chicken manure, each with a difference of 2.78 g and 2.23 g in fresh weight (Table 4).

Cow manure has several benefits, such as balancing low substrate parameters, pH, C/N ratio, and nutrient content in the soil (Tallou et al., 2020). According to Musdalifah et al. (2021), widely distributed organic fertilizers are cow manure, which has not been used optimally. Cow manure has a high nutrient content of C, N, P, Ca, and Mg. By increasing the photosynthetic rate, chemical fertilizers particularly N and P can improve the growth characteristics and output of plants (Iqbal et al., 2019). It was claimed that when 50% chemical fertilizer and nano-chelated fertilizer were applied, the proportion of menthol increased and the percentage of benzofuran decreased, which was correlated with the quality of peppermint essential oil (Ostadi et al., 2020). The research results of Biswas et al. (2022) show that applying cow manure to mint plants can increase plant height, main branches, and secondary branches. Carbohydrates will be synthesized into protein by nitrogen so that the cell division process increases, which impacts the formation of stems and branches and is followed by an increase in the fresh weight of mint.

## Dry Weight

Various compositions of soil and husk charcoal and various types of manure independently affected the dry weight of mint plants. The composition of soil and husk charcoal with a ratio of 3:1 produced the lowest dry weight of mint plants compared to other treatments (Table 3). Meanwhile, goat manure was applied to the type of manure, which resulted in the lowest dry weight of mint plants compared to cow manure and chicken manure. In addition to its high nutrient content, organic manure has a lot of vitamins, plant development hormones, and beneficial microorganisms. Applying organic fertilizer has a positive impact on the biomass production of mint (Olumide, 2022). By enhancing soil quality and structure, plant nutrient availability, and biomass C input through improved crop development, manure application can sustain high levels of crop yield. A sustainable increase in soil productivity is probably the cause of higher crop productivity when manure is applied (Du et al., 2020).

Dry weight shows the biomass plants produce from the photosynthesis process while the plant is growing. According to Amani et al. (2019), Enhancing biomass yield and the quality of essential oils in aromatic and medicinal plants depends on nutrient availability. The use of husk charcoal in the planting medium increased the dry-weight yield of mint (Table 3). Rice husk charcoal can improve soil structure so that it becomes loose. Pratama et al. (2020) state that husk charcoal functions to bind and release water and is a source of N, P, and K. The result of research by Biswas et al. (2022) showed that the application of organic manure had a significant effect on dry weight and fresh weight of mint.

Different mint species have different physical traits and chemical compositions depending on their surroundings (Koutsoukis et al., 2019). Genetic variables from distinct species influence the growth, synthesis, and accumulation of secondary metabolisms in medicinal and aromatic plants (Li et al., 2020). The research showed that the application of cow manure could increase the dry weight of mint. Organic fertilizers can influence plant growth through improving soil physical properties, C, N, P, and K status, and microbial biomass. The availability of macro-nutrients like N, P, and K is very important for the growth of mint plants because if a deficiency occurs, it will have an impact on plant production, such as reducing shoot root ratio, leaf area, and dry weight (Janpen et al., 2019).

## Leaf Area

Various compositions of soil, husk charcoal, and various types of manure independently showed an effect on the leaf area of mint plants. Based on Table 3, the composition of soil and husk charcoal with a ratio of 1:3 produced the largest plant leaf area compared to the ratios of 1:1 and 3:1, each with a difference of 0.35 $cm^2$ and 0.37 $cm^2$. The application of cow manure showed the largest plant leaf area compared to goat manure and chicken manure, each with a difference of 0.41 $cm^2$ and 0.27 $cm^2$ (Table 4).

The production of dry matter and photosynthesis are strongly correlated with leaf area, therefore a larger leaf area boosts the plant's ability for photosynthesis. As a result, a significant portion of photosynthetic energy is used by plants to make leaves. Because they are involved in a variety of physiological processes (Shanmugabhavatharani et al. 2021). This research shows that husk charcoal and manure affect the leaf area of mint. Husk charcoal and manure contain a lot of organic material, which is beneficial for plant growth, including leaf area. Amri's research results show that using organic fertilizer can increase the growth of mint because the presence of microorganisms in the soil changes the ability to mobilize unavailable elements into available elements that are efficiently utilized directly by plants.

Using husk charcoal as a mint planting medium provides the highest mint plant leaf area yield. Adding husk charcoal affects increasing plant growth. Applying materials such as rice husk, wood ash, sawdust, and charcoal results in a higher dry matter yield in mint, presumably because they provide essential plant nutrients. It appears that the increased biomass is caused by the addition of certain essential nutrients to the soil after the decomposition of waste materials (Haque & Sakimin, 2022). Following the research by Syahda (2019) on kailan plants, which showed that the use of husk charcoal increased the yield of leaf area, leaf color, fresh weight of the crown, and dry weight of the roots, Wijaya et al. (2020) state that husk charcoal as a planting medium is very good at increasing leaf area because it contains good macro- and micronutrients. Macronutrients and micronutrients are crucial for the growth and development of plants. The availability of nutrients in manure has been proven to improve plant growth. Kaho et al. (2020) mention that cow manure contains balanced nutrients to supply the nutrients needed for plant growth. The nutrients contained in cow manure undergo complete decomposition, and they can release the nutrients needed to increase leaf area. The leaf area will be higher if the nutrient content in the planting medium is sufficiently available because most of the assimilated allocated is used for leaf formation, thereby increasing leaf area. According to (Olumide, 2022), such a promoting effect is optimal when applying organic fertilizer concerning the number of leaves, number of tendrils, fresh and dry weight compared with Inorganic fertilizer. Thus, using organic fertilizer positively impacted the biomass production of *Mentha piperita*.

## CONCLUSION

The interaction of a 1:3 soil and husk charcoal composition with cow manure could increase the number of mint plant branches to a total of 47 branches. The composition of soil and husk charcoal in a ratio of 1:3 showed the highest number of leaves (219.75), leaf area (209.50$cm^2$), fresh weight (11.77g), and dry weight (3.24g) compared to all treatments. The application of cow manure resulted

in the highest number of leaves, leaf area, and dry weight compared to all treatments, with their respective values of 223.33, 211.21cm$^2$, and 2.96.

## ACKNOWLEDGEMENT

The author thanks the Sebelas Maret University Group Research Grant for providing financial assistance for this research. The author also thanks the Faculty of Agriculture, Sebelas Maret University, for the laboratory facilities that helped with the analysis process.

## AUTHORS CONTRIBUTIONS

PDN coordinated research activities such as preparing outputs and activity reports. AIN coordinated research activities in the field to collect research data. PH provided guidance in preparing reports and research outputs. The experiment was conducted by MMFN. All authors provided critical feedback and contributed to the development of the research, analysis, and manuscript.

## COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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# Effects of Cricket and Fruit Fly Flour in Growth Media on *Beauveria bassiana* (Bals.) Vuill Pathogenicity Against *Zeugodacus cucurbitae* (Coquillet) Prepupae

10.18196/pt.v13i1.21463

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**ABSTRACT**

*Zeugodacus cucurbitae* (Diptera: Tephritidae) is a major insect pest of horticultural crops, causing significant yield losses.The efficacy of *Beauveria bassiana*, a potential biocontrol agent, can be influenced by growth media composition. This study examined the effects of cricket and fruit fly flour on *B. bassiana* growth and pathogenicity against *Z. cucurbitae*. The methods added *B. bassiana* growth media with different concentrations (0%, 0.5%, 1%, and 1.5%) of cricket and fruit fly flour and assessing its pathogenicity against *Z. cucurbitae* at a spore density of $10^6$ and $10^8$ spores/mL. The results showed that 1% cricket flour combined with a spore density of $10^8$ spores/mL resulted in the highest mortality rate (29.33%) and the fastest infection (2 days). The lowest average mortalities were found in treatments without flour addition which were 21.3%, 19.3%, and 19%, respectively. The longest time to cause infection was observed in 7 days. Infection symptoms are marked by the emergence of white fungal mycelia covering the cuticle, while infected adults exhibit deformed, wrinkled, and smaller wings. These findings highlight the potential of growth media optimization to enhance *B. bassiana* virulence, contributing to the development of more effective and sustainable biocontrol strategies against *Z. cucurbitae*.

**Keywords:** Biological control; Entomopathogenic fungi; Spore density; Insect Flour; Virulence

## INTRODUCTION

The fruit fly *Zeugodacus cucurbitae* (Diptera: Tephritidae) is important in horticultural crop cultivation (Lei Li et al., 2019). The host plants of *Z. cucurbitae* belong to the families Cucurbitaceae (Nair & Pal., 2020), Caricaceae, Fabaceae, Loganiaceae, Malvaceae, Myrtaceae, Pandanaceae, Passifloraceae, Rhamnaceae, Saptoaceae, Solanaceae, Agavaceae, Capparidaceae, Moraceae, Rutaceae, and Vitaceae. *Z. cucurbitae* is widely distributed in regions with temperate, tropical, and subtropical climates. The damage caused by fruit fly infestations can reach up to 100% (Sari et al., 2020).

Integrated pest management components to control *Z. cucurbitae* can be achieved using entomopathogenic fungi. According to Altinok et al. (2019), one of the commonly employed entomopathogenic fungi for pest-insect control is *Beauveria bassiana*. *B. bassiana* fungus produces toxins such as beauvericin, beauverolite, bassianolite and isorolite that act by damaging tissues, which leads to mortality, as reported by Bagariang et al. (2023). This entomopathogenic fungus is effective in sup-

Article History
Received : 25 January 2024
Revised : 05 October 2024
Accepted : 28 November 2024

pressing the development of pest insects in the orders Hemiptera (Sani et al., 2020; Atta et al., 2020), Orthoptera (Romero-Arenas et al., 2020), and Diptera (White et al., 2021).

The decline in the quality of entomopathogenic fungal spores and virulence can occur during the in vitro subculture process (Hussien et al., 2021). The reduction in the spore quality of entomopathogenic fungi may be attributed to the diminished sources of carbon, chitin, starch, and protein in the propagation media (Islam et al., 2021). Adding carb and nitrogen sources in the growth media can aid in preserving the infectivity of entomopathogenic fungi by activating chitinase enzymes crucial for the penetration of the host cuticle. Therefore, a propagation technique that can maintain the fungus's quality and virulence is essential (Sari & Khobir, 2020).

Adding protein and chitin sources to growth media can maintain the virulence of entomopathogenic fungi and activate protease and chitinase enzymes involved in the degradation of insect cuticles (Susila et al., 2023). Chitin and protein sources are most abundant in insect integuments (Khayrova et al., 2021). Incorporating insect flour into the growth media can enhance spore density and fungal viability due to its nutrient content closely resembling the natural host (Mascarin et al., 2024). The types of insect flour used in this study originated from adult crickets and fruit flies. This research investigated the effects of adding cricket and fruit fly flour to *B. bassiana* growth media with varying concentrations and spore densities on the infection symptoms, development, mortality, and the speed of infection onset in *Z. cucurbitae* pupae.

## MATERIALS AND METHODS

### Propagation of *Zeugodacus cucurbitae*

The mating process of *Z. cucurbitae* adults occurred within the rearing cage (30 cm x 30 cm x 30 cm). The hatching substrate consisted of 3 × 3 cm pieces of black cloth moistened with yellow pumpkin juice. *Z. cucurbitae* adults were provided with an artificial diet of sugar and yeast, which plays a role in sexual maturity and egg production (Gokulanathan et al., 2023). Adults deposited their eggs on the black cloth after being provided with the artificial diet. Eggs attached to the cloth were harvested by dipping the black cloth into 150 ml of distilled water until they detached.

The eggs poured into the artificial feed of wheat bran hatched after 24 hours and developed into larvae. Larvae reaching the final instar stage crawled onto the sawdust and transformed into pupae. The pupae within the sawdust were sifted for subsequent placement into the rearing cage to propagate the next generation.

### The Diet for *Z. cucurbitae*

The artificial diet for *Z. cucurbitae* larvae combined 185 grams of wheat bran, 180 ml of distilled water, 43.2 grams of sugar, 10.8 grams of yeast, 0.3 grams of nipagin, and 0.3 grams of sodium benzoate. Wheat bran was poured into a tray measuring 20 × 15 cm. Distilled water, sugar, nipagin, and sodium benzoate were mixed and stirred until homogeneous, then allowed to stand for 5 minutes. After swelling, this solution was poured into the wheat bran tray and stirred until well distributed. The artificial feed containing eggs was placed in a 35 × 25 × 10 cm container containing 300 grams of sterilized sawdust. It was then covered with black cloth and secured with a string to prevent any interference from pests.

## The Production of Cricket and Fruit Fly Flour

The production of cricket flour and fruit fly flour was carried out based on previous research by Septiani et al. (2020). Adult crickets and fruit flies were dried and sterilized simultaneously using a Memmert UN 30 oven at 100°C for 3 hours. Subsequently, crickets and fruit flies were finely ground into flour and sifted through a sieve with a mesh size of 1 mm.

## The Production of Growth Media

PDA (Potato Dextrose Agar) growth media are commonly used for cultivating fungal microorganisms. The composition of the ingredients in 250 ml of PDA consists of 250 ml of distilled water, 62.5 grams of potatoes, 5 grams of dextrose, and 5 grams of agar. The potatoes were peeled, cut into small diced pieces, placed in an Erlenmeyer flask containing distilled water, and boiled until soft. The potato extract was then supplemented with dextrose and agar (Wahyu et al., 2021).

The growth media were supplemented with cricket flour and fruit fly flour at concentrations of 0.5%, 1%, and 1.5%. Determining the media composition with the addition of cricket flour and fruit fly flour utilized the dilution formula of $M_1V_1=M_2V_2$. In 250 ml of PDA, 1.25, 2.5, and 3.75 grams of flour were added for a concentration of 0.5%, 1%, and 1.5%, respectively. The solution was then boiled until homogeneous. The homogenized medium was sterilized in an autoclave at a temperature of 121°C for 25 minutes and a pressure of 1 atm.

## Inoculation of *B. bassiana*

The isolates of *B. bassiana* fungi used in this study are from the collection of the Biological Control Laboratory, Plant Pest and Disease Program, Faculty of Agriculture, Universitas Gadjah Mada. These isolates were obtained initially from the Pest and Disease Observation Laboratory in Banyumas, with *Leptocorisa oratorius* as the host insect. The fungal isolates were cultured using the streaking technique within a Laminar Air Flow cabinet on all tested growth media. Subsequently, they were incubated for 14 days.

## The Calculation of Spore Density

The 14-day-old isolates were harvested by adding 10 ml of distilled water (aquades) and taking them from the growth media. The resulting solution was transferred to sterile test tubes and vortexed. One milliliter of the fungal suspension was pipetted into another test tube containing 9 ml of distilled water, and it was vortexed again. This step can be repeated to achieve the desired dilution level. The fungal suspension was then placed onto a hemocytometer chamber and covered with a cover slip. Spore density calculations were carried out manually using a binocular microscope and were computed using the formula from Gabriel & Riyanto (1989).

$$K = \times \frac{n}{t \times 0{,}25} \times 10^6 \qquad (1)$$

K represents the spore density per ml of solution. T is the number of conidia in the observed square. N is the number of hemocytometers counting squares (5 large squares × 16 small squares), and 0.25 is the correction factor for using the small-scale sample squares on the hemocytometer.

## The application of *B. bassiana* to *Z. cucurbitae* Prepupae

This research was arranged in a Completely Randomized Factorial Design (CRFD). The factors involved in the study include the type of insect diet, diet concentration, and spore density. There were 21 treatment combinations, each replicated 3 times, resulting in 63 experimental units, as shown in Table 1.

**Table 1.** Treatment Combination

| Code | Treatment of spore/mL |
|---|---|
| B1V1 | *B. bassiana*on pure PDA with spore density $10^8$ |
| B1V2 | *B. bassiana*on pure PDA with spore density $10^7$ |
| B1V3 | *B. bassiana* on pure PDA with spore density $10^6$ |
| B2V1 | *B. bassiana*onPDA + Cricket Flour 0.5% spore density $10^8$ |
| B2V2 | *B. bassiana*on PDA + Cricket Flour 0.5% spore density $10^7$ |
| B2V3 | *B. bassiana*on PDA + Cricket Flour 0.5% spore density $10^6$ |
| B3V1 | *B. bassiana* on PDA + Cricket Flour 1% spore density $10^8$ |
| B3V2 | *B. bassiana* on PDA + Cricket Flour 1% spore density $10^7$ |
| B3V3 | *B. bassiana* on PDA + Cricket Flour 1% spore density $10^6$ |
| B4V1 | *B. bassiana* on PDA + Cricket Flour 1,5%spore density $10^8$ |
| B4V2 | *B. bassiana* on PDA + Cricket Flour 1,5% spore density $10^7$ |
| B4V3 | *B. bassiana* on PDA + Cricket Flour 1,5% spore density $10^6$ |
| B5V1 | *B. bassiana* on PDA + Fruit Flies Flour 0,5% spore density $10^8$ |
| B5V2 | *B. bassiana* on PDA + Fruit Flies Flour 0,5% spore density $10^7$ |
| B5V3 | *B. bassiana* on PDA + Fruit Flies Flour 0,5% spore density $10^6$ |
| B6V1 | *B. bassiana*on PDA + Fruit Flies Flour 1% spore density $10^8$ |
| B6V2 | *B. bassiana*on PDA + Fruit Flies Flour 1% *spore* density $10^7$ |
| B6V3 | *B. bassiana*on PDA + Fruit Flies Flour 1% spore density $10^6$ |
| B7V1 | *B. bassiana*on PDA + Fruit Flies Flour 1,5% spore density $10^8$ |
| B7V2 | *B. bassiana* on PDA + Fruit Flies Flour 1,5% spore density $10^7$ |
| B7V3 | *B. bassiana on* PDA + Fruit Flies Flour 1,5% spore density $10^6$ |

The fungal suspension had $10^8$, $10^7$, and $10^6$ spore/mL spore densities. 30 prepupae of *Z. cucurbitae* per replication were placed in sterilized sawdust-filled Petri dishes. The application of *B. bassiana* fungus was carried out using a spray technique with a manual sprayer. Each treatment received 5 ml of suspension, administered only once. Observations were conducted daily for 14 days. Samples were collected for microscopic identification in the presence of *B. bassiana* symptoms.

## Data Analysis

The observation results were analyzed using analysis of variance (ANOVA). Means were separated by Duncan's Multiple Range Test (DMRT) at the 5% significance level using the SPSS software.

# RESULTS AND DISCUSSION

## Symptoms *of B. bassiana* Infection

The pre-pupal stage appeared normal, with a milky white color and a still soft larva-like texture (Figure 1A). Based on this research, *B. bassiana, when* applied to the pre-pupal stage, started to

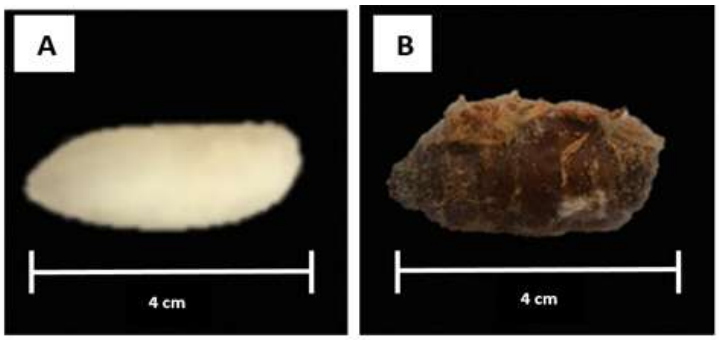


**Figure 1.** Healthy Prepupae of *Z. cucurbitae* (A) and Pupae Infected with *B. bassiana* (B)

exhibit symptoms during the pupal stage. Pupae infected with *B. bassiana* were characterized by the appearance of white fungal mycelium. Initially, the mycelium was only on one side of the pupal cuticle. However, as they aged, the *B. bassiana* fungus spread to cover the entire surface of the *Z. cucurbitae* pupal cuticle (Figure 1B). Microscopic observations were conducted to confirm that the entomopathogenic fungus infecting *Z. cucurbitae* was *B. bassiana*. Based on the observations, it was found that the entomopathogenic fungus *B. bassiana* exhibited characteristic morphology, which is hyaline and unicellular (Figure 2).

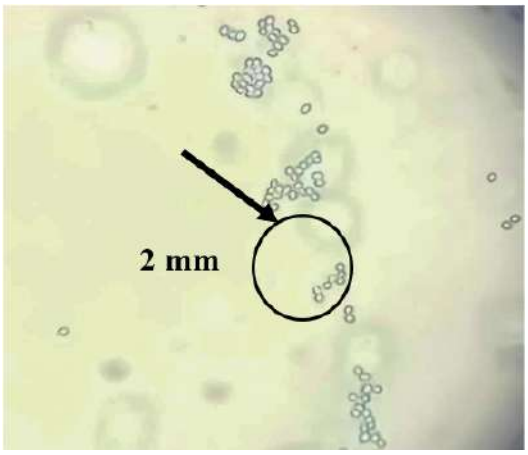


**Figure 2.** Conidia of *B. bassiana*

The presence of toxins within the *B. bassiana* was acknowledged for its potential to inflict tissue damage, particularly to muscles and the nervous system of *Plutella xylostella*, as stated by Ardan et al. (2020). These toxins are believed to impede the development of pupae into adults. The characteristics of *B. bassiana,* which is hyaline and unicellular, align with the statement by Sopialena et al. (2022) that *B. bassiana*, under microscopic examination, exhibits hyaline, round-shaped conidia that are unicellular.

## The Growth of *Z. cucurbitae* Adults

Observations on day 14 indicated that pupae successfully developed into the adult stage. There are distinct characteristics between normal and abnormal adults. Normal adults have wings with a distinctive dark-colored costal band narrowing in the R2+3 area and widening towards the wing's apex. Another characteristic feature is a dark transverse line along the dm-cu area and a spot at the

wing's apex that does not reach the M vein and narrows along r-m (Figure 3A). In contrast, adults infected with the *B. bassiana* fungus exhibit morphological deviations, primarily in the wing area. Observable characteristics include wrinkled wings and relatively smaller size (Figure 3B). These adults experienced imperfect growth due to the entomopathogenic fungus applied during the pre-pupal stage, which did not exhibit infection symptoms initially because the infection process took longer, and symptoms only became apparent in the subsequent stage, the adult.

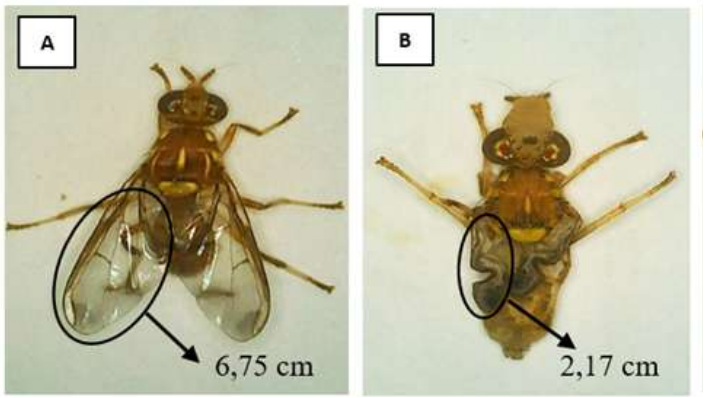


**Figure 3.** Morphology healthy adult of *Z. cucurbita*e (A) and adult infected with *B. bassiana* (B)

Normal adult *Z. cucurbitae* adults typically engage in activities such as flying, foraging, and reproduction, with a life cycle lasting up to 29 to 58 days in the wild (Vasudha et al., 2019; Wei et al., 2020). Conversely, abnormal adults face limitations in activities and are unable to fly, forage, or mate, resulting in a decline in *the Z. cucurbitae* population, as stated by Steck et al. (2022). Entomopathogenic fungi are known to impact insect growth and development, leading to reduced fertility, fitness, and the induction of abnormalities (Ullah et al., 2023).

## The Average Mortality of *Z. cucurbitae*

Each treatment showed varying average mortality (Figure 4). The average mortality of *Z. cucurbitae* was affected by the application of *B. bassiana* with the addition of 1% cricket flour and a spore density of $10^8$ was 29.33%. Adding 1 % cricket flour at a spore density of $10^7$ resulted in an average mortality of 28.3 %. Treatment with adding fruit fly flour at a concentration of 1% and spore densities of $10^8$ and $10^7$ showed average mortalities of 27.3 % and 27 %, respectively. The treatment of adding 1.5% flour exhibited a lower average mortality. Cricket flour and fruit fly flour added at a concentration of 1.5 % with a spore density of $10^6$ resulted in an average mortality of 24%. The lowest average mortalities of *Z. cucurbitae* were found in treatments without flour addition at spore densities of $10^8$, $10^7$ and $10^6$, which were 21.3 %, 19.3 %, and 19 %, respectively (Table 3).

The research findings underscore the significant impact of adding cricket flour and fruit fly flour to *B. bassiana* growth media, resulting in significantly different average mortalities of *Z. cucurbitae* compared to treatments without flour addition. Insect flours, rich in chitin compounds, stimulate the growth of chitinase enzymes crucial for maintaining the entomopathogenic fungus's infection ability, particularly during penetration into the cuticle of test insects (Islam et al., 2021; Prayogo et al., 2019). Chemical analysis reveals that cricket flour contains 54.68 % protein and 27.49 % chitin.

Chitin and protein are abundant energy sources found in insect integuments, so their addition to the propagation medium can inhibit viability decline (Hirsch et al., 2019). Similar content is expected in fruit fly flour, influencing the entomopathogenic fungus's ability to degrade the cuticle and penetrate the insect's body.

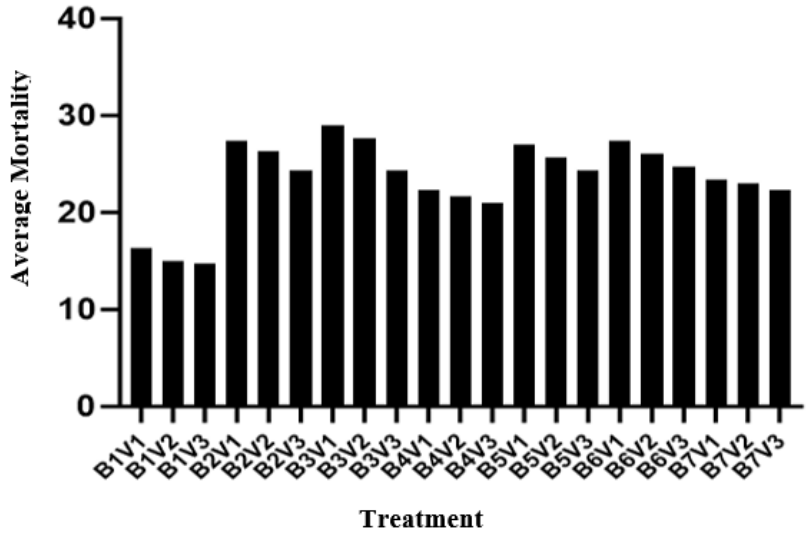


**Figure 4.** Mortality of *Z. cucurbitae* as affected by the application of *B. bassiana*

**Table 3.** Average Mortality of *Z. cucurbitae* as affected by the application of *B. bassiana*

| Code | Treatments | Average Mortality |
|---|---|---|
| B1V1 | *B. bassiana*on Pure PDA and spore density $10^8$ | 21.33±1.52$^{ab}$ |
| B1V2 | *B. bassiana*on Pure PDA and spore density $10^7$ | 19.33±4.04$^{a}$ |
| B1V3 | *B. bassiana*on Pure PDA and spore density $10^6$ | 19.00±4.35$^{a}$ |
| B2V1 | *B. bassiana*on PDA + Cricket Flour 0,5% spore density $10^8$ | 27.33±2.51$^{de}$ |
| B2V2 | *B. bassiana* on PDA + Cricket Flour 0,5% spore density $10^7$ | 26.33±2.30$^{de}$ |
| B2V3 | *B. bassiana* on PDA + Cricket Flour 0,5% spore density $10^6$ | 26.67±1.15$^{de}$ |
| B3V1 | *B. bassiana* on PDA + Cricket Flour 1% spore density $10^8$ | 29.33±1.15$^{de}$ |
| B3V2 | *B. bassiana* on PDA + Cricket Flour 1% spore density $10^7$ | 28.33±1.52$^{de}$ |
| B3V3 | *B. bassiana* on PDA + Cricket Flour 1% spore density $10^6$ | 26.67±1.15$^{de}$ |
| B4V1 | *B. bassiana* on PDA + Cricket Flour 1,5% spore density $10^8$ | 25.33±0.57$^{de}$ |
| B4V2 | *B. bassiana* on PDA + Cricket Flour 1,5% spore density $10^7$ | 24.67±0.57$^{cd}$ |
| B4V3 | *B. bassiana* on PDA + Cricket Flour 1,5% spore density $10^6$ | 24.33±2.08$^{cd}$ |
| B5V1 | *B. bassiana* on PDA + Fruit fly Flour 0,5% spore density $10^8$ | 27.33±2.08$^{de}$ |
| B5V2 | *B. bassiana* on PDA + Fruit fly Flour0,5% spore density $10^7$ | 25.67±1.52$^{de}$ |
| B5V3 | *B. bassiana* on PDA + Fruit fly Flour0,5% spore density $10^6$ | 25±1.00$^{cd}$ |
| B6V1 | *B. bassiana* on PDA + Fruit fly Flour1% spore density$10^8$ | 27.33±2.08$^{de}$ |
| B6V2 | *B. bassiana* on PDA + Fruit fly Flour1% spore density $10^7$ | 27.00±2.00$^{de}$ |
| B6V3 | *B. bassiana* on PDA + Fruit fly Flour1% spore density $10^6$ | 26.33±1.52$^{de}$ |
| B7V1 | *B. bassiana*on PDA + Fruit fly Flour1,5% spore density $10^8$ | 25.33±0.57$^{de}$ |
| B7V2 | *B. bassiana*on PDA + Fruit fly Flour1,5% spore density $10^7$ | 24.33±2.51$^{cd}$ |
| B7V3 | *B. bassiana*on PDA + Fruit fly Flour1,5% spore density $10^6$ | 24±1.00$^{bc}$ |

Remarks: Means followed by the same superscript letters are not significantly different based on DMRT at 5%.

