**Indigofera tinctoria** L. growth at various light intensities and shading time intervals

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Abstract. Plant production and changes in physiological aspects during the growing season can be influenced by climate change, one of which is the light factor in *Indigofera tinctoria*. The production of secondary metabolites *Indigofera tinctoria* as a source of natural dyes is responsive to light. This study examines the effect of shading time intervals and light intensity on the growth of *Indigofera tinctoria* L. The study used a Randomized Complete Block Design with a split-plot design consisting of 2 treatment factors, namely: the shading time interval as the main plot with five levels, namely 1-4, 1-8, 1-12, 8-12 and 4-12 weeks after planting. Light intensity as a subplot with three levels, namely the light intensity of 50%, 25%, and 10%. The results showed that combination shading time interval and light intensity significantly affected the number of nodia, leaf area of 8 WAP root biomass. Shade time of 1-4 weeks with a light intensity of 50% showed the highest number of nodia was 45.67 nodia, root fresh weight was 137.00 g, and root biomass was 60.10 g. The shading time interval had a significant effect on the net assimilation rate of the vegetative phase and root fresh weight. The vegetative phase's net assimilation rate in the 8-12 WAP shading time treatment was 0.029 g.cm⁻².day⁻¹. The longer the shading time interval with the lower the light intensity can increase the area index and decrease plant growth.

1. Introduction

*Indigofera tinctoria* or Indian indigo/common indigo produce blue and blue-black colour from its leaf. The blue colorant in this plant is indigo dyes. As natural dyes, the characteristic colors are less poisonous, less contaminating, less wellbeing perilous, non-carcinogenic and non-poisonous. Included to this, they are harmonizing colours, tender, delicate and unpretentious, and make a tranquil impact. Over all, *Indigofera tinctoria* can be used as an alternative to synthetic dyes due to environment neighborly and can be reused after utilize. Beside used as natural dyes, *Indigofera tinctoria* extract’s also used used in epilepsy and other nervous; in the form of ointment used for sores, old ulcers and piles. Root used in urinary complaints and hepatitis [1]. Indigofera plants are composed of a huge number of complex natural substances counting alkaloids, flavonoids, phenols, tannins, saponins, glycosides and terpenoids [2].

Climate change within light affects plant production and physiology such as secondary metabolites. Light is one of most significant environmental alert for growth and physiological plant processes. During growth in most plants naturally produced indoles, glucosinolates and pigments (secondary metