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# Mycorrhizal symbiosis and natural dye waste organic fertilizer: Enhancing growth and yield in *Indigofera tinctoria*

**Nor Isnaeni Dwi Arista[1]*, Maria Theresia Sri Budiastuti[1], Supriyono[1], Aprilia Ike Nurmalasari[1], Desy Setyaningrum[1], Ida Rumia Manurung[1]**
[1] *Department of Agrotechnology, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta 57126, Indonesia*
*Correspondence: dewi.arista99@student.uns.ac.id

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## ABSTRACT
**Background:** *Indigofera tinctoria*, commonly known as true indigo, is a plant widely used in the textile industry for its natural indigo dye, which produces a rich blue color for fabrics. *I. tinctoria*, known for its natural bluish-purple dye, generates 10% dye and 90% waste during extraction, requiring effective waste management. This study aimed to optimize organic fertilizer and mycorrhiza doses from *I. tinctoria* waste for enhancing soil fertility in arid regions. **Methods:** The study was conducted from May to December 2020 in Puron Village, Bulu District, Sukoharjo Regency. A factorial Completely Randomized Block Design (CRBD) with 2 factors was employed. The first factor consisted of 5 levels of *I. tinctoria* organic fertilizer treatments: 0, 100, 200, 300, and 400 g per plant. The second factor included 3 levels of mycorrhiza treatments: 0, 10, and 20 g per plant. Variables observed included mycorrhizal infection on roots, plant growth rate, and yield (fresh leaf and shoot weight of *I. tinctoria* ). Data analysis utilized ANOVA at a 5% significance level followed by Duncan Multiple Range Test (DMRT). **Results:** The results indicated that organic fertilizer from natural dye waste at a dosage of 200 g per plant increased the percentage of mycorrhizal-infected roots and boosted shoot weight by 63.27% at 8 weeks after planting (WAP). Mycorrhiza at 10 g per plant increased mycorrhizal infection percentage and enhanced shoot weight by 45.98% at 4 WAP. The combination of *I. tinctoria* extraction waste organic fertilizer at 200 g per plant and mycorrhizal at 10 g per plant showed interaction, significantly increasing the growth of root nodules of *I. tinctoria* by 84.04% at 12 WAP. **Conclusion:** The integration of organic fertilizer derived from indigo dye waste and mycorrhiza presents a promising strategy for enhancing *I. tinctoria* growth and productivity. **Novelty/Originality of this Study:** This study is distinctive in its demonstration of the effective use of *I. tinctoria* extraction waste as an organic fertilizer, aligning with zero-waste principles and contributing to improvements in plant growth and soil fertility. Furthermore, it investigates the synergistic effects of mycorrhizal associations on enhancing nutrient absorption and overall productivity of *I. tinctoria* , an aspect that has not been thoroughly explored in prior research.

**KEYWORDS**: *Indigofera* waste; mycorrhiza; organic fertilizer; soil fertility.

## 1. Introduction

*Indigofera tinctoria*, known for its historical significance and traditional use, is a plant that produces a natural indigo dye widely valued for its rich blue color and cultural heritage in textile dyeing practices. The textile industry utilizing natural dyes derived from *Indigofera tinctoria* generates significant waste that remains unutilized. The abundance of

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waste is particularly high in the form of solid waste resulting from the extraction of *Indigofera tinctoria*. The extraction process of *Indigofera tinctoria* yields only 10% natural indigo dye, with the remaining 90% comprising unused waste from the extracted stems and leaves (Sihta et al., 2018). One way to manage organic waste is through the production of organic fertilizer. Organic wastes such as water hyacinth, urban organic waste, and sawdust can be recycled into organic fertilizer. The production of organic fertilizer is initiated by adding EM4 bioactivator to accelerate decomposition. Additionally, cow manure can be added because it contains phosphorus, which can accelerate the decomposition of waste mixtures (Unuofin & Siswana, 2019). The application of organic fertilizer to plants can increase the biomass of legume plants (Christophe et al., 2019). Organic material from the waste of *Indigofera tinctoria* extraction can also be utilized as a raw material for organic fertilizer production.

The management of organic waste from natural dye extraction can be processed into organic fertilizer (Budiastuti et al., 2020a). Organic fertilizer from the waste of *Indigofera tinctoria* extraction can be applied to the cultivation of *Indigofera tinctoria*, ensuring that no waste is generated from the extraction of natural dyes, in line with the zero-waste principle. This organic fertilizer can enhance soil fertility. Increasing soil fertility in arid lands can be achieved by using organic fertilizer and technology. The application of technology in the cultivation of *Indigofera tinctoria* is necessary to achieve optimal plant growth. The growth and yield of *Indigofera* are determined by the nutrient content of the growing medium. Thus, technological innovations that can increase the nutrient content in the growing medium are essential. One applicable technology is the use of Arbuscular Mycorrhizal Fungi (AMF).

Organic fertilizer has numerous benefits, including improving soil structure, facilitating nutrient absorption, and providing beneficial bacteria in the soil (Minardi et al., 2011). The application of AMF helps increase the growth of fresh and dry matter production of *Indigofera tinctoria* (Budiastuti et al., 2021; Setyaningrum et al. 2020). The application of organic fertilizer and AMF results in the highest mycorrhizal spore count in the soil rhizosphere layer. The symbiosis between plants and mycorrhizae can influence plant metabolism, new root formation, and the permeability of root membranes to absorb nutrients (Sari & Indrawati, 2019).

Mycorrhiza is a structure resulting from a mutualistic symbiotic relationship between soil fungi and the roots of higher plants. The advantages mycorrhiza provides to its host plants include improved nutrient absorption from the soil, protection against root pathogens, enhanced resilience to drought, stimulation of growth-promoting hormones, and maintenance of biogeochemical cycles. Mycorrhizal fungi, a type of biological fertilizer, are vital for plant growth by forming symbiotic associations with plant roots, which aid in energy transfer within plant cells, membrane formation, and increased nitrogen use efficiency (Wahab et al., 2023). Moreover, mycorrhiza enhances the availability and uptake of natural phosphates (P), thus requiring the application of biological fertilizers like mycorrhiza to improve phosphate availability (Laksono & Karyono, 2017).

Mycorrhiza can develop a mutualistic relationship with plant roots, benefiting both the fungi and the plants. Mycorrhiza acquires carbon through photosynthesis, while the plant gains essential nutrients from the Arbuscular Mycorrhizal Fungi (AMF). Research has demonstrated that AMF can boost plant productivity by 25%-50%, including improvements in plant health, yield quality, tolerance to water stress, fertilization efficiency, and suppression of soil-borne pathogenic microbes (Ansiga et al., 2017).

Mycorrhiza infects the root system of host plants, particularly young roots, and subsequently produces an extensive network of hyphae. Mycorrhizal plants thus enhance their capacity to absorb water and nutrients (Chauhan et al., 2022). The fine size of the hyphae allows them to penetrate even the smallest soil pores (micro-pores), enabling them to absorb water under extremely low moisture conditions. By aiding in water and nutrient uptake such as N and P, mycorrhiza accelerates plant growth and development, thereby enhancing overall plant growth (Putri et al., 2019). Based on these findings, mycorrhiza enhances the growth and yield of *Indigofera tinctoria* due to its extensive hyphal network, ensuring continuous water and nutrient supply to the plant roots. Previous research has investigated the effects of NPK fertilization combined with the application of biofertilizers such as rhizobium and mycorrhiza in *Indigofera tinctoria* (Setyaningrum et al., 2020). This studies have typically underscored the importance of nutrient availability but have not comprehensively examined the role of symbiotic relationships in plant growth. This study is unique in that it investigates the specific role of mycorrhiza in enhancing the growth and yield of *Indigofera tinctoria*.

## 2. Methods

*2.1 Materials, research tools, and experimental design*

The materials utilized include seeds of *Indigofera tinctoria*, waste from the extraction process of *Indigofera tinctoria*, mycorrhizal inoculum, soil, cow manure fertilizer, rice bran, molasses, EM4 solution, and distilled water. The equipment employed comprises a shredder, scale, plastic drums for composting, sprayer, blender, wooden trays, seedling polybags, tarpaulin, knife, hoe, dibble stick, shovel, drill, oven, label paper, ruler/measuring tape, and writing utensils. The procedure involves initially processing organic fertilizer from natural dye waste, predominantly sourced from *Indigofera tinctoria* waste. The biological fertilizer treatment incorporates mycorrhiza, utilizing an inoculum obtained from Gadjah Mada University; mycorrhiza inoculum is generally accessible at agricultural supply outlets.

