Effect of light intensity on growth, yield and indigo content of *Indigofera tinctoria* L.

M T S Budiastuti¹,², D Purnomo¹, Supriyono¹, B Pujiasmanto¹ and D Setyaningrum²

¹Department of Agrotechnology, Faculty of Agriculture, Universitas Sebelas Maret, Jl. Ir. Sutami 36 A, Kentingan, Surakarta, 57126, Indonesia
²Department of Magister Agronomy, Faculty of Agriculture, Universitas Sebelas Maret, Jl. Ir. Sutami 36 A, Kentingan, Surakarta, 57126, Indonesia
³Corresponding author: mariatheresia@staff.uns.ac.id

Abstract. Synthetic dyes can increase the amount of pollutants that become a serious problem in the environment. The use of synthetic dyes can be replaced with dyes from natural ingredients, namely *Indigofera tinctoria*. These plants are a source of blue natural dyes because it contains indigo. The content of indigo is very responsive to light. The impact of climate change is a serious threat to the supply of natural dyes. So, judge the suitability of the environment and indigo content under climate change are essential for the sustainable production of natural dyes *Indigofera tinctoria*. The research aimed to examine the role of light on the growth, yield, and indigo content in *Indigofera tinctoria*. The study was conducted using a randomized complete design with one factor, namely light intensity with 3 levels namely light intensity 100%, 50%, and 25%, with 9 replications. Light intensity affected the number of leaves, nodes, fresh weight, and indigo content. The highest number of leaves, nodes, and fresh weight were at 100% light intensity, while the highest indigo content was at 25% light intensity. The fresh weight with indigo paste is positively correlated. The higher the fresh weight of the plant, the more paste will be produced. However, the content of indigo was negatively correlated with indigo paste.

1. Introduction

Synthetic dye waste is toxic because it contains dyes, chemical compounds, chromium compounds, and heavy metals that cause environmental pollution [1] Annually a total of 280,000 tonnes of synthetic dye waste in the textile industry are disposed of worldwide [2]. This waste can cause water pollution, reduce light penetration in water bodies, and thus affect the photosynthetic activity of aquatic flora [3]. The large scale and extensive production of synthetic dyes can cause significant environmental pollution and become a serious health risk factor [4]. The use of synthetic dyes can be replaced with environmentally friendly natural dyes.

*Indigofera tinctoria* L. is a source of natural dyes. These plants contain indigo which produces a blue color [5]. Indigo is produced from plant leaves that contain indican secondary metabolites (indoxyl-β-D-glucoside) as a precursor to indigo which is produced in leaf vacuoles [6]. Indican is hydrolyzed to indoxyl and glucose by β-lucosidase in chloroplast mesophyll cells [7]. Indoxyl-β-D-glucoside is hydrolyzed to indoxyl and is oxidized by air become indigo.

Environmental conditions play an important role in influencing the yield of indigo-producing plants. Based on research by Angelini et al. [8] that environmental climatic conditions, namely light, and rainfall, highly affect indican production. Indigo compound production is very responsive to sunlight [9]. The results of the study by Stoker et al. [9] showed that a greater indigo content was obtained from plants.
harvested after a period of increased sunlight. Meanwhile, Sharma and Chandraprabha [10] reported that the increase in the indigo content of *Isatis tinctoria* was affected by light. The quality of light affects the production of indigo precursors in *Isatis tinctoria* L. and *Isatis indigotica* [11]. The results of the research by Campeol et al. [12] show that the indigo content in *Polygonum tinctorium* was affected by the temperature and intensity of sunlight.

The problem of indigo content encourages the development of natural dyes by optimizing the growth of *I. tinctoria*, the resulting paste production, and indigo content. Most of the pigments that attached specifically to the thylakoid protein and the photosynthetic system are strongly affected by the availability of light. The level of light intensity on the yield of *Indigofera tinctoria* and indigo content is unknown. The novelty of this study was to use several levels of light intensity to determine plant yield and indigo content in *Indigofera tinctoria* in the tropics. The purpose of this study was to examine the role of light intensity on the growth, yield, and content of indigo in *Indigofera tinctoria* L.

2. Materials and methods

The research was conducted in Puron Village, Bulu District, Sukoharjo, Central Java, Indonesia. The research location is 1100 51'49.44 'BT and 70 48' 54.3 " LS. This study used a complete randomized complete block design (RCBD) with one treatment factor, namely the level of light intensity with 3 levels, namely: N1 = 100% light intensity (63,200 lx.m2), N2 = 50% light intensity (32,400 lx.m2) and N3 = light intensity 25% (5,300 lx.m2). Each experimental unit was repeated 9 times.

The equipment in this research was a crop net as an application of light intensity, luxmeter (Shanghai, China), analytical scales, oven (Binder ED 56, Indonesia), and UV Vis spectrophotometry (Shimadzu Uv-1700 Pharmaspec, Japan). The materials in this study were *Indigofera tinctoria* green seeds and came from CV. Indigo Biru Baru, the indigo standard is indigo charmine and aquades. Materials used for the paste-making process: Ca(OH)₂ and aquades.

The indigo paste was made at the age of 12 WAP (Weeks After Planting). The process of the leaves into a natural dye paste using the maceration extraction method, that is the leaves are soaked for 48 hours, water and leaves are separated, then add CaCO₃ to the water that has been separated, then stir (oxidize) until it turns blue, then it is deposited for 24 hours. The indigo content of *Indigofera tinctoria* paste was analyzed spectrophotometrically [13]). The indigo concentration was calculated using the indigo calibration curve. Calibration curves were prepared by dissolving indigo standard (Sigma) in ethyl acetate [14]. The calibration curves were performed using various amounts of indigo standard, obtained by dissolving 8 mg of indigo standard in 20 ml of H₂SO₄ and diluting to 500 ml with distilled water. The solution was then diluted to different concentrations with H₂SO₄ solution (H₂SO₄: distilled water; 1:24) and the absorbance was measured at 611 nm.

Observation variables include growth variables such as the number of leaves, nodes, and leaf area measured at 10 WAP, yield variables include plant fresh weight measured at harvesting (12 WAP), paste yield, and indigo content. The research data were analyzed using analysis of variance with a test level of α 5% (95% confidence level). If the significant effect is carried out further analysis using Duncan's Multiple Range Test (DMRT). Correlation analysis was conducted to determine the relationship between the observed variables.

3. Results and discussion

3.1 Growth of *Indigofera tinctoria* L.

Light intensity significantly affected the number of leaves, nodes, and leaf area of *Indigofera tinctoria* (Table 1). The number of leaves reaches 265 strands at 100% light intensity. The number of leaves at each intensity showed significantly different. The decrease in light intensity causes a decrease in the number of leaves. The number of leaves at a light intensity of 50% and 25% decreased by 31% and 50.94% compared to the light with 100% intensity. This indicates that the lower the light intensity causes lower leaf growth. This is because net photosynthesis is reduced in low light [15].
Low light intensity causes reduced growth of nodes. The number of nodes in full light intensity reached 23 nodes, whereas in light 50% and 25% the number of nodes was reduced by 40.20% and 47.18% compared to 100% light intensity. Plants respond to low light by elongation internodes and petioles which results in reduced nodes [16]. The number of nodes was positively correlated with the number of leaves (Table 4). This is caused by low light respond plants by lengthening internodes and petioles which results in reduced nodes and leaf numbers [17].

Leaf area 8 WAP reaches 38.81 cm² at 25% light intensity. Leaf area decreased 36.27% at 50% light intensity and 64.17% less at 100% light intensity compared to 25% light intensity. This suggests that the plant responds to low light by increasing leaf area. The results of this study are in line with Li et al. [18] that the leaves are narrower at a high light intensity and the leaves are wider at a low light intensity. Leaf area shows the ability of plants to carry out photosynthesis because it is related to the use of light and the use of growing facilities [19].

### Table 1. Effect of light intensity on the number of leaves, nodes and leaf area.

| Light intensity (%) | Number of leaves | Number of nodes | Leaf area (cm²) |
|---------------------|-----------------|----------------|----------------|
| 100                 | 265,55b         | 23,78b         | 13,93 a        |
| 50                  | 181,44a         | 14,22a         | 24,73 b        |
| 25                  | 130,78a         | 12,56a         | 38,81 c        |

Note: Numbers that followed by the same letter in the column showed are not significantly different based on DMRT (α = 0.05)

### 3.2. Fresh weight and indigo paste yield

The light intensity had a significant effect on the fresh weight of *Indigofera tinctoria* (Table 2). 100% light intensity showed the highest fresh weight, namely 430 g. Full light intensity helps the maximum photosynthesis process, causing an increase in plant size or weight. This is because full light intensity increases the growth of roots, stems, and leaves associated with cell division and elongation and the formation of meristem tissue [20]. Light affects the allocation of photosynthetic products so that it significantly affects plant fresh weight [21].