Optimal concentrations of insect flour, specifically at 0.5 % and 1 %, demonstrated the most favorable results, enhancing *B. bassiana* conidia production and maintaining a stable count. Conversely, 1.5 % insect flour addition did not significantly differ from the treatment without flour, potentially inhibiting conidia formation due to a denser growth medium, resulting in a narrower growth space for *B. bassiana* (Rosana et al., 2021). According to Saidah & Asri (2019), conidia formation was inhibited due to the accumulation of metabolites resulting from adding a chitin source with a concentration that was too high.

Spore density of the entomopathogenic fungus during application also influences *Z. cucurbitae* mortality. Higher spore density increases the likelihood of active spores germinating, penetrating the cuticle, and causing Infection (Hardiansyah et al., 2023). Spores will penetrate the cuticle and develop within the tissue, causing Infection in *Z. cucurbitae*. Sumikarsih et al. (2019) stated that the spore density, whether high or low, significantly affected the effectiveness of *B. bassiana* in the field.

## Time of *B. bassiana* Infection in *Z. cucurbitae*

Based on Table 2, the shortest time required by *B. bassiana* to infect *Z. cucurbitae* was observed in treatment B3V1 (1 % cricket flour and a spore density of $10^8$), which was 2 days, followed by treatment of 1% cricket flour at spore densities of $10^7$ (B3V2) and $10^6$ (B3V3), as well as 1% fruit fly flour at spore densities of $10^8$ (B6V1) and $10^7$ (B6V2), which required 3 days for infection. Meanwhile, the longest time to cause Infection was observed in treatments B1V1, B1V2, B1V3 (without flour

**Table 2.** Infection Time

| Treatment | Infection Time (Day) |
|---|---|
| B1V1 | 7 |
| B1V2 | 7 |
| B1V3 | 7 |
| B2V1 | 5 |
| B2V2 | 5 |
| B2V3 | 5 |
| B3V1 | 2 |
| B3V2 | 3 |
| B3V3 | 3 |
| B4V1 | 6 |
| B4V2 | 6 |
| B4V3 | 7 |
| B5V1 | 5 |
| B5V2 | 5 |
| B5V3 | 5 |
| B6V1 | 3 |
| B6V2 | 3 |
| B6V3 | 4 |
| B7V1 | 6 |
| B7V2 | 6 |
| B7V3 | 7 |

addition at spore densities of $10^8$, $10^7$, and $10^6$ spore/mL), and B7V3 (1.5 % fruit fly flour addition with a spore density of $10^6$), which was 7 days.

Time is a crucial factor, as observed by Dannon et al. (2020) and Friska et al. (2023). *B. bassiana* requires time to infect *Spodoptera litura* larvae because fungal conidia need time to germinate and form hyphae before they can eventually penetrate the insect's cuticle. Hence, *Z. cucurbitae* infection symptoms appeared one day after application and entered the pupal stage. The time of infection is influenced by the germination rate, which is affected by nutrient content in the growth medium, with cricket flour and fruit fly flour contributing high levels of chitin and protein (Rehman et al., 2023). The high content of chitin and protein in the growth medium of entomopathogenic fungi can accelerate germination because they play a vital role in the formation of fungal cell structures during the germination process to infect *Z. cucurbitae.*

## CONCLUSION

The effectiveness of *B. bassiana* in controlling *Z. cucurbitae* is significantly influenced by the interaction between fungal toxins, insect flours, and spore density. Adding 1% cricket flour at a spore density of $10^8$ spores/mL enhance *B. bassiana* infection, accelerating mortality and reducting infection time. Infection symptoms in *Z. cucurbitae* pupae are marked by the emergence of white fungal mycelia covering the cuticle, while infected adults exhibit deformed, wrinkled, and smaller wings. The highest average mortality of *Z. cucurbitae* was found in the treatment with 1% cricket flour and a spore density of $10^8$ m/L, reaching 29.3 %, with an infection time of 2 days. In contrast, the lowest average mortality was observed in the treatment without flour addition at a spore density of $10^6$, which was 19%, with an infection time of 7 days. These findings highlight the potential of optimized fungal growth media in enhancing *B. bassiana* virulence, providing valuable insights for improving entomopathogenic fungus-based biocontrol strategies.

## AUTHORS CONTRIBUTIONS

NR played a pivotal role in shaping the research methodology by contributing to the design and development of models, ensuring a strong foundation for the study. She also provided essential oversight and mentorship throughout the project, guiding the external team in planning and execution. Additionally, she prepared the initial draft of the publication, incorporating detailed translations to ensure clarity and precision. UUM focused on conducting experiments and collecting critical data, playing a hands-on role in the investigation process. She conceptualized the research goals and managed data-related activities, including annotation, cleaning, and preparing the data for reuse. She also ensured the accuracy and replicability of the results through validation processes while creating impactful visual presentations of the data. Her contributions extended to project administration and formal analysis, applying statistical and computational techniques to synthesize findings. RMK enhanced the publication's quality through a meticulous review and editing process, offering insightful revisions and commentary to refine the work during both pre-and post-publication stages. Together, their combined efforts ensured the project's success and impactful dissemination.

## COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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*Penelitian dan Pengabdian Kepada Masyarakat LPPM Universitas' Aisyiyah Yogyakarta, 1*, 137-142.

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# Antagonistic activity of *Trichoderma harzianum* against *Aspergillus parasiticus* and *Mucor circinelloides* in corn plant (*Zea mays* L.)

10.18196/pt.v13i1.21507

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**ABSTRACT**

*Aspergillus parasiticus*, the causative agent of *Aspergillus* cob rot, and *Mucor circinelloides*, which causes *Mucor* stem rot, are types of pathogenic fungi that have the potential to reduce the quality of corn (*Zea mays* L) harvests. An effective and environmentally friendly approach to control these pathogens is to use biological agents, such as *Trichoderma harzianum*. This study aims to evaluate the inhibitory potential of *T. harzianum* against *A. Parasiticus* and *M. circinelloides* using antagonism tests with dual culture methods in vitro. The parameters used in this study were measurements of the percentage of the inhibition rate of the biological agent *T. harzianum* against pathogenic fungi. The results of the antagonism test showed that the biological agent *T. harzianum* had moderate inhibition against *A. parasiticus* and *M. circinelloides* with the percentage of each inhibition during the five days incubation period are 32.5% and 42.38%. This indicates that *T. harzianum* has the potential as a biological agent in controlling *A. parasiticus* and *M. circinelloides* in corn plants.

**Keywords:** Biocontrol; Cob rot; Fungal disease; Stem rot

## INTRODUCTION

Corn is a staple food and has the potential to become a strategic commodity that plays an important role in increasing income in Indonesia. Plant Disturbing Organisms (PESTs) are one of the main obstacles in efforts to maintain and improve the quality of corn crop productivity (Lestari et al., 2021). One of the pests that often attack are disease-causing microbes that can affect the production of corn plants, such as pathogenic fungi (Suriani et al., 2021). Pathogenic fungi such as *Aspergillus parasiticus* and *Mucor circinelloides* are major fungal pathogens that decrease corn productivity (Sari et al., 2024).

*A. parasiticus* is a type of fungus that often contaminates cereals such as corn kernels, growing well at 80% humidity with a temperature of 25-40°C (Bagus et al., 2017). Another symptom of the pathogenic fungus *Aspergillus* that attacks corn seeds is the growth of green mycelium, which gradually covers the seeds (Fitria et al., 2020). *Aspergillus* pathogens on corn kernels can produce several types of mycotoxins, including aflatoxins, ochratoxins, patulin, and sterigmatocystin (Hanif et al., 2019).

Article History
Received : 30 January 2024
Revised : 01 November 2024
Accepted : 11 December 2024

*M. circinelloides* is a type of fungus that can cause rot from puncture wounds in the cuticle on stems and fruit petals, with symptoms of white or gray colonies appearing with dark spore heads, also irregular fungi colonies and gray-white thread-like strands of mycelia appear (Figure 3) (Ling et al., 2023). *Mucor circinelloides* can cause rot disease in plants with symptoms of skin on the slices containing fine granules with irregular white-gray colonies (Simamora et al., 2022).


**Figure 3.** Corn stem infected by *Mucor circinelloides* (1) The symptoms that arise are indicated by the formation of thin grayish-black colonies that form spots

Controlling pathogenic fungi in agricultural land have been widely practiced using chemical fungicides that reported to be more effective and faster in inhibiting the growth of these pathogenic fungi (Umboh & Rampe, 2019). However, chemical fungicides on agricultural land have a negative impact for environment if used continuously which is used intensively increase the risk of poisoning in consumers from the harvest of agricultural land (Kusumaningtyas et al., 2021). Fungicide application can also cause decreased soil quality, reduced biomass and soil microbial activity, and increased potential pathogen resistance to fungicides (Wu et al., 2023). As for the economic side, using fungicides tends to be expensive. Chemical fungicides also have the potential to kill microorganisms that are not their targets and can trigger the emergence of pathogen groups with new strains that are more resistant to the fungicides themselves. Therefore, a biologically environmentally friendly (eco-friendly) control effort is needed, namely using antagonistic endophytic microorganisms that can spur growth and increase plant resistance (Rotasouw et al., 2020).

One effective solution in controlling diseases caused by pathogenic fungi in plants is using biological agents, such as *Trichoderma* sp. (Hamidson et al., 2020). The *Trichoderma* sp. fungus is the most promising technology for sustainable agriculture because it can break down organic matter well, increase plant growth, and control diseases that attack plants (Nawaal et al., 2022). In its natural habitat, *Trichoderma* sp. has antagonistic activities that can attack and inhibit the growth of pathogenic fungi that cause disease in plants by releasing toxins in the form of cellulase and chitinase enzymes (Ruswandari et al., 2020). *Trichoderma* sp. can live and develop in healthy plant tissue and form colonies well. Although colony formation occurs in the host plant tissue, this does not harm the host plant because of its nature as an endophytic fungus. This type of fungus can be found in

plants, such as roots, stems, leaves, and flowers (Rotasouw et al., 2020). *Trichoderma harzianum* is classified as antagonistic fungi because these fungi have an antagonistic antibiosis mechanism against pathogenic fungi by producing secondary metabolite compounds, such as peptaibol, pyrone, and viridol (Singh et al., 2018). Despite the well-documented efficacy of *Trichoderma harzianum* as a biocontrol agent, research on its antagonistic effects against *Aspergillus parasiticus* and *Mucor circinelloides*, the causative agents of corn cob rot and stem rot, remains limited.

This study aims to fill this research gap by evaluating the inhibitory potential of *T. harzianum* against these fungal pathogens using the dual culture in vitro method. The dual culture method involved co-cultivation of *T. harzianum* with each pathogen in a single petri dish to assess competitive interactions. Diseased corn plants were used as a source for fungal isolation, followed by morphological and growth characterization. The antagonistic potential of *Trichoderma harzianum* was determined by measuring the inhibiting rate of fungal growth. The findings of this study will contribute to the advancement of sustainable and eco-friendly disease management strategies in corn production.

## MATERIALS AND METHODS

### Research Design

The study was experimental research conducted at the Biotechnology Laboratory of the Department of Biology, Diponegoro University, from October 2$^{nd}$ to November 20$^{th}$, 2023. The analysis of antagonistic fungi against pathogens in corn was performed using the dual culture method in the antagonistic test. The research was arranged in a Completely Randomized Design (CRD), and the results were analyzed based on the inhibition category of the data on the inhibition of antagonistic fungi against pathogenic fungi obtained.

### Corn Sampling

Corn seed samples suspected of being infected with pathogenic fungi were taken from a corn field in Brumbung Village, Mranggen Sub-District, Demak Regency, Central Java, Indonesia. Plant samples were taken from the infected organs, the corn seed cobs. Plant samples were then put in plastic for further testing in the laboratory.

### Preparation of PDA Media

The PDA media were prepared using 3.9 g PDA powder and 100 ml distilled water put in an Erlenmeyer flask, which was then homogenized, heated in microwave for 3 minutes until clear PDA media was obtained in the Erlenmeyer flask, and covered for further sterilization in autoclave 121$^{o}$C 1 atm for 15 minutes. Sterile PDA media added with Chloramphenicol were poured into sterile Petri dishes and allowed to solidify.

### Isolation and Koch's Postulate Test

Pathogenic fungi were isolated from infected corn parts (seed and stem) by soaking them in 70% alcohol for 15-30 seconds, which were rinsed in distilled water, dried, and placed on sterile PDA media to be incubated for 7 days, then purified by culturing them on new sterile PDA media (Wakhidah

et al., 2021) and incubating them for 5 days (Safitri et al., 2023). Koch's Postulate Test was carried out, in which healthy corn cobs were peeled off, sterilized with 70% alcohol, pierced and filled with purified fungi suspension using sterile ose, and then incubated until the symptoms were seen, and the corn cob decayed. The grown colonies were isolated and incubated for 5 days to identify the fungi (Safitri et al., 2023). The *Trichoderma* sp. isolates were obtained by rejuvenating the cultures from the Laboratory of Pest and Disease Observation Temanggung, Central Java, by culturing them on a PDA medium in a new Petri dish and incubating them for 3 days.

## Identification

The fungi were identified macroscopically in pure colonies on petri dishes (Sholihah et al., 2019). The observed characteristics include color, shape, symmetry/asymmetry, and direction of colony growth. The identification was also carried out microscopically in object glass with Lactophenol Cotton Blue (LCB) under a microscope at 400 times magnification with observed characteristics including the form of shape, color, concentrated/unconcentrated spores or conidia, hyphae (Safitri et al., 2023), conidiospores, and phialids (Sholihah et al., 2019).

## Antagonistic Test using Dual Culture Method

The antagonistic test was performed using a dual culture method carried out with the inoculum of pathogenic fungal isolates and *Trichoderma* sp. grown at a distance of 3 cm on the same PDA growth medium with a diameter of 9 cm. The inoculum of both fungi was taken with a 4-mm-diameter cork borer (Safitri et al., 2023). The test was conducted with 3 replications and 2 treatments: control and antagonist. The culture was then incubated at room temperature, and the growth colonies and inhibition zones between the two fungal colonies were observed.

The final result of the antagonistic test in this study was to determine the ability of *Trichoderma harzianum* to inhibit the growth of pathogenic fungi. The inhibitory rate of antagonistic fungi was calculated based on the following formula (Halwiyah et al., 2019):

$$I = \frac{(r1-r2)}{r1} \times 100\% \qquad (1)$$

Remarks:
I : Inhibitory rate (%)
r1 : Radius of pathogenic fungi colonies that move away from antagonistic fungi colonies
r2 : Radius of pathogenic fungi colonies approaching antagonistic fungi colonies

According to Win et al. (2021), the antagonistic mechanism of *Trichoderma* sp. against phytopathogens studied in vitro can be grouped into 4 categories based on the inhibitory rate:

1 (low) = 1-25%,
2 (medium) = 26-50%
3 (high) = 51-75%
4 (extremely high) = 76-100%

## RESULTS AND DISCUSSION

### Symptoms of *Aspergillus* Cob Rot in Corn

Corn cob rot in this study was indicated by the appearance of fungi colonies on the corn cob samples used. The kernels on the corn samples showed a blackened color, with the cobs starting to soften and rot (Figure 1). Common symptoms of *Aspergillus* infection on corn kernels include discoloration of the kernels and increased mycotoxin content. Morphologically, corn kernels infected with this fungus can be identified by green to black sections with a granular texture. *Aspergillus* infection of corn kernels results from conditions suitable for this fungus to grow on corn. Several factors can influence the growth of this fungus, such as temperature, humidity, and the use of chemicals in corn cultivation. The growth medium most often contaminated with *Aspergillus* is agricultural food. High organic content in agricultural products, suitable humidity, and the ease of *Aspergillus* spores growth and development. *Aspergillus* morphology is generally characterized by green to dark green or yellowish-green colonies with a granular colony texture. The young colonies will be white and turn yellowish-green after the conidia are formed (Kapli et al., 2022). *Aspergillus* in infected corn will cause the accumulation of mycotoxins that are harmful when consumed. This will affect the yield and production quality of the corn kernels. If the infected corn is consumed, the kernels will affect human health due to harmful mycotoxins, such as carcinogenic aflatoxins. Aflatoxin compounds are secondary metabolite compounds produced by *Aspergillus* that produce aflatoxin toxins of AFB1, AFB2, AFG1, and AFG2. This compound can be lethal when consumed in high concentrations. In addition, even if consumed in low concentrations over a long period, it can also cause liver cancer and kidney cancer (Sasongkowati et al., 2024). *A*flatoxin is a secondary metabolite produced by *Aspergillus parasiticus, w*hich is toxic, carcinogenic, mutagenic, stable, and resistant to degradation. The commonly found groups of aflatoxins are AFB2, AFG1, and AFG2, and the most toxic are AFB1 (Abdelaziz et al., 2022). Aflatoxins are often contaminants in agricultural products from tropical countries, such as nuts, rice, wheat, cotton seeds, and corn seeds (Rajarajan et al., 2021). This compound has the potential to cause several serious diseases in humans, such as liver cancer, immunosuppression, and stunting (Navale et al., 2021).

**Figure 1**. Corn seeds cobs infected by *Aspergillus parasiticus* shown in blue circle

### *Koch's Postulate Test of Aspergillus sp.*

Koch's Postulate test was carried out on healthy corn cobs, and the inoculation results showed the same symptoms between the seeds on the inoculated corn cobs and the symptoms on the plants from which the fungi isolates were isolated. The results of re-isolation of inoculated corn seeds also showed the same type of fungi. Koch's Postulate Test aims to prove and ensure that the isolated microbes are pathogenic by looking at the similarity of symptoms (Kumala et al., 2023). Colonies that grow in the Koch Postulate Test results showed a granular green color, both in the seeds in the inoculated healthy corn cobs and the results of their re-isolation. The color and texture of the colonies formed were similar to the symptoms on the original plant. If the results of pathogen isolation show the same type of microbe, it can be said that the microbe has met the requirements of Koch's Postulate Test so that it can be known that the microbe is indeed pathogenic to the original plant (Budi et al., 2022).

### *Identification of Aspergillus sp.*

Macroscopic observations on PDA media showed that the growing colonies were green with a colony diameter of 5.2 cm, granular colony texture, white inverted colonies, growth zones and radial grooves, and no exudate drops (Figure 2). Microscopically, this fungus was characterized by apparent, transparent hyphae, septate hyphae (hyphae with partitions), clear, transparent conidiophores without branches, vesicles, metula, fialid, and round (globose) conidia, and no ascus or ascospores. According to the identification book by Watanabe (2002), the species was identified as *A. parasiticus*, with the key determination of *Aspergillus* Mich : Fr. is 1a - 2b, with morphological characteristics in the form of erect conidiophores, simple, rough surface, having foot cells at the base, expanding at the apex to form round vesicles, and bearing conidia heads made up of uniseriate or, in rare cases, biseriate phialides, which were yellowish green, radiate, columnar, and concentrated, with pale green colonies. *A. parasiticus* is characterized by a dark green colony color and microscopic features in globular vesicles with a set of sterigmata (Nikolić et al., 2021).

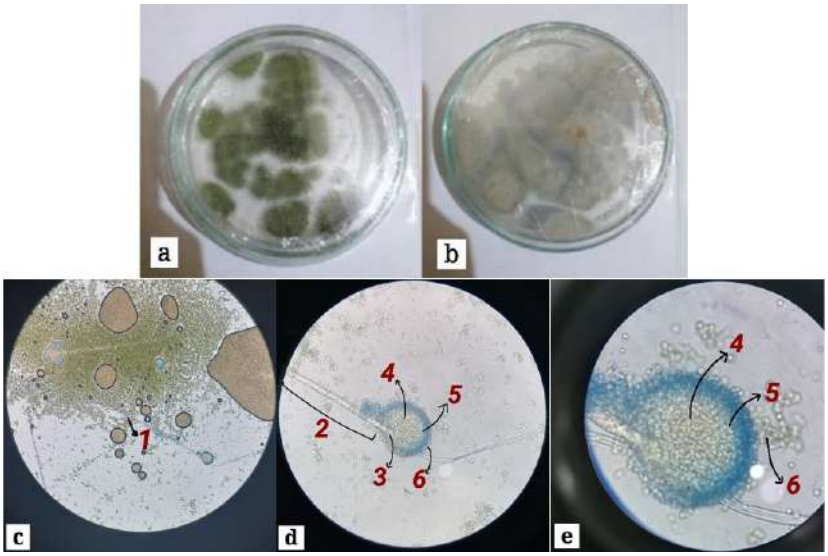


**Figure 2.** Characteristic Appearance of Pathogenic Fungi Isolates (*Aspergillus parasiticus*). a. upper colony, b. reverse colony, c. 40X microscopy, d. 100X microscopy, e. 400X microscopy (1: foot cell, 2: conidiophore, 3: vesicle, 4: metula, 5: fialid, 6: conidia)

## Symptoms of Mucor Stem Rot in Corn

Mucor stem rot is characterized by symptoms of light brown to golden tissue. In addition, gray colonies with a cotton-like texture also appear. The pathogenic fungus *M. circinelloides* causes rot with symptoms of white or gray colonies with dark spore heads and cottony texture (Ali & Samosir 2021).

### *Mucor circinelloides*

Microscopic identification was carried out on corn plants showing stem rot symptoms to ensure that they were in accordance with the characteristics of the fungus *M. circinelloides*. The hyphae are non-concentrated based on the identification results (Figure 4). It also has sporangium and sporangiophore. Sporangiophore of *M. circinelloides* is transparent and has a smooth surface. In addition, there are also spherical, single or branched sporangiums. The sporangium of *M. circinelloides* is located at the top of the sporangiophore. The microscopic characteristics of *M. circinelloides* are that the hyphae are not insulating, have single sporangiophores and no rhizoids are visible. In addition, *M. circinelloides* also has sporangium and columella, which are round with round and smooth spores (Izzatinnisa' et al., 2020). Macroscopic identification showed the characteristics of gray-white colonies (Figure 5). *M. circinelloides* have white colonies, in which the increasing age of the fungus will cause the color changes to gray. The colonies grow densely with cotton-like mycelium. In addition, *M. circinelloides* has a flat colony surface and no concentric radial lines. *M. circinelloides* has no exudate points, and the colony can grow to a diameter of 9 cm. The genus *Mucor* is a Zygomycetes fungus, which is white with a cotton texture without exudates, and its colonies can grow to fill Petri dishes.

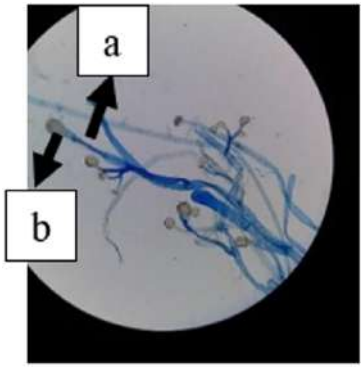


**Figure 4.** Microscopic *Mucor circinelloides* (a) sporangiophore, (b) sporangium

**Figure 5.** Macroscopic *Mucor circinelloides*

## Antagonistic Fungi (*Trichoderma harzianum)*

Based on the microscopic observation, *Trichoderma* sp. isolates on PDA growth media (Figure 6) showed a dark green colony color with a colony diameter of 8.3 cm. The texture was granular with a whitish-green reverse colony without a growing zone, radial furrows, or exudate drop. The microscopic characteristics obtained include septate apparent transparent hyphae without foot cells or Hulle cells, branched conidiophores with mono-verticillate branching type, no vesicles, round conidia of bright yellow color, phialids, and no metula, ascus, or ascospores. Identification was carried out using the identification book "Pictorial Atlas Of Soil And Seed Fungi: Morphologies Of Cultured Fungi And Key To Species" by Watanabe (2002), and the results confirm that the type of *Trichoderma* sp. is *T. harzianum* with the key determination of *Trichoderma* Pers. : Fr. 1b - 2a. Its morphological characteristics include hyaline, upright, branched conidiophores, with a mass of spores at the apical end in verticillate short and thick phialids; insulated conidia, hyaline, round, spherical, subglobose, or ovoid, single-celled, and brown chlamydospores that are subglobose. The initial growth of *T. harzianum* on the culture media was indicated by the characteristics of dense and thick mycelium, greenish white, then developed into green in the middle and white at the edges. White and green circles were formed with clear boundaries. The green color looked more significant and more intense, and the color looked clearer. Colony color is influenced by phial phosphorus pigment, the number of spores, and the pH of the medium. Colonies appear transparent on media with limited nutrients, while on media with more nutrients, colonies appear whiter. In the growth media, color changes also occur due to *T. harzianum* degrading the media as nutrients for its growth (Suharni et al., 2023).

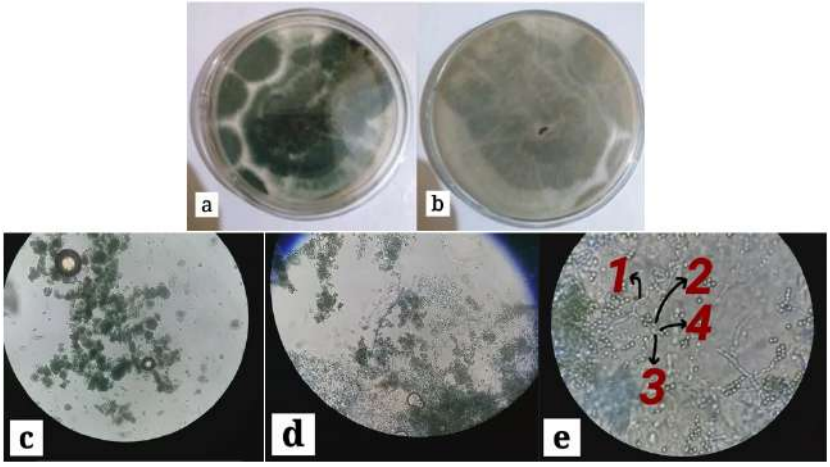


**Figure 6.** Characteristic appearance of *Trichoderma harzianum* isolates a. upper colony, b. reverse colony, c. 40X microscopy, d. 100X microscopy, e. 1000X microscopy (1: conidiophore, 2: conidiophore branch, 3: phialid, 4: conidia/phialospore)

## Growth of Pathogenic Isolates

The growth phase of fungi occurs through several stages. The stages in this phase are often represented on the fungi growth curve. The fungi growth phase consists of the lag, acceleration, exponential (log), stationary, and autolysis phases (Heirina et al., 2020). The lag phase is the growth phase of the fungus adapting to its environmental conditions. The acceleration phase is the phase when cell

division begins. The log or logarithmic phase of fungi growth occurs between days 7 and 14, where biomass increases. During the lag phase, the fungi cells can adapt to their environment and produce enzymes that degrade the substrate. The stationary phase occurs between days 15 and 21, where fungi growth is relatively constant and balanced by the number of dead cells. The autolysis or death phase is when the number of cells decreases or cell death occurs.