The study employs a Complete Randomized Block Design (CRBD) factorial design comprising 2 treatment factors with 3 replications. The first factor is the dose of organic fertilizer derived from *Indigofera tinctoria* extraction waste, consisting of 5 levels: (1) 0 g/plant (O0), (2) 100 g/plant (O1), (3) 200 g/plant (O2), (4) 300 g/plant (O3), and (5) 400 g/plant (O4). The second factor is the dose of mycorrhizal inoculum, which includes 3 levels: (1) 0 g/plant (M0), (2) 10 g/plant (M1), and (3) 20 g/plant (M2). Mycorrhiza infection observations are conducted at 4 and 8 weeks after planting (WAP). The technique involves root staining followed by microscopic examination to observe spores, hyphae, vesicles, and/or arbuscules.

*2.2 Implementation of the study*

Organic fertilizer production from Indigofera tinctoria waste is guided by previous research findings (Budiastuti et al., 2020a). The organic fertilizer is produced from 142 kg of dry waste obtained from CV. Indigo Biru Baru, mixed with 2.37 kg of rice bran, 14.2 kg of compost, 500 ml of molasses, and 500 ml of EM4 solution. The mixture is thoroughly blended, covered with tarpaulin, and stirred periodically to maintain a temperature of 40-50°C. After one month, the matured organic fertilizer, characterized by its black color, friability, lack of heat, and odor, is ready for use. Seed preparation involves selecting seeds visually inspected from CV. Indigo Biru Baru based on physical morphology: round-flat, plump, unwrinkled, and shiny seeds soaked in distilled water for 24 hours to break dormancy.

Seedlings are planted in a 1:1 mixture of soil and compost in 5 cm x 5 cm polybags, with each bag containing 3-5 seeds, watered twice daily for one month. Soil preparation for transplanting includes plowing, weeding, and digging 50 cm x 50 cm planting holes to a depth of 10 cm. One month after germination, the transplanting is preceded by applying Indigofera tinctoria extraction waste organic fertilizer dosages. Additionally, mycorrhizal inoculum is applied to the roots during transplantation. Maintenance involves daily initial watering, transitioning to evening watering in the second week after planting (WAP), manual weeding, and soil aeration with a shovel. Topdressing is done at 6 WAP using Indigofera tinctoria extraction waste organic fertilizer by creating shallow furrows beside the plants and covering them with soil. Harvesting occurs at 4, 8, and 12 WAP by uprooting plant roots for examination in the laboratory.

### 2.3 Variables of observation, observing plant yield, and data analysis

Soil Analysis The soil analyzed was the soil before and after planting. The soil after planting was taken from each treatment after harvesting 12 MST. The analysis was conducted at the Laboratory of Soil Chemistry and Physics, Faculty of Agriculture, UNS, based on Balittan (2009). Observation of mycorrhizal infection is conducted on plants aged 4 and 8 weeks after planting (WAP). The technique used is root staining followed by microscopic examination. The observations include spores, hyphae, vesicles, and/or arbuscules. Plant Growth Rate (PGR) (g/week) is the increase in dry weight of plants over a specific time interval. PGR is used to measure the efficiency of plant biomass productivity. The formula for PGR is as follows, where w is the final dry weight, and t is the time (Equation 1).

$$PGR = \frac{w2-w1}{t2-t1} \quad \text{(Eq. 1)}$$

Observation of plant yield includes measuring the fresh leaf weight of Indigofera tinctoria (g) at 12 WAP. The fresh leaf weight is determined using an analytical balance. The fresh shoot weight (g) is also measured at 4, 8, and 12 WAP using an analytical balance. Meanwhile, the research data will be analyzed using an F-test with a significance level of α = 5%. If the F-test indicates significant differences, further analysis will be conducted using the DMRT (Duncan Multiple Range Test). This method determines substantial differences among treatments concerning the observed variables, such as fresh leaf weight and fresh shoot weight of Indigofera tinctoria.

## 3. Results and Discussion

### 3.1 Mycorrhizal infection

The site is situated in the middle of rice fields that have been actively used to cultivate Indigofera tinctoria since 2016, with physical modifications to the planting area, including creating 13.5 m long and 2.1 m wide beds. The research area is open, allowing Indigofera tinctoria to receive direct sunlight. Regular environmental observations monitored light intensity, pH, and soil moisture. Based on the ecological observations (Table 1), the highest light intensity was recorded at noon, ranging from 76,928.6 to 73,757.1 lux, which supports optimal photosynthesis in Indigofera tinctoria, resulting in high biomass production. According to Setyaningrum et al. (2020), 100% light intensity increases the wet weight and biomass of Indigofera tinctoria because high light intensity supports optimal photosynthesis, producing high carbohydrates. The soil pH measurements ranged from 5.6 to 7, indicating slightly acidic to neutral conditions, while soil moisture ranged from 5.21 to 5.57%, considered adequate.

Table 1. Average environmental conditions at the research site

| Block | Environmental Variable | Observation Time (WIB) | | |
|---|---|---|---|---|
| | | 07.00 | 12.00 | 17.00 |
| 1 | Light Intensity (lux) | 14800 | 76928.6 | 8142.86 |
| | pH | 5.8 | 5.8 | 5.6 |
| | Soil Moisture (%) | 4.71 | 5.21 | 5.57 |
| 2 | Light Intensity (lux) | 13885.7 | 76942.9 | 7657.14 |
| | pH | 6 | 5.54 | 5.7 |
| | Soil Moisture (%) | 5 | 5.35 | 5.42 |
| 3 | Light Intensity (lux) | 12485.7 | 73757.1 | 8300 |
| | pH | 6 | 5.9 | 5.7 |
| | Soil Moisture (%) | 5.2 | 4.7 | 5.5 |

*Indigofera tinctoria* research utilized dry grumusol soil, typically found in marginal agricultural lands. Grumusol soil is characterized by its black color, clay texture, clumpy structure, and high adsorption capacity. Prior to the research treatment, the land was previously used for organic *Indigofera tinctoria* cultivation using manure at a dose of 10-15 g per plantapplied before planting.

The initial soil analysis (Table 2) showed a pH of 6 (slightly acidic) with moderate nitrogen (N) content of 0.22%, high potassium (K) content of 0.69 me/100 g, but very low phosphorus (P), organic carbon, and organic matter contents of 1 ppm, 0.6%, and 1.1%, respectively. Organic matter content in the soil depends on several factors such as soil depth and texture, and soil type can also affect nutrient content, especially clay content; higher clay content generally leads to lower organic matter content (Palupi, 2015). These low nutrient levels can inhibit plant growth, thus necessitating soil fertility management through fertilization.

Soil fertility management using Arbuscular Mycorrhizal Fungi (AMF) technology can help increase available phosphorus. According to Hernández et al. (2019), mycorrhiza application can increase available phosphorus; roots infected by mycorrhiza can utilize the organic matter in the soil by releasing root exudates, making unavailable organic phosphorus available to plants. Mycorrhizal biofertilizers can be combined with organic fertilizers. Sindhu et al. (2016) found that the application of manure and biofertilizers to *Indigofera tinctoria* resulted in high indigo yield and quality, increased soil nitrogen and potassium, and reduced production costs.

Table 2. Initial soil analysis

| Treatment | Soil Chemical Properties Analysis Results |
|---|---|
| Moisture Content (%) | 6.91 |
| pH | 6 (Slightly Acidic) |
| N (%) | 0.22 (Moderate) |
| P (ppm) | 1 (Very Low) |
| K (me/100 g) | 0.69 (High) |
| Organic Carbon (%) | 0.6 (Very Low) |
| Organic Matter (%) | 1.1 (Low) |

The final soil analysis of moisture content reveals varying responses to different levels of mycorrhiza and organic fertilizer applications (Table 3). At control, the moisture content increases with 100 g and 200 g of organic fertilizer, reaching a peak at 14.50%, before declining sharply at higher fertilizer levels. When 10 g of mycorrhiza is applied per plant, the moisture content is highest with no organic fertilizer (14.87%) and shows a general decline with increasing fertilizer amounts, albeit with a slight increase at 200 g. With 20 g of mycorrhiza per plant, moisture content varies less dramatically, generally maintaining moderate levels, with the highest content recorded at 400 g of organic fertilizer. These findings indicate that both mycorrhiza and organic fertilizer significantly influence soil moisture content, but the optimal combination for maximum moisture retention varies.