The lower light intensity received by the plant causes a decrease in fresh weight. Fresh weight at a light intensity of 50% and 25% decreased by 31.38% and 47% compared to light 100%. This is consistent with the research of Wu et al. [20] that low light intensity causes the decrease of plant fresh weight. This is due to plant competition for light by reducing stem diameter, number of nodes, and number of leaves. Plant fresh weight correlated positively with leaf number (Table 4). Light is an environmental factor that plays a role in metabolic processes. Fresh weight is the result of metabolic activity and the value of fresh weight is influenced by tissue water content, nutrients, and metabolic products [22].

Light intensity does not affect the result of indigo paste. The highest paste yields were at 100% light intensity. The yield of indigo paste had a positive correlation with plant fresh weight and the number of leaves. The more leaves quantity makes the higher the plant fresh weight so that the indigo paste that produced is higher (Table 4). However, the paste yields at each light intensity were not significantly different (Table 2). The paste yield reached 55.64 g at 100% light intensity. The yield of paste in this study was higher than Chanayath [23] which was 26.83 g.

### Table 2. Fresh weight of *Indigofera tinctoria* at several light intensities

| Light intensity (%) | Fresh weight (g) | Paste yield |
|---------------------|------------------|------------|
| 100                 | 430,76b          | 55,64 a    |
| 50                  | 295,56 ab        | 54,42 a    |
| 25                  | 226,80 a         | 55,02 a    |

Note: Numbers that followed by the same letter in the column showed are not significantly different based on DMRT (α = 0.05)
3.3. Content of indigo

The UV-Vis absorption of indigo dye using a UV-Vis spectrophotometer showed that the absorption peak was at a wavelength of 611 nm (Figure 1). Based on the results of research by Wahyuningsih et al. [24] showed that the UV-Vis absorption of indigo dye using a UV-Vis spectrophotometer is in the wavelength range of 550-700 nm, the maximum absorption is reached at a concentration of 0.008 mg/mL. In general, the concentration of indigo affects color intensity. Indigo is a group of carbonyl dyes, a colorless derivative of glucosia from the enol indoxyl form [7].

Light intensity affects indigo content (Table 3). The highest indigo content was at 25% light intensity, which was 6.52 mg/l and decreased with the increase of light intensity. The indigo content decreased by 18% at 50% light intensity and decreased by 69% at 100% light intensity compared to 10% light intensity. According to Stoker et al. [9] that the concentration of indigo in *Isatis tinctoria* is affected by light intensity. However, the concentration of indigo per unit leaf weight in *Polygonum tinctorium* increased to higher light intensity before harvest [12] while the results of Tozzi et al. [11] showed that the production of indigo precursors in *Isatis tinctoria* L. and *Isatis indigotica* are affected by the quality of light.

![Figure 1. UV-Vis absorbance indigo standard.](image)

Indigo is a synthesis of the indoxyl-β-D-glucoside precursor molecule derived from plant secondary metabolites. These precursors are thought to have originated as indole from the shikimic acid pathway, via tryptophan or indole-3-pyruvate [25]. Shikimic acid is a major intermediate in the aromatic and essential amino acid pathways. Based on the biosynthetic pathway, the production of secondary metabolites is classified into three major groups, namely, terpenes (or isoprenoids), phenolic compounds (phenylpropanoids and flavonoids), and compounds containing nitrogen (alkaloids, glucosinolates, and cyanogenic glycosides) [26]. Indigo precursor is a metabolite compound containing nitrogen. In the shikimic acid pathway, the precursors derived from glycolysis and pentose phosphate are converted into aromatic amino acids [27]. Nitrogen-containing metabolites (alkaloids, glucosinolates, and cyanogenic glycosides) increase with decreasing light [28].

The results showed that indigo production increased with decreasing light intensity. These results suggest that low light stimulates biosynthesis or accumulation of secondary metabolites. Low light intensity is considered an environmental stimulus in the production of secondary metabolites. Low light intensity significantly increases the concentration of glycyrrhizic acid and liquidity [29]. Environmental
factors such as temperature and light have a strong influence on the accumulation of plant pigments and glucosinolates [30][31]. Identified alkaloid content in *I. tinctoria*. Light is also an important factor in alkaloid synthesis and accumulation, in *Mahonia bodinieri* found that the synthesis and accumulation of alkaloids are effective at 50% and 30% light intensity while decreasing at 100% light [32].

**Table 3.** The concentration of indigo *Indigofera tinctoria* at several light intensities.

| Light intensity (%) | Indigo Concentration (mg/l) |
|---------------------|-----------------------------|
| 100                 | 2.02a                       |
| 50                  | 5.31b                       |
| 25                  | 6.52c                       |

Note. Numbers that followed by the same letter in the column showed are not significantly different based on DMRT (α = 0.05)

**Table 4.** Correlation between growth, yield and indigo content in *Indigofera tinctoria*

|                         | Number of leaves | Number of leaves | Leaf area | Plant fresh weight | Paste yield | Indigo content |
|-------------------------|------------------|------------------|-----------|--------------------|-------------|----------------|
| Number of leaves        | 1                | 0.014ns          | -0.467*   | 0.489**            | 0.527**     | -0.543**       |
| Number of nodes         | 0.014ns          | 1                | 0.027ns   | 0.121ns            | 0.130ns     | -0.333ns       |
| Leaf area               | -0.467*          | 0.027ns          | 0.160ns   | 0.085ns            | 0.829**     |                |
| Plant fresh weight      | 0.489**          | 0.121ns          | 0.160ns   | 1                  | 0.800**     | 0.062ns        |
| Paste yield             | 0.527**          | 0.130ns          | 0.085ns   | 0.829**            | 1           | -0.070ns       |
| Indigo content          | -0.543**         | -0.333ns         | 0.829**   | 0.062ns            | -0.070ns    | 1              |

Note. ns = not significant; * = significantly different; ** = significantly very different.

4. Conclusion

100% light intensity increases the number of leaves, nodes, and plant fresh weight. However, the highest leaf area and indigo content were at 25% light intensity. The yield of indigo paste had a positive correlation with the number of leaves and plant fresh weight.

Acknowledgments

The authors wish to express sincere thankfulness to Universitas Sebelas Maret for financial support on the Community Partnership Program with Non-Tax State Revenue funds for the 2020 budget year with contract number: 452/UN27.21/PN/2020 in this study.