### *Aspergillus parasiticus*

The growth of *A. parasiticus* isolates can be seen in the growth curve of the isolates (Figure 7). The curve shows that the three replicates of *A. parasiticus* isolate experienced a consistent increase for 5 days after isolation. *A. parasiticus* isolates after 2 days of isolation experienced a significant increase in colony diameter because cell growth began to enter the logarithmic phase. *A. parasiticus* growth can be influenced by factors such as temperature and relative humidity. The growth of *Aspergillus* sp. on bread showed that the fungus grew faster at room temperature (25 °C-28°C) than in the refrigerator (10 °C-15 °C). The maximum growth of *Aspergillus* sp. on corn and soybean was achieved at 20 °C with 90 % relative humidity or at 40 °C with 70 % relative humidity (Agriopoulou et al., 2020). In addition, *Aspergillus* sp. grew well on various media, such as PDA, and waste products, such as tempeh wastewater and coconut water. The main nutrient required for Aspergillus growth is carbohydrate.

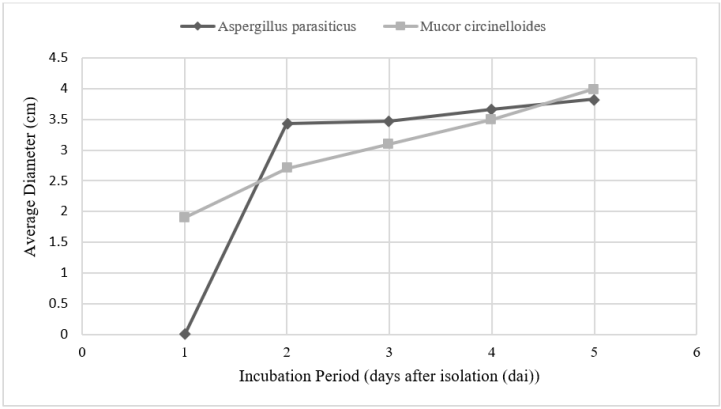


**Figure 7**. Growth of pathogenic fungi isolates *Aspergillus parasiticus* and *Mucor circinelloides* from corn seed cobs (*Zea mays* L.)

### *Mucor circinelloides*

The results of observations for 5 days regarding the branches of the pathogenic fungus *Mucor circinelloides* (Figure 7) showed that the pathogenic branches continued to increase from day to day. *Mucor circinelloides* growth is influenced by several factors, such as warm temperatures, high humidity, and appropriate soil pH. High humidity will increase the risk of infection in plant tissues because the potential for *Mucor circinelloides* to live is higher. *Mucor circinelloides* is a dimorphic mold genus that requires growth of carbon dioxide and hexose sugar (Widiyantini & Kumoro, 2017).

## In Vitro Antagonistic Test

The antagonistic test in this study was carried out in vitro with a double culture method on a 9-cm-diameter petri dish with a distance of 3 cm between isolates. This test aims to determine the amount

of ability of antagonistic fungi to inhibit the growth of pathogenic fungi. The test isolates selected as antagonistic fungi are isolates that can inhibit > 70 % (Safitri et al., 2019).

### *Trichoderma harzianum with Aspergillus parasiticus*

The inhibitory rate of antagonistic fungi from the first day to the seventh day order was 22.86 %, 34.19 %; 36.19 %; 32.78 %; 32.22 %; 33, 73 %; and 32.41 % (Figure 8), with an average value of 32.5%. The inhibitory rate is classified into three categories, namely low (0%-30 %), medium (31 %-40 %), and high (higher than 40 %) (Arti et al., 2021). Based on this, the inhibitory rate of *T. harzianum* against *A. parasiticus* is in the medium category. Fungi with more than 50 % inhibition rate can be used as biological agents to control pathogenic fungi, so *T. harzianum* has no less potential as a biological agent in inhibiting the growth of pathogenic fungi *A. parasiticus growth*. The inhibition of *T. harzianum* against *A. parasiticus* growth can also be seen directly in the petri dish. The antagonistic mechanism formed from the treatment is an antibiosis mechanism, seen from the formation of an inhibition zone around *T. harzianum,* which is marked with an orange circle (Figure 9). The formation of the inhibition zone is thought to be due to the formation of secondary metabolites or active compounds. *T. harzianum* produces metabolites and filtrates that can inhibit the growth of the pathogenic fungus *A. parasiticus* (Rahmadanty et al., 2023).

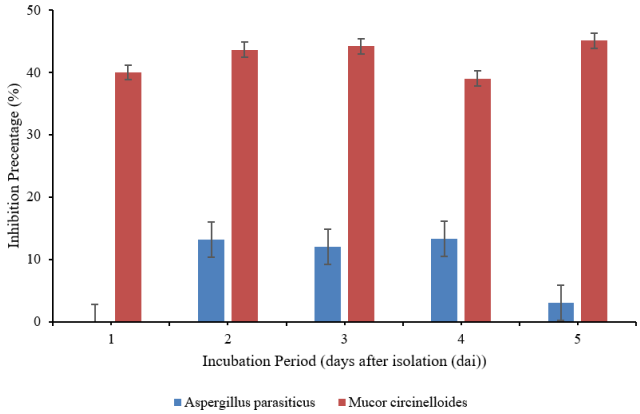


**Figure 8.** Inhibitory Rate of *Trichoderma harzianum* against *Aspergillus parasiticus* and *Mucor circinelloides* 5 days after incubation (dai)

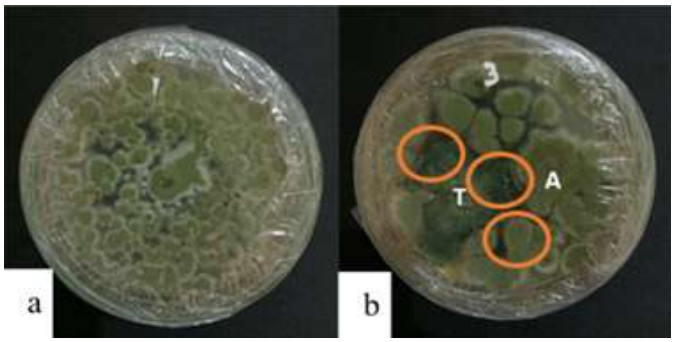


**Figure 9.** Antagonistic Test of *Trichoderma harzianum* against the pathogenic fungus *Aspergillus parasiticus* at 7 days after isolation shown in orange circle. (a) Control, (b) Antagonistic Test, (A) *Aspergillus parasiticus* (T) *Trichoderma harzianum*; orange circles indicate clear zones.

### *Trichoderma harzianum with Mucor circinelloides*

*Trichoderma sp.* performed optimal inhibition starting from day 2 after inoculation (Figure 8). The growth on day 2 resulted in an inhibitory rate of 43.60 % and increased on day 3 to 44.20 %. However, on the 4th day, it decreased to 39 %. The decrease can be caused by the growth of filaments that are less than optimal, causing the percentage value to decrease from the previous day. The length of the radius of pathogenic colonies affects the percentage of endophytic fungi inhibition (Pasalo et al., 2022). However, the inhibitory rate started to increase again on day 5, which is enough to prove that the antagonistic fungus *Trichoderma harzianum* is able to inhibit the growth of the fungus *Mucor circinelloides*.

The values of the inhibitory rate of *Trichoderma harzianum* against *Mucor circinelloides* were 39-45.10 % from day 1 to day 5, and the average value was 42.38 %. This inhibitory rate value is included in the medium category. The inhibitory rate values are divided into 4 categories, namely low (1-25 %), medium (26-50 %), high (51-75 %), and extremely high (76-100 %) (Win et al., 2021). Although the inhibitory rate value obtained tended to increase, the inhibitory effect was not significant. The inhibitory effect of *Trichoderma harzianum* is still relatively low but can inhibit the growth of *Mucor circinelloides*. The less than 60 % inhibitory rate value means that the antagonistic fungus only has a minimal inhibitory effect on the development of pathogenic fungi that attack (Halwiyah et al., 2019). Meanwhile, if the inhibitory rate value is more than 60 %, it can be said that antagonistic fungi can inhibit the growth of pathogens to the maximum.

## CONCLUSION

Antagonistic test results showed that the application of biological agents *Trichoderma harzianum* was classified as moderate against *Aspergillus parasiticus* and *Mucor circinelloides* with an average inhibitory rate of 32.5 % within five days and 42.38 %, respectively. An antagonist agent can be said to be a biological agent if it can inhibit pathogens > 50 %. Therefore, it can be said that *Trichoderma harzianum* only has a minimal inhibitory effect on the growth of pathogenic fungi that attack corn plants.

## ACKNOWLEDGMENTS

Working on and preparing this study is inseparable from the help, guidance, and support of various parties. Therefore, on this occasion, the authors would like to express gratitude to Dr. Dra. Susiana Purwantisari, M.Si as the supervisor who has accompanied and guided the authors during the research process.

## AUTHORS CONTRIBUTIONS

FAN, MFIS, and MC contributed to the design of the topic, methods, and implementation of the research with SP guiding, giving direction during the research, and financing the research needs. FAN and MFIS were responsible for determining the sampling areas, finding corn samples infected with pathogenic fungi, and antagonistic tests against *Aspergillus* cob rot. MC was responsible for the antagonistic test and fungicide mechanism against *Mucor* Stem Rot. This manuscript was primarily

composed by FAN, and all authors provided critical feedback and contributed to the development of the research, analysis, and manuscript.

## DECLARATION OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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# Formulation and Market Acceptability of Dragon Fruit (*Selenicerius undatus*) Flavored Mead

10.18196/pt.v13i1.22318

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**ABSTRACT**

This paper discusses the development and acceptability of Mead wine flavored with Dragon Fruit. A quantitative descriptive analysis survey was utilized among forty (40) panelists who are experts in alcohol. At the same time, 75 respondents were randomly selected as participants in the sensory analysis using a Hedonic rating scale with a 5-point Likert scale. The results of the study have shown that the product was acceptable, considering its color, aroma, taste, and even the alcohol content of both treatments. In the findings, the highest response on color is ruby with 62.5%, and responses on aroma are regarded as sweet, tangy, and even zesty nuance. The taste reveals that it is fairly acceptable, with a 2.30 mean, which accounts for its acidity and is good for food pairing. On the overall acceptability level of the three coded samples, the wine-coded control got the highest description of high acceptable and a mean of 4.38. As for the willingness to purchase the product, the commercial product got the highest mean of 4.13, acceptable, and wine with code treatment 1 got a high score of 4.03, which is also acceptable. The results of this study imply that mead with added dragon fruit has the potential to produce mead that is preferred by respondents.

**Keywords:** Acceptability; Dragon Fruit; Mead; Physico-Chemical; Wine

## INTRODUCTION

Mead is an alcoholic drink primarily made from the combination of honey as its main ingredient, added with water and yeast and set for fermentation for two to three weeks. Its alcoholic content ranges from 8% to 18% (Harder et al., 2021). Mead is dedicated to its medicinal properties because of the high-antioxidant properties that the honey contains. It is also believed that drinking a glass of this drink each day may lower the cholesterol levels in the blood and may reduce the risks of cardiovascular diseases, atherosclerosis, hypertension, type 2 diabetes, neurological disorders, some metabolic syndromes, and certain types of cancer. Aside from that, this drink also gained attention in economic parlance due to the honey's therapeutic properties (Romano et al., 2021).

The desired quality of taste and aroma of mead depends on a primary factor, which encompasses the proportion of water, yeast, and honey. It says that the more honey you add to the mixture, the stronger the taste, while a small quantity of honey combined with the mixture along with a higher quantity of water would give a lighter taste of the mead. Variety of honey, pH level, yeast strain, and yeast nutrition also affect the outcome of mead (Senn et al., 2021).

Meanwhile, the ingredient present in mead is honey. Honey is a natural food substance that is sweet since it is composed mainly of sugars and other natural chemicals such as vitamins, aromatic

Article History
Received : 15 January 2025
Revised : 11 February 2025
Accepted : 18 February 2025

substances, organic acids, enzymes, amino acids, and carotenoids (Edo et al., 2023). It is a substance produced by honey bees from the nectar of plants. Accordingly, honey contains large benefits to all living things. It is rich in phenolic acids and flavonoids, which produce biological effects and antioxidants (Afroz et al., 2023; Mărgăoan et al., 2020)

Another notable factor that contributes to the successful production of mead is the kind of yeast that will be used in the fermentation. According to Franceschetti, (2023), some strains of yeasts are not suitable for honey mead production since they contribute to the production of off-flavor, delayed, and arrested fermentations. Literature suggests that for yeast to effectively turn sugar into alcohol and make wine, it is good to identify which yeast is to be added. Although *Saccharomyces cerevisiae* is widely popular among bakers because of its effectivity in dough emulsification, it must be noted that it has a lot of strains to which they act differently.

Meanwhile, dragon fruit is selected as flavoring because of its availability in the area where the mead is produced. This fruit has been getting so much attention worldwide because of its red-purple color and the economic benefit it provides, as well as the antioxidative activity from the betacyanin it contains (Iffah et al., 2024). There is considerable scientific and public interest in the important role that antioxidants play in health care, such as by acting as cancer chemopreventive and anti-inflammatory agents and by reducing the risk of cardiovascular mortality 2 (Iffah et al., 2024).

Traditionally, although mead is made from the combination of three ingredients, namely, honey, water, and yeast, flavoring is also added to give a twist to the mead (Saša et al., 2022). Some recorded mead styles are Braggot, Capsicumel, Cyser, Melomel, Metheglin, Morat, Omphacomel, Oxymel, Pymet, Rodomel, Tej, and Ypocras. Each of the mead styles written is unique depending on the added flavoring present on it. Braggot, for instance, is a mead flavored with malt; Capsicumel is a mead made from Chile pepper and honey; Cyser is a honey and apple extract/apple juice which is combined; Melomel is a mixture of honey and other fruit juices, Metheglin is a mead combined with herbs and spices such as cloves and cinnamon; Morat is a mead with the addition of mulberry; Omphacomel mead with verjus; Oxymel is with wine vinegar; Pymet is mead with honey and wine juice and may sometimes refer as wine sweetened with honey; Rodomel is a combination of honey and rose petal or the rose petal oil called attar; Tej somehow is a mead that came from Ethiopia and referred as white wine; and the last is Ypocras a mead added with spices. No matter what flavoring is added to the mead, the final strength and sweetness of this drink depend on the proportion of the honey and water a mether or mead maker used.

However, there is no recorded mead flavored with dragon fruit yet. Hence, the study is focused on developing a mead flavored with dragon fruit and assessing its acceptability in the market. In order to do that, the researcher has made use of two treatments to see which one is preferred by consumers, which will enable the researcher to set a standard ingredient for mead.

## MATERIALS AND METHODS

### Preparation of the Dragon Fruit Must

The fruit must of the Dragon fruit was obtained by washing the fruit, cutting it in half, scooping it, and crushing it until the juice came out. Making the must of the dragonfruit is the first process in making the mead. You can also include its peeling if you desire since it is smooth and can be added.

## Honey Preparation

The honey added to the treatment was bought from the province of Quezon, Philippines. Based on the literature, the honey must be simmered at 185 degrees Fahrenheit with water to eliminate the scummed floating in the honey. Besides, the selection of honey must be taken into consideration because there are those honey that are tampered with water or added with sugar or citrus, making it adulterated. The honey used was produced by a natural beehive of the cliffs and trees in the forests. It was harvested by the local bee hunters from the place. The honey is unprocessed, unfiltered, unheated, and unpasteurized, ensuring that all natural ingredients, living enzymes, and other nutritional elements are preserved.

## Yeast Inoculation

The yeast used in the study was a commercialized Red Star Premier Blanc Wine Yeast, which was manufactured in Belgium by Fabriqué en Belgique par Algist Bruggeman N.V. Langerbruggekaai 37, 9000 GENT for pour. Its ingredients are *Saccharomyces bayanus*, emulsifier: sorbitan monostearate (E941). This yeast is an all-purpose and vigorous, moderately foaming, and sulfite-tolerant strain. This brand is suitable for making Mead, Cider, Cabernet, Dry Whites, other Fruits and Sodas. It has a 15% alcohol tolerance with a fast rate of fermentation.

## Dragon Fruit Mead Fermentation Process

Two compositions are selected for the treatments during the fermentation process, as shown in Figure 1. The fermentation process was done for about 3 weeks in 3 gallons of water or 13.64 liters, considering the favorable conditions of the environment, where temperature must not exceed thirty degrees Celsius to avoid possible risk of off-flavor or spoilage. The fermentation process was conducted from sterilization of materials used up to bottling. Necessary steps were conducted before the mixture of the must, inoculation of yeasts, and simmering of honey-water. Observation of the fermentation process was employed, and changes in the appearance and aroma were recorded.

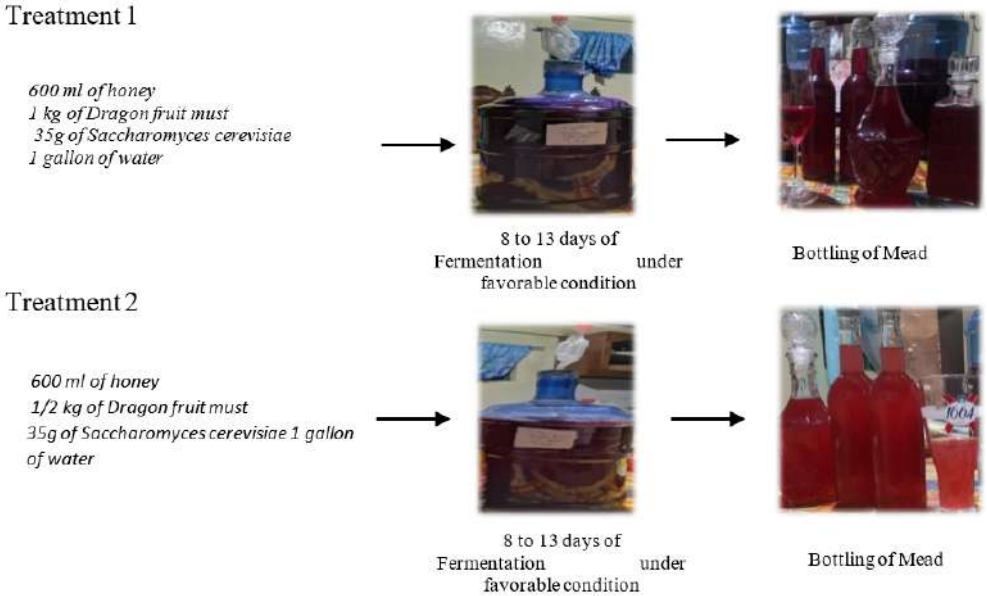


**Figure 1.** Dragon Fruit Mead Fermentation Process

## Data Gathering Tool

The researcher utilized a quantitative descriptive analysis survey, which was clustered into two parts. The first part is intended to elicit the panelists' experience and knowledge of drinking alcoholic beverages, while the second part is to determine the physical characteristics of the product based on its aroma/odor, color/appearance, texture, and taste. The survey was conducted among 40 panelists who are alcohol experts. Another tool used in gathering needed data is the Consumer Preference Test where panelists and the other participants were assessed for their willingness to purchase the product and the total acceptability of the product. Furthermore, the consumer preference test was given to the 75 panelists who are not alcohol experts. The tools used in this study underwent pilot testing to make sure that the data gathered were valid and reliable.

## Data Gathering Procedures

The researcher was not able to secure the Institutional Review Board Approval; however, before conducting the study, the samples were tested at the Department of Agriculture - Cagayan Valley Integrated Laboratory for safe consumption. Before gathering data, a consent form was presented and given to them, along with the assurance that their responses would be solely used in the study.

## Data Analysis

The first part of the problem reflects the demographic profile of the respondents, which was analyzed using frequency and percentage to determine the distribution of the participants in the study. The quantitative descriptive analysis and the acceptability level of the participants were computed using a weighted mean. The use of the mean as the statistical tool is beneficial in determining the average responses and their variation from one another. In the study, there were forty (40) panelists composed of 10 females and 30 males, ranging in age from 18 to 65 years, who were treated as experts and asked to give their judgment on the mead. They were purposively selected because of their knowledge and expertise to give constructive evaluative judgement when it comes to food and beverage. There were three samples of Dragon fruit mead with 5 ml presented to them, including the controlled sample, which is a commercialized mead. Each sample was given a code like WCo1 (Treatment 1), WCo2 (Treatment 2) and WCo3 (Control). There were another 75 panelists who were randomly selected in the sensory analysis using a Hedonic rating scale with a 5-point Likert scale. They were treated as non-experts in the wine tasting, and somehow, their level of acceptability of the produced mead is equivalently important in assessing their overall impression of the mead. The hedonic rating scale was chosen because of its popularity in eliciting responses or the degree of consumer acceptance of a product (Ribeiro et al., 2024). The study employed 2 to 3 sensory evaluation tools. This technique somehow provides substantial information compared to just using a single sensory evaluation like a Hedonic rating. Integration of 2 or 3 techniques can be a powerful tool in quality evaluation.

## RESULTS AND DISCUSSIONS

The percentage of alcohol present in the two-sample mead is presented in Table 1. The US standard drink, however, recommends that wine must have 12% Alcohol by Volume (12% ABV). The alcohol by volume of the two-sample wine complements the result of the test conducted by the Department of Science and Technology (DOST), where Dragon Fruit Wine A (Red) has 9.01 % alcohol, and Dragon Fruit Wine A (Yellow) has 8.88% alcohol content. This implies that the results of the two sample wines are the same and will still fall within the alcohol content in wine ranges of 5.5% to 25% ABV. This result is supported by the argument (Berger & Zelman, 2022) that the higher the concentration of alcohol, the lower the number of consumers who will choose the product because many believe that it is associated with health damage and social responsibilities (Petticrew et al., 2020; Gutan, 2024).

**Table 1.** Percentage of Alcohol present in the two-sample mead

| Sample code | Sample Description | Result (g/100g) |
|---|---|---|
| CHE – 0695 | Dragon Fruit Wine A(Red) | 9.01 |
| CHE – 0696 | Dragon Fruit Wine A(Yellow) | 8.88 |

The frequency and percentage distribution of the respondents as to their experience drinking alcoholic beverages were illustrated in Table 2. As shown in the table above, the percentage of males, which is 75%, compared to females, which is only 25%, implies that males are more into drinking alcoholic beverages. Although this was purposively selected, it implicitly implies that the male status when it comes to drinking is greater than the female. This supports the many studies composed mostly of men that focus on alcohol consumption and alcohol-related problems compared to women (Jaswal et al., 2025). Drinking per se and high-volume drinking were consistently more prevalent among men than among women, but lifetime abstention from alcohol was consistently

**Table 2.** Frequency and Percentage distribution of the respondents as to their Experience Drinking Alcoholic Beverages.

| Gender | | Frequency | Percent |
|---|---|---|---|
| | Male | 30 | 75.0 |
| | Female | 10 | 25.0 |
| | **Total** | **40** | **100.0** |
| Type of Drinker | | Frequency | Percent |
| | Alcoholic Beverage Consumer | 28 | 70.0 |
| | Expert Wine Consumer | 3 | 7.5 |
| | Non Expert Wine Consumer | 9 | 22.5 |
| | Total | 40 | 100.0 |
| Age | | Frequency | Percent |
| | 18 - 25 years old | 10 | 25.0 |
| | 26 to 35 years old | 7 | 17.5 |
| | 36 to 45 years | 12 | 30.0 |
| | 46 to 55 years old | 2 | 5.0 |
| | 56 to 65 years old | 9 | 22.5 |
| | Total | 40 | 100.0 |
| Experienced Drinking Alcoholic Beverage | | Frequency | Percent |
| | Yes | 39 | 97.5 |
| | No | 1 | 2.5 |
| | Total | 40 | 100.0 |

more prevalent among women (Zhao et al., 2023). On the Type of Drinker Variable, 70% of the respondents are Alcoholic Beverage Consumers. While 7.5% have said that they are experts when it comes to wine, 22.5% of them are expert wine consumers. As the literature has said, flavor and sensory perception are highly variable across individuals (Bertelsen et al., 2020). Although there was a minimal percentage of non-expert respondents compared to non-expert wine consumers, the data still supports the findings of the researcher that their responses are helpful in the overall taste impression and acceptability of the mead product. When it comes to age as an indicator of the general profile of the respondents, the findings revealed that the respondents are mostly from ages 36 to 45 years old. A higher prevalence of high-frequency drinking in older age groups of drinkers is reported also in at least a few other surveys (Ranker et al., 2023). On their experience of drinking alcohol, almost all the respondents, which accounts for 97.5% of the population, have said that they already consumed alcohol. Kilian et al. (2022) conducted a large population survey between men's and women's drinking behavior, showed that the prevalence of high drinking is higher in men than women and the high volume of frequent drinking is higher in the oldest age group, and finally, the frequency of drinking did not decline instead it tends to become increasing especially in Europe and other English-speaking countries like the Philippines.

**Table 3.** Responses of the respondents in rank style as to the type of Alcoholic Beverage they consumed

| Data | Rank |
|---|---|
| Beer | 1 |
| Brandy | 4 |
| Mead | 8 |
| Rum | 5 |
| Spirits | 3 |
| Tequila | 7.5 |
| Vodka | 6 |
| Whiskey | 7.5 |
| Wine | 2 |

The responses of the respondents in rank style as to the type of alcoholic beverage they consumed are shown in Table 3. The result shows that among the alcoholic beverages included in the choices on the type of alcohol they consume, beer got the first rank and the least Mead. In a study, 'Influence of information about manufacturing process on beer acceptability,' the results show the mean acceptability liking for beer has been confirmed by the author's findings (Hernández-Mora et al., 2022). The study where two-way ANOVA was used to assess the differences between actual liking mean scores and the baseline shows that manufacturing processes have a significant effect on beer acceptability (Orden et al., 2023).

The frequency and percentage distribution of the respondents as to how often they consumed alcoholic beverages were revealed in Table 4. The results have shown the frequency of the respondents drinking alcohol, and obviously, they are only more into drinking alcohol on special occasions, which garnered 60%, while the least have 2.5% who drink alcohol every day. This only implies the awareness of the respondents on the effect of alcohol on our health, especially if they do it on a daily basis of consuming it. High levels of alcohol consumption (2 drinks per day) are associated with an increased risk of hypertension (Vacca et al., 2023). The frequency and percentage distribution of the respondents as to their knowledge about mead were shown in Table 5. Gleaned from the table

above is the response of the respondents regarding their knowledge of Mead. It shows that there were 34 respondents who knew this wine already, which accounts for 85% of the respondents, while 6 of them, which accounts for 15%, have said that they do not know about Mead. The decision to consume alcohol has been associated with factors like variety seeking, experience, product involvement, demographic characteristics, and sources of information. Additionally, subjective knowledge is related to one's own preferences and other sources like acquaintances, sales personnel, and friends (Pickering, 2024; Gorman et al., 2024).

**Table 4.** Frequency and Percentage distribution of the respondents as to how often they consumed Alcoholic Beverage

| | Frequency | Percent |
|---|---|---|
| Everyday | 1 | 2.5 |
| 3 to 5 times a week | 7 | 17.5 |
| Once a week | 4 | 10.0 |
| Only on weekend | 4 | 10.0 |
| On special occasion | 24 | 60.0 |
| Total | 40 | 100.0 |

**Table 5.** Frequency and Percentage distribution of the respondents as to their knowledge about Mead

| | Frequency | Percent |
|---|---|---|
| Yes | 34 | 85.0 |
| No | 6 | 15.0 |
| Total | 40 | 100.0 |

The frequency and percentage distribution of the respondents as to their assessment of the color of the product are presented in Table 6. Among the color options associated with red, Ruby got the highest, at 62.5%. According to research, the color red is eye-catching and triggers appetite. It's useful for packaging design; this is likely because the color, when found in natural foods like berries, indicates ripeness or sweetness (Romeh et al., 2024). The aroma/odor of mead was revealed in Table 7. Generally, the respondents associated the aroma or odor of the mead product with a red berry aroma, which was sweet, tangy, and even zesty nuance. In a study of the aroma of wine, various volatile compounds have been seen to interact with each other and create the final aroma and flavor palette of the product. The aroma also is affected by the amount of ethanol present in a wine. It shows that a decrease in the ethanol concentration in a model wine from 10 to 9% had no effect on the flavor or aroma profile. When the ethanol was lowered to 7%, there was an increase in the strength of the flowery, fruity, and acid flavors and aromas. However, when ethanol concentration was lowered to 3%, the wine no longer resembled a wine anymore (Gabler et al., 2024). In another study, the reduction of the alcohol levels in wine affects the bouquet by intensifying the fruity odor and woody odor of wine. Consequently, modification of their chemical ratio also affects the odor of the alcoholic beverage (Silva, 2024).

The results on product taste acceptance are shown in Table 8. The findings revealed that the product's taste was fairly acceptable, with 1.83 as a categorical mean. In assessing the results, the researcher is supposed to enhance the quality of the product's taste by considering factors such as the correct proportion of ingredients, the quality of the ingredients, and even the condition of fermentation. This

is because a 1.83 hgb using a qualitative technique to explore the perception of the drinkers when it comes to quality wine, and they found out that quality is based on intrinsic and extrinsic factors. Extrinsic factors include the kind of fruits, production, and marketing, while Intrinsic factors are appearance, pleasure, and gustatory (taste, smoothness, body, drinkability, balance, concentration, complexity, and interest).

**Table 6.** Frequency and Percentage distribution of the respondents as to their Assessment on the color of the product

| | Frequency | Percent |
|---|---|---|
| Purple | 5 | 12.5 |
| Ruby | 25 | 62.5 |
| Garnet | 6 | 15.0 |
| Orange | 4 | 10.0 |
| Total | 40 | 100.0 |

**Table 7.** Aroma/odour of Mead

| Data | Rank |
|---|---|
| Citrus | 2 |
| Tropical | 4/5 |
| Red berry | 1 |
| Blue berry | 5.5 |
| Black berry | 5.5 |
| Apple | 3 |
| Pear | 6.5 |
| Stone Fruit | 6.5 |

**Table 8.** Product Taste

| Variable | Mean | Description |
|---|---|---|
| Sweetness | 1.60 | Fairly Acceptable |
| Acidity | 2.30 | Fairly Acceptable |
| Mouthful/Tannin | 1.65 | Fairly Acceptable |
| Alcohol | 1.53 | Fairly Acceptable |
| Finish | 1.63 | Fairly Acceptable |
| Food Pairing | 2.30 | Fairly Acceptable |
| Categorical Mean | 1.83 | Fairly Acceptable |

The acceptability level of the three coded samples is shown in Table 9. Control treatment or the commercialized product garnered the highest mean average when it comes to color and appearance. Between the two mead products, Treatment 1 got higher, with 4.11, than Treatment 2, with 3.93 as the mean. This implies that color affects the acceptability of the respondents when it comes to choosing a product, and the two created samples from Treatment 1 tend to have a ruby color, which is brighter and reflects the vibrant color of the wine. When it comes to the aroma/smell, Control treatment still got the highest, well, obviously, since this was already commercialized. Between treatments 1 and 2, the latter got 1 point higher, 3.88, acceptable, while treatment 1 got 3.87, acceptable. The difference is not by far big. Hence, it would still imply that the two created mead products are acceptable when it comes to their Aroma/Smell. On the other hand, regarding taste/flavor, the control treatment still got the highest, with 4.41, which is highly acceptable. Treatment 1 is higher in terms of mean, which is 4.04, acceptable; however, Treatment 2 was also noted to be acceptable at the same time,

although the mean is lowered compared to the former code. Generally, respondents have assessed control treatment to be highly acceptable. This is because people tend to accept a product when they have experienced it already (Rauschnabel et al., 2024).

**Table 9.** Acceptability level of the three coded samples

| Treatment 1 | | |
|---|---|---|
| Variable | Mean | Description |
| Color/Appearance | 4.11 | Acceptable |
| Aroma/Smell | 3.87 | Acceptable |
| Taste/Flavor | 4.04 | Acceptable |
| Categorical Mean | 4.00 | Acceptable |
| Treatment 2 | | |
| Color/Appearance | 3.93 | Acceptable |
| Aroma/Smell | 3.88 | Acceptable |
| Taste/Flavor | 3.81 | Acceptable |
| Categorical Mean | 3.88 | Acceptable |
| Control | | |
| Color/Appearance | 4.35 | Highly Acceptable |
| Aroma/Smell | 4.39 | Highly Acceptable |
| Taste/Flavor | 4.41 | Highly Acceptable |
| Categorical Mean | 4.38 | Highly Acceptable |

The willingness of the respondents to purchase the product is presented in Table 10. An alcoholic beverage is a product that is noted to be an information-experience product because the quality cannot be assessed until one has actually been involved in consuming it (Faro, 2021; Petticrew et al., 2020). The olfactory factors of alcohol are part of the total experience of a consumer (Betancur et al., 2020). Hence, marketers must have a deep understanding of the consumers' sensory preferences when it comes to the alcoholic beverage that they are buying, and this somehow is an area that has not yet been extensively researched (Betancur et al., 2020). Surprisingly, the findings above have shown that the three coded samples have an acceptable impression among respondents, with a categorical mean of 3.97. Although Control got the highest, treatment 1 is not by far, with 4.03 as the mean average. This shows that respondents are willing to purchase the products, although 85% know mead.

**Table 10.** Willingness to purchase scale

| Variable | Mean | Description |
|---|---|---|
| Treatment 1 | 4.03 | Acceptable |
| Treatment 2 | 3.75 | Acceptable |
| Control | 4.13 | Acceptable |
| Categorical Mean | 3.97 | Acceptable |

The nutritive value content of mead is listed in Table 11. With the use of proximate analysis, nutrient content on mead was identified. In comparison, Treatment 1 has a total kcal content of 71 while Treatment 2 has a 90 kcal content. This means that Treatment 1B is greater when it comes to its intensity of providing more energy to the body. The nutritional information of the mead product is necessary for giving nutritional data of the products to potential consumers. The results have shown that while mead is not commonly known to many, findings revealed that it is a source of vitamin C, has anti-oxidant, and calorie content which is at the minimum level (Medina & Medina, 2025; Essiedu & Kovaleva, 2024). The relationship between the level of willingness of the consumers and

the overall taste acceptability is exemplified in Table 12. The three samples have illustrated highly significant results in the respondents' willingness to buy the products. Furthermore, there is a positive correlation between the overall taste preferences and their willingness to purchase the product.

**Table 11.** The Nutritive Value content of Mead

| | | |
|---|---|---|
| **Treatment 1**<br>Sample net. Weight: 1000ml | | |
| Nutrition Facts | | |
| Serving size: 147 ml<br>No.of servings per container:7<br>% of alcohol: 9% | | |
| Amount per serving | | |
| | | %RENI |
| | | 3% |
| Calories (kcal) 71 | | |
| Calories from fat 0 kcal | | |
| Total fat (g) | 0.0 | - |
| Total Carbohydrates (g) | 2.3 | - |
| Sugar (g) | 2.3 | - |
| Total Protein (g) | 0.0 | 0% |
| *Percent RENI values are based on 2015 RENI PDRI reference male adult requirement of 19-29 years old | | |
| **Treatment 2**<br>Sample net. Weight: 1000ml | | |
| Nutrition Facts | | |
| Serving size: 147 ml<br>No.of servings per container:7<br>% of alcohol: 12% | | |
| Amount per serving | | |
| | | %RENI |
| | | 3% |
| Calories (kcal) 90 | | |
| Calories from fat 1 kcal | | |
| Total fat (g) | 0.1 | - |
| Total Carbohydrates (g) | 3.4 | - |
| Sugar (g) | 3.4 | - |
| Total Protein (g) | 0.0 | 0% |
| *Percent RENI values are based on 2015 RENI PDRI reference male adult requirement of 19-29 years old | | |

**Table 12.** Test of Relationship between the Level of Willingness of the Consumers and the Over-all Taste Acceptability

| Variable | | Willingness to Purchase Scale |
|---|---|---|
| Treatment 1 | r – value | .760* |
| | p – value | .000 |
| Treatment 2 | r – value | .743* |
| | p – value | .000 |
| Control | r – value | .564* |
| | p – value | .000 |

## CONCLUSION

In the present study, only when these two treatments were being correlated to a commercially available product, undeniably, the commercialized product was highly accepted by the respondents. This is because, according to literature, people tend to purchase a good that they have already experienced, considering the extrinsic and intrinsic factors that affect the product. On the other side of the findings, the physicochemical properties of meads were also analyzed, and chemical elements were found to be responsible for sensory properties like color, taste, and aroma. This method is essential in determining the quality of mead. When it comes to the respondents' willingness to purchase the product, it was noted that there is a positive correlation, which is highly significant for the respondents. The results of this study imply that mead with added dragon fruit has the potential to produce mead that is preferred by respondents.

## RECOMMENDATION

The researcher highly recommends further investigation of mead wine, particularly on the possibility of mixing those fruits available in the study setting. Furthermore, since the foregoing study has a high chance of being an alternative option among consumers, future research may include a return on investment.

## ACKNOWLEDGEMENT

The author is grateful to the Department of Agriculture - Cagayan Valley Integrated Laboratory for their expert support and technical assistance in letting the researcher use their laboratory facilities to analyze data needed to elicit information on the samples. Gratitude is also given to the Isabela State University - San Mariano Campus for financing the expenses of this research study.

## COMPETING INTEREST

The author declares that she has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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# Effects of Biocontrol Product Bio P60 and Liquid Organic Fertilizer on The Development of Fusarium Wilt and Yield of Shallot

10.18196/pt.v13i1.24558

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**ABSTRACT**

Shallot production is frequently threatened by Fusarium wilt. Chemical control has proven ineffective, highlighting the need for environmentally friendly alternatives such as the application of liquid organic fertilizer combined Bio P60. The study evaluated the effectiveness of the fertilizer and Bio P60 in controlling the disease and their impact on shallot growth and yield. A completely randomized block design with 16 treatments and three replicates was used, testing Bio P60 application (control, 20 mL per plant applied 1, 3, or 5 times) and the fertilizer (control, 3, 5, or 7 mL $L^{-1}$). Results showed that the five times application of Bio P60 significantly delayed the incubation period by 61.71%, suppressing disease incidence by 66.67% and reducing AUDPC by 69.84%. Bio 60 also increased plant height by 30.75%, number of leaves by 40.7%, number of bulbs by 75.6%, bulb fresh weight by 104.53%, blub dry weight by 51.1%, and total biomass compared to control. However, the fertilizer application has no significant effect on all variables, and no interaction was found between Bio P60 and the fertilizer. These findings suggest Bio P60 is an effective biocontrol agent for suppressing Fusarium wilt and improving shallot yield, offering a sustainable alternative to chemical treatments.

**Keywords:** Eco-friendly method; *Fusarium oxysporum*; *Pseudomonas fluorescens*; Shallot

## INTRODUCTION

Shallots are a type of vegetable crop in incredible demand by the community and have great potential to be cultivated in Indonesia (Saptana et al., 2021). Shallots are utilized in different ways, including conventional pharmaceuticals, crude materials for the nourishment industry, and flavors (Moldovan et al., 2022). The dietary substance contained in 100 g of shallots is 39 calories, phosphorus 40 mg, iron 0.8 mg, protein 1.5 g, hydrate charcoal 0.3 g, fat 1.2 g, calcium 36 mg, and vitamin C 2 g (Sun et al., 2019). Indonesia's shallot production in 2023 is 1,985,233 tons, which is still low compared to the shallot production data for 2021, which is 2,004,590 tons (BPS, 2024). The low shallot production is caused by several factors, including bad weather that does not support plant development, wasteful utilization of production facilities, and plant pests and diseases (Manwan et al., 2020; Ortiz-Bobea et al., 2021).

Article History
Received : 23 October 2024
Revised : 20 February 2025
Accepted : 21 February 2025

Plant pests and diseases reduce shallot yield (Rovicky et al., 2024). Fusarium wilt is a plant disease caused by *Fusarium oxysporum* f. sp. *cepae,* a primary disease in shallot plantations (Sakane et al., 2024). This disease can harm shallot plants. Twisting leaves are caused by shallots infected with Fusarium. Fusarium wilt may cause Indonesian shallot producing centers to lose at least 50% of their yield, according to Hadiwiyono et al. (2020). Harvest failure is another consequence of infected shallot plants (Sharma et al., 2024). Link first defined the genus Fusarium in 1809, citing the specific conidia that had a banana or canoe shape (Samiksha & Kumar, 2021). The most harmful species are Fusarium species (Ajmal et al., 2023). Soil-borne pathogens are another name for them.

Control of Fusarium wilt now depends on chemical fungicides (Tang et al., 2020; Baloch et al., 2021), along with measures such crop rotation (Theron et al., 2023) and fumigation (El-Aswad et al., 2023). Inappropriate utilize of chemical fungicides can hurt the environment and result in hurtful buildups in agrarian items, posturing a risk to human wellbeing (McDonald et al., 2019; Okagu et al., 2023). To address the inconvenient impacts of chemical fungicides, it is vital to investigate elective ecologically inviting control strategies, counting organic specialists (Bubici et al., 2019; Tang et al., 2020). One of the organic operators utilized to combat contagious and bacterial pathogens is the opposing microscopic organisms known as *Pseudomonas fluorescens*. One strain of the adversarial microscopic organisms, *P. fluorescens* P60, has illustrated viability in controlling a run of plant pathogens, especially those found within the soil. This has been watched in research facility, nursery, and field ponders (Bhetwal et al., 2021). *P. fluorescens* P60 created auxiliary metabolites with bioactive compounds. The fluid detailing for the opposing microscopic organisms is called Bio P60 (Soesanto et al., 2019). The item incorporates bioactive compounds in auxiliary metabolites gotten from *P. fluorescens* P60, which produces different compounds, counting anti-microbials, lytic chemicals, and siderophores.

Expanded shallot generation is affected by inside components, such as plant hereditary qualities, and outside variables, such as soil and water reasonableness, climate, and intelligent with other living life forms (Zhang et al., 2022a). An increment in shallot generation can be upheld by utilizing natural fertilizers, such as NASA, which has been commercially accessible for this reason (Lasmini et al., 2019; Hayati, 2020). The fluid natural fertilizer could be a characteristic fertilizer made from extricates of characteristic fixings sourced from animals and poultry excrement, plants, and other natural materials. It is prepared utilizing ecologically neighborly innovation based on the Zero Emanation Concept guideline (Sopha & Lukman, 2019; Husen et al., 2022). NASA fluid natural fertilizer quickens plant development, diminishes bother assaults, has no hurtful impacts on plants or the environment, and is secure for people. The nutrient content of NASA liquid organic fertilizer is as follows: N 0.12%, P2O5 0.03%, K 0.31%, S 0.12%, and Cl 0.29% (Wanimbo & Tuhuteru, 2020; Serdani et al., 2023). The combined effect of Bio P60 and NASA has not been studied. A study assessed the effectiveness of NASA liquid organic fertilizer and Bio P60 in controlling Fusarium wilt and its impact on shallot growth and yield in plants. The benefits of this research ensure that the Fusarium wilt of shallots can be tackled organically.

## MATERIALS AND METHOD

The research was conducted in Banjarsari Wetan Village, Sumbang Subdistrict, Banyumas Regency, with coordinates of 7°21'47" S 109°15'04" E, altitude of 220 m above sea level and tropical climate, and at the Plant Protection Laboratory, Faculty of Agriculture, Universitas Jenderal Soedirman for five months.

### Preparation of Bio P60

Bio P60 production initiates by inoculating *P. fluorescens* P60 in as much as 1 test tube into 1 L of liquid King's B. incubated with shaking at 135 rpm at room temperature for 3 days (Han et al., 2012). Preparation of propagation media with 200 g of boiled catfish and 80 g of shrimp paste in 20 L of water. Filter and transfer to a sterile jerry can, then cool. After cooling, 40 mL of liquid *P. fluorescens* P60 was added to the media and shaken at 135 rpm at room temperature for 3 days. The density was calculated to be $10^9$ cfu $mL^{-1}$ (Wang et al., 2024).

### Preparation of *Fusarium oxysporum* f.sp. *cepae*

The pathogenic organism *F. oxysporum* f.sp. *cepae* was disconnected from onion bulbs showing Fusarium shrivel side effects. The bulbs were surface sterilized with 70% alcohol, washed with sterile water three times, and dried on sterile filter paper. Next, the tubers were cut into small pieces, placed on PDA media in Petri dishes, and incubated at room temperature for 5 days (Kalman et al., 2020). To confirm *F. oxysporum* f.sp. *cepae*, the fungus was identified microscopically and compared with the literature (Sharma et al., 2024). The fungus was cultured in PDB liquid media by placing 2 plugs (diameter 1 cm) of *F. oxysporum* f.sp. *cepae* into a 250 mL Erlenmeyer flask filled with 125 mL of cold, sterile PDB and incubated with shaking at 135 rpm for 7 days at room temperature. The density of fungal microconidia was counted using a hemocytometer until it reached a density of $10^6$ conidia $mL^{-1}$.

### Preparation of growth media

The planting medium was a mixture of soil and manure (2: 1, w/w), and the mixture was pasteurized at 80 °C, then put into polybags (25 × 40 $cm^2$), and the number of polybags was 144. The bottom of the polybags was given some drainage holes (Haase et al., 2021).

### Preparation of shallot seeds and planting

Seeds of the shallot variety Bima Brebes were prepared. The seeds were cut off the top 1/3 with a sterile knife and planted 1-2 cm deep, with the cut marks facing upwards, then covered with thin soil. Additional fertilizer, such as base fertilizer, was given in the form of TSP in the form of as much as 3 g per polybag (Purba et al., 2017).

### Application of treatments

The *F. oxysporum* f.sp. *cepae* suspension was inoculated by pouring into shallot seeds as much as 10 mL plant-1. Furthermore, two days after *F. oxysporum* f.sp. *cepae* inoculation, Bio p60 application was carried out by sprinkling it to plants as much as 20 mL plant-1 with a concentration

of 5 mL solution$^{-1}$ and repeated according to the treatment. NASA liquid organic fertilizer was applied with a concentration according to the treatment and sprinkled as much as 40 mL plant$^{-1}$ at the age of shallot plants 7, 17, 27, and 37 days after planting (Buton et al., 2019).

## Research design

This study used a completely randomized design consisting of two factors. The first factor was the intensity of Bio P60 application, which was the control and Bio P60 watering of 20 mL plant$^{-1}$ repeated 1, 3, and 5 times. The second factor was NASA liquid organic fertilizer, the control, and 3, 5, and 7 mL L$^{-1}$ concentrations. The combined results of the two factors resulted in 16 treatment combinations, which were repeated 3 times, resulting in 48 experimental units. One experimental unit contained 3 plants, resulting in 144 plants (Piepho et al., 2022).

## Variables observed

The variables observed were the incubation period with units of days after inoculation disease incidence using the formula (Nathawat et al., 2020): DI = (n ÷ N) ×100%, where: DI = Disease incidence (%); n = number of plant samples or plant parts that were damaged or considered damaged; N = number of plant samples or plant parts observed. The area under disease progress curve (AUDPC) was calculated by the formula (Alves & Del Ponte, 2021):

$$\text{AUDPC} = \frac{\left(\frac{I1+I2}{2} x\, T\right)+\left(\frac{I1+I2}{2} x\, T\right)+\left(\frac{I1+I2}{2} x\, T\right)+\left(\frac{I1+I2}{2} x\, T\right)}{n-1} \qquad (1)$$

Notes: I = Intensity of disease attack at different observations ($I_1$, $I_2$, .... $I_n$); T = Interval of observation interval; n = Number of observations. Plant growth and yield components observed were crop height, number of leaves, number of bulbs, crop fresh weight, bulb fresh weight, crop dry weight, and bulb dry weight.

## Data Analysis

Data were analyzed with ANOVA at the 95% confidence level. If the analysis results show a significant effect, continue with DMRT (Duncan's Multiple Range Test) at a 5% error level (Midway et al., 2020).

# RESULT AND DISCUSSION

## Incubation Period

The observation of the incubation period of Fusarium wilt on shallot plants revealed that using Bio P60 could prolong the onset of disease symptoms and was noticeably different from the control (Table 1). Bio P60 applications of 1, 3, and 5 times can delay the incubation period of the fungal pathogen *F. oxysporum* f. sp. *cepae*. Compared to the control, the suppressive effects were observed at 50.81%, 55.18, and 61.71%. Bio P60 has been shown to reduce the incubation period of fungal pathogens. Bio P60 is a formulated product containing secondary metabolites from the antagonistic bacteria *P. fluorescens* P60. Cell-based antagonistic bacteria, *P. fluorescens,* suppressed pathogen development by producing antibiotic compounds like phenazine carboxylic acid (PCA), pyrrolnitrin, oomycin A, 2,4- diacetylphloroglucinol (Phl), and pyoluteorin (Plt) (Saeed et al., 2021; Raio, 2024).

**Table 1.** Pathosystem components of Fusarium wilt of shallot plants

| Treatments | Incubation period (dai) | Disease incidency (%) | AUDPC (% days) |
|---|---|---|---|
| NASA Liquid organic fertilizer | | | |
| Control | 36.64 a | 47.22 a | 156.94 a |
| Con 3 mL | 37.51 a | 41.67 a | 144.44 a |
| Con 5 mL | 41.35 a | 33.33 a | 112.50 a |
| Con 7 mL | 42.42 a | 27.78 a | 106.94 a |
| Bio P60 | | | |
| Control | 27.71 b | 66.67 b | 248.61 b |
| Bio P60 1x | 41.79 a | 35.55 a | 109.71 a |
| Bio P60 3x | 43.61 a | 30.55 a | 87.50 a |
| Bio P60 5x | 44.81 a | 22.22 a | 74.99 a |
| Interaction of NASA x Bio P60 | | | |
| Control | 22.47 a | 77.78 a | 305.56 a |
| Bio P60 1x | 42.53 a | 33.33 a | 94.44 a |
| Bio P60 3x | 43.90 a | 33.33 a | 83.33 a |
| Bio P60 5x | 37.67 a | 44.44 a | 144.43 a |
| Con 3 mL, Control | 25.57 a | 77.78 a | 261.11 a |
| Con 3 mL, Bio P60 1x | 44.13 a | 22.22 a | 83.33 a |
| Con 3 mL, Bio P60 3x | 35.43 a | 44.44 a | 177.78 a |
| Con 3 mL, Bio P60 5x | 44.90 a | 22.22 a | 55.55 a |
| Con 5 mL, Control | 32.57 a | 55.56 a | 199.99 a |
| Con 5 mL, Bio P60 1x | 37.40 a | 44.44 a | 155.55 a |
| Con 5 mL, Bio P60 3x | 47.43 a | 22.22 a | 44.45 a |
| Con 5 mL, Bio P60 5x | 48.00 a | 11.11 a | 50.00 a |
| Con 7 mL, Control | 30.23 a | 55.56 a | 227.78 a |
| Con 7 mL, Bio P60 1x | 43.10 a | 22.22 a | 105.55 a |
| Con 7 mL, Bio P60 3x | 47.67 a | 22.22 a | 44.44 a |
| Con 7 mL, Bio P60 5x | 48.67 a | 11.11 a | 50.00 a |

Notes: Numbers in the same column followed by different letters indicate significant differences at the DMRT 5% error level; dai = days after inoculation.

In addition, *P. fluorescens* bacteria produce bioactive compounds in the secondary metabolites, such as the enzyme pectinase (Raio, 2024). The enzyme degrades the conidia wall of *F. oxysporum* f.sp. *cepae*, so that ruptures the cell fluid, causing the pathogenic fungus to die. This condition is causing a delay in the incubation period. Other factors include the adaptation of pathogenic fungi in new environments over time. The pathogenic fungi inoculated into the growing medium are produced in a laboratory under controlled conditions. When pathogenic fungi are introduced into the growing medium, they must acclimate to the new surroundings to flourish (Xiao et al., 2022). The inoculation of the pathogenic fungus F. oxysporum f.sp causes the emergence of disease symptoms. *cepae* despite the beneficial impact of the Bio P60 application.

On the other hand, the onset of Fusarium wilt symptoms was quicker in control plants compared

to Bio P60-treated plants. The fungus has adapted to the new environment, allowing it to develop without inhibiting factors (Nnadi & Carter, 2021). Symptoms of Fusarium wilt disease include alterations in the growth pattern of shallot plants. The growth of shallot plants becomes grooved and lopsided, and the color of the leaves turns yellow (Marianah et al., 2024). The disease symptoms appeared more quickly in control plants, partly due to the aggressiveness of the pathogenic fungus (El-Baky & Amara, 2021; Peng et al., 2021). This demonstrates that using Bio P60 can postpone the onset of Fusarium wilt symptoms, resulting in lower disease severity.

## Disease incidence

Bio P60 at all dosages effectively reduces Fusarium wilt disease in shallot, as evident from the markedly varied results in Table 1. Bio P60 application of 1, 3, and 5 times can reduce disease incidence by 46.68, 54.18 and 66.67%, respectively, compared to the control. The greater the application doses of Bio P60, the higher the disease suppression. This condition aligns with the incubation period. The prevention of disease when using Bio P60 may be due to its role as a secondary metabolite of *P. fluorescens, which* can inhibit the growth of harmful fungi through its bioactive compounds. The hindrance of pathogenic parasites Various bioactive compounds is found within the secondary metabolites of *P. fluorescens*, which create lysis proteins and poisons, as detailed by Sharma et al. (2019). These secondary metabolites can affect plant resistance against pathogenic organisms, progressing plant development and defense mechanisms. Secondary metabolites are delivered utilizing water-soluble substances taken up by plant roots and conveyed all through the plant, where they can repress the development of pathogenic parasites (Soesanto et al., 2019; Zhou et al., 2023). The need of characteristic inhibitors in control plants comes about in a better infection rate. Concurring to Nnadi & Carter (2021), the quick emergence of Fusarium shrink indications within the control bunch is ascribed to the forcefulness of the pathogen in causing illness and the need of hindrance of pathogen development and advancement. Besides, the pathogen's compatibility with shallot plants comes about in prior side effect sign, leading to increased infection rate. *F. oxysporum* f.sp. *cepae* may be a soil-borne pathogen that taints shallot plants. The organism within the soil will adjust and create well after vaccination (Hadiwiyono et al., 2020). The development and improvement of soil-borne parasites are backed by natural conditions within the soil, especially soil dampness. This leads to quick contamination of plant roots by the organism, causing illness rapidly (Wang et al., 2019).

## Area Under Diseases Progress Curve (AUDPC)

The application of Bio P60 brought about in essentially diverse AUDPC values for Fusarium shrink in shallot plants, as appeared in Table 1. All Bio P60 applications effectively decreased the AUDPC esteem of Fusarium shrivel on shallot plants compared to the control. Bio P60 applications of 1, 3, and 5 times decreased AUDPC values by 55.87, 64.8, and 69.84%, separately, compared to the control. This adjusts with the information on the incubation period and plant infection rate. The more Bio P60 is connected, the more prominent the concealment of illness. The Bio P60 application decreases AUDPC, coming about in lower values than the control since the secondary metabolites in Bio P60 can hinder the improvement of the pathogen *F. oxysporum* f.sp. *cepae* (Maurya et al., 2024). The littler the AUDPC esteem, the way better the malady concealment (Bock et al., 2022). The moor

**Table 2.** Application of Bio P60 and NASA liquid organic fertilizer on the growth and yield components of shallots

| Treatments | Crop height (cm) | Number of leaves | Frech weight of crop(g) | Fresh weight of bulb (g) | Number of bulbs | Dry weight of crop (g) | Dry weight of bulb (g) |
|---|---|---|---|---|---|---|---|
| NASA Liquid organic fertilizer | | | | | | | |
| Control | 41.09 a | 44.35 a | 107.99 a | 69.94 a | 10.24 a | 54.83 a | 50.17 a |
| 3 mL | 43.06 a | 49.14 a | 103.31 a | 70.28 a | 10.33 a | 54.08 a | 49.75 a |
| 5 mL | 43.54 a | 52.92 a | 139.53 a | 90.90 a | 11.53 a | 74.01 a | 67.91 a |
| 7 mL | 44.76 a | 51.34 a | 121.50 a | 85.14 a | 11.34 a | 64.40 a | 58.96 a |
| Bio P60 | | | | | | | |
| Control | 35.48 b | 39.36 b | 72.06 b | 47.01 b | 7.09 b | 37.78 a | 33.89 b |
| 1x | 43.93 a | 51.48 a | 13.88 a | 90.86 a | 13.00 a | 71.65 a | 66.73 a |
| 3x | 46.65 a | 51.53 a | 12.17 a | 82.25 a | 10.90 a | 62.69 a | 56.67 a |
| 5x | 46.39 a | 55.38 a | 139.23 a | 96.14 a | 12.45 a | 75.21 a | 69.50 a |
| Interaction of NASA x Bio P60 | | | | | | | |
| Control | 30.63 a | 31.90 a | 49.43 a | 27.00 a | 4.57 a | 19.43 a | 17.23 a |
| 1x | 43.93 a | 50.57 a | 136.53 a | 86.20 a | 13.70 a | 68.80 a | 62.80 a |
| 3x | 47.07 a | 47.13 a | 128.00 a | 86.67 a | 12.47 a | 67.67 a | 60.97 a |
| 5x | 42.73 a | 47.80 a | 118.00 a | 79.90 a | 10.23 a | 63.43 a | 59.67 a |
| 3 mL, Contrl | 36.07 a | 38.57 a | 64.13 a | 44.80 a | 6.80 a | 34.53 a | 32.23 a |
| 3 mL, 1x | 45.83 a | 55.43 a | 138.43 a | 92.53 a | 14.20 a | 75.23 a | 69.90 a |
| 3 mL, 3x | 42.63 a | 50.20 a | 86.43 a | 62.10 a | 9.20 a | 44.20 a | 41.10 a |
| 3 mL, 5x | 47.70 a | 52.37 a | 124.23 a | 81.67 a | 11.10 a | 62.37 a | 55.77 a |
| 5 mL, Contrl | 36.33 a | 42.87 a | 85.77 a | 61.90 a | 7.90 a | 50.33 a | 45.53 a |
| 5 mL, 1x | 41.70 a | 50.67 a | 143.23 a | 96.03 a | 12.10 a | 80.67 a | 74.43 a |
| 5 mL, 3x | 48.77 a | 58.00 a | 168.57 a | 93.90 a | 11.23 a | 74.13 a | 65.90 a |
| 5 mL, 5x | 47.37 a | 60.13 a | 160.57 a | 111.77 a | 14.90 a | 90.90 a | 85.77 a |
| 7 mL, Contrl | 38.90 a | 44.10 a | 88.90 a | 54.33 a | 9.10 a | 46.80 a | 40.57 a |
| 7 mL, 1x | 44.23 a | 49.23 a | 117.33 a | 88.67 a | 12.00 a | 61.90 a | 59.77 a |
| 7 mL, 3x | 48.13 a | 50.80 a | 125.67 a | 86.33 a | 10.70 a | 64.77 a | 58.70 a |
| 7 mL, 5x | 47.77 a | 61.23 a | 154.10 a | 111.23 a | 13.57 a | 84.13 a | 76.80 a |

Notes: Numbers in the same column followed by different letters indicate significant differences at the DMRT 5% error level.

AUDPC esteem in plants treated with Bio P60 may be ascribed to secondary metabolites that seem restrain the pathogen *F. oxysporum* f.sp. *cepae* and influence plant resistance (Anjali et al., 2022).

In differentiate, the tall AUDPC values within the control plants adjusted with the brooding period and illness rate. In controlled situations, parasitic pathogens can effectively create and taint crops due to the need of components that inhibit their development, beside the nearness of aggressive pathogens (Jian et al., 2024). The pathogenic organism *F. oxysporum* f.sp. *cepae* may be a term commonly utilized in plant pathology to allude to a particular shape of the organism that taints certain plant species. The quick foundation of infection indications within the have plant shows compatibility with the pathogenic organism, driving to tall illness rate and AUDPC values (Bock et al., 2022). The quick incubation period and high disease rate contribute to the speedy malady.

## Crop height

Plant stature shown critical contrasts when Bio P60 was connected compared to the control, but there was no critical distinction between distinctive Bio P60 applications (Table 2) improvement, affecting the AUDPC esteem. The highest shallot plant height was observed when Bio P60 was applied 3 times, followed by Bio P60 applications 5 times and 1 time, resulting in heights of 31.48, 30.75, and 23.82% compared to the control. The decreased plant height in the control group results from the plants infected by the pathogen *F. oxysporum* f.sp. *cepae* causing insufficient plant growth and can lead to mortality in shallot plants. This follows the opinion of Lal et al. (2024) that plants affected by Fusarium wilt will collapse and die in advanced attacks.

The application of NASA liquid organic fertilizer and the interaction between Bio P60 and NASA were not significantly different (Table 2) but tended to increase the height of shallot plants compared to the control. The presence of NASA is believed to enhance the nutrient content in the soil, particularly macro-nutrients like N, benefiting plant growth. The element N is recognized as essential for the growth of leaves, stems, and roots in the vegetative stage (Ye et al., 2022). The growth enhancement in plants treated with Bio P60 is attributed to the secondary metabolites of *P. fluorescens* P60, which produces antibiotics that suppress Fusarium wilt and generate growth hormones for plants. *P. fluorescens* is a bacterium that falls under the category of plant growth-promoting rhizobacteria (PGPR), according to Saeed et al. (2021). *P. fluorescens* P60 produces growth hormones, including IAA, in its secondary metabolites that promote plant growth (Sah et al., 2021). IAA hormone, an endogenous auxin, contributes to cell enlargement, inhibits side shoot growth, stimulates abscission, aids in xylem and phloem network formation, and influences root development and elongation, ultimately promoting increased plant growth (Zhang et al., 2022b; Mishra et al., 2023).

## Number of leaves

The results indicated significant differences in the number of leaves for the Bio P60 application, with no significant differences observed between different Bio P60 applications. The highest increase in leaf quantity, up to 40.7%, was achieved by applying Bio P60 five times, as shown in Table 2. No significant difference was observed in the NASA application and interaction with Bio P60. However, the NASA treatment tended to increase the number of leaves compared to the control. It is believed that the presence of growth regulators and macro- and micro-nutrients in NASA can stimulate the growth and yield of shallots. According to Wanimbo & Tuhuteru (2020), NASA provides a comprehensive mix of macro- and micro-nutrients, which can enhance crown growth and leaf count in plants.

The abundance of leaves in the Bio P60 treatment is attributed to *P. fluorescens,* a biological PGPR agent that enhances shallot growth by synthesizing auxin (Khoso et al., 2024). Leaves, being crucial vegetative organs responsible for photosynthesis, play a vital role in plant function (Wang et al., 2021). *P. fluorescens* possesses the capability to degrade phosphate. Adequate phosphorus is crucial for optimal plant growth and development, increasing the number of shallot leaves (Li et al., 2023).

## Number of bulbs

The number of shallot bulbs produced significantly varied with Bio P60 applications but not with Bio P60 intensities (Table 2). The highest number of bulbs was observed when using Bio P60

once, then with Bio P60 applied 5 times, and lastly with Bio P60 applied 3 times. The increase in the number of bulbs compared to the control was 83.35, 75.6, and 53.74%, respectively. The application of NASA and the interaction of Bio P60 with NASA did not have a significant effect, but they did lead to a higher number of tubers than the control. Liquid organic fertilizers are more effective than solid fertilizers because they are readily available and easily taken up by plants (Tian et al., 2022). The bulbs are believed to be linked with the number of leaves; more leaves generally result in a higher yield of tubers. The high number of leaves in the Bio P60 treatment is due to the secondary metabolites of *P. fluorescens* P60 contains growth-stimulating hormones such as IAA, auxin, gibberellin, and cytokinin (Gupta et al., 2023). This ability is evident in its impact on the parameters linked to the function of every hormone. Cytokinin and gibberellin promote plant growth by stimulating cell division and elongation, ultimately affecting the development of shallot bulbs (Barbosa & Dornelas, 2021).

## Bulb fresh weight

The results indicated that all Bio P60 applications resulted in a significant difference in bulb fresh weight compared to the control. However, there was no significant difference between the Bio P60 applications themselves. This suggests that more applications can enhance bulb weight in shallots. The highest bulb fresh weight was observed when Bio P60 was applied 5 times, resulting in a 51.1% increase in fresh bulb weight. The interaction application of Bio P60 and NASA showed no significant difference but tended to increase the fresh weight of the bulbs compared to the control (Table 2). The increased tuber weight when using Bio P60 5 times is believed to be caused by the secondary metabolites of *P. fluorescens* P60, which contains bioactive compounds, specifically growth hormones. The findings of a study conducted by Soesanto et al. (2019) demonstrated that using Bio P60 led to a 68.07% increase in fresh weight of pakcoy plants compared to the control. This is attributed to the plant regulator properties of Bio P60 secondary metabolites, particularly growth hormones like IAA. The application of NASA increases the fresh weight of bulbs compared to the control. NASA increases nutrient availability and uptake by shallot plants, which also contain growth regulators, promoting plant growth and high bulb production (Tian et al., 2022). The low fresh bulb weight in the control treatment is believed to be linked to the high intensity of the disease. This is consistent with the incubation period and high disease incidence in the control, preventing plant growth and leading to bulb rot. This aligns with the statement of Lal et al. (2024). In young plants, Fusarium wilt leads to death. In adult plants, symptoms include smaller bulb size and rotting. This will impact the fresh weight of the bulbs.

## Bulb dry weight

The dry weight of bulbs showed no significant difference when NASA was applied or in the interaction between Bio P60 and NASA. The only noticeable distinction was observed in the Bio P60 application, as shown in Table 2. The heaviest bulb dry weight was achieved by applying Bio P60 five times, resulting in a weight of 69.5 g, a 51.24% increase compared to the control. All Bio P60 applications showed a significant difference in the dry weight of shallot bulbs compared to the control. However, there were no significant differences between Bio P60 applications, which is

consistent with the results for the fresh weight of the bulbs. Dry weight increases as the fresh weight of bulbs increases at the same drying temperature. The dry weight of the bulbs increases as a result of applying Bio P60 due to the secondary metabolites of *P. fluorescens* P60 in Bio P60 contain bioactive compounds in the form of growth hormones, such as IAA and cytokinin (Gupta et al., 2023). The cytokinin hormone impacts the yield of shallot plants (Barbosa & Dornelas, 2021). Using Bio P60 (Table 2) results in a higher yield of fresh bulb shallots. This is attributed to the impact of growth hormones on secondary metabolites and the prevention of the pathogenic fungus *F. oxysporum* f.sp. *cepae* attack Data on the dry weight of bulbs is utilized for more precise measurements compared to data on the fresh weight of bulbs. The freshness of bulbs is influenced by various factors, particularly water content and tissue structure, especially when using quantitative data (Levinsh, 2023). The dry weight of the tuber represents the true yield weight in comparison to the fresh weight of the tuber (Huang et al., 2019).

## Crop fresh weight

There was a significant difference in crop fresh weight with the application of Bio P60, as shown in Table 2. The data analysis results indicated that applying Bio P60 five times was the most effective, resulting in a 48.24% increase in crop fresh weight compared to the control. The application of NASA liquid organic fertilizer and the interaction between Bio P60 and NASA showed no significant differences. The increased crop weight when using Bio P60 is believed to be a result of the secondary metabolites produced by *P. fluorescens* P60 not only suppresses pathogens but also promotes plant growth. *P. fluorescens* is a PGPR bacterium that produces IAA, cyanide acid (HCN), siderophores, and phosphate solubilizing compounds beneficial for plant growth (Li et al., 2023; Khoso et al., 2024). *P. fluorescens* can indirectly supply crucial plant nutrients like nitrogen, phosphate, sulphur, and potassium, along with iron and ions (Sah et al., 2021). The availability of nutrients affects the number of leaves on shallot plants, which impacts the number of shallot tillers or bulbs. The increase in leaves resulted in more shallot bulbs and higher production (Rahmawati & Ladewa, 2023).

NASA application showed a slight increase in plant fresh weight compared to the control treatment. This is likely due to NASA's ability to supply plants with both macro- and micro-nutrients. The nutrients in NASA have a balanced content of macro- and micro-nutrients. Plant growth depends on plants' capacity to uptake macro- and micro-nutrients from the nutrient solution (Kumar et al., 2021). According to Levinsh (2023), the plant body's water content significantly affects plants' fresh weight. Applying NASA liquid organic fertilizer at the correct concentration can enhance plant nutrients, light, and water absorption, promoting optimal growth and influencing organ development (Liu et al., 2024).

## Crop dry weight

Crop dry weight varied significantly with Bio P60, as shown in Table 2. The most noteworthy edit dry weight was watched by applying Bio P60 five times, coming about in a 49.77% increment compared to the control. The affect of applying Bio P60 on edit dry weight is accepted to be caused by a secondary metabolite delivered by *P. fluorescens*, particularly siderophores. Siderophores can

tie iron ($Fe^{3+}$) to create siderophore-iron bonds, which plants can get to whereas remaining blocked off to pathogens (Xie et al., 2024). The investigate conducted by Timofeeva et al. (2022) shows that siderophores help in making iron accessible for plants, advancing plant development. Iron is pivotal for plant nourishment, photosynthesis, respiration, and defense against pathogens.

NASA application and the interaction between Bio P60 and NASA had no critical impact. This adjusts with the investigate conducted by Oktaviani et al. (2020), which found that treating NASA concentration on shallot plants did not have a noteworthy affect on any of the parameters watched. The need of interaction between Bio P60 and NASA is likely due to NASA's imperfect dosing, coming about in an uneven affect on the two medicines. On the off chance that one calculate includes a more grounded impact than others, its impact is prioritized. In the event that each figure includes a particular nature of impact and work, it'll result in a relationship that does not altogether bolster plant development (Xiaoqin et al., 2022). This investigate appears that controlling the Fusarium shrivel of shallots can be done utilizing auxiliary metabolites determined from *P. fluorescens* P60. The secondary metabolites can control other plant infections (Muarifah et al., 2023). Fusarium wilt is one of the most important diseases of shallot and is difficult to control by any means. However, the results of this study prove that Fusarium wilt can be overcome organically.

## CONCLUSION

The application of Bio P60 was able to suppress Fusarium wilt in shallots. The best application was in the Bio P60 treatment 5 times which was able to delay the incubation period by 61.71%, suppress the disease intensity by 66.67% and reduce the AUDPC value by 69.84%, which was able to increase growth and yield components such as plant height by 40.7%, number of leaves by 30.75%, number of bulbs by 83.35%, bulb fresh weight by 51.1%, bulb dry weight by 51.24%, plant fresh weight by 48.24% and plant dry weight by 49.77% compared to the control. The application of NASA liquid organic fertilizer has not been able to significantly affect the development of Fusarium wilt and shallot growth and yield. There is no interaction between the application of Bio P60 and NASA on the development of Fusarium wilt and shallot growth and yield. Fusarium wilt of shallots can be overcome organically, and it provides hope for the community to overcome Fusarium wilt disease organically.

## AUTHORS CONTRIBUTIONS

LS and WSS designed and conceived the experiments. AMY and EM experimented. LS, AMY, and WSS contributed to the preparation of samples and interpretation of the results. The manuscript was primarily composed by LS and MWRS. All authors provided critical feedback and contributed to developing the research, analysis, and manuscript.

## COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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# Antagonistic Effect of Nitrogen Fertilizer and Rhizobium on Growth, Nodulation and Yield of Peanut (*Arachis hypogaea* L.) in Acidic Soil

10.18196/pt.v13i1.23242

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**ABSTRACT**

Acid soil is widely distributed in Indonesia but underexploited for agriculture due to limited nitrogen availability and aluminium toxicity. Nitrogen fertilizer and rhizobium are crucial to improving plant growth, especially in peanut cultivation. This study examines the antagonistic effects of nitrogen fertilizer and rhizobium on the growth, nodulation, and yield of peanuts cultivated in acidic soil. A factorial randomized complete block design with two factors: nitrogen fertilizer application (0, 50, 100, 150 kg $ha^{-1}$) and rhizobium inoculation (without rhizobium, rhizobium at 10 g $kg^{-1}$ seed, and rhizobium sourced from peanut plantations). The combination of 100 kg $ha^{-1}$ nitrogen and rhizobium from peanut plantations resulted in the highest leaf count (675.33 leaves per plant). A nitrogen dose of 50 kg $ha^{-1}$ produced the highest effective number of nodules and total nodules. The optimum nitrogen fertilizer dose is 44 kg $ha^{-1}$ for nodule growth. 50 kg $ha^{-1}$ nitrogen dose produced the highest number of pods and seed weight, namely 48.67 pods and 407.79 g of seeds. These findings suggest that when applied at an appropriate dose, nitrogen fertilizer enhances peanut growth, nodulation, and yield in acidic soil. However, excessive nitrogen application may induce antagonism with the nodulation process, reducing overall yield.

**Keywords:** Legume plant; Nitrogen fixation; Nodules; Rhizobium

## INTRODUCTION

Acidic soil covers 25% of Indonesia's total land area, about 45.79 million hectares, with 5.22 million hectares used for crop cultivation (BPS, 2020). Acidic soil is suboptimal, so harvest yields are below the national average each season. However, acidic soil is widely used for crop cultivation because the optimal area of agricultural land is decreasing. According to the BPS (2018), the conversion of rice fields reaches 100,000 to 150,000 hectares per year, which is not comparable to the creation of new rice fields, which is only 60,000 hectares per year. However, agricultural extensification efforts utilize suboptimal land, such as acid soil (Arista et al., 2023). However, soil acidity is considered a key variable in soil chemistry because of its significant impact on chemical reactions involving essential plant nutrients (Gerke, 2022; Javed et al., 2022; Raza et al., 2021; Sintorini et al., 2021).

Agricultural practices can accelerate the process of soil acidification during soil weathering (Bolan et al., 2023; Chen et al., 2022). Agricultural practices with the continuous addition of chemical

Article History
Received : 17 July 2024
Revised : 18 February 2025
Accepted : 20 February 2025

fertilizers can increase the concentration of H+ ions in the soil (Han et al., 2021; Wan et al., 2021). An increase in H+ ions results in soil acidity, which hurts soil microbial and plant activity (Daba et al., 2021; Raza et al., 2020). Additionally, soil acidity makes metals more soluble and mobile, preventing plants from accessing vital nutrients (Alves et al., 2019; Wang et al., 2023a; Zhang et al., 2019). Low soil pH makes certain essential plant nutrients insoluble and less accessible, including phosphorus, calcium, magnesium, and molybdenum (Abdul Halim et al., 2018; Wang et al., 2023b) and can affect the decline in plant growth (Baccari & Krouma, 2023; Barrow & Hartemink, 2023). Planting plants from the Leguminaceae family is a strategy to utilize acidic soil, such as peanuts (Abd-Alla et al., 2023), because it is supported by the ability of legume plants to form a symbiotic relationship with soil microorganisms such as rhizobium (Yang et al., 2022). Legumes can fix nitrogen in the atmosphere and increase available nitrogen through biological nitrogen fixation (Basile & Lepek, 2021; Goyal & Habtewold, 2023; Ramoneda et al., 2021).

An estimated 1.75 × 1011 kilograms of nitrogen are fixed globally each year, with 8.0 × 1010 kg coming from legume symbiosis and an average of 20–200 kg of fixed N $ha^{-1}$ $year^{-1}$ (Kebede, 2021). Thus, legume-rhizobium symbiosis-mediated biological nitrogen fixation is an attempt to alter soil organism activity and boost nutrition availability (Goyal et al., 2021; Grzyb et al., 2021; Mesfin et al., 2020). The lack of rhizobium bacteria causes nodules to form on peanut roots, so plants cannot independently fix free nitrogen in the air through nitrogen fixation. Land that lacks nitrogen and does not contain rhizobium bacteria will result in the vegetative growth of peanut plants being hampered because they lack the nutrient nitrogen. The lack of rhizobium bacteria in the soil causes farmers to spend more on inorganic fertilizers to meet the need for nitrogen nutrients (Etesami, 2022; Vanlauwe et al., 2019). Rhizobium bacteria can infect the roots of peanut plants and create colonies to form nodules that trap free nitrogen in the air. Nitrogen available in the soil causes the rhizobium to be ineffective in collecting free nitrogen in the air; conversely, if nitrogen is not available in the soil, the rhizobium effectively increases free nitrogen in the air (Ramoneda et al., 2021). Optimal doses of nitrogen fertilizer are needed to ensure the presence of rhizobium bacteria so that mutualistic symbiosis with peanuts can occur in peanut growth. The research examines the effect of antagonism and nitrogen fertilizer on peanuts' growth, nodulation, and yield in sour planting.

## MATERIALS AND METHODS

### Study site and soil characteristics

At a height of 148 meters above sea level, the study was conducted at the Laboratory Experiment Field of the Faculty of Agriculture, Sebelas Maret University, Jumantono, Karanganyar Regency. It was situated at 7º37'48.82" South Latitude and 110º56'52.17" East Longitude. The research used alfisol soil with soil acidity characteristics of 5.6 (acid category); C-organic 0.65 % (very low); total nitrogen 0.06 % (deficient); P2O5 total 16 ppm (medium); K2O total 12.26 mg/100g (low); C/N ratio 4.84 (very low). Planting was carried out in polybags measuring 35 x 35 cm, and the distance between the polybags was 25 x 25 cm. The planting medium used is acid soil and cow dung fertilizer in a ratio of 1 : 1. The seeds used are Kancil variety peanuts. Basic fertilizer is applied twice before planting, using SP36 fertilizer at 200 kg $ha^{-1}$ or 0.8 g $polybag^{-1}$ and KCl fertilizer at 50 kg $ha^{-1}$ or 0.2 g $polybag^{-1}$.

## Experimental design

The research used a factorial complete randomized block design with two factors. The first factor is the dose of Nitrogen fertilizer with four levels, namely 0, 50, 100, and 150 kg $ha^{-1}$. The second factor is the application of rhizobium sources at three levels: without rhizobium, rhizobium dose of 10 g $kg^{-1}$ seeds, and source of rhizobium in soil used from peanut planting. The research was repeated three times. Nitrogen fertilizer treatment was carried out at planting, and rhizobium treatment was carried out on peanut seeds before planting. Treatment of rhizobium from former peanut plantings was carried out by mixing the soil weighing 15 g into the planting medium.

Observation variables include growth variables, namely plant height four weeks after planting (WAP) and number of leaves at twelve WAP. The nodulation variables observed were the number of nodules and the effective number of nodules carried out at 10 WAP. The outcome variables are the number of pods and the weight of 1000 seeds. Outcome variables were observed at 90 days after planting.

## Data Analysis

Analysis of Variant level 5% was used to examine the observational data. If it was significant, the 5% Duncan Multiple Range Test was used to determine whether there were significant differences between treatments. The ideal nitrogen dosage was found by regression.

## RESULTS AND DISCUSSION

The research results showed that the combination of nitrogen fertilizer doses with the application of rhizobium sources affected peanut plant height four weeks after planting (Table 1). The optimum nitrogen fertilizer dose was 54.83 kg $ha^{-1,}$ with rhizobium inoculum from soil used for peanut plantations to produce the highest plant height, 15.70 cm, with a correlation coefficient of 0.99. The addition of nitrogen can stimulate the growth of rhizobium bacteria (Shome et al., 2022). Also, rhizobium bacteria from used peanut soil can form a symbiotic relationship with the perfect peanut root system to maximize the nitrogen fixation process (Boivin et al., 2020; Yang et al., 2022). The combination of nitrogen fertilizer doses with the application of rhizobium sources affects the number of leaves (Table 2). The dosage of 100 kg $ha^{-1}$ of nitrogen fertilizer with rhizobium inoculum from soil used to plant peanuts showed the highest number of leaves, namely 675.33. However, the number of leaves in this treatment combination was similar to the treatment combination of 100 kg $ha^{-1}$ of nitrogen and rhizobium. The compatibility of legin rhizobium and rhizobium from soil used for peanut plantations is high. Rhizobium can associate with the host to produce many nodules and fix nitrogen for plant growth (Fahde et al., 2023; Mathenge et al., 2019). The efficacy of nitrogen-fixing bacteria in peanuts will be increased by applying a specific amount of nitrogen fertilizer (Jaiswal et al., 2021). The availability of nitrogen will influence cell division in the apical meristem, resulting in the formation of tall leaves (Sun et al., 2020). Rapid leaf growth is impacted by cell division, which can also result in more leaves because it produces more new leaves (Sakakibara, 2021; Shi & Vernoux, 2022).

**Table 1.** Combination of nitrogen fertilizer doses and rhizobium sources on plant height four weeks after planting

| Nitrogen Fertilizer Dosage (kg.ha$^{-1}$) | Source of Rhizobium | | | Average |
|---|---|---|---|---|
| | Without Rhizobium | Rhizobium | Land Used by Peanut Plantings | |
| 0 | 14.30abcde | 15.33abc | 13.00bcde | 14.21a |
| 50 | 13.13abcde | 13.80abcde | 15.73a | 14.22a |
| 100 | 15.70ab | 13.53abcde | 14.33abcd | 14.52a |
| 150 | 12.67cde | 11.40de | 8.50e | 10.86b |
| Average | 13.95a | 13.52a | 12.89a | + |

Note: Numbers followed by the same letter notation in columns and rows are not significantly different in the DMRT test at the 5% level. (+): there is interaction

**Table 2.** Combination of nitrogen fertilizer doses and rhizobium sources on leaf number 10 weeks after planting

| Nitrogen Fertilizer Dosage (kg.ha$^{-1}$) | Source of Rhizobium | | | Average |
|---|---|---|---|---|
| | Without Rhizobium | Rhizobium | Land Used by Peanut Plantings | |
| 0 | 606.00 | 552.00 | 629.00 | 595.67 |
| 50 | 555.67 | 569.33 | 571.33 | 565.44 |
| 100 | 598.33 | 636.00 | 675.33 | 636.56 |
| 150 | 529.67 | 543.67 | 427.00 | 500.11 |
| Average | 572.42 | 575.25 | 575.67 | + |

Note: Numbers followed by the same letter notation in columns and rows are not significantly different in the DMRT test at the 5% level. (+): there is interaction

**Table 3.** The role of nitrogen fertilizer dosage and rhizobium source on nodule growth

| Treatment | Number of nodules | Effective number of nodules |
|---|---|---|
| Nitrogen Fertilizer Dosage (kg.ha$^{-1}$) | | |
| 0 | 358.56a | 18.89a |
| 50 | 363.00a | 19.33a |
| 100 | 351.33a | 18.67a |
| 150 | 138.00b | 13.67b |
| Source of Rhizobium | | |
| No Inoculant | 311.17 | 17.25 |
| Rhizobium inoculant | 301.17 | 17.67 |
| Land Used by Peanut Plantings | 295.83 | 18.00 |
| Average | 302.72 | 17.64 |
| Interaction | - | - |

Note: Numbers followed by the same letter notation in one column indicate that they are not significantly different in the DMRT test at the 5% level. (-): no interaction

The number of nodules and the number of effective nodules were shown to be influenced by the nitrogen fertilizer dosage (Table 3). The number of peanut nodules formed by a nitrogen dose of 50 kg ha$^{-1}$ was 225, significantly different from 150 kg ha$^{-1}$. With a coefficient of 0.97, the most significant number of peanut nodules, or 391.56 grains, were produced by the optimal nitrogen fertilizer dose of 44 kg ha$^{-1}$ (Figure 1). Peanuts are a family of legumes capable of forming nodules for nitrogen fixation. Low nitrogen fertilizer doses produce high nodules. Legumes have been proven to significantly increase the abundance of rhizosphere soil microorganisms (Malviya et al., 2021). Through nitrogen-fixing enzymes, nitrogen-fixing bacteria control the nitrogen-fixing process (Lai et al., 2022). Based on Abd-Alla et al. (2023), giving the highest dose of nitrogen fertilizer produces

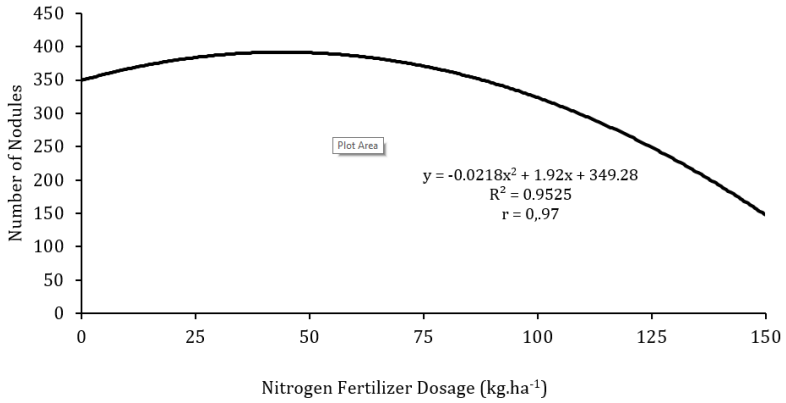


**Figure 1.** Regression test for the number of peanut nodules at 4 WAP with the administration of several doses of nitrogen fertilizer

**Table 4.** Effect of nitrogen fertilizer dose and rhizobium source on peanut yield

| Treatment | Number of Pods | Weight of 1000 Seeds (g) |
|---|---|---|
| Nitrogen Fertilizer Dosage (kg.ha$^{-1}$) | | |
| 0 | 36.11b | 380.13a |
| 50 | 48.67a | 407.79a |
| 100 | 46.33a | 383.35a |
| 150 | 38.56a | 337.16b |
| Source of Rhizobium | | |
| No Inoculant | 45.58 | 380.90 |
| Rhizobium inoculant | 44.42 | 370.63 |
| Land Used by Peanut Plantings | 37.25 | 379.80 |
| Average | 42.42 | 377.11 |
| Interaction | - | - |

Note: Numbers followed by the same letter notation in one column indicate that they are not significantly different in the DMRT test at the 5% level. (-): no interaction

the lowest number of nodules because when there is excess nitrogen in the soil, rhizobium bacteria cannot fix nitrogen in the air. A nitrogen fertilizer dose of 50 kg ha$^{-1}$ can produce 5.66 effective peanut nodules and more than a nitrogen fertilizer dose of 150 kg ha$^{-1}$ (Table 3). The optimum nitrogen fertilizer dose of 49 kg ha$^{-1}$ produced the highest number of effective peanut nodules, namely 19.93 grains, with a correlation coefficient of 0.98. Low nitrogen fertilizer doses can increase the activity of active rhizobium bacteria in the nodules to provide nitrogen for peanut plants. Peanut plants will produce enzymes in soil conditions low in nitrogen, so the rhizobium actively fixes nitrogen and results in effective active nodules (Etesami, 2022; Solanki et al., 2020).

The level of nitrogen use has an essential influence on rhizosphere microorganisms and changes in community growth and development (Li et al., 2022; Ren et al., 2020). Numerous studies have shown that the amount of nitrogen in the soil significantly impacts the makeup of the bacterial population and that short-term fertilization also alters the composition of the rhizobia community (Yu et al., 2021). Fertilization increases the abundance of nitrogen-fixing bacterial species (Wassermann et al., 2023). In Northeast China's black soil region, it helps to increase the quantity and diversity of nitrogen-fixing bacterial genes (Liu et al., 2021). However, the results showed that the source of rhizobium did not affect the number of nodules and the number of effective nodules (Table 3), which

can be caused by rhizobium incompatibility. The availability of organic carbon can be a source of energy for rhizobium bacteria to work effectively. Previous research showed that the organic C content at the three research locations was low and produced the lowest rhizobium bacteria population compared to locations with moderate organic C content (Li et al., 2022). Therefore, it is necessary to add organic materials in the form of compost, manure, and green manure to increase the population of rhizobium bacteria in the soil. The soil used to plant peanuts contains rhizobium bacteria, which can infect roots and form nodules on legume plants (Jach et al., 2022). Rhizobium in soil used to harvest peanuts can still survive under environmental conditions supporting rhizobium bacteria growth (Neelipally et al., 2020).

The results showed that the dose of nitrogen fertilizer affected the number of pods and the weight of 1000 seeds (Table 4). A nitrogen fertilizer dose of 50 kg $ha^{-1}$ significantly differs from a nitrogen fertilizer dose of 0 kg $ha^{-1}$ regarding the number of pods and weight of 1000 peanut seeds. The optimum nitrogen fertilizer dose of 78.75 kg $ha^{-1}$ produced the highest number of peanut pods, namely 49 pods, with a coefficient of 0.97 (Figure 1). The results of this research align with research by El-sherbeny et al. (2023) that the highest number of pods were produced in the treatment of urea and animal manure fertilizer. Peanut pod formation requires high nitrogen levels to form new cells composed of photosynthate. Forming peanut pods requires more nitrogen because it forms new cells composed of photosynthate. The ability of peanut plants to accumulate photosynthate when filling the pods is a factor that influences the formation of complete pods (Arsovski et al., 2018; Yavari et al., 2021). Nitrogen nutrients given during the pod-filling phase at the right dose have better pod yields with seeds inside. The ability of peanut plants to accumulate photosynthate when filling the pods is a factor that influences the formation of complete pods. Nitrogen is the structural component of protein and amino acids used by peanut plants for seed formation (Ahanger et al., 2021). Urea contains high nitrogen, namely 46%, to increase growth. However, the source of rhizobium did not affect the number of pods and the weight of 1000 seeds. Rhizobium bacteria use organic matter available in the soil for growth and metabolism so that the rhizobium can survive and increase its population (Santachiara et al., 2019).

## CONCLUSION

The combination of 100 kg $ha^{-1}$ of nitrogen with a source of used peanut rhizobium showed the highest number of plant leaves. The optimum nitrogen fertilizer dose is 44 kg $ha^{-1}$ for nodule growth. The 50 kg ha-1 nitrogen dose produced the highest pods and seed weight. Nitrogen fertilizer at the correct dose can increase peanuts' growth, nodulation, and yield in acid soil. However, a dose that is too high may cause antagonism with the nodulation process and reduced yield.

## AUTHORS CONTRIBUTIONS

DS and S designed and conceived the experiments. DS and RNP experimented. DS, S, and RNP contributed to the preparation of samples and interpretation of the results. The manuscript was primarily composed by DS. All authors provided critical feedback and contributed to developing the research, analysis, and manuscript.

## COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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# Assessing Soil Nutrient and Biomass Contributions to Peatland Formation

10.18196/pt.v13i1.24233

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**ABSTRACT**

Peat formation is a key factor in carbon sequestration in Peat Swamp Forest (PSF). The study aims to analyze alternative pathways for peat formation based on soil nutrient availability and dried biomass accumulation. A randomized complete block design was used with two treatment factors across three blocks: (A) sampling plots representing land covers and (B) dried biomass levels. Data were analyzed using two-way ANOVA and Tukey Honestly Significant Difference (HSD) test at a 5 % significance level. Results showed a high supply of dried below-ground biomass did not correspond to increased rooting litter production under high soil nutrient conditions. Instead, most of the biomass was transported upwards into above-ground biomass. All land cover types generated above-ground biomass with significant differences in peat formation potential across all measured parameters. Peat formation was strongly influenced by land cover type (e.g., peat forest), environmental factors, seed bank composition, and species competition. Restoration strategies, including revegetation, rewetting, and revitalization, are crucial to promoting the establishment of peat-forming species. This research provides valuable insights for enhancing PSF restoration efforts and facilitating recovery toward a near-natural condition.

**Keywords:** Addition; Organic carbon; Peat development; Soil nutrients

## INTRODUCTION

The Peat Swamp Forest (PSF) belongs to a unique territorial ecosystem (Imanudin et al., 2019), with groundwater that delays the complete decomposition of dead leaves and timber. This produces a thick layer of acidic peat over time. These forests are being heavily logged in large regions. PSF retain and accumulate enormous amounts of carbon, far more than trees on mineral soil or non-peatland. As organic matter decomposes more slowly than it is produced, the excess material builds up as peat, which acts as a natural carbon sink. As the greatest near-surface stocks of terrestrial organic carbon, their stability has significant implications for climate change. Ecologically significant tropical peat swamp forests are among the most endangered, least researched, and poorly known biotypes (Syakina et al., 2024a; Syakina et al., 2024b).

There are at least five PSF ecosystem values (benefits): direct values, indirect values, optional values, bequest values, and existence values (Wildayana & Armanto, 2017; Wildayana & Armanto, 2018b). Direct values include using PSF as food sources, building materials, bridges, firewood, fences, medicines, climbing sticks, and fodder. Indirect values include PSF functions, such as keeping food cycles, road infrastructure, flora/fauna habitats, flood, drought, erosion, and climate control.

Article History
Received : 24 September 2024
Revised : 05 February 2025
Accepted : 16 February 2025

Protection of biodiversity, habitat, and agro-tourism can be optional values (Wildayana & Armanto, 2018a; Wildayana & Armanto, 2018c). Bequest values consist of the uses of PSF as shoreline safety, forestry nurseries, and inheritance. Existence values include the uses of PSF as buffering zones, protected forests, and habitats for wild, rare, and protected species (Armanto & Wildayana, 2022).

The impact of human intervention on PSF is increasingly negative and can lead to the extinction of PSF (Armanto, 2019a). Most PSFs are degraded due to drainage, logging, fire, oxidation, and pollution (Zuhdi et al., 2019). In South Sumatra, Indonesia, human exploitation has destroyed almost 25% of PSF, in which this destruction was caused by 50% of plantations, 27% by forestry, 10% by food agriculture, and 7% by industrial development and settlements (Wildayana & Armanto, 2021; Armanto, 2019b). This is receiving a lot of international attention, and, at the same time, policy and funding initiatives for restoration from the local to the landscape scale are being promoted (Byg et al., 2023).

The understanding of ecological restoration is still in its infancy, especially in its application, resulting in an imbalance between PSF restoration activities and good ecological applications (Armanto et al., 2023a; Armanto et al., 2023b). In addition, despite the many activities currently underway and information being acquired, the results of ecological restoration research have not been published (Wildayana & Armanto, 2018d; Wildayana & Armanto, 2018e). The Indonesian government has established the Peat Restoration Agency (BRG) to restore at least 2 million hectares of PSF between 2016 and 2020, with an additional 1.2 million hectares scheduled for 2020 to 2024. This agency will now be known as the Peat Restoration Agency and Mangroves (PMRA, 2022; Armanto et al., 2022). This is in response to the problems caused by PSF degradation.

PSF ecological restoration generally aims to maintain and increase carbon sequestration, prevent fires, protect water and air quality, ecosystems, and species, and protect all living things (Holidi et al., 2019; Wildayana, 2017). Hence, peat formation is the primary key to carbon sequestration in the PSF area. The development of peat-forming vegetation is a prerequisite for forming PSF (Barry et al., 2021). Swamp grass and swamp bush spread rapidly after restoration or fire. Under favorable conditions, these species can cover the entire PSF surface in 2-3 years, depending on the extent to which natural PSF species survive (Armanto et al., 2025a; Armanto et al., 2024). Research on surface peat accumulation has been carried out in many restored PSF places, and a comprehensive study on this issue is currently being carried out (Armanto et al., 2025b; Armanto, 2019c). Field surveys of the restored PSF carried out ten years after the restoration revealed varying degrees of waterlogging on the PSF surface (Kaban et al., 2024; Jing et al., 2020), with the new peat surface becoming thicker where fluctuations in the Ground Water Table (GWT) were minimal and shallow (Armanto & Wildayana, 2023).

The availability of nutrients characterizes vegetation growth. The availability of these nutrients can be caused naturally, for example, in river valleys (Lázaro-Lobo et al., 2023), or can be caused by anthropogenic eutrophication. Nutrient availability can be used to control patterns of biodiversity and the movement of dominant species. A fertile PSF can accumulate peat effectively, depending on other supporting factors, such as hydrological, biogeochemical, or microbiological constraints. The research aimed to analyze alternative possibilities for peat formation based on the available soil

nutrients and dried biomass. The research novelty is to give an alternative for forming PSF based on soil nutrients and biomass availability.

## MATERIALS AND METHODS

### Location and Time of Research

The research was conducted in Pedamaran Sub-district, OKI District, South Sumatra, Indonesia, which is included in the Peat Hydrological Unit (KHG) of the Sibumbung-Burnai River, covering an area of 87,000 ha (Figure 1). The research was conducted from 2022 to 2024.

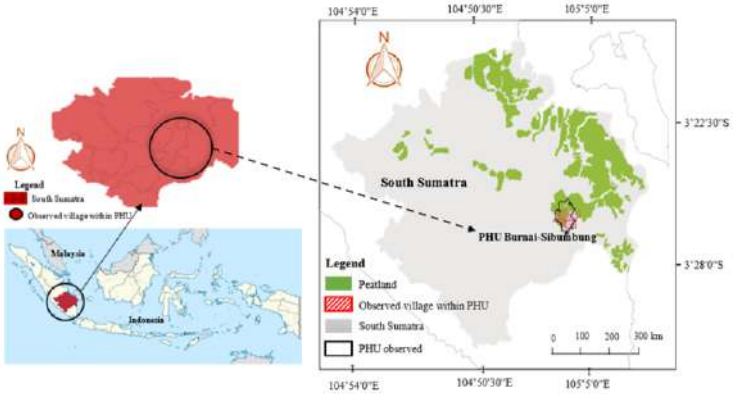


**Figure 1.** Research location in South Sumatra Province, Indonesia

### Research Methods and Experiment Design

In a complete randomized block design, a factorial trial was applied using two treatment combinations in three blocks: factor A (sampling plots, land cover) and Factor B (dried biomass). Blocks were made according to the three-peat depths, including shallow, moderate, and deep. Factor A (sampling plots, land cover) consisted of four sampling plots as natural treatments, namely peat forest (L0), restored PSF (L1), swamp bush (L2), and swamp grass (L3). Factor B (dried biomass) consisted of above-ground dried biomass, namely dried leaves (T0); grass stalks, dried twigs, wicker, wood (T1); and below-ground dried biomass, namely root litter (T2).

### Soil and Biomass Sampling

All sampling plots were located in adjacent areas with a distance of $< 2000$ m and elevation in the 0-3% range. Soil sampling and dried biomass data were acquired using the quadratic approach located in each treatment combination. Plot sizes were determined to be $10 \times 10$ m for peat forest and restored PSF and 5 m x 5 m for swamp grass and bush.

### Data Analysis

Plant tissue samples were taken from sampling plots, and all soil samples were analyzed in the laboratory. The collected plant tissues were weighed to obtain the fresh weight. Before analysis, they were washed with ion-free water to remove dust and other impurities, dried in an oven with a fan, and cut into pieces for faster drying. The oven was set at 70 oC. Dried samples were weighed to obtain dried biomass and milled using a machine grinder with a filter with a fineness of 0.5 mm. Methods of plant tissue analysis is summarized in Table 1. Dried biomass content was calculated from the organic C content with the following formula:

$$\text{Dried biomass (\%)} = 1.74\% \times \text{organic C (\%)}$$

The conversion factor was obtained by assuming 58% organic C in the dried biomass.

Collected data were analyzed using two-way ANOVA with the SPSS Statistics 26 and the Tukey HSD (Honestly Significant Difference) Test at a significance level of 5% to determine whether there was a significant difference between treatments according to the parameters observed, namely dried biomass, organic C, total N, P, K, Ca, and Mg. The collected data and field phenomena were then extensively described and explained using tables and figures and then compared to the parameters measured from each research plot and a more detailed comparison of above-ground biomass with below-ground biomass.

**Table 1.** Methods of Plant Tissue Analysis

| Parameter and units | Methods |
|---|---|
| Water content (%) | Oven drying |
| Dried biomass (kg $ha^{-1}$) | Weigh, oven drying |
| Total biomass (kg $ha^{-1}$) | Calculation: 1.74% x C organic (%) |
| Organic C(kg $ha^{-1}$) | Ash method |
| Total N (kg $ha^{-1}$) | Wet ash with $H_2SO_4$, spectrophotometer |
| C/N ratio | ratio calculation |
| Total P, K, Ca, Mg (kg $ha^{-1}$) | Wet ash with $HNO_3$ & $H_2SO_4$, spectrophotometer |

Note: C (Carbon); N (Nitrogen); P (Phosphorus); K (Potassium); Ca (Calcium): Mg (Magnesium); H2SO4 (Sulfuric acid); HNO3 (Nitric acid)

## RESULTS AND DISCUSSION

### Specific Descriptions of Sampling Plots

The climate of the research area is categorized as a wet climatic type of B since it has roughly seven to nine rainy months, an annual average precipitation of 2,600 to 3,300 mm, and an uneven distribution of rainfall (Stasiun Klimatologi Kayu Agung, 2025). The plains physiographic group included all sampling plots, which ranged in elevation from 0 to 3 m above sea level and in peat depth from 1.20 to 7.50 meters. Table 2 provides a detailed description of sample plots.

**Table 2.** Specific explanation of sampling plots

| Description | Peat forest (L0) | Restored PSF (L1) | Swamp bush (L2) | Swamp grass (L3) |
|---|---|---|---|---|
| Wildfires | 1 time | 2 times | 3 times | 4 time |
| Sites | 104°57′54.65″ E 3°25′20.43″ S | 104°57′52.38″ E 3°25′22.84″ S | 104°53'35.12" E 3°25'53.37" S | 104°57'18.58" E 3°26'37.31" S |
| Age (years) | 10-15 | 5-10 | 3-5 | 1-5 |
| Height (m) | 6.00-9.00 | 3.25-6.00 | 1.50-3.00 | 0.25-1.00 |
| Main species | Meranti, Punak, Jelutong, Ramin, Pelawan, Medang | Jelutong, Ramin, Bush, Grass | Gelam, Pakis Udang, Kumpai, Medang, Belidang, Seduduk | Purun Tikus, Kumpai, Teki-Tekian, Seduduk, Belidang |
| Peat depth | 3-7 m | 2-5 m | 1-4 m | 1-4 m |
| GWT*/ | -50 cm | -55 cm | -45 cm | -50 cm |
| Status | Undrained, undisturbed, unrestored | Undrained, undisturbed, restored | Drained, disturbed, unrestored | Drained, disturbed, unrestored |
| Field condition |  |  |  |  |

Note: */ GWT (Ground Water Table)

### *Peat Forest (L0)*

The peat forest experienced a fire once in 2006, then was restored, even though it was not optimal, by planting Red Meranti (*Shorea balangeran*) and Punak (*Tetramerista glabra* Miq). Other growing species are Jelutong (*Dyera lowii* L.), Ramin (*Gonystylus bancanus* L.), Pelawan (*Tristaniopsis merguensis* Griff.), and Medang (*Blumeodendron kurzii* (Hook.f.) J.J.Sm). The peat forest is stable and can be protected due to degradation processing energy (rainfall and drainage).

### *Restored PSF (L1)*

These PSF experienced two fires in 1997 and 2006 and have experienced less than optimal restoration with the planting of Jelutong (*Dyera lowii* L.) and Ramin (*Gonystylus bancanus* L.). The lower part of the tree is overgrown with swamp grass and swamp bush in the form of Kumpai *(Hymenachine amplexicaulis Rudge),* Pakis Udang (*Stenochlaena palustris*); Belidang *(Eleusine indica),* Seduduk *(Melastoma malabatrihcum);* and Teki-tekian *(Cyperus rotondus).* PSF restoration showed a low leaching process because the restored PSF had not been burnt since it was restored. The restored PSF is stable and can be protected due to degradation processing energy (rainfall and drainage).

### *Swamp Bush (L2)*

Swamp bush has burned three times in 1997, 2006, and 2015. Swamp bush is dominated by Gelam (*Melaleuca cajuputi* Powell); Medang (*Litsea* spp); Kumpai *(Hymenachine amplexicaulis Rudge);* Pakis Udang (*Stenochlaena palustris*); Belidang *(Eleusine indica);* Seduduk *(Melastoma malabatrihcum);* and Teki-tekian *(Cyperus rotundus).* Once it was used by native farmers for subsistence farming (sonor system) to grow paddy and regional vegetables, swamp shrubs in peat domes finally developed after being neglected for five to ten years.

### *Swamp Grass (L3)*

Swamp Grass has experienced fires four times in 1997, 2006, 2012, and 2015. Native farmers once used the sonor system of subsistence farming to grow paddy and regional vegetables on swamp grass under peat domes. After being abandoned for five to ten years, the grass eventually turned into swamp shrubs. The energy of rainfall, which severely reduces soil fertility and increases the risk of fires, mainly contributes to the ongoing degradation of peat. The natural plants that makeup swamp grass are mostly Purun Tikus (*Eleocharis dulcis* Hensch), Kumpai *(Hymenachine amplexicaulis Rudge);* Belidang *(Eleusine indica);* Pakis Udang (*Stenochlaena palustris*); Seduduk *(Melastoma malabatrihcum);* Teki-tekian *(Cyperus rotondus); Zalaca* spp, *Pandanus* spp, *Crunis* spp, and creeping species (namely *Uncaria* spp).

## Dried Biomass Production

The amount of soil-dried biomass is influenced by the degree of decomposition, type of land use, and soil characteristics. Biomass, C, and N produced by plant residues (kg $ha^{-1}$ $year^{-1}$) are presented in Table 3. Annual dried biomass production increased to natural levels within ten years after restoration, mainly due to the rapid growth of peat forest, swamp grass, and swamp bush. The average annual above-ground dried biomass production rate was around 9.985 - 25.275 kg $ha^{-1}$ $year^{-1}$ during the first ten years. The peat forest could supply around 25.275 kg $ha^{-1}$ $year^{-1}$ of dried biomass, compared to

swamp grass supplying only 9.985 kg ha$^{-1}$ year$^{-1}$ (only 40% compared to dried biomass supply from peat forest), and dried leaves providing the most dominant contribution to dried biomass supply.

**Table 3.** Biomass, C-organic, and N contributed by plant residues (kg ha$^{-1}$ year$^{-1}$)*/

| Interactions | Biomass | Organic C | N total | C/N |
|---|---|---|---|---|
| L0 T0 | 11,260±451$^{g}$ | 6,526±267$^{i}$ | 326.54±44.01$^{g}$ | 19.98±6.09$^{a}$ |
| L0 T1 | 11,250±443$^{g}$ | 6,525±266$^{i}$ | 34.88±11.23$^{f}$ | 187.10±34.01$^{d}$ |
| L0 T2 | 2,765±161$^{b}$ | 1,604±156$^{a}$ | 21.57±8.04$^{b}$ | 74.36±22.23$^{b}$ |
| L1 T0 | 9,430±267$^{f}$ | 5,471±201$^{h}$ | 188.64±35.45$^{e}$ | 29.00±10.43$^{a}$ |
| L1 T1 | 8,173±234$^{e}$ | 4,740±190$^{g}$ | 25.34±10.11$^{d}$ | 187.10±35.66$^{e}$ |
| L1 T2 | 2,567±172$^{a}$ | 1,489±192$^{a}$ | 20.02±7.42$^{c}$ | 74.35±23.77$^{b}$ |
| L2 T0 | 3,240±178$^{c}$ | 2,236±169$^{c}$ | 12.96±4.21$^{a}$ | 172.50±32.89$^{d}$ |
| L2 T1 | 5,100±204$^{d}$ | 2,958±168$^{e}$ | 20.40±7.96$^{c}$ | 144.00±30.78$^{c}$ |
| L2 T2 | 6,700±207$^{e}$ | 3,886±187$^{f}$ | 23.45±8.71$^{d}$ | 165.71±31.66$^{cd}$ |
| L3 T0 | 2,115±171$^{a}$ | 1,459±89$^{a}$ | 8.46±3.33$^{a}$ | 172.49±33.79$^{d}$ |
| L3 T1 | 3,120±177$^{bc}$ | 1,810±92$^{b}$ | 12.48±4.44$^{b}$ | 145.00±28.99$^{c}$ |
| L3 T2 | 4,750±189$^{b}$ | 2,755±173$^{d}$ | 16.63±5.67$^{c}$ | 172.50±30.66$^{d}$ |

Note: */ Mean values followed by the same superscript within the same column are not significantly different at the 5% (level p < 0.05).
Source: Results of two-way ANOVA and Tukey HSD Test

At the 5% significance level, almost all of the interactions between treatment combinations were statistically significant. The maximum total dried biomass supply was shown by the treatment combination of peat forest with dried leaves (L0 T0) and peat forest with grass stalks, dried twig, wicker, and wood (L0 T1) each valued around 11.260 kg ha$^{-1}$ year$^{-1}$ and 11.250 kg ha$^{-1}$ year$^{-1}$, respectively. The minimum value of the treatment combination of swamp grass with dried leaves (L3 T0) was around 2.115 kg ha$^{-1}$ year$^{-1}$. From this, it was illustrated that dried leaves dominated the supply of total dried biomass to the PSF.

## C Fixation and C/N Ratio

### *C fixation*

C fixation describes how much C can be bound by PSF over ten years. Almost all treatment combinations were significantly different at the 5% significance level. The maximum total supply of C fixation was shown by the treatment combination of peat forest with dried leaves (L0 T0) and peat forest with grass stalks, dried twig, wicker, and wood (L0 T1), which were around 6,526 and 6,525 kg C ha$^{-1}$ year$^{-1}$, respectively. The treatment combination of restored PSF with root litter (L1 T2) of about 1.489 kg C ha$^{-1}$ year$^{-1}$ showed the minimum value. It was concluded that dried leaves dominated the supply of total C fixation to the PSF. C fixation contributed by root litter was generally very low, with the lowest starting order of restored PSF, peat forest, swamp grass, and swamp bush, which was around 1,489; 1,604; 2,755; and 3,886 kg C ha$^{-1}$ year$^{-1}$, respectively.

### *C/N Ratio*

The C/N ratio pattern did not follow the C distribution pattern and was more likely to follow the land covers. A high C/N ratio was indicated by swamp grass and swamp bush. Almost all treatment interactions had significant differences at the 5% significance level. The maximum C/N ratio was shown by the treatment combination of swamp grass with root litter (L3 T2) and swamp grass with dried leaves (L3 T0) of around 172.50 and 172.50, respectively. The treatment combination of peat forest and dried leaves (L0 T0) showed the minimum value, valuing 19.98.

## Addition Potential of Soil Nutrients

One of the functions of surface peat accumulation was to increase soil nutrients and form peat. In nutrient-poor PSF, the annual nutrient supply increased to natural levels within ten years of restoration, mainly due to the rapid growth of peat forest and swamp grass from swamp bush.

Figure 2 illustrates that all treatment combinations were significantly different from one another at the 5% significance level. The maximum values of all parameters observed (N, P, K, Ca, and Mg) were generally shown by the treatment combination of peat forest with dried leaves (L0 T0), and the minimum values were demonstrated by the treatment combination of swamp grass with grass stalks, dried twig, wicker, and wood (L3 T2) or swamp bush with grass stalks, dried twig, wicker, and wood (L2 T2).