Table 3. Final soil analysis of moisture content (%)

| Mycorrhiza (g/plant) | Organic Fertilizer (g/plant) | | | | |
|---|---|---|---|---|---|
| | 0 | 100 | 200 | 300 | 400 |
| 0 | 7.89 | 10.13 | 14.50 | 12.73 | 6.82 |
| 10 | 14.87 | 10.33 | 12.12 | 10.51 | 7.24 |
| 20 | 9.44 | 11.71 | 9.31 | 10.38 | 11.02 |

The soil prior to treatment had a pH of 6, categorized as slightly acidic (Table 2). The final soil analysis for pH (Table 4) indicated an increase to 6.6 (neutral) under treatments without organic fertilizer and with mycorrhizal applications of 10 and 20 g/plant. However, not all treatments resulted in a pH increase. The combination of 100 g/plant organic fertilizer and 20 g/plant mycorrhiza, as well as the sole treatment of 200 g/plant organic fertilizer, showed a pH decrease, although still within the same qualitative range as the initial slightly acidic pH. The sole application of organic fertilizer only increased pH by 1.64% from the initial pH. Sole mycorrhiza application increased the pH by up to 9.1% from the initial pH. The combination of organic fertilizer and mycorrhiza increased the pH by 3.2% from the initial value. According to research by Della et al. (2020), mycorrhiza inoculation can alter soil pH, increasing it in acidic soils, whereas control treatments without microbial inoculation had the lowest pH compared to other treatments.

Table 4. Final soil analysis of pH

| Mycorrhiza (g/plant) | Organic Fertilizer (g/plant) | | | | |
|---|---|---|---|---|---|
| | 0 | 100 | 200 | 300 | 400 |
| 0 | 6.5 (SA) | 6.0 (SA) | 5.9 (SA) | 6.0 (SA) | 6.1 (SA) |
| 10 | 6.6 (N) | 6.1 (SA) | 6.0 (SA) | 6.1 (SA) | 6.0 (SA) |
| 20 | 6.6 (N) | 5.9 (SA) | 6.1 (SA) | 6,1 (SA) | 6.2 (SA) |

Notes: Neutral (N), Slightly Acidic (SA)

The available nitrogen (N) content before treatment was 0.22% (medium). According to the final soil analysis (Table 5), available N increased in most treatments compared to before treatment (Table 2). The combination treatment of 400 g/plant organic fertilizer and 10 g/plant mycorrhiza resulted in the highest N content. This is attributed to the adequate N content in organic fertilizer (Table 3). Research by Minardi et al. (2011) indicates that organic fertilizers improve soil structure, facilitate nutrient absorption, and provide beneficial soil bacteria. By employing effective rhizobial strains with compatible legume cultivars, it is possible to decrease the need for nitrogenous fertilizers, as well as the energy inputs and greenhouse gas emissions related to their production and use (Abd-Alla et al., 2023). In contrast, the control treatment showed a decrease in available N, influenced by suboptimal conditions such as nutrient loss due to the mobile nature of nitrogen (Sieczko et al., 2023).

Table 5. Final soil analysis of available N content (%)

| Mycorrhiza (g/plant) | Organic Fertilizer (g/plant) | | | | |
|---|---|---|---|---|---|
| | 0 | 100 | 0 | 300 | 0 |
| 0 | 0.19 (L) | 0.24 (M) | 0.30 (M) | 0.29 (M) | 0.27 (M) |
| 10 | 0.28 (M) | 0.22 (M) | 0.21 (M) | 0.20 (M) | 0.30 (M) |
| 20 | 0.27 (M) | 0.31 (M) | 0.21 (M) | 0.27 (M) | 0.27 (M) |

Notes: L=Low, M=Medium.

The available phosphorus (P) content before treatment was 1ppm (very low) (Table 2). Post-treatment, available P generally increased, including in the control treatment (Table 6). This increase is likely due to Indigofera tinctoria, a leguminous plant that easily forms symbiotic relationships with soil microbes, enhancing soil quality. Legumes naturally establish symbiosis with rhizobium bacteria, which improves soil conditions. These bacteria release root exudates that facilitate legume-rhizobia symbiosis, boosting the presence of beneficial microbes (Alemneh et al., 2020).

Table 6. Final soil analysis of available P content (ppm)

| Mycorrhiza (g/plant) | Organic Fertilizer (g/plant) | | | | |
|---|---|---|---|---|---|
| | 0 | 100 | 0 | 300 | 0 |
| 0 | 3.5 (VL) | 3.0 (VL) | 4.4 (VL) | 3.7 (VL) | 2.3 (VL) |
| 10 | 6.2 (L) | 2.3 (VL) | 3.8 (VL) | 2.6 (VL) | 3.3 (VL) |
| 20 | 3.5 (VL) | 1.7 (VL) | 4.5 (VL) | 3.9 (VL) | 3.3 (VL) |

Notes: VL=Very Low, L=Low.

The highest P content, 6.2 ppm (low), was observed with the application of 10 g mycorrhiza per plant. Aleixo et al. (2020) found that leguminous plants inoculated with mycorrhiza form effective root symbioses. These symbioses produce external hyphae that release phosphatase enzymes, converting phosphorus into accessible forms for plants.

The available potassium (K) content before treatment was 0.69 l/100 $g^{-1}$ (high) (Table 2). Post-treatment, available K increased across all treatments (Table 7). The combination of 400 g/plant organic fertilizer and 20 g/plant mycorrhiza resulted in the highest available K content. The combination of organic materials and mycorrhiza in legume plants can enhance available K in soil due to the decomposition of organic materials releasing organic acids that increase available K. AMF (Arbuscular Mycorrhizal Fungi) are obligate symbionts, not parasites, that require a host plant to complete their life cycle. They enhance crop productivity by boosting the uptake of water and nutrients, including nitrogen (N), phosphorus (P), and potassium (K) (Anderson et al., 2018). It is crucial in protein and carbohydrate formation, strengthening plant organs to prevent shedding of flowers, fruits, and leaves.

Table 7. Final soil analysis of available K content (l/100 g)

| Mycorrhiza (g/plant) | Organic Fertilizer (g/plant) | | | | |
|---|---|---|---|---|---|
| | 0 | 100 | 0 | 300 | 0 |
| 0 | 0.70 (H) | 0.81 (H) | 0.80 (H) | 0.92 (H) | 0.85 (H) |
| 10 | 0.83 (H) | 0.78 (H) | 0.79 (H) | 0.94 (H) | 0.81 (H) |
| 20 | 0.74 (H) | 0.77 (H) | 0.85 (H) | 0.91 (H) | 0.94 (H) |

Notes: H=High. Ratings based on Soil Research Institute 2009

Organic fertilizer from *Indigofera tinctoria* extraction waste, mycorrhiza, and their combination can increase organic carbon and organic matter content in soil. Before treatment, the organic carbon content was 0.6% (very low) due to intensive cultivation of *Indigofera tinctoria* (Table 2). Post-treatment, organic carbon content generally increased (Table 8). The combination of 200 g/plant organic fertilizer and 10 g/plant mycorrhiza resulted in the highest organic carbon content of 3.5% (medium). This increase is due to the organic fertilizer made from natural dye plant waste, containing sufficient organic carbon and organic matter (Table 3). Adding organic matter from *Indigofera tinctoria* extraction waste and mycorrhiza increased organic carbon after treatment. This aligns with research by Budiastuti et al. (2020b), which found that using organic fertilizers can enhance soil productivity, prevent land degradation, and increase soil humus content.

Table 8. Final soil analysis of organic carbon content (%)

| Mycorrhiza (g/plant) | Organic Fertilizer (g/plant) | | | | |
|---|---|---|---|---|---|
| | 0 | 100 | 0 | 300 | 0 |
| 0 | 1.9 (L) | 2 (L) | 3.5 (M) | 2.1 (M) | 1.9 (L) |
| 10 | 1.8 (L) | 1.6 (L) | 2.6 (M) | 1.5 (L) | 1.8 (L) |
| 20 | 2 (L) | 1.2 (L) | 1.9 (L) | 1.7 (L) | 1.8 (L) |

Notes: L=Low, M=Medium.

Soil organic matter content correlates with increases in soil organic carbon, meaning higher organic carbon results in higher organic matter content in soil (Table 9). Research by Setiawati et al. (2020) found that adding rice plant residues to soil can increase organic carbon from 1.25% to 2.43% due to improved microbial quantity and activity, enhancing soil nutrients. Mycorrhiza significantly influences soil organic carbon storage by controlling organic matter decomposition, modifying the amount of nitrogen available to free-living microbes (Tatsumi et al., 2020). Organic matter decomposition products are partially used and absorbed by microbes, while some transform into humus and organic compounds, increasing soil organic carbon (Simanungkalit et al., 2006).

Table 9. Final soil analysis of organic matter content (%)

| Mycorrhiza (g per plant) | Organic Fertilizer (g/plant) | | | | |
|---|---|---|---|---|---|
| | 0 | 100 | 0 | 300 | 0 |
| 0 | 3.2 (H) | 3.4 (H) | 6.1 (H) | 3.5 (H) | 3.2 (H) |
| 10 | 3.1 (H) | 2.7 (M) | 4.5 (H) | 2.7 (M) | 3.1 (H) |
| 20 | 3.4 (H) | 2.1 (M) | 3.3 (H) | 3.0 (H) | 3.0 (H) |

Notes: H=High, M=Medium.

### 3.2. Mycorrhizal infection

Based on the analysis of variance using an F-test with a 5% significance level, it was found that there is no interaction between organic fertilizer from *Indigofera tinctoria* extraction waste and mycorrhiza. Application of organic fertilizer from *Indigofera tinctoria* extraction waste alone significantly affects the percentage of *Indigofera tinctoria* roots infected by mycorrhiza at 4 WAP. The dosage of mycorrhiza significantly influences the percentage of *Indigofera tinctoria* roots infected by mycorrhiza at 4 and 8 WAP.

Based on the variance analysis at 4 WAP as shown in Table 10, it is evident that organic fertilizer from *Indigofera tinctoria* extraction waste at 300 g/plant significantly differs from the treatment without organic fertilizer in terms of the percentage of mycorrhizal-infected roots of *Indigofera tinctoria*. The application of organic fertilizer significantly increased the percentage of mycorrhizal-infected roots by 50.18% at 4 WAP. This is attributed to the compatibility between mycorrhiza and the host plantinfluenced by the amount of organic fertilizer applied. Organic fertilizer from *Indigofera tinctoria* extraction waste contains comprehensive organic materials. According to research by Sari & Indrawati (2019), organic fertilizer can improve the physical, chemical, and biological properties of soil. Additionally, organic fertilizers contain organic materials that create a favorable rhizosphere environment for microbial survival.

Table 10. Mean percentage of *Indigofera tinctoria* roots infected with mycorrhiza at 4 WAP

| Mycorrhiza (g/plant ) | Organic Fertilizer (g/ plant$^{-1}$) | | | | | Average |
|---|---|---|---|---|---|---|
| | 0 | 100 | 200 | 300 | 400 | |
| 0 | 1,67 | 0,00 | 0,00 | 1,67 | 3,33 | 1,33b |
| 10 | 13,33 | 30,00 | 20,00 | 35,00 | 46,67 | 29,00a |
| 20 | 18,33 | 28,33 | 36,67 | 45,00 | 45,00 | 34,67a |
| Average | 11,11b | 19,44ab | 18,89ab | 27,22a | 31,67a | - |

Note: The symbol (-) indicates no interaction between factors. Numbers followed by the same letter within the same row and column indicate no significant difference according to the DMRT test at 5% significance level.

Further DMRT (Duncan Multiple Range Test) analysis at a 5% significance level indicated that the single treatment of mycorrhiza at a dose of 10 g/plant significantly differs from the treatment without mycorrhiza, but does not significantly differ from the dose of 20 g/plant regarding the percentage of mycorrhizal infection in the roots of *Indigofera tinctoria* at 4 WAP (Table 11). However, at 8 WAP, the highest percentage of mycorrhizal-infected roots of *Indigofera tinctoria* was observed in the single treatment of mycorrhiza at 20 g plant-1, showing significant differences from the treatments with 10 g/plant and without mycorrhiza. The application of mycorrhiza increased mycorrhizal infection in roots by 95.41% at 4 WAP and 81.82% at 8 WAP. This high mycorrhizal infection rate is influenced by soil fertility. This is consistent with the findings of Sari & Indrawati (2019) that soil conditions significantly affect the presence of mycorrhiza, particularly in terms of soil physical and chemical fertility. Moreover, the symbiosis between plants and mycorrhiza can influence plant metabolism, root formation, and root membrane permeability. Mycorrhiza can form mutualistic symbiosis with plant roots, thereby benefiting both parties where mycorrhiza obtains carbon from photosynthesis while providing nutrient supply to the plant roots. Additionally, mycorrhiza aids in decomposing organic matter. This aligns with the research of Hernández et al. (2019) indicating that mycorrhiza application can enhance the availability of phosphorus (P). Mycorrhizal-infected roots can utilize organic matter in the soil by releasing root exudates containing phosphatase enzymes to dissolve insoluble and unavailable P into an available form. Furthermore, mycorrhiza possesses external hyphae to increase the nutrient absorption surface area.

Table 11. Mean percentage of *Indigofera tinctoria* roots infected with mycorrhiza at 8 weeks after planting

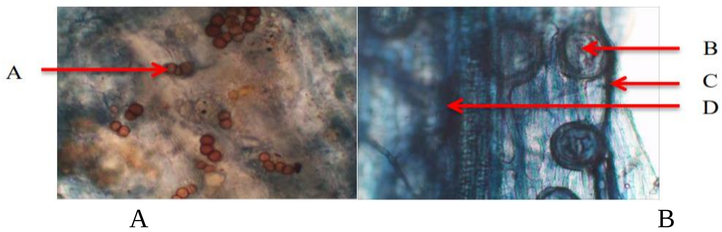
| Mycorrhiza (g/plant ) | Organic Fertilizer (g/plant ) | | | | | Average |
|---|---|---|---|---|---|---|
| | 0 | 100 | 200 | 300 | 400 | |
| 0 | 11.67 | 10.00 | 5.00 | 10.00 | 10.00 | 9.33c |
| 10 | 21.67 | 30.00 | 30.00 | 56.67 | 45.00 | 36.67b |
| 20 | 41.67 | 48.33 | 53.33 | 58.33 | 55.00 | 51.33a |
| Average | 25.00 | 29.44 | 29.44 | 41.67 | 36.67 | - |

Note: The symbol (-) indicates no interaction between factors. Numbers followed by the same letter in the same column indicate no significant difference according to the DMRT test at 5% significance level.

Both the control treatment and the treatment without mycorrhizal inoculation showed mycorrhizal infection in the roots of *Indigofera tinctoria* (Tables 10 and 11). This occurrence is due to the presence of indigenous mycorrhiza in the soil medium. According to Zulya et al. (2016), mycorrhizal infection was also found in treatments without mycorrhizal inoculation because the soil naturally contained indigenous mycorrhiza. Therefore, mycorrhizal infection was observed in the control treatment during the observation period.

Based on observations, mycorrhiza associated with infected roots exhibit structures such as arbuscules, vesicles, hyphae, and spores within the roots (Figure 1). At 4 WAP, only mycorrhizal spores were found, whereas at 8 WAP, mycorrhiza with arbuscular structures, vesicles, and hyphae were clearly observed. This is because after 4 WAP, mycorrhiza becomes active in spreading, infecting, and forming hyphae. Arbuscules establish a mutually beneficial symbiosis with specific fungal groups. Moreover, *Indigofera tinctoria* is a leguminous plant that naturally forms symbiosis with soil microbes, including rhizobia.

According to Setyaningrum et al. (2020), mycorrhiza application on *Indigofera tinctoria* synergistically interacts with rhizobia to enhance soil nutrients. This aligns with the findings of Budiastuti et al. (2020b), indicating that combining 100% light intensity treatment with dual inoculation of mycorrhiza and rhizobia increases root biomass and nodules in *Indigofera tinctoria*, as plants receive more nutrients while mycorrhiza obtains photosynthates from the plants. The application of mycorrhiza results in infection on the roots of *Indigofera tinctoria*. The observed mycorrhizal infection, both with organic fertilizer treatment and mycorrhiza treatment, can be seen in the following table.

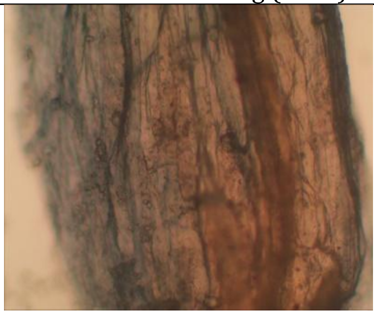
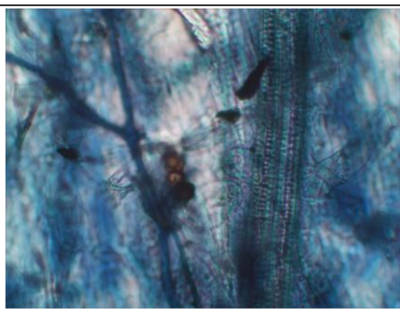
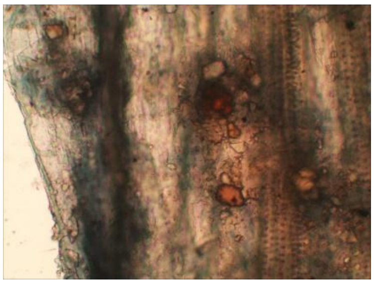
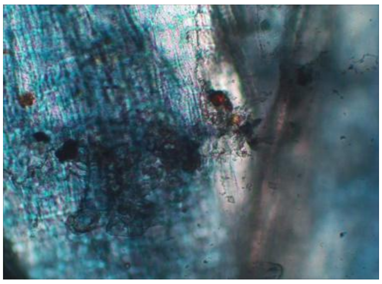
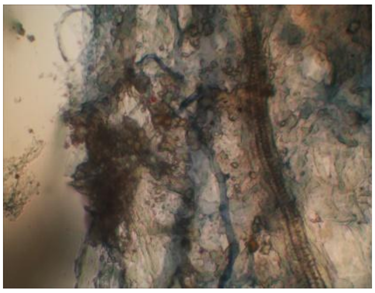
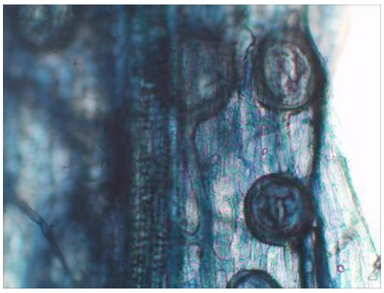
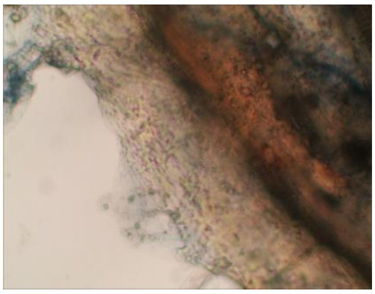
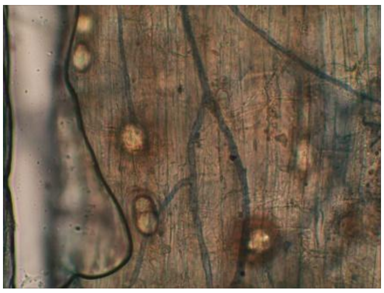



Note: A = spores, B = vesicles, C = hyphae, and D = arbuscules

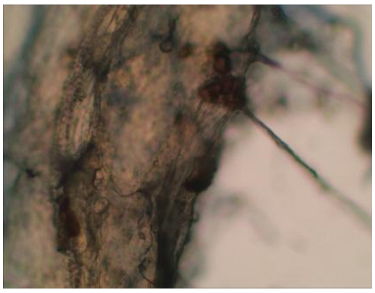
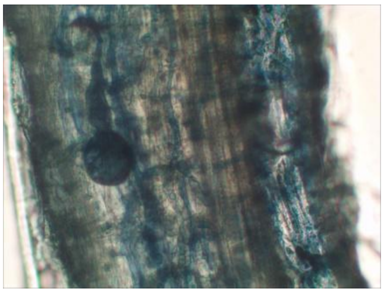
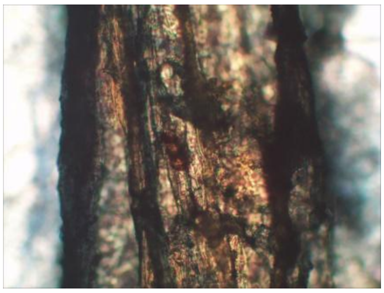
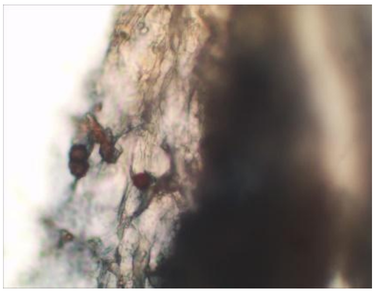
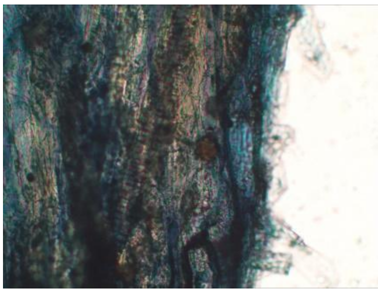
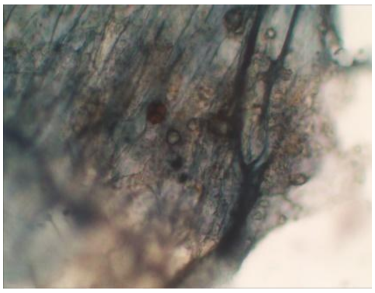
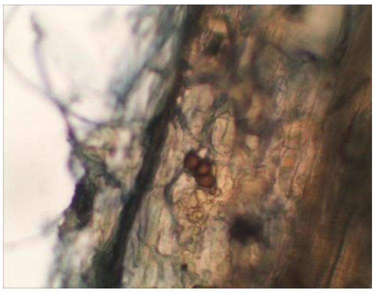
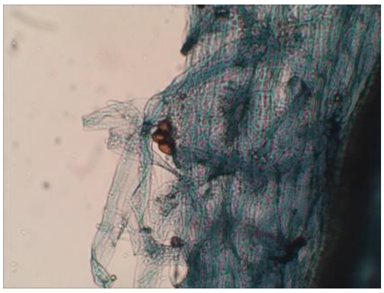
Fig. 1. (a) Mycorrhiza at 4 WAP, (b) Mycorrhiza at 8 WAP - observations under a microscope at 40x magnification.

