The role of organic fertilizer from natural dye waste and mycorrhizal inoculation on the growth of *Indigofera tinctoria* L.

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Abstract. Management of *Indigofera tinctoria* as a natural dye produces organic waste that has not been utilized. One of the proper managements of organic waste is to process it into organic fertilizer. This study examines the role of organic fertilizer waste and mycorrholiae on the growth and yield of *Indigofera tinctoria*. The study used a completely randomized block design with two factors: organic waste fertilizer and mycorrhizae. The results showed that organic fertilizers and mycorrhizae did not affect the net assimilation and leaf area indexes. The combination of organic fertilizers with mycorrhizae supports leaf growth. The combination of 400 g.plant⁻¹ organic fertilizer and 10 g.plant⁻¹ mycorrhizal fertilizer increased the number of leaves by 257%. Organic fertilizer has a significant effect on the fresh weight of the crown, fresh weight and root biomass. Organic fertilizer dose of 200 g.plant⁻¹ increased fresh root weight, root biomass and fresh crown weight by 68.5%, 68.29% and 63.27% respectively. Mycorrhizae 10 g.plant⁻¹ increased root length by 23.54%. Leaf growth correlated with length, fresh weight and root biomass. Organic fertilizer from the extraction of *Indigofera tinctoria* is an effort to achieve zero waste to support plant growth.

1. Introduction

Natural dyes are dyes that come from nature. *Indigofera tinctoria* is a source of natural blue dye because it contains indigo pigment [1]. Indigo content is produced from the extraction of the leaves of this plant [2]. The extraction process of the natural colour from *Indigofera tinctoria* only yield 10% of the total raw materials; the remaining 90% is in the form of stem and leaf waste [3]. Previous research reported that *Indigofera tinctoria* waste was processed into biogas and was capable of producing electrical power at the maximum of 700 W [4]. The extraction waste contains organic materials that can be processed and utilized further. *Indigofera tinctoria* natural dye extraction waste can be used as organic fertilizer because it comes from legumes. Organic matter is derived from the weathering of legumes through biological processes with the help of decomposing organisms capable of binding N in the air so that it can increase N levels in plants [5]. One way to utilize organic waste is by making it into organic fertilizers.
Natural dye waste organic fertilizer was applied to *Indigofera tinctoria* cultivation to increase soil fertility. In order to improve soil fertility in dry land, organic fertilizers and several other technologies can be used. One technology that can be applied is the provision of mycorrhizae, which is expected to increase the growth and yield of *Indigofera tinctoria*. The combination of the use of organic fertilizers and mycorrhizae can be applied simultaneously so that plants can absorb P in more significant quantities than plants without mycorrhizae [6]. In addition, the application of mycorrhizae can act in mutual symbiotic manners with the roots of *Indigofera tinctoria* plants and microorganisms in the soil [7]. Based on the above discussion, there is a need for research to support the application of organic fertilizers from *Indigofera tinctoria* waste and mycorrhizae in increasing the growth and yield of *Indigofera tinctoria*; this is because there are still limited references related to the application of these technologies.

2. Materials and methods

The research was conducted from May to December 2020 at CV. Indigo Biru Baru, located in Puron Village, Bulu District, Sukoharjo Regency; the research location is at 110°51'49.44"E and 7°48'54.3"S with an altitude of 125 meters above the sea level. Based on the results of soil analysis, the soil had a neutral pH of 6.0 with a slightly acidic category, 0.22% nitrogen (N), one ppm phosphorous (P), and 0.69 me 100 g of soil potassium (K). C-organic was low at 0.6%, and organic matter was low at 1.1%. The materials used were *Indigofera tinctoria* seeds, waste extracted from *Indigofera tinctoria*, mycorrhizal inoculum, soil, cattle manure, bran, molasses, EM4 and distilled water. The tools used are a chopper machine, scales, plastic drums for composting containers (barrels), sprayer, blender, wooden trays, seedling polybags, tarpaulins, knives, hoe, watering can and oven. The fertilization processing method used was anaerobic composting.