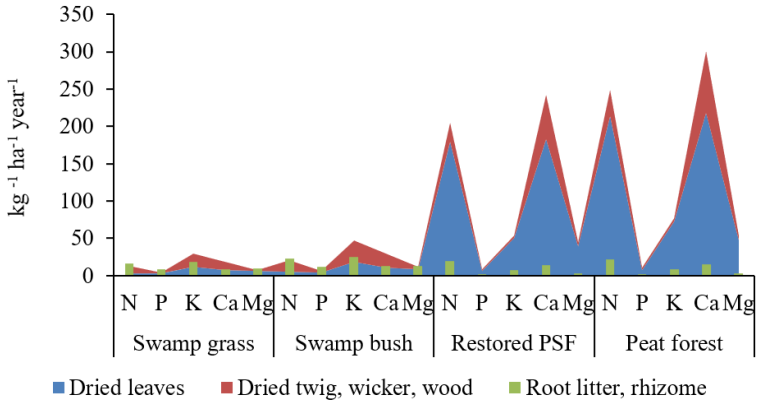


**Figure 2.** Amount of soil nutrients contributed by dried biomass (kg $ha^{-1}$ $year^{-1}$)

## Comparing Dried Biomass Supply in the Above and Below Ground

Production of above-ground dried biomass increased as nutrient levels increased by over 500-800% in peat forests and restored PSF. Dried leaves from peat forests showed significantly higher dried biomass production at the 5% significance level than other land covers. These findings were comparable to those of Hinzke et al. (2021a).

Total production of dried biomass (above-ground and below-ground) of all land covers increased with an increase in nutrients by more than 200-300%. The pattern of total dried biomass production was in peat forests with higher levels of nutrients. In contrast, land covers (swamp bush and swamp grass) showed low levels of soil nutrients, so dried biomass production was also low based on the 5% significance level. Hinzke et al. (2021b) reported comparable work for PSF in Kalimantan. As seen in Figure 3 at the 5% significance level, the pattern of total dry biomass production revealed a significant difference between above and below-ground/soil surface layers.

Total production of dried biomass below ground/ (represented by root litter and rhizome weight) of all land covers showed increased saturation, except for peat forest. However, the length and "number of rhizomes differed between land covers. Overall, the dried biomass production below ground showed the same pattern, which was lower than the above-ground dried biomass. These findings were comparable to those of Hinzke et al. (2021a).

A comparison of above-ground biomass production (represented by dried leaves) with below-ground biomass production (shown by rhizomes and root litter) showed an increasing trend of

improving soil nutrient levels. This was supported by the works of Hagan et al. (2023). Dried leaves were found to predominate in accumulating an increasing share of the total dried biomass production with improved soil nutrients (Table 4).

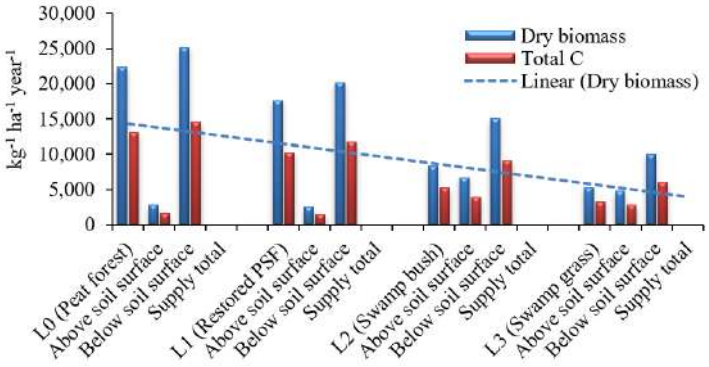


**Figure 3.** Total supply of dried biomass and C fixation (kg ha$^{-1}$ year$^{-1}$)

**Table 4.** Percentage of supply total for dried biomass and C fixation

| Land covers | Above ground | Below ground | Total supply |
|---|---|---|---|
| L0 (Peat forest) | | | |
| Dried biomass (%) | 88.97 | 11.03 | 100.00 |
| C fixation (%) | 44.49 | 4.96 | 49.45 |
| L1 (Restored PSF) | | | |
| Dried biomass (%) | 87.27 | 12.73 | 100.00 |
| C fixation (%) | 43.64 | 5.73 | 49.36 |
| L2 (Swamp bush) | | | |
| Dried biomass (%) | 55.45 | 44.55 | 100.00 |
| C fixation (%) | 24.95 | 20.05 | 45.00 |
| L3 (Swamp grass) | | | |
| Dried biomass (%) | 52.43 | 47.57 | 100.00 |
| C fixation (%) | 23.59 | 21.41 | 45.00 |

Source: Field and laboratory results (2024)

Although C fixation in root litter was low, especially in peat forests and restored PSF, the contribution was high (very dominant) to C fixation by above-ground dried biomass. In contrast, the contribution of root litter in other land covers was not optimal in supplying C fixation to PSF. The opposite was seen in swamp grass and bush, where C fixation in root litter was high (2,755 and 3,886 kg C ha$^{-1}$ year$^{-1}$, respectively), which could not supply above-ground dried biomass. This phenomenon proved that swamp grass and bush were less able to supply dried biomass for peat formation. This work was positively correlated with that reported by Zhang (2023).

The potential of the below-ground dried biomass (rhizomes and root litter) forming peat was similar to dried biomass production despite increased soil nutrients. This was proven by the fact that even though peat forests and restored PSF were the richest in soil nutrients, there was no additional peat-forming dried biomass below the ground. Yan et al. (2023) showed the same result with this research. The increase in the potential of root litter dried biomass to form peat was dominant in the dried biomass above the ground, followed by an increase in soil nutrient levels. This means that the supply of soil nutrients is more than 200-600% played by above-ground dried biomass compared to below-ground dried biomass. The same result was given by He et al. (2023).

## Comparing Soil Nutrients Supply in the Above and Below Ground

From this description, it was clear that swamp grass and swamp bush were minimal in supplying soil nutrients to PSF. These soil nutrients were the main prerequisite for increasing the productivity of dried biomass production for the peat formation. The observed nutrient contents of N, P, K, Ca, and Mg increased back to levels observed in similar natural PSF in about ten years of restoration. These nutrients showed a distinctive distribution pattern in the different topsoil and subsoil layers. The surviving parts of PSF vegetation recycled nutrients released from the root litter exudate; the nutrient contents were the highest above the ground compared to nutrients below the ground.

The production of soil nutrient supply (represented by N, P, K, Ca, and Mg) above the ground increased as the nutrient level increased by more than 300%. In comparison, the production of soil nutrients below the ground only increased slightly (less than 100 %). Dried leaves from peat forests showed significantly higher dried biomass production at the 5% significance level than other dried biomass types (Figure 4). These results supported the work of Ribeiro et al. (2021).

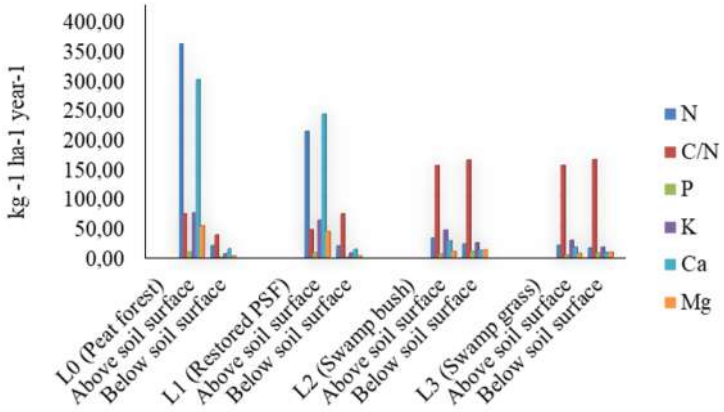


**Figure 4.** Total supply of soil nutrients to PSF (kg $ha^{-1}$ $year^{-1}$)

The production of soil nutrient supply below the ground was relatively saturated, even though the dried biomass was slightly higher than the ground. It can be concluded that the increase in dried biomass below the ground did not automatically increase above-ground dried biomass (Figure 4). There is a tendency for an increase in above-ground biomass, which is also accompanied by an increase in soil nutrients, especially for all parameters observed; however, this trend does not apply to below-ground biomass because an increase in root and rhizome biomass does not automatically increase soil nutrients. This phenomenon is interesting to study in more detail, as peat formation is more dominant from above-ground biomass. Lin et al. (2020) have found the similar results.

It can be concluded that dried leaves were able to lower the C/N ratio due to the high N content in the dried leaves, while root litter increased the C/N ratio. There is a tendency that the higher the dried leaves, the lower the C/N ratio. Conversely, the higher the root litter, the higher the C/N ratio. The additional potential of soil nutrients can only be contributed dominantly by peat forests or restored PSF and dried leaves. In contrast, root litter contributed minimally (less than a quarter of the total addition potential of soil nutrients). This inability of root litter to reach the additional potential of soil nutrients was due to the very high C/N ratio of root litter, making it difficult for soil microbes to decompose, becoming soil nutrients. These results are in line with Michaelis et al. (2020).

## CONCLUSION

This research contributes to sharpening, perfecting thinking, and implementing ecological restoration so that the results of environmental restoration applications can restore or bring Peat Swamp Forest (PSF) closer to its original condition. There is potential for the formation of peat originating from native species, followed by an increase in soil nutrients. Hence, the production of root litter at a high level of soil nutrients was not followed by a higher supply of dried below-ground biomass. Most dried biomass is transported above the ground into dried above-ground biomass. All types of land covers can produce above-ground dried biomass and have the potential to form peat, with significant differences in all parameters studied. The potential for peat formation is highly dependent on the different types of land covers (e.g., peat forests) and environmental factors, the composition of the seed bank, and the ability of species to compete. Restoration actions (replanting, rewetting, and revitalization) aim to accelerate the development of peat-forming species.

## ACKNOWLEDGMENTS

The authors would like to thank anyone who helped with this research, from the design to the writing of this manuscript. Special thanks go to all the students in the field who collect and analyze data.

## AUTHORS CONTRIBUTIONS

Three authors here have equal and fair duties in developing this research. The first author (MEA) conceptualized and created the primary concept and framework for the manuscript, conducted the comprehensive review and literature review, wrote the original draft preparation, and oversaw the collection and analysis of data from field studies. The second author (EW) contributed to methodology, data analysis, and writing regarding review and editing. The last author (MSI) carried out the field research coordination, qualitative data collection, writing in sections on field research and findings, and ethical considerations in ensuring that all field research activities adhered to ethical standards.

## COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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# Effects of Arbuscular Mycorrhiza-Enriched Bio-compost and Organic Fertilizer on Reducing Heavy Metal Absorption in Shallots

10.18196/pt.v13i1.21684

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**ABSTRACT**

Shallots have physiological and pharmacological effects on the human body and have been cultivated intensively using bio-compost, which might be contaminated with heavy metals. The main objective of this study is to enrich arbuscular mycorrhizal fungi (AMF) in bio-compost and add biological organic fertilizer (BOF) to reduce the absorption of heavy metals in shallot bulbs. The study was conducted in Enrekang District, South Sulawesi, where the components of heavy metal content in plant organs and the level of infection of AMF in plant roots were observed. The study was a filed research arranged in a Randomized Block Design with five treatments, including shallots cultivation method by indigenous farmers/control(B0) and using various doses of bio-compost enriched with AM fungi of 100kg plot$^{-1}$(B1); 200kg plot$^{-1}$(B2); 100kg plot$^{-1}$+BOF(B3); and 200kg plot$^{-1}$+BOF). The results showed that bio-compost enriched with AMF at a dose of 200 kg plot$^{-1}$ and 100kg plot$^{-1}$+BOF could increase Cr, Cu, and Pb accumulation in roots and leaf, as well as reduce metal accumulation in shallot bulbs, with the level of AMF infection classified as very high. The novelty of this study is that organic fertilizer enriched with AMF can reduce the absorption of heavy metals accumulated in shallot bulbs.

**Keywords:** Colonization; Endomycorrhizae; Organic fertilizer; Spice plant

## INTRODUCTION

Shallots (*Allium cepa* L) are functional plants (Bamba et al., 2020; Hawayanti et al., 2021) that contain carbohydrates (16.80 g), protein (2.5 g), fiber (3.2 g), fat (0.1 g), vitamin A (9 IU), vitamin C (31.2 mg), thiamin (0.20 mg), riboflavin (0.11 mg), niacin (0.7 mg), pyridoxine (1.2 mg), folic acid (3 ug) and several minerals, such as phosphorus, calcium, sodium, iron and potassium (Amare, 2020; Aryanta, 2019). Shallots also contain active compounds that have pharmacological effects on the body, including flavonoids, saponins, quercetin, essential oils, and allicin (Marefati et al., 2021; Setiawandari et al., 2021), which have been used as traditional medicine for the treatment of ulcers, stomach, cholesterol, diabetes mellitus, and respiratory disorders (Mustofa et al., 2020; Zafran et al.,2021).

Article History
Received : 20 February 2024
Revised : 17 December 2024
Accepted : 06 February 2025

Shallots as a vegetable commodity have been cultivated intensively by farmers. Organic fertilizer used as a source of plant nutrition has long been campaigned to create healthy and environmentally friendly agriculture. Shallots are a vegetable commodity cultivated intensively by horticulture farmers. Organic fertilizers that provide plant nutrients have long been promoted to support sustainable, healthy, and environmentally friendly agricultural practices. However, several research results show that organic fertilizer use has a negative impact because it contains heavy metals (Li et al., 2023; Subiksa et al., 2020). Heavy metals contained in organic fertilizers include boron (B), cadmium (Cd), Cobalt (Co), Chromium (Cr), copper (Cu), mercury (Hg), Manganese (Mn), molybdenum (Mo), Nickel (Ni), lead (Pb), and zinc (Zn), which are also contained in manure (El- Shabasy et al., 2023; Gong & Tian, 2019; Priyadi et al., 2021) and compost (Pinto et al., 2020; Ramísio et al., 2023).

Heavy metals, such as Cu, Zn, Mn, Fe, Mg, and Mo, can play an important role in plant metabolic processes (Angulo-Bejarano et al., 2021; Bhat et al., 2020) within a certain concentration range and become harmful at concentrations exceeding the maximum limit, especially for heavy metals such as Hg, Pb, Cd, As, Cr, and Ni (Ali et al., 2019; Balali-mood et al., 2021). Heavy metals such as Hg, Pb, Cd, As, and Cr are potentially toxic to plants, animals, and humans when contaminated soil is used for crop production. Lindawati et al. (2023) stated that Cd concentration in local Palu shallots in the planting area of Oloboju Village reached the concentration of 1.68 – 101.34 mg/kg in the roots and 0.01 – 0.04 mg/kg in the tubers, while in the planting area of Solove Village, the Cd concentration was 3.78 – 107.18 mg/kg in the roots and 0.01 – 0.03 mg/kg in the tubers; therefore, an environmentally friendly technology is needed as an effort to reduce the absorption of heavy metals by plants. One of the biological technologies that can reduce the absorption of heavy metals is arbuscular mycorrhizal fungi (AMF).

Plant roots with AMF association are known to reduce heavy metal contamination accumulated in plant organs (Dhalaria et al., 2020; Riaz et al., 2021). The results of preliminary research on post-nickel mining land have found three types of AMF spores that are resistant to heavy metals, namely *Acaulospora* sp, *Gigaspora* sp, and *Glomus* sp with different spore abundances, and they have been bred to increase the number of spores.

Several researchers have revealed that the AMF application can reduce Cd levels in *Brassica juncea* plant tissue in soil contaminated with Cadmium (Cd) (Nurlaili et al., 2021). Colonization of indigenous AMF *Glomus* sp. and *Acaulospora tuberculata* can limit Fe, Mn, and Ni levels in *Nauclea orientalis* L (Husna et al., 2021). The combination of compost and AMF applied to annual plants, such as *Nauclea orientalis,* can reduce levels of heavy metals Cr, Ni, Mn, Fe, and Zn in the soil and tissues or organs of plants that humans do not consume (Boorboori & Zhang, 2022; Putra et al., 2022). These three findings reveal the ability of tree plants to absorb metals to prepare phytoremediation plants in post-mining land but do not reveal the concentration of heavy metals accumulated in the organs of consumptive plants, such as shallots plants supported by AMF.

The mechanism of protection from heavy metal provided by AMF decomposition is carried out through the mechanism of hyphal secretion by external hyphal secretions (Chulikavit et al., 2023; Dhalaria et al., 2020); therefore, it is necessary to conduct research aiming to determine the proper dose of bio-compost enriched with AMF and biological organic fertilizer (BOF) to reduce the

absorption of heavy metals accumulated in shallots bulbs; which is also the novelty of this research, supporting organic farming and a healthy environment, especially in areas central where shallots are planted which is essential information for farmers.

## MATERIALS AND METHODS

### Research Site

Horticultural research activities were carried out at the shallot development center in Pekalobean-Sipate Village, Enrekang District, South Sulawesi, at an altitude of 1022 m asl in the 2021-2022 dry season with an average air temperature of 30.0$^{O}$C and RH of 51.0%. The land area used is 1,125 m$^2$, with facilities including sprinkler irrigation and pest control equipment. The land area is divided into 15 sections, each with an area of 75 m$^2$.

### Experimental design

The research was arranged in a Randomized Block Design (RBD) with three replications and five treatments: shallots cultivation method used by local farmers/control (B0); Bio-compost doses enriched with AMF of 100kg.plot$^{-1}$(B1); 200kg.plot$^{-1}$(B2); 100kg.plot$^{-1}$+ BOF (B3); and 200kg. plot$^{-1}$+ BOF (B4).

### Preparation and application of bio-compost enriched with AMF

The bio-compost was goat dung produced by local breeders in the Enrekang District. Meanwhile, the AMF used resulted from early-stage research activities isolated from post-nickel mining land and bred in the Agrotechnology Greenhouse, Muhammadiyah University of Parepare. AMF are propagules containing Glomus spores, *Acaulospora* sp, *Gigaspora* sp, host plant root pieces, and carrier media (sand, zeolite, and biochar). Every 10 g of propagules contains 80-100 spores.

Enriching AMF in bio-compost for treatment B1 was carried out by mixing 100 kg of bio-compost with 7.5 kg of AMF propagules until homogeneous, then weighing 100 kg of the homogenized material for application in the field. The same thing was also done for treatment B3. Meanwhile, for treatment B2, 200 kg of bio-compost and 7.5 kg of AMF propagules were mixed until homogeneous, then 200 kg of the homogenized material was weighed for application in the field, and the same thing was also done for treatment B4.

### Preparation and application of biological organic fertilizer

The biological organic fertilizer (BOF) used in this study was produced by the Indonesian Institute of Sciences (LIPI), which is now the National Innovation Research Agency (BRIN), with Patent No: 00201601284. BOF containing a population of root microbes (*Bacillus* sp., *Pseudomonas* sp., *Burkholderia* sp., *Brevundimonas* sp., and *Brucellaceae* sp) at a concentration of $10^6$-$10^7$ CFU ml$^{-1}$ was used to support the growth of shallot plants in treatments B3 and B4 at a dose of 10-15 L ha$^{-1}$ applied 3 times a week.

### Heavy metal analysis

The concentration of heavy metals (Cr, Cu, and Pb) in the soil and shallot tissue was analyzed

at the chemistry and soil fertility laboratory at Hasanuddin University, Makassar, using an Atomic Absorption Spectrophotometer (AAS) with the HClO4:HNO3 method.

## Mycorrhiza arbuscular fungi (AMF) infection analysis

The roots infected with AMF were observed and analyzed in the Biotechnology laboratory of Makassar Environmental and Forestry Research and Development Center (BP2LHK Makassar). Mycorrhizal infections were observed using the root staining technique, which involves selecting fine, fresh roots from the roots of the sample plants. The roots were placed in a tube containing FAA solution for 24 hours. The FAA solution was discarded, and the roots were washed until clean. Next, the roots were soaked in 10% KOH solution for 24 hours. The KOH solution was discarded, and the roots were cleaned and washed. Next, the roots were soaked in a hot $H_2O_2$ solution for 24 hours and washed thoroughly. The roots that had been washed thoroughly were soaked in a 2% HCl solution for 24 hours. The HCl solution was discarded, and the fine roots were washed with running water. Next, the roots were soaked in 0.05% trypan blue solution for 24 hours. Then, one root sample with a length of 1 cm was taken from the colored roots and arranged on a glass slide. Root pieces on slides were observed at each angle. The percentage of root infection was calculated using the following formula (Brundrett et al., 1996):

$$\textbf{Percentage of AMF infection} = \frac{\sum \textbf{number of fields of view infectied}}{\sum \textbf{total observed field of view}} \times 100\% \qquad (1)$$

The percentage of MA fungi infection was classified based on the following criteria (Rajapakse & Miller, 1992):

<5% = extremely low category
6 – 25% = low category
26 – 50% = medium category
51 – 75% = high category
>75% = extremely high category

## Statistical analysis

Observation data were statistically analyzed using Microsoft Excel software. Effects of AMF dose on the variables observed were determined using analysis of variance (ANOVA). Differences between treatments are categorized as significant if the P-value is less than 0.05 or 0.01 in the Duncan test.

## RESULTS AND DISCUSSION

Heavy metal analysis before planting showed that the concentrations of Cr, Cu, and Pb in the shallot planting area were within normal limits for farming activities (Table 1). However, suppose the land is to be planted. In that case, caution is needed because heavy metals can be absorbed and accumulated in plant organs, reducing shallots' quality and causing health problems for other organisms (Briffa et al., 2020a; Briffa et al., 2020b).

**Table 1.** Concentration of heavy metals in soil and plants before and after planting shallots

| Component | Heavy metal concentration (ppm) | | |
|---|---|---|---|
| | Cr (Chromium) | Cu (Copper) | Pb (Lead) |
| Critical limits in soil | 2.5[a]-100[b] | 60-125[ab] | 100[ab]-400[b] |
| Critical limits in plants | 5-30[c] | 20-100[c] | 50[c] |
| The concentration of heavy metals in the soil before planting | 185.721 | 58.956 | 112.147 |
| The concentration of heavy metals in the soil after planting | | | |
| Plot B0 | 45.26 | 56.32 | 185.32 |
| Plot B1 | 63.32 | 85.63 | 211.25 |
| Plot B2 | 55.85 | 74.32 | 218.63 |
| Plot B3 | 69.32 | 65.32 | 197.64 |
| Plot B4 | 71.25 | 48.32 | 255.25 |

Notes: a (Ministry of State for Population and Environment Republic of Indonesia and Dalhousie University Canada, 1992); b (Kabata & Pendias, 2011); c (Alloway, 2013).

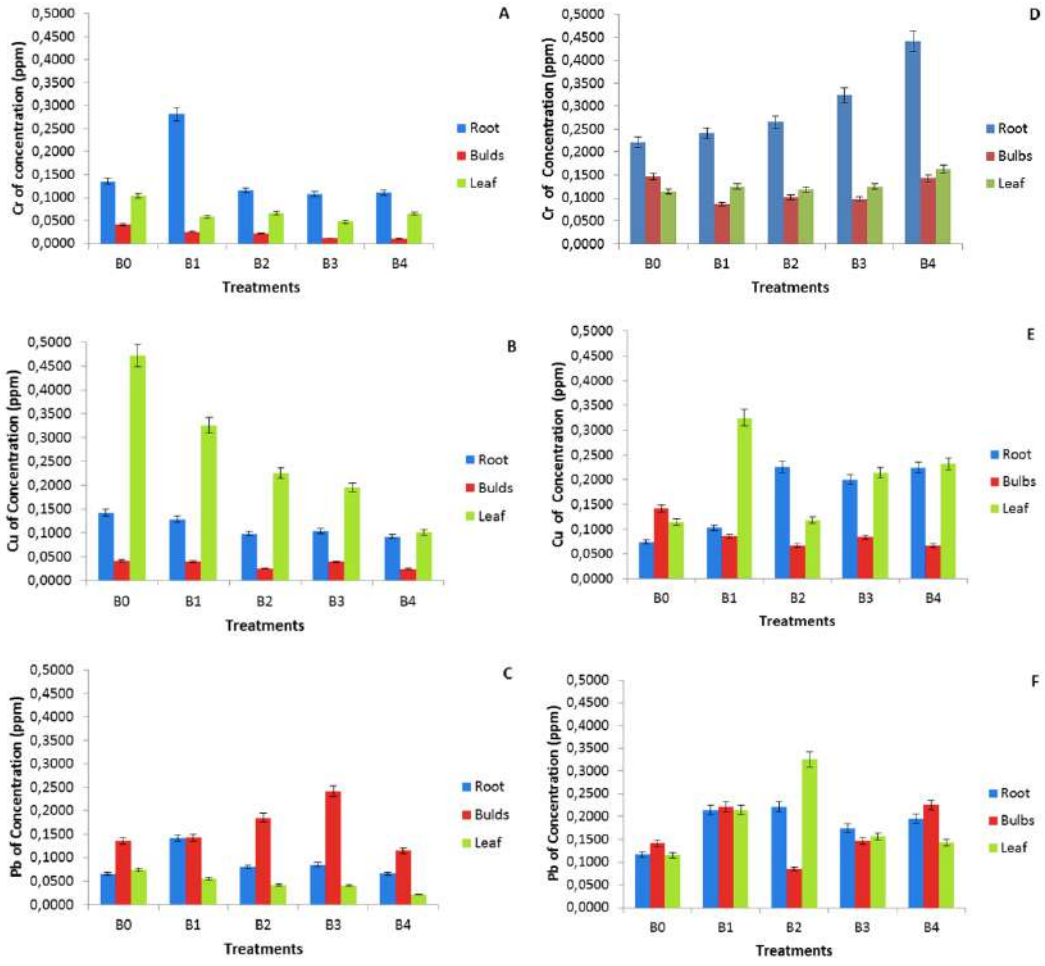


**Figure 1.** Average heavy metal concentrations of Cd, Cu, and Pb in shallot treated with treatment bio-compost enriched with AMF and BOF application at 30 (A, B, C) and 75 DAP (D, E, F)

The concentration of Cu and Pb in the planting area has increased after planting shallots. This increase can be caused by the use of chemical compounds (pesticides and fertilizers) by local farmers around the research area through the flow of rainwater and wind so that they can accumulate on the surrounding land. Rasman & Hasmayani (2018) stated that the types of pesticides used by

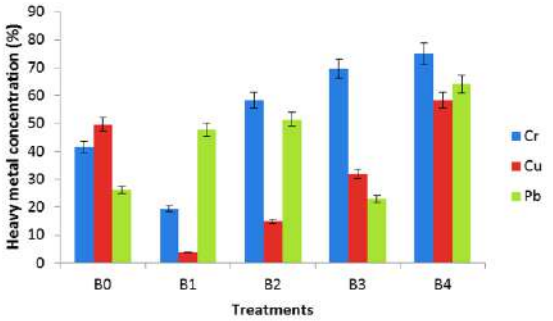


**Figure 2.** Percentage of the increase in heavy metal concentrations in shallot plants treated with bio-compost enriched with AMF and BOF application from 30 to 75 DAP

farmers in Enrekang that contain Pb include Antracol WP, Dithane M 45 80 WP, and Buldok EC, with Pb contents of 12.4800 ppm, 19.3710 ppm, and 2.0420 ppm, respectively, with application doses reaching 500-900 L $ha^{-1}$ applied every 1-2 days. Meanwhile, the type of fertilizer used contains lead (Pb), namely 4.4511 ppm urea and 2.1620 ppm Nitro Ponska, which can also contribute to the heavy metals Zn and Cu. Ruhban & Kurniati (2017) stated that shallot plantations in Enrekang District, Indonesia, showed average Pb content in agricultural land aged 10 - 25 years of 15,224 - 17,523 ppm, and the cadmium content in shallots reached 0.2386 ppm. Dewi et al. (2021) added that there are 11 brands of pesticides from the avermectin, dithiocarbamate, glycine, imidazole, carbamate, organophosphate, pyrethroid, pyrrole, and triazole groups containing Pb of 2.70-22.31 mg $kg^{-1}$ and Cd of 0.04-0.50 mg $kg^{-1}$ and 5 brands of inorganic fertilizer containing 10.53-28.09 mg $kg^{-1}$ of Pb and 0.07-0.52 mg $kg^{-1}$ of Cd.