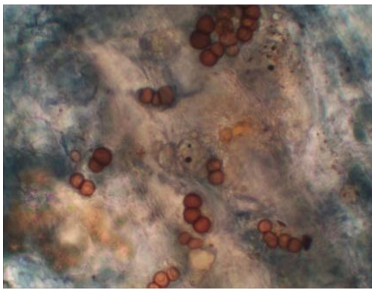
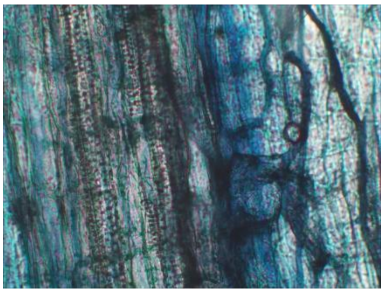
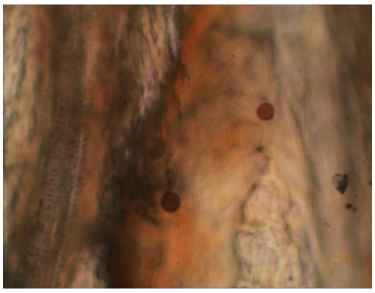
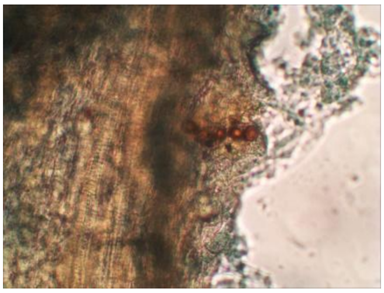
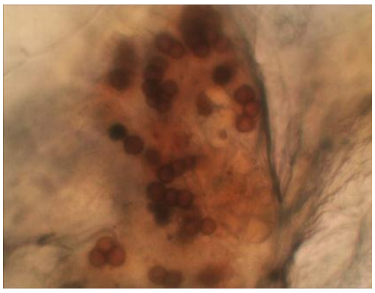
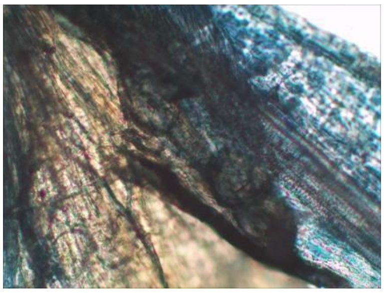
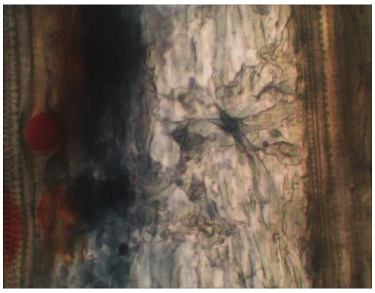
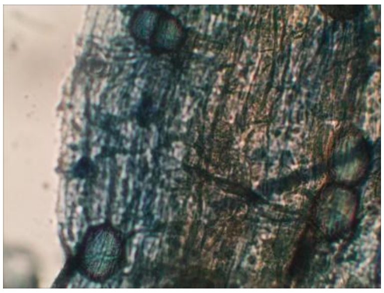
The application of mycorrhiza in the study involved inoculum (material containing mycorrhizal fungus spores). According to the research, by 8 weeks after planting (WAP), mycorrhizal infection had spread extensively, indicated by clearly visible mycorrhizal structures (Table 12). Based on research by Rini et al. (2020), mycorrhizal spores infect maize roots within 1 to 2 weeks after application, and these spores can persist in the soil for up to 6 months without a host plant, with some even surviving for up to two years. The mycorrhiza infecting *Indigofera tinctoria* roots belongs to the endomycorrhizal type, as the fungal hyphae penetrate the roots into the cortex, which is observed under a microscope. According to Hajoeningtijas (2009), endomycorrhiza heavily depends on the energy flow provided by the host planting the form of photosynthates for survival and reproduction. However, further research is needed to understand the ability of spores in the soil to effectively infect plants in the absence of a host.

Table 12 Observation of mycorrhizal infection on *Indigofera tinctoria* roots under the microscope for each treatment

| Treatment | Mycorrhizal infection on plant roots | |
|---|---|---|
| | 4 Weeks After Planting (WAP) | 8 WAP |
| Control |  Infected |  Infected |
| 10g mycorrhiza |  Infected |  Infected |
| 20g mycorrhiza |  Infected |  Infected |
| 100g organic fertilizer |  Not infected |  Infected |

 https://doi.org/10.61511/jek.v2i1.2024.930

| Treatment | Mycorrhizal infection on plant roots | |
|---|---|---|
| | 4 Weeks After Planting (WAP) | 8 WAP |
| 100g organic fertilizer+20g mycorrhiza | <br>Infected | <br>Infected |
| 200g organic fertilizer | <br>Not infected | <br>Infected |
| 200g organic fertilizer+10g mycorrhiza | <br>Infected | <br>Infected |
| 200g organic fertilizer+20g mycorrhiza | <br>Infected | <br>Infected |
| Organic fertilizer 300g | <br>Infected | <br>Infected |

| Treatment | Mycorrhizal infection on plant roots | |
|---|---|---|
| | 4 Weeks After Planting (WAP) | 8 WAP |
| 300g organic feltilizer+20g mycorrhiza | <br>Infected | <br>Infected |
| 400g organic fertilizer | <br>Infected | <br>Infected |
| 400g organic fertilizer+ 10g mycorrhiza | <br>Infected | <br>Infected |
| 400g organic fertilizer+20g mycorrhiza | <br>Infected | <br>Infected |

### *3.3 Plant growth*

Based on the results of the analysis of variance with the F test at the 5% level, it shows that there is no interaction between extracted waste organic fertilizer and mycorrhiza. Single application of extracted waste organic fertilizer has no real effect (Table 13). The dose of mycorrhiza did not give a real effect on the height of *Indigofera tinctoria*. The observation results of *Indigofera tinctoria* height treated with organic fertilizer and mycorrhiza can be seen in the following table and graph.

Table 13. Effect of organic fertilizer and mycorrhiza treatment on *Indigofera tinctoria* height (cm) in 12 WAP

| Mycorrhiza (g/plant) | Organic Fertilizer (g/plant) | | | | | Average |
|---|---|---|---|---|---|---|
| | 0 | 100 | 200 | 300 | 400 | |
| 0 | 86.50 | 95.13 | 93.80 | 109.97 | 87.03 | 94.49 |
| 10 | 93.53 | 112.63 | 129.80 | 89.93 | 112.83 | 107.74 |
| 20 | 78.10 | 104.87 | 108.10 | 99.17 | 95.13 | 97.07 |
| Avrage | 86.04 | 104.21 | 110.57 | 99.69 | 98.33 | - |

Notes: The sign (-) indicates no interaction between factors.

Based on the results of the study, the height of *Indigofera tinctoria* plants in each treatment showed an increase in each week which can be seen in Figure 2. The treatment of natural dye waste organic fertilizer and mycorrhiza did not show significant differences. *Indigofera tinctoria* plant height in 12 WAP that showed higher results than all treatments was in the combination of organic fertilizer of *Indigofera tinctoria* waste as much as 200 g/plant and mycorrhiza 10 g/plant producing plant height of 129.80 cm (Table 13). This is because organic fertilizers contain complete nutrients. Plant height growth requires macro and micro nutrients. The availability of these elements is provided by organic fertilizer of *Indigofera tinctoria* extraction waste, based on laboratory analysis of the fertilizer has a pH content, moisture content, N, P, K, C-organic, and organic matter in fertilizer that is in accordance with the provisions of the Ministry of Agriculture regarding the requirements of organic fertilizer (Table 3). Based on the research, organic fertilizer has no effect on plant height. In contrast to the research of Paulus et al. (2020) that the provision of organic fertilizers derived from raw materials of gamal leaf legume plants (*Gliricia sepium*) has a real effect on increasing plant height, because organic fertilizers have complete nutrients.