References

[1] Kant R 2012 Textile dyeing industry an environmental hazard *Nat. Sci.* 4 22–6
[2] Varjani S, Rakholiya P, Ng H Y, You S and J A Teixeira 2020 Microbial degradation of dyes: An overview *Bioresour. Technol.* 314 123728
[3] Ali H 2010 Biodegradation of synthetic dyes - A review *Water. Air. Soil Pollut.* 213 251–73
[4] Oros G, Cserháti T and Forgács E 2003 Separation of the strength and selectivity of the microbiological effect of synthetic dyes by spectral mapping technique *Chemosphere* 52 185–93
[5] Hariri M R, Chikmawati T and Hartana A 2017 Genetic diversity of indigofera tinctoria L. In java and Madura islands as natural batik dye based on inter-simple sequence repeat markers,” *J. Math. Fundam. Sci.* 49 105–15
[6] Inoue S, Moriya T, Morita R, et al. 2017 Characterization of UDP-glucosyltransferase from Indigofera tinctoria *Plant Physiol. Biochem.* 121 226–33
[7] Minami Y, Takao H, Kanafuji T *et al.* 1997 β-Glucosidase in the indigo plant: Intracellular localization and tissue specific expression in leaves *Plant Cell Physiol.* 38 1069–74
[8] Angelini L G, Tozzi S and o Di Nasso N N 2004 Environmental factors affecting productivity,
indicant content, and indigo yield in Polygonum tinctorium Ait., a subtropical crop grown under temperate conditions J. Agric. Food Chem. 52 7541–7
[9] Stoker K G, Cooke D T and Hill D J 1998 Influence of light on natural indigo production from woad (Isatis tinctoria) Plant Growth Regul. 25 181–5
[10] Sharma S and Chandraprabha 2016 Present Status of Plant Derived Indigo Dye - a Review,” Int. J. Res. Eng. Technol. 5 42–7
[11] Tozzi S, Lercari B and Angelini L G 2005 Light Quality Influences Indigo Precursors Production and Seed Germination in Isatis tinctoria L. and Isatis indigotica Fort Photochem. Photobiol., 81 914
[12] Campeol E, Angelini L G, Tozzi S and Bertolacci M 2006 Seasonal variation of indigo precursors in Isatis tinctoria L. and Polygonum tinctorium Ait. as affected by water deficit Environ. Exp. Bot. 58 223–33
[13] Wu E, Komolpis K and Wang H Y 1999 Chemical extraction of indigo from Indigofera tinctoria while retaining biological integrity Biotechnol. Tech. 13 567–9
[14] Sales E, Kanhonou R, Baixauli C, et al. 2006 Sowing date, transplanting, plant density and nitrogen fertilization affect indigo production from Isatis species in a Mediterranean region of Spain Ind. Crops Prod. 23 29–39
[15] Yang X, Jost A P T, Weiner O D and Tang C 2013 A light-inducible organelle-targeting system for dynamically activating and inactivating signaling in budding yeast Mol. Biol. Cell 24 2419–30
[16] Lukitasari M 2012 Pengaruh intensitas cahaya matahari terhadap pertumbuhan tanaman kedelai (Glycine Max) J. Pembelajaran Biol. 2 1–11
[17] González N C and Kröger M 2020 The potential of Amazon indigenous agroforestry practices and ontologies for rethinking global forest governance For. Policy Econ. 118 102257
[18] Li T, Liu L N, Jiang C D, Liu Y J and Shi L 2014 Effects of mutual shading on the regulation of photosynthesis in field-grown sorghum J. Photochem. Photobiol. B Biol. 137 31–38
[19] Pujiwati I, Aini N, Sakti S P and Guritno B 2018 The effect of harmonic frequency and sound intensity on the opening of stomata, growth and yield of soybean (Glycine max (L.) Merril),” Pertanika J. Trop. Agric. Sci. 41 963–74.
[20] Wu Y S, Yang F, GONG C Z, et al. 2017 Shade adaptive response and yield analysis of different soybean genotypes in relay intercropping systems J. Integr. Agric. 16 1331–40
[21] Zhang J, Liu J, Yang C, Du S and Yang W 2016 Photosynthetic performance of soybean plants to water deficit under high and low light intensity South African J. Bot. 105 279–87
[22] Baek K H and Skinner D Z 2012 Production of reactive oxygen species by freezing stress and the protective roles of antioxidant enzymes in plants J. Agric. Chem. Environ. 1 34–40
[23] Chanayath N, Lhieochaiphant S and Phutarakul S 2002 Pigment Extraction Techniques from the Leaves of Indigofera tinctoria Linn. and Baphicacanthus cusia Brem . and Chemical Structure Analysis of Their Major Components Agris 1 149–60
[24] Wahyuningsih S, Ramelan A H, Wardani D K, et al., 2017 Indigo Dye Derived from Indigofera Tinctoria as Natural Food Colorant,” IOP Conf. Ser. Mater. Sci. Eng. 193 012048
[25] Xia Z Q and Zenk M H 1992 Biosynthesis of indigo precursors in higher plants zhi-qiang Phytochemistry, 31 2695–7
[26] Xin F, Yang C, Wei Y, et al. 2011 Genomics Grand for Diversified Plant Secondary Metabolites,” Plant Diversity and Resources 33 53–64
[27] Sher A, Khan A, Hussain S, et al. 2017 Significance of chemical priming on yield and yield components of wheat under drought stress Am. J. Plant Sci. 8 1339–44
[28] Coelho G C, Rachwal M F G, Dedekew R A, et al. 2007 Effect of light intensity on methylxanthine contents of Ilex paraguariensis A. St. Hil. Biochem. Syst. Ecol. 35 75–80
[29] Hou J, Li W, Zheng Q, Wang W, Xiao B and Xing D 2010 Effect of low light intensity on growth and accumulation of secondary metabolites in roots of Glycyrrhiza uralensis Fisch,” Biochem. Syst. Ecol. 38 160–8
[30] Lefsrud M G, Kopsell D A, Kopsell D E and Curran-Celentano J 2006 Irradiance levels affect growth parameters and carotenoid pigments in kale and spinach grown in a controlled environment Physiol. Plant. 127 624–31

[31] Sindhu P V, Kanakamany M T and Beena C 2016 Effect of organic manures and biofertilisers on herbage yield, quality and soil nutrient balance in Indigofera tinctoria cultivation J. Trop. Agric. 54 16–20

[32] Takshak S and Agrawal S B 2019 Defense potential of secondary metabolites in medicinal plants under UV-B stress,” J. Photochem. Photobiol. B Biol. 193 51–88