The phenomenon of Cr, Cu, and Pb metal concentrations appears to increase and decrease as the plants reach harvest time (75 DAP) (Figure 2), which are accumulated in the roots, tubers, and leaves of shallots (Figure 1). However, plants' average metal concentration is still within normal limits (Table 1).

Cr, Cu, and Pb accumulation in organ tissue occurs in two ways: absorption through plant leaves and roots. Because Cr, Cu, and Pb particles in the air fall and settle on the leaf surface, absorption through leaves happens (Hardiyanti et al., 2020). Shallot leaves have large stomatal size (long, 20-35 µm and wide, 5-15 µm) (Nur et al., 2020; Saparso et al., 2021) compared to the size of Pb particles (less than 4 µm), which allows the metal to enter leaf tissue through the stomatal pore (Hamidah et al., 2020). Once the metal enters the tissue, a buildup will occur between the cells of the leaf or root tissue (Ejaz et al., 2023; Febrita, 2020). Therefore, the content of heavy metals (Pb and Cu) in leaf tissue is higher than in other tissues, such as tubers and roots.

Metals of Cr, Cu, and Pb absorbed by hairy roots will experience binding, inactivation, and deposition as a protective strategy for plants (Giannakoula et al., 2021; Srivastava et al., 2021; Zulfiqar et al., 2019). Meanwhile, AMF protects host plants from absorbing toxic heavy metal elements through filtration, complexation, and accumulation effects (Chot & Reddy, 2022; Dhalaria et al., 2020).

Arbuscular mycorrhizal fungi can boost absorption and accumulation of heavy metal while limiting excessive absorption that enters plant cells, so they can function as a biological agent in situations when land is contaminated with heavy metals (Boorboori & Zhang, 2022; Dhalaria et al., 2020; Haider et al., 2021). Metal elements absorbed by AMF will be stored in vacuoles, cell walls, hyphae,

vesicular and arbuscular (Begum et al., 2019; Dhalaria et al., 2020; Doyama et al., 2021). Thus, the concentration of heavy metals in root tissue will be higher than in the plant canopy.

The analysis of variance showed that bio-compost enriched with AMF significantly affected the percentage of shallot roots infected with AMF at the ages of 30 and 75 DAP. The Duncan test at 30 DAP demonstrated that bio-compost enriched with AMF of 200 kg plot$^{-1}$ + BOF had a significant effect compared to the other treatments (Figure 3).

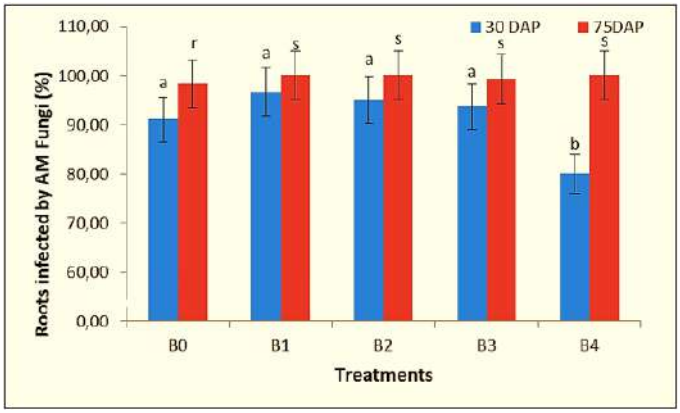


**Figure 3.** Levels of shallot root infection treated with bio-compost enriched with AMF and BOF application at 30 and 75 DAP

The availability of nutrients causes this phenomenon to occur through the application of bio-compost and BOF, which is at quite high doses, so it can reduce the effectiveness of AMF infections at 30 DAP, especially at a dose of 200 kg plot$^{-1}$+BOF. According to Muchoka et al. (2020), Robifahmi et al. (2020), and Prosanti et al. (2023), there is an inhibition of the performance of the AMF in fertile soil, especially the availability of P. In contrast, in soil with infertile conditions, the AMF is very active (Huey et al., 2020; Robifahmi et al., 2020). When the plants reached 75 DAP, all bio-compost treatments enriched with AMF responded similarly. AMF enriching bio-compost is thought to show positive performance. In addition to the availability of nutrients for plants and the ability of AM fungal spores to adapt to heavy metals, it is also possible that AM fungal spores have developed and grown in more significant numbers. However, this did not happen in the control treatment, even though many indigenous AM fungal spores were found.

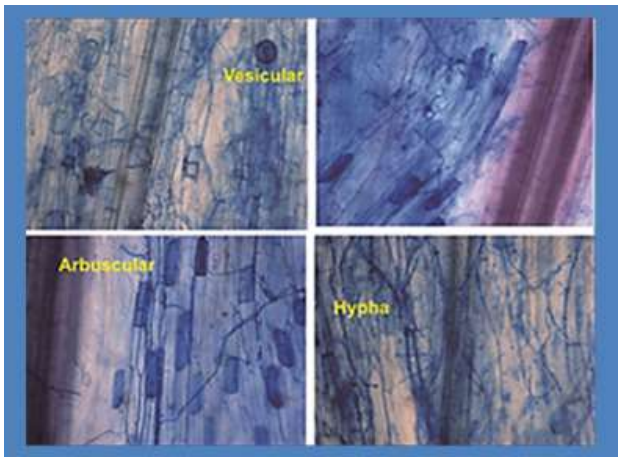


**Figure 4.** Cross section of shallot plant roots infected with AMF

The results of observations of roots infected with AMF at 75 DAP reached 76% - 100% and were classified in the highly high category. The performance of the AMF in carrying out infections from 30 to 75 DAP increased in each treatment, namely 7.34% (B0), 3.33% (B1), 5.00% (B2), 5.70% (B3), and 20.00% (B4). Figure 4 illustrates that the AMF organelles in vesicular, arbuscular, and hyphae found in root epidermal cells infecting shallot roots may bind heavy metals. This happens because the AMF is known to be able to bind heavy metals in carboxyl groups and pectic compounds (cellulose chemicals) in the matrix between the contact the surface of the AMF and the host plant, in the polysaccharide sheath and hypha cell walls (Begum et al., 2019; Dhalaria et al., 2020). AMF can bind metal ions to the cell walls of their hyphae and can protect plants from metal ions. Heavy metals are deposited in crystalloids in the fungal mycelium and mycorrhizal plant cortex cells (Dhalaria et al., 2020; Li et al., 2023).

## CONCLUSION

Bio-compost enriched with AMF of 200 kg plot$^{-1}$ and 100 kg plot$^{-1}$+BOF increased metal accumulation in the roots and leaves and reduced Cr, Cu, and Pb accumulation in shallot bulbs.

## ACKNOWLEDGMENTS

The research team thanks the Ministry of Education, Culture, Research and Technology for funding research through the Program of National Research Priority to Higher Education, 2021 (Program Unggulan Prioritas Riset Nasional Perguruan Tinggi, 2021).

## AUTHORS CONTRIBUTIONS

MAA, SY, and SA designed and conceived the experiments. MAA and SY conducted the experiment. MAA, TK, TKD, and ES contributed to the preparation of samples and interpretation of the results. The manuscript was primarily composed of MAA, TK, and SA. All authors provided critical feedback and contributed to developing the research, analysis, and manuscript.

## COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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# Effects of Soil Ameliorants on Growth And Yield of Elephant Grass (*Pennisetum Pupureum*) in Post-Tin Mining Land

10.18196/pt.v13i1.21066

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**ABSTRACT**

Post-tin mining land is dominated by sand fractions and low in organic C content and essential macronutrients. A high yield of elephant grass can be produced on land containing sufficient and constantly available nutrients. This research aimed to determine the effects of mycorrhizae and NPK fertilizer ameliorants on the growth and yield of elephant grass plants in post-tin mining land. This research was arranged in a randomized complete block design with a combination of mycorrhizae and NPK fertilizer doses. Treatments consisted of control (without mycorrhizae and NPK), NPK 100%, mycorrhizae + NPK 25%, mycorrhizae + NPK 50%, and mycorrhizae + NPK 100%. The recommended dose of NPK 100% was 300 kg/ha. The research results demonstrated the significant effects of the combination of mycorrhizae and NPK fertilizer doses on the plant height, number of clumps, and crop production per plot. Applying NPK fertilizer 100% to elephant grass plants resulted in the highest ratoon yields in post-tin mining land.

**Keywords:** Mycorrhizae; NPK fertilizer; Organic matter; Soil quality.

## INTRODUCTION

Post-tin mining land is often found in Bangka Island. According to Asmarhansyah & Hasan (2020); Asmarhansyah (2020), post-tin mining land is considered poor, indicated by the dominance of the sand fraction, low organic C content, low cation exchange capacity, low essential macronutrients, and low exchangeable bases (Agus et al., 2018). Despite having a very low cation exchange capacity, a very low nutrient content (available and total-N, P, K, Ca, Mg), and high toxicities of Zn, Cu, B, Cd, and Ti, Agus et al. (2019) found that the tropical post-tin-mined acid soil (pH 5.34), which was primarily composed of sand particles (88%), still had low toxicities of B, Zn, Cu, Ti, and Cd.Tailings have a low pH of 4 to 5, are deficient in nutrients and microorganisms, and include almost 95% quartz sand (Khodijah et al., 2019a). By raising the pH, accessible P and K, and cation exchange capacity, organic paramagnetic humus and compost additives can enhance soil quality while remaining minimally harmful (Agus et al., 2019). Within the tin mining area of Dwi Makmur Merawang Village, Bangka Regency, the soil content is 7.53% clay, 0.001% total N, 10.88 cmolkg-1 CEC, 51.78% sand texture, 40.69% dust, and 0.097% organic C (extremely low) (Lestari et al., 2019). These results show that post-tin mining land is included in the category of not ideal for agriculture. According to Harwanto et al. (2023), organic matter and pH are important for soil quality because they affect the soil's

Article History
Received : 13 May 2024
Revised : 29 August 2024
Accepted : 21 January 2025

ability to bind and provide plant nutrients and water. Good soil quality maintains and improves plant growth and yield and prevents degradation.

Adding ameliorants and using palm oil waste for bioremediation can boost the productivity of post-tin mining soil (Lestari et al., 2019; Lestari et al., 2020). Providing organic material on post-tin mining land in the form of empty oil palm fruit bunches of 60 tons/ha can reduce the metal Pb content in eggplants cultivated in tailings (Inonu et al., 2020). Providing 25 tons/ha of cow dung compost in the tailings soil can also increase the production and yield of porang plants, which are safe for consumption (Inonu et al., 2023). The use of post-tin-mining land for livestock feed crop cultivation has been widely carried out, one of which is the cultivation of elephant grass.

Elephant grass (*Pennisetum purpureum*) is a type of grass that grows in clumps. Elephant grass is a forage/grass with high nutritional content and is used as cattle feed (Syaiful & Utami, 2020). Elephant grass can adapt very well to various soil types with low fertility levels. Elephant grass can still produce green food crops. Based on the research results of Khodijah et al. (2019b), a growth medium composed of 50% tailings, 40% ultisol, 5% organic material, and 5% NPK is the best for elephant grass growth. Production of elephant grass will increase when planted on land containing sufficient nutrients that are always available. NPK fertilizer, compost, and mycorrhiza can help improve post-tin mining soil. Mycorrhiza is the symbiotic association between fungi and plants that colonize the root cortex tissue. According to Aqma et al. (2020), mycorrhizal fungi have a symbiotic relationship with plant roots to obtain carbohydrates from plants in the form of simple sugar or glucose. Mycorrhizal fungi on plants can help absorb the nutrients that plants need.

NPK fertilizer is a compound fertilizer made by mixing N, P, and K. Providing NPK Mutiara fertilizer (16:16:16) at the correct dose can improve the physical, chemical, and biological properties of the soil, thereby improving the soil structure so that it becomes more fertile (Ramadhan et al., 2022). Providing organic chicken manure and 100% N, P, and K fertilizer can improve the growth and yield of sorghum on post-tin mining land (Lestari et al., 2021). The recommended NPK fertilizer application for livestock feed crops with low nutrient status is 300 kg/ha.

Post-tin mining land use, the dose of mycorrhiza and NPK fertilizer will influence elephant grass plants growth and yield. Providing mycorrhiza to elephant grass on post-tin mining land is one of the efforts to increase elephant grass production on post-tin mining land. The success of elephant grass cultivation is determined by the amount of NPK fertilizer utilized. The purpose of this study was to ascertain how the growth and production of elephant grass plants on post-tin mining ground were affected by combination doses of NPK fertilizer and mycorrhiza. Reforestation in places affected by tin mining can benefit from this research.

## MATERIALS AND METHODS

### Experimental Site, Time of the Research and Plant Materials

This study was conducted in tin-mining land, Kampoeng Reklamasi Air Jangkang, Riding Panjang, Merawang, Bangka. The study was conducted in September 2022 – March 2023. The materials used were water, elephant grass, lime, compost, mycorrhiza (10 g/plant), and NPK 16:16:16 fertilizer (300 kg/ha).

## Experimental Design

The study was set up using a Randomized Block Design and included five treatments: NPK 100% (30 g/plant); control (no NPK or Mycorrhiza); Mycorrhiza 10g+ NPK 25% (7.5 g/plant); Mycorrhiza 10g + NPK 50% (15 g/plant); and Mycorrhiza 10g + NPK 100% (30 g/plant). The 100% NPK dose was 300 kg/ha (30 g/plant). Each treatment was made into 6 replications, resulting in 30 experimental plots.

## Procedures

The land used was plotted to be 1.5 m x 1.5 m. The number of research plots was 30 plots. The distance between plots was 1 m. One week before planting, lime and compost were applied. The elephant grass planting materials were made from cuttings that were 1 month old after being sown in polybags. The cuttings were planted directly on the field. Fertilization was carried out three times, at 2, 6, and 9 weeks after planting, using hole fertilization in the root zone. Harvesting was done when the plants were 4 months old by cutting using a sickle.

Observations were made on the plant height, number of clumps, stem diameter, root length, and yield. Plant height was measured from the shoot stem's base to the longest leaf's tip. The number of clumps was obtained by counting the clumps/shoots. The diameter of the rod was measured using a caliper. Root length was measured after pulling out the plant. Yield was observed at the harvest.

## Data Analysis

The data obtained were analyzed using Analysis of variance (ANOVA). Significant effects of the treatments were tested using Duncan's Multiple Range Test (DMRT) at the 5% level.

# RESULTS AND DISCUSSION

## First Harvest Results (H1)

The analysis results of the first harvest showed that applying a combination of mycorrhiza and NPK fertilizer had significant effects on plant height, the number of clumps, stem diameter, and yield of elephant grass cultivated in post-tin mining land (Table 1). Tin mining activities cause a decrease in land quality, including physical, chemical, and biological soil properties (Lestari et al., 2019). The soil analysis revealed that the post-tin mining land in Air Jangkang (the research location) had 66.11% sand, 29.08% silt, and 4.81% clay. Its pH was 6.10, its organic C content was 0.16%, its total N content was 0.09%, its K-dd was 0.01 Mol(+)/kg, its Na-dd was 0.05 Mol(+)/kg, its Ca-dd was 0.17 Mol(+)/kg, and its Mg-dd was 0.16 Mol(+)/kg (Oktaviani et al., 2020). Kurnia and Rohaendi's (2023) study revealed that the Bangka Belitung Islands' post-tin mining land had a moderate to low heavy

**Table 1**. The growth and yield variations (H1) of elephant grass on post-tin mining land

| Observed Variables | Pr > F | Coefficient value (%) |
|---|---|---|
| Plant height (cm) | 0.0001** | 5.39 |
| Number of leaves | 0.0039* | 18.10 |
| Stem diameter (mm) | 0.3927ns | 10.17 |
| Fisrt harvest result (kg/plot) | 0.0001** | 10.90 |

Remarks: * significant at α 5%, ** significant at α 1%, ns not significant

metal level, which allowed it to be utilized as agricultural land and other productive land. Providing nitrogen fertilizer at various doses significantly increased plant height, leaf length, leaf width and number of leaves, and yield of Pak Chong grass (Rinduwati et al., 2023). According to Khodijah et al. (2019a), this is thought to be due to the greater efficiency of Pb uptake in S.spontaneum compared to the increase in absorption efficiency in *P. purpureum* and *H. acutigluma.*

The results of further test analysis of the first harvest of elephant grass are shown in (Table 2). The 100% NPK fertilizer treatment showed significant effects on all variables. The control treatment gave the lowest results compared to all treatments. The combination treatment of mycorrhiza and NPK fertilizer gave lower results than applying 100% NPK fertilizer without mycorrhiza on the growth and yield of elephant grass plants. This is thought to be due to the direct effect of NPK fertilizer on plants, thereby reducing the role of mycorrhiza. Applying compound fertilizers influences elephant grass's vegetative growth and production. The research results of Rinduwati et al. (2023) showed that applying nitrogen fertilizer at a dose of 225 kg N/ha = 2.5 g N/polybag) provided optimal results on elephant grass. Revegetation of post-tin mining land can use animal feed plants such as elephant grass (Lestari et al., 2022). Elephant grass are forages that have the potential to be used as a source of feed ingredients for livestock (Hakim et al., 2023).

**Table 2.** Analysis of growth and yield (H1) of elephant grass in post-tin mining land

| Treatment | Plant height (cm) | Number of clumps | Stem diameter (mm) | First harvest result (kg/plot) |
|---|---|---|---|---|
| Control | 64.65 d | 3.50 c | 1.52 | 14.08 d |
| NPK 100% | 134.87 a | 5.62 a | 1.55 | 36.86 a |
| Mycorrhizal + NPK 25% | 109.74 c | 4.17 bc | 1.53 | 19.01 c |
| Mycorrhizal + NPK 50% | 114.01 bc | 4.45 bc | 1.41 | 21.99 c |
| Mycorrhizal + NPK 100% | 118.30 b | 4.58 b | 1.51 | 26.94 b |

Remarks: Means followed by same letters in the same row are not significantly different based on DMRT with a 5% significance level.

## Ratoon Harvest Result (H2)

The application of mycorrhiza and different amounts of NPK fertilizer significantly impacted the variables of plant height, the number of clumps, stem diameter, root length, root weight, and elephant grass production in post-tin mining ground, according to the analysis of variance results (Table 3).

**Table 3.** The growth and yield variations (H2) of elephant grass on post-tin mining land

| Observed Variables | Pr > F | Coefficient value (%) |
|---|---|---|
| Plant height (cm) | 0.0001** | 7.89 |
| Number of clumps | 0.0250* | 10.78 |
| Stem diameter (mm) | 0.0042* | 1.28 |
| Second harvest result/ratoon (kg/plot) | 0.0001** | 23.57 |
| Root length (cm) | 0.3594ns | 22.18 |

Remarks: * significant at α 5%, ** significant at α 1%, ns not significant

The results of the further tests for the second harvest/ratoon of elephant grass are shown in Table 4. The 100% NPK fertilizer treatment showed significant effects on all variables. Mycorrhiza treatment + NPK 50% significantly impacted the number of clumps. The control treatment significantly affected the stem diameter variable, and the control treatment showed the lowest results on the variables of plant height, number of clumps, plant yield, root length, and root weight in post-tin mining land.

**Table 4.** Analysis of growth and yield (H1) of elephant grass in post-tin mining land

| Treatment | Plant height (cm) | Number of clumps | Stem diameter (mm) | Second harvest result (kg/plot) | Root length (cm) |
|---|---|---|---|---|---|
| Control | 61.55 c | 5.75 c | 1.53 a | 3.20 d | 34.50 |
| NPK 100% | 87.50 a | 18.37 a | 1.56 a | 15.29 a | 66.50 |
| Mycorrhizal +NPK 25% | 66.38 c | 11.94 b | 1.32 c | 7.20 c | 43.08 |
| Mycorrhizal +NPK 50% | 76.08 b | 17.45 a | 1.41 b | 9.65 bc | 60.88 |
| Mycorrhizal +NPK100% | 78.94 b | 15.91 ab | 1.46 b | 11.59 b | 60.58 |

Remarks: Means followed by same letters in the same row are not significantly different based on DMRT with a 5% significance level.

The appropriate N, P, and K fertilizer dose will affect plant growth and yield. Providing 15 tons/ha of chicken manure and 100% NPK fertilizer (Urea 300 kg/ha, SP-36 200 kg/ha, and KCl 150 kg/ha) showed the best growth and production of sorghum on post-tin mining land compared to applying NPK 50 fertilizer % (Urea 150 kg/ha, SP-36 100 kg/ha and KCl 75 kg/ha (Lestari et al., 2021).

The 100% NPK fertilizer treatment without using mycorrhiza applications gave higher results for all variables than other treatments. This is because NPK fertilizer has provided sufficient nutrients for elephant grass plants on post-tin mining land. Hasibuan et al. (2018), claim that consuming nitrogen (N), potassium (K), and phosphate (P) nutrients will improve the growth and formation of plant vegetative organs, such as stems, leaves, and roots. Elephant grass plant growth and development on post-tin mining ground were not significantly. Basri (2018) added that plant type and variety, soil type, fertilizer type, AMF type, and environmental factors, such as light and temperature, also influenced the effects of mycorrhiza fungal inoculation on growth, P uptake, and plant yield. Rakotoarimanga et al. (2023) stated that cultivation of *Intsia bijuga* with endomycorrhizal host plants made effective endomycorrhizal symbiosis in *Intsia bijuga*.

The mycorrhiza and various doses of NPK fertilizer significantly affected the plant yields. The highest plant yield was found in the plants treated with 100% NPK fertilizer. The percentage of reduction in plant yield after treatment was 77.27% for the control treatment, 58.52% for 100% NPK treatment, 62.12% for mycorrhiza treatment + NPK 25%; 56.12% for mycorrhiza + 50% NPK treatment, and 56.96% for mycorrhiza + 100% NPK treatment.

The results of the first and second harvest/ratoon showed decreasing yields. The percentage of yield reduction from the first to the second harvest was 77.27% for control, 58.52% for 100% NPK treatment, 62.12% for 25% mycorrhiza + NPK treatment, 56.12% for mycorrhiza treatment + 50% NPK, and 56.96% for mycorrhiza + 100% NPK treatment. This decrease in yield is thought to be due to the decreasing organic nutrient content in the soil because the main plant has used it. Hence, the micronutrient content in the soil is insufficient for the needs of elephant grass plants in the second/ratoon harvest. The short vegetative phase before the reproductive phase causes imperfect growth and development of ratoons, which can be seen from the reduction in the number and area of leaves during photosynthesis. The research results of Buyana et al. (2019) showed that the need for nitrogen fertilizer for rice plants was 45-135 kg/ha.

## Growth of Elephant Grass Roots in Post-Tin Mining Land

The average root length and root fresh weight of elephant grass plants were obtained after the second ratoon harvest when the plants were 5 months old. The 100% NPK treatment resulted in higher root length and weight values than other treatments. The 100% NPK treatment without using

mycorrhiza showed higher results than without mycorrhiza. The control treatment showed the lowest results compared to the different treatments.

Root growth and development were greater in the 100% NPK fertilizer and the mycorrhiza + 100% NPK treatments compared to the other treatments. The mycorrhiza + 25% NPK and the mycorrhiza + 50% NPK treatments showed fewer roots than the 100% NPK and the mycorrhiza + 100% NPK treatments. The control treatment showed the fewest roots compared to the other treatments. Root growth and development were greater in 100% NPK fertilizer and 100% mycorrhiza + NPK treatments compared to other treatments. The mycorrhiza + 25% NPK and the mycorrhiza + 50% NPK treatments showed fewer roots, while the control treatment showed the fewest roots compared to the other treatments. Good root penetration ability in the 100% NPK fertilizer treatment is characterized by a greater root weight and a more significant number of roots, making it easier for the roots to obtain nutrients and air so that the plants do not lack nutrients. A large number of roots indicates better root development. Mycorrhiza application is thought to have no significant effect on all variables because the level of compatibility of mycorrhiza with plants is low, and the mycorrhizae do not originate from the area of origin of the research. Basri (2018) states that the mycorrhiza used in this study are from different regions. Their adaptation and compatibility with host plants are low when planted on post-tin mining land. Plant type and variety, soil type, fertilizer type, AMF (arbuscular mycorrhizal fungi) type, and other environmental factors, such as light and temperature, play a role in the effects of mycorrhizal fungus inoculation on the growth, P uptake, and plant yield. In tropical post-mining sites, the application of AMF and reforestation with exotic, rapidly growing pioneer legume species of *P. pinnata* significantly enhanced certain chemical soil qualities appropriate for rehabilitation initiatives. According to Lestari et al. (2024), mycorrhiza enhances the growth and yield of pakchong plants on post-tin mining sites in comparison to its absence.

Microscopic observation results showed mycorrhizal infection on plant roots (image not shown). Mycorrhizal infection can be seen on the roots, characterized by hyphae. Applying mycorrhiza can improve the physical and chemical properties of the soil. Still, this study had no significant effect on the growth and yield of elephant grass plants in post-tin mining land. Applying mycorrhiza to elephant grass has not effectively infected plant roots in post-tin mining fields. It is suspected that the presence of mycorrhiza in elephant grass plant roots is not efficient. It is thought that the mycorrhiza used is incompatible with infecting elephant grass plants' roots. Hadianur et al. (2018) added that the compatibility of mycorrhiza with host plants varied greatly depending on the mycorrhizal species and host plant species. Adding organic material to the planting medium before planting and applying NPK fertilizer can increase the pH value of the soil, thereby inhibiting mycorrhizal activity. According to Santana et al. (2022), AMF fungi are often found in dormant conditions at suitable soil pH due to their acidophilic nature. Applying mycorrhiza + a dose of NPK fertilizer did not have a significant effect, presumably because mycorrhiza could not interact with the roots in absorbing nutrients in elephant grass plants in post-tin mining land. According to Thepu et al. (2021), plant nutrient uptake is influenced by a specific dose; an excessive dose will not increase nutrient uptake because plants limit nutrient absorption. Budiastuti et al. (2024) stated that applying NPK on the surface reduced the highest rice biomass.

The land used for previous research had been planted with green beans with the addition of rhizobium treatment in the study of Oktaviani et al. (2020). Treatment of 100% NPK fertilizer showed higher results than Mycorrhiza + 50% NPK; it is suspected that the land used is predominantly sandy, and the organic material in the land is sufficient so that the mycorrhizae do not work. The combination of 10 g mycorrhizae/plant and 50% NPK fertilizer showed the lowest percentage of reduction in yield after ratooning, which was 56.12%, compared to the 100% NPK fertilizer treatment, showing a decrease of 58.52%. It is suspected that giving mycorrhiza 10 g/plant is not practical in promoting the growth of elephant grass in post-tin mining land. Nurmas et al. (2024) showed that the Liquid Organic Fertilizer (LOF) treatment of LOF 30 mL $L^{-1}$ water and AMF 15 g/plant was the best treatment for local corn production on marginal land. The research results of Agus et al. (2018) showed that administering a mycorrhiza at a dose of 4 g/plant had an ineffective effect on *Pongamia pinnata* plant seedlings compared to a dose of 2 g/plant in ex-coal mining soil media. The results of the analysis of animal feed (silage) resulting from elephant grass cultivation on post-tin mining land are <0.75, indicating that it is safe for livestock consumption (Lestari et al., 2022).

## CONCLUSION

The 100% NPK fertilizer treatment (300kg/ha) provided the best growth and highest yield of elephant grass (*Pennisetum purpureum*) in post-tin mining land compared to the mycorrhiza + NPK combination treatment. The results of the first and second harvest/ratoon of elephant grass in post-tin mining land showed decreased yields ranging from 56.12%.

## ACKNOWLEDGMENTS

This work was supported by the Ministry of Research, Technology, and Higher Education of Indonesia through Universitas Bangka Belitung in 2022.

## AUTHORS CONTRIBUTIONS

TL designed and conceived the experiments. TL and NSP experimented. NSK and NSP contributed to the preparation of samples and interpretation of the results. The manuscript was primarily composed by TL and DP. All authors provided critical feedback and contributed to the development of the research, analysis, and manuscript.

## COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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