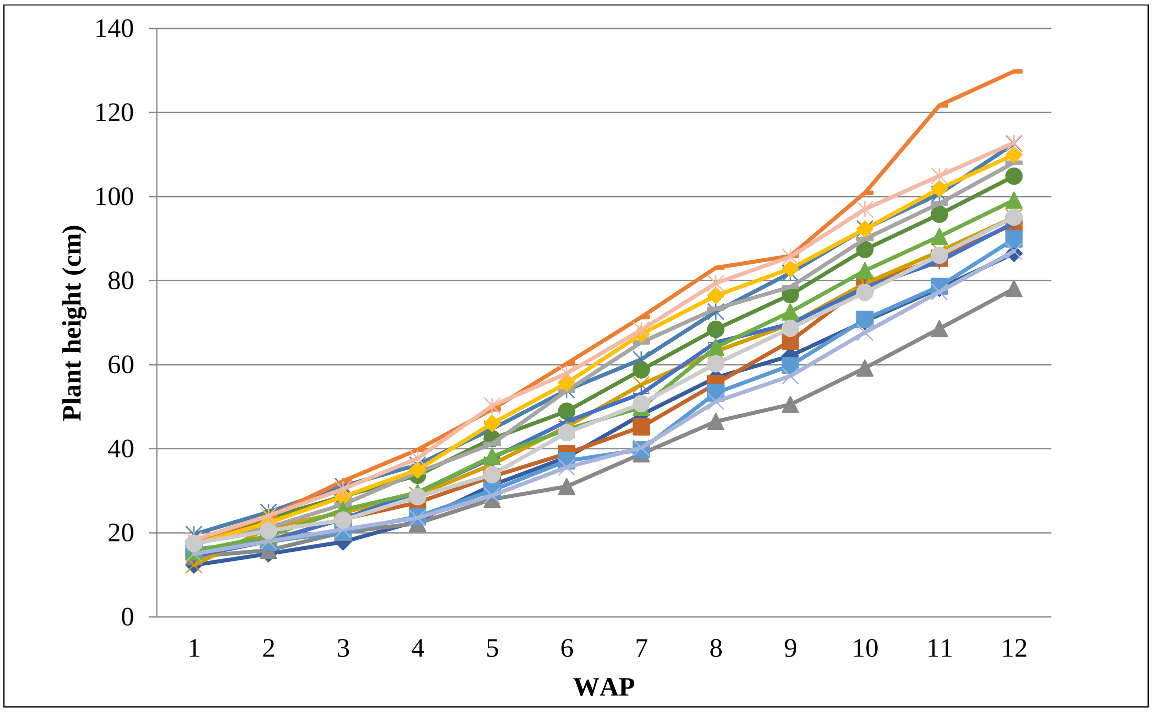



Fig. 2. Graph of *Indigofera tinctoria* plant height

The provision of mycorrhiza is used to increase the available P element. Based on research conducted by Laksono and Karyono (2017) on *Indigofera zollingeriana* that the provision of 10 g/plant mycorrhiza has an effect on plant height. This is because mycorrhiza is able to produce hyphae to expand the field of nutrient absorption. Therefore, the combination of organic fertilizer and mycorrhiza can support the growth of *Indigofera* *tinctoria* plant height because plants can absorb complete macro and micro nutrients available in the soil so that photosynthesis can run optimally.

Based on the dry weight of *Indigofera tinctoria*, the growth rate over a 4-week interval can be determined. The variance analysis using an F-test at a 5% significance level showed no interaction between organic fertilizer from *Indigofera tinctoria* extraction waste and mycorrhiza. The application of organic fertilizer from *Indigofera tinctoria* extraction waste alone did not have a significant effect. Similarly, different doses of mycorrhiza did not significantly affect the growth rate of *Indigofera tinctoria*.

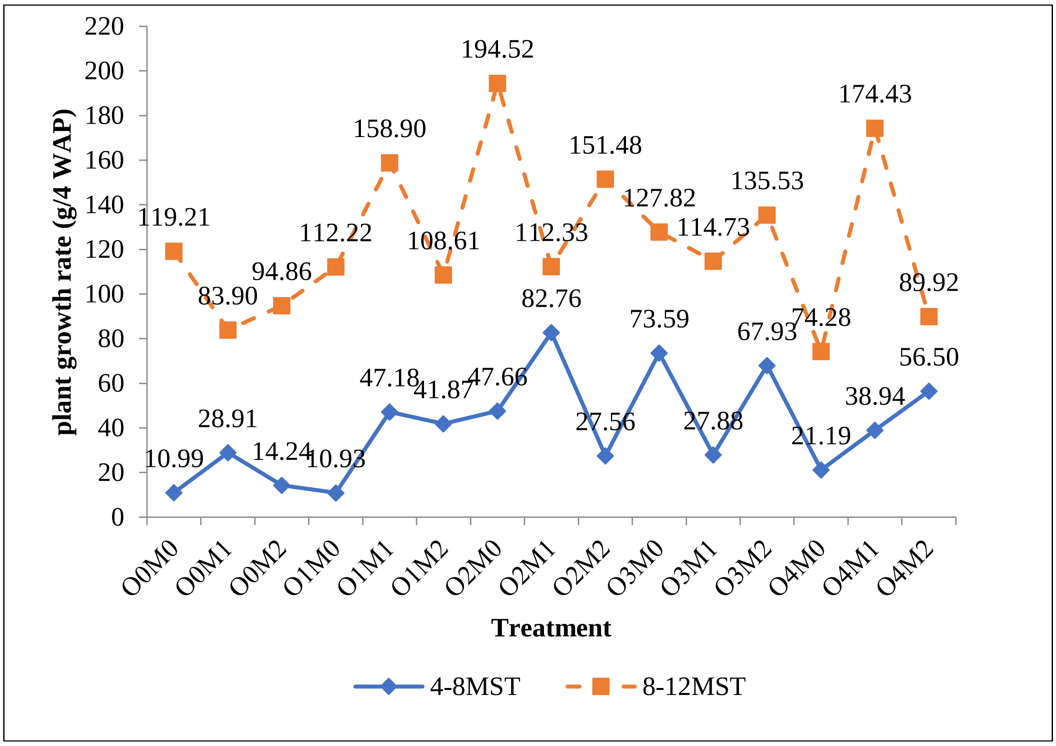



Fig. 3. Growth rate based on dry weight of *Indigofera tinctoria*.

Based on the observations, the application of mycorrhiza has positively impacted the growth of *Indigofera tinctoria*, as evidenced by the increased dry weight every 4 weeks. The combination of 200 g/plant of organic fertilizer from *Indigofera tinctoria* extraction waste and 10 g/plant of mycorrhiza resulted in higher plant growth rates, specifically 82.96 g/4 weeks between 4-8 WAP, while the application of 200 g/plant of organic fertilizer alone resulted in the highest growth rate of 194.52 g per 4 weeks between 8-12 WAP. This increase is attributed to *Indigofera tinctoria*'s ability to effectively absorb nutrients due to the organic fertilizer from *Indigofera tinctoria* extraction waste containing essential nutrients. According to Li et al. (2023), organic fertilizers can improve soil conditions and enhance soil fertility in terms of physical, chemical, and biological aspects. Biological fertilizers have a better impact on plant growth rates compared to controls.

### *3.4 Production*

Based on the analysis of variance using an F-test at a 5% significance level, there was no interaction observed between organic fertilizer from extraction waste and mycorrhiza. Single application of organic fertilizer from extraction waste significantly influenced the fresh weight of *Indigofera tinctoria* at 8 weeks after planting (WAP). Mycorrhiza dosage significantly affected the fresh weight of *Indigofera tinctoria* at 4 WAP (Table 14).

Sole application of 10 g/plant mycorrhiza significantly differed from treatment without mycorrhiza and the 20 g/plant mycorrhiza dose regarding the fresh weight of *Indigofera tinctoria* at 4 weeks after planting (WAP) (Table 14). The mycorrhiza dose of 10 g/plant resulted in the highest fresh weight of *Indigofera tinctoria* at 4 WAP, with an increase of 36.93% compared to the treatment without mycorrhiza. This is because mycorrhiza produces hyphae to expand the absorption area and can increase the availability of phosphorus (P) in the soil, thereby increasing the fresh weight of plants. This aligns with research by Hernández et al. (2019) which suggests that mycorrhiza can enhance the availability of phosphorus in the soil. The total fresh weight of *Indigofera tinctoria* is related to the metabolic yield that occurs in the plants. According to research by Rahman et al. (2020), the provision of mycorrhiza in legumes can also improve the availability of other nutrients such as iron (Fe), which plays a crucial role in chlorophyll formation and in making the photosynthesis process efficient. In addition, environmental factors such as light also have an impact on the fresh weight of plants. This is consistent with research by Setyaningrum et al. (2020) that light greatly affects the fresh weight of *Indigofera tinctoria*, with the highest fresh weight observed under 100% light intensity due to its role in photosynthesis. Low light can reduce photosynthesis and affect plant fresh weight production.

Table 14. Mean fresh weight of *Indigofera tinctoria* at 4 WAP

| Mycorrhiza (g/plant ) | Organic Fertilizer (g/plant ) | | | | | Average |
|---|---|---|---|---|---|---|
| | 0 | 100 | 200 | 300 | 400 | |
| 0 | 9.98 | 11.97 | 8.11 | 8.2 | 10.02 | 9.65b |
| 10 | 15.78 | 13.25 | 13.89 | 13.8 | 20.06 | 15.3a |
| 20 | 12.82 | 7.91 | 10.38 | 12.03 | 15.72 | 11.77b |
| Average | 12.86 | 11.04 | 10.79 | 11.34 | 15.27 | - |

Note: (-) indicates no interaction between factors. The same letters in the same column indicate no significant difference based on the DMRT test at 5% significance level.