The study used a factorial Completely Randomized Block Design (RCBD) with 2 treatment factors. The first factor was the dose of organic fertilizer extracted from *Indigofera tinctoria*, consisted of 5 levels, namely 0, 100, 200, 300, and 400 g.plant-1. The second factor was the dose of mycorrhizal inoculum consisted of 3 levels, namely 0, 10, and 20 g.plant-1. Hence, there were 15 treatments combinations and three replicates. Net Assimilation Rate (NAR) (g/cm²/week) represented the dry weight accumulation rate per unit time. The period for measuring NAR was carried out according to the parameter period for measuring leaf area and dry weight. The NAR formula is as follows:

\[ \text{NAR} = \frac{(w_2 - w_1)(\ln A_2 - \ln A_1)}{(IA_2 - IA_1)(t_2 - t_1)} \]

where:
- \( w \) = plant dry weight
- \( \ln \) = natural logarithm,
- \( IA \) = leaf area
- \( t \) = time

Leaf area index was estimated using the gravimetric method. The variables observed were growth variables for leaf number, leaf area index, net assimilation rate, crown fresh weight, fresh root weight, root biomass and root length. The obtained data were analyzed using Analysis of Variance (ANOVA) at the confidence level of 5% level. Post hoc tests were carried out with the Duncan's Multiple Range Test (DMRT) at the significance level of 5%.

3. Results and discussion

The results showed that the combination of organic fertilizers and mycorrhizae did not affect the leaf area index (Table 1). Leaf area index is influenced by the formation of leaf organs that requires available nitrogen to make up chlorophyll and support photosynthesis. Leaf area is strongly influenced by nitrogen to stimulate vegetative growth, including leaves; nitrogen has an important role in plant photosynthesis, metabolism, and respiration [8].

Organic fertilizer and mycorrhizal did not have any effect on the net assimilation rate (Table 2). This is because the leaf area in all treatments was not significantly different, so the leaves on the plants caught the sun and produced the same net assimilation rate. Based on the research of Ronga et al. [9] the growth
of the leaves of the plant was exposed to direct solar radiation so that the speed of CO\(_2\) assimilation increased in all treatments resulting in high net assimilation rate values. The organic fertilizer treatment of 200 g.plant\(^{-1}\) produced a higher net assimilation rate than the other treatments, which was 2.26 g/cm\(^2\)/week. Net assimilation rate showed a measure of the efficiency of photosynthesized leaf area to increase plant dry weight, so it can be interpreted as an increase in dry weight per unit time and per unit leaf area [10].

**Table 1.** Combination of organic fertilizers and mycorrhizal on leaf area index

| Mycorrhizae (g.plant\(^{-1}\)) | Organic fertilizer (g.plant\(^{-1}\)) | Average |
|-------------------------------|-------------------------------------|---------|
|                               | 0        | 100     | 200     | 300     | 400     |         |
| 0                             | 0.0674   | 0.0710  | 0.0650  | 0.0566  | 0.0690  | 0.0658  |
| 10                            | 0.0750   | 0.0755  | 0.0775  | 0.0799  | 0.0719  | 0.0740  |
| 20                            | 0.0639   | 0.0718  | 0.0706  | 0.0714  | 0.0719  | 0.0697  |
| Average                       | 0.0688   | 0.0728  | 0.0710  | 0.0660  | 0.0706  | -       |

**Table 2.** Combination of organic fertilizers and mycorrhizal on the net assimilation rate

| Mycorrhizae (g.plant\(^{-1}\)) | Organic fertilizer (g.plant\(^{-1}\)) | Average |
|-------------------------------|-------------------------------------|---------|
|                               | 0        | 100     | 200     | 300     | 400     |         |
| 0                             | 1.37     | 1.18    | 2.26    | 1.95    | 0.80    | 1.44    |
| 10                            | 0.89     | 1.47    | 1.19    | 1.16    | 1.88    | 1.32    |
| 20                            | 1.21     | 1.19    | 1.53    | 1.37    | 0.89    | 1.24    |
| Average                       | 1.16     | 1.28    | 1.66    | 1.37    | 1.19    | -       |

The results showed that the combination of organic fertilizers and mycorrhizae had significant effects on the number of leaves (Table 3). Organic fertilizer 400 g.plant\(^{-1}\) with mycorrhizal 10 g showed the highest number of leaves, which was 1,027.33 leaves. This was because one of the leguminaceae family so that the roots can have symbiosis with microbes in the soil [7]. Organic fertilizers also affect the number of leaves. These results indicated that the number of plant leaves is increasing with the application of organic fertilizer from the extraction waste. This is because the organic fertilizer from the extraction of *Indigofera tinctoria* has met the standards according to the Ministry of Agriculture No.70/Permentan/SR.140/10/2011 as organic fertilizer [11]. Organic fertilizers contain complete nutrients, including N nutrients which play role in stimulating the growth of stems, branches and leaves [12]. The element of N plays an important role in the formation of leaf chlorophyll which is useful in the photosynthesis process [13]. Leaf growth was positively correlated with root growth (Table 8). The results showed that the number of leaves was positively correlated with root length, root fresh weight and root biomass. Optimum root growth can promote optimum leaf and canopy growth [14].

**Table 3.** Combination of organic fertilizers and mycorrhizal on the number of leaves

| Mycorrhizae (g.plant\(^{-1}\)) | Organic fertilizer (g.plant\(^{-1}\)) | Average |
|-------------------------------|-------------------------------------|---------|
|                               | 0        | 100     | 200     | 300     | 400     |         |
| 0                             | 287.33\(^{a}\) | 569.33\(^{ab}\) | 528.00\(^{ab}\) | 910.33\(^{ab}\) | 717.67\(^{ab}\) | 602.53  |
| 10                            | 375.33\(^{a}\) | 778.67\(^{ab}\) | 746.00\(^{ab}\) | 749.67\(^{ab}\) | 1,027.33\(^{b}\) | 735.40  |
| 20                            | 343.67\(^{a}\) | 686.67\(^{ab}\) | 667.00\(^{ab}\) | 682.33\(^{ab}\) | 473.00\(^{ab}\) | 570.53  |
| Average                       | 335.44\(^{a}\) | 678.22\(^{b}\) | 647.00\(^{b}\) | 780.78\(^{b}\) | 739.33\(^{b}\) | +       |

Note: Number followed by the same letters and columns showed no significant difference based on the DMRT level of 5%, (+) is an interaction between Indigofera tinctoria organic fertilizer and mycorrhizae

Organic fertilizer from the extraction waste significantly affected the crown fresh weight of *Indigofera tinctoria* (Table 4). Organic fertilizer 200 g.plant\(^{-1}\) increased the fresh weight of the crown by 63.27% from the treatment without organic fertilizer. This was because the application of organic fertilizers can add nutrients and improve soil fertility. Soil fertility is an important factor in providing nutrients in the soil. This is because legumes that planted on fertile land will provide optimal
productivity, in contrast to less fertile land where N fixation occurs but N is difficult to absorb into plants due to poor aeration [15]. Therefore, the availability of sufficient soil N that is easily absorbed by plants is very important to help the formation of the crown of *Indigofera tinctoria*. Crown fresh weight was positively correlated with plant root growth (Table 8). This showed that the application of organic matter can improve soil physical properties and increase soil fertility, because organic matter increases the availability of nutrients to increase plant growth [16].

**Table 4.** Combination of organic fertilizers and mycorrhizal on crown fresh weight

| Mycorrhizae (g.plant⁻¹) | Organic fertilizer (g.plant⁻¹) | Average |
|------------------------|-------------------------------|---------|
|                        | 0                             | 100     | 200     | 300     | 400     |
| 0                      | 23.89                         | 38.00   | 87.37   | 48.23   | 45.09   | 68.52   |
| 10                     | 60.55                         | 99.12   | 149.13  | 63.67   | 79.70   | 90.41   |
| 20                     | 32.55                         | 74.70   | 82.08   | 126.81  | 128.41  | 88.91   |
| **Average**            | **39.00**                     | **70.61**| **106.19**| **112.87**| **84.40**| **ab**   |

Note: Number followed by the same letters and columns showed no significant difference based on the DMRT level of 5%.

Mycorrhizal inoculation had a significant effect on the root length of *Indigofera tinctoria* (Table 5). Soil fertility is influenced by organic matter contained in the soil, increasing organic matter can be done through the provision of organic fertilizers so that it can affect soil fertility on its physical, chemical, and biological properties [17]. This is in line with the research of Budiastuti et al. [18] that giving 10 g.plant⁻¹ of mycorrhiza and 10 g.plant⁻¹ of rhizobium have a synergistic relationship with the soil which caused maximum absorption of nutrients. The administration of mycorrhiza 10 g.plant⁻¹ increased root length 23.54% compared to without mycorrhizae. Mycorrhizae have the ability to infect plant roots and form hyphae, thereby increasing the length of plant roots. In addition, *Indigofera tinctoria* is a legume that can act symbiotically with bacteria in the soil, namely Rhizobium which can fixate nitrogen [7].

**Table 5.** Combination of organic fertilizers and mycorrhizal on root length

| Mycorrhizae (g.plant⁻¹) | Organic fertilizer (g.plant⁻¹) | Average |
|------------------------|-------------------------------|---------|
|                        | 0                             | 100     | 200     | 300     | 400     |
| 0                      | 42.07                         | 44.77   | 37.83   | 50.67   | 41.17   | 43.30 b |
| 10                     | 45.00                         | 58.00   | 62.33   | 56.83   | 61.00   | 56.63 a |
| 20                     | 47.00                         | 55.83   | 68.67   | 53.33   | 56.00   | 56.17 a |
| **Average**            | **44.67**                     | **52.87**| **56.28**| **53.61**| **52.72**| -        |

Note: Number followed by the same letters and columns showed no significant difference based on the DMRT level of 5%.

Mycorrhizae dose did not have a significant effect on the fresh weight of *Indigofera tinctoria* roots. Organic fertilizer from the extraction of *Indigofera tinctoria* 200 g.plant⁻¹ was significantly different from the treatment without organic fertilizer, but not significantly different at the
doses of 300 and 400 g.plant\(^{-1}\). The addition of organic fertilizer from the extraction of *Indigofera tinctoria* 200 g.plant\(^{-1}\) was optimal enough to provide nutrients and was able to increase the fresh weight of *Indigofera tinctoria* roots by 68.5%. This is because the organic fertilizer contains sufficient available nutrients. Organic matter produced from weathering of legumes through biological processes with the help of decomposing organisms provides high available N in the soil and increases the activity of nitrogen-fixing bacteria to bind air N [19]. The organic fertilizer had a significant effect on the root biomass of *Indigofera tinctoria* (Table 7). Extracted organic fertilizer of 200 g.plant\(^{-1}\) produced root biomass of *Indigofera tinctoria* which was significantly different from the treatment without organic fertilizer, but not significantly different from that of 300 g.plant\(^{-1}\). The application of 200 g organic fertilizer was able to increase root biomass by 68.29% compared to no fertilizer because organic fertilizer was very useful for improving soil fertility.

**Table 7. Combination of organic fertilizers and mycorrhizal on root biomass**

| Mycorrhizae (g.plant\(^{-1}\)) | Organic fertilizer (g.plant\(^{-1}\)) | Average |
|-------------------------------|--------------------------------------|---------|
|                               | 0 | 100 | 200 | 300 | 400 |         |
| 0                             | 2.34 | 4.10 | 11.63 | 15.82 | 5.17 | 7.81 |
| 10                            | 7.25 | 11.20 | 19.95 | 5.68 | 8.68 | 10.53 |
| 20                            | 3.27 | 8.55 | 9.01 | 14.93 | 13.61 | 9.87 |
| Average                       | 4.29 \(^{b}\) | 7.95 \(^{ab}\) | 13.53 \(^{a}\) | 12.14 \(^{a}\) | 9.15 \(^{ab}\) |         |

Note: Number followed by the same letters and columns showed no significant difference based on the DMRT level of 5%.

**Table 8. Correlation between number of leaves, fresh weight of crown and root growth**

| Number of leaves | Crown fresh weight | Root length | Root fresh weight | Root biomass |
|------------------|--------------------|-------------|-------------------|--------------|
| 1                | 0.526**            | 0.535**     | 0.487**           | 0.468**      |
| Crown fresh weight | 0.526**            | 0.487**     | 0.937**           | 0.960**      |
| Root length      | 0.535**            | 1           | 0.500**           | 0.467**      |
| Root fresh weight| 0.487**            | 0.937**     | 0.500**           | 1            |
| Root biomass     | 0.468**            | 0.960**     | 0.467**           | 0.961**      |

Note: ** Correlation is significant at the 0.01 level (2-tailed).

**4. Conclusion**

The combination of organic fertilizer with mycorrhizal supports the growth of *Indigofera tinctoria* leaves. The highest number of leaves was 1,077.33 leaves in a combination of organic fertilizer 400 g.plant\(^{-1}\) with mycorrhizal 10 g/plant. Organic fertilizer dose of 200 g.plant\(^{-1}\) can increase fresh root weight 68.5%, root biomass 68.29%, and shoot fresh weight by 63.27%. Mycorrhizae 10 g.plant\(^{-1}\) increased root length by 23.54%. Organic fertilizer from the extraction of *Indigofera tinctoria* is an attempt to achieve zero waste to support plant growth.

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