Based on the results shown in Table 15, organic fertilizer dose of 200 g/plant significantly differed from treatment without organic fertilizer, but not significantly different from dose of 300 g/plant regarding the fresh weight of *Indigofera tinctoria* at 8 WAP. Sole application of organic fertilizer 200 g/plant is effective in increasing the fresh weight of *Indigofera tinctoria* at 8 WAP by 64.54%. This is due to the organic fertilizer from extraction waste of *Indigofera tinctoria* containing complete available nutrients (Table 3). This is supported by research by Christophe et al. (2019) that the provision of organic fertilizer to plants can increase biomass from legumes.

Table 15. Mean Fresh Weight of *Indigofera tinctoria* at 8 WAP

| Mycorrhiza (g/plant) | Organic Fertilizer (g/plant) | | | | | Average |
|---|---|---|---|---|---|---|
| | 0 | 100 | 200 | 300 | 400 | |
| 0 | 30.44 | 49.11 | 112.46 | 188.85 | 58.84 | 87.94 |
| 10 | 77.44 | 129.47 | 199.42 | 81.20 | 104.71 | 118.45 |
| 20 | 41.14 | 94.99 | 108.44 | 165.06 | 166.40 | 115.21 |
| Average | 49.68b | 91.19ab | 140.11a | 145.04a | 109.98ab | - |

Note: (-) indicates no interaction between factors. The same letters in the same row indicate no significant difference based on the DMRT test at 5% significance level.

Based on the analysis of variance using an F-test at a 5% significance level, there was no interaction observed between organic fertilizer from extraction waste and mycorrhiza. Sole application of organic fertilizer from extraction waste did not significantly affect the dry weight of *Indigofera tinctoria*. Mycorrhiza dosage significantly affected the dry weight of *Indigofera tinctoria* at 4 weeks after planting (WAP).

Based on the results of the research in Table 16. shows that the single treatment of mycorrhiza doses of 10 and 20 g/plant is not significantly different, but the mycorrhiza dose of 10 g/plant is significantly different from without mycorrhiza on the average dry weight of *Indigofera tinctoria* at 4 weeks after planting. The highest dry weight of 4.93 grams was in the application of mycorrhiza 10 g/plant, so that the dose of mycorrhiza 10 g/plant was effective to increase plantdry weight by 43.61%.

Table 16. Mean dry weight of *Indigofera tinctoria* at 4 WAP

| Mycorrhiza (g/plant) | Organic Fertilizer (g/plant) | | | | | Average |
|---|---|---|---|---|---|---|
| | 0 | 100 | 200 | 0 | 100 | |
| 0 | 2.22 | 3.21 | 2.69 | 2,53 | 3,26 | 2,78b |
| 10 | 4.79 | 4.03 | 5.37 | 4.9 | 5.56 | 4.93a |
| 20 | 3.96 | 3.42 | 3.63 | 3.76 | 4.88 | 3.93a |
| Average | 3.66 | 3.55 | 3.9 | 3.73 | 4.57 | - |

Note: (-) indicates no interaction between factors. The same letters in the same row indicate no significant difference based on the DMRT test at 5% significance level.

The results in Table 16 are in line with Putri et al. (2019) that the provision of mycorrhiza has a significant effect on the biomass of mung bean plants. Supported by the research of Setyaningrum et al. (2020) that mycorrhiza can symbiotic well with the roots of *Indigofera tinctoria* plants so that mycorrhiza can increase the biomass of *Indigofera tinctoria* because mycorrhiza and plant roots have a mutualistic symbiosis, namely roots infected with mycorrhiza help plants to absorb and provide nutrients, while plants provide some of their photosynthates. This is in line with Brigido et al. (2019) that various types of soil microbiota can increase plant growth by different amounts in each location, because endophytic bacteria can also symbiotic with rhizobia to fix $N_2$, this nitrogen is very useful for forming plant growth so as to increase plant biomass.

## 4. Conclusions

The application of organic fertilizer from natural dye extraction waste alone on *Indigofera tinctoria* at a dose of 200 g/plant (equivalent to 8 tons/ha) or mycorrhiza at a dose of 10 g/plant (equivalent to 0.4 tons/ha) with a planting distance of 50x50 cm is more effective for farmers in increasing *Indigofera tinctoria* yields. Additionally, the processing of *Indigofera tinctoria* extraction waste into organic fertilizer should be continued to create sustainable agriculture. Further research is needed on mycorrhiza application to *Indigofera tinctoria* regarding mycorrhiza availability in the soil when the host plantis absent.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## Author Contribution

Conceptualization, M.T.S.B., S.S., A.I.N., & D.S.; Methodology, N.I.D.A., M.T.S.B., S.S., A.I.N., & D.S.; Software, N.I.D.A., D.S., & I.R.M.; Validation, S.S., & A.I.N.; Formal Analysis, N.I.D.A.; Investigations, N.I.D.A., D.S., & I.R.M.; Resources, M.T.S.B & N.I.D.A.; Data Curation, N.I.D.A., S.S., & A.I.N.; Writing – Original Draft Preparation, N.I.D.A.; Writing – Review & Editing, N.I.D.A., S.S., & A.I.N.; Visualization, N.I.D.A.; Supervision, M.T.S.B., S.S., A.I.N., D.S., & I.R.M.; Project Administration, N.I.D.A. & D.S.; and Funding Acquisition, M.T.S.B.


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## Ethical Review Board Statement

Not applicable.

## Informed Consent Statement
Not available.

## Data Availability Statement
Not available.

## Conflicts of Interest
The authors declare no conflict of interest.

## Open Access


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## Biographies of Authors

**Nor Isnaeni Dwi Arista,** Department of Agrotechnology, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, 57126, Indonesia

- Email : dewi.arista99@student.uns.ac.id
- ORCID: 0000-0001-7196-2838
- Web of Science ResearcherID: JKI-9867-2023
- Scopus Author ID: 58185882900
- Homepage: N/A

**Maria Theresia Sri Budiastuti,** Lecturer, Department of Agrotechnology, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, 57126, Indonesia

- Email : mariatheresia@staff.uns.ac.id
- ORCID: 0000-0002-4144-4041
- Web of Science ResearcherID: N/A
- Scopus Author ID: 57194587611
- Homepage: https://iris1103.uns.ac.id/profil-0005125905.asm

**Supriyono,** Lecturer, Department of Agrotechnology, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, 57126, Indonesia

- Email : supriyono59@staff.uns.ac.id
- ORCID: 0000-0002-5970-9204
- Web of Science ResearcherID: N/A
- Scopus Author ID: 57201778008
- Homepage: https://iris1103.uns.ac.id/profil-0011075907.asm

**Aprilia Ike Nurmalasari,** Lecturer, Department of Agrotechnology, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, 57126, Indonesia

- Email : ikeaprilia@staff.uns.ac.id
- ORCID: 0000-0002-1390-854X
- Web of Science ResearcherID: AAD-8024-2022
- Scopus Author ID: 57216670665
- Homepage: https://iris1103.uns.ac.id/profil-6606.asm

**Desy Setyaningrum,** Lecturer, Department of Agribusiness, Vocational School, Universitas Sebelas Maret, Surakarta, 57126, Indonesia

- Email : desy_setyaningrum@staff.uns.ac.id
- ORCID: 0000-0001-6345-7241
- Web of Science ResearcherID: N/A
- Scopus Author ID: 57221447417
- Homepage: https://iris1103.uns.ac.id/profil-7617.asm

**Ida Rumia Manurung,** Lecturer, Department of Agrotechnology, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, 57126, Indonesia

- Email : idarumia@staff.uns.ac.id
- ORCID: 0000-0002-4164-3462
- Web of Science ResearcherID: N/A
- Scopus Author ID: 57220777112
- Homepage: https://iris1103.uns.ac.id/profil-6745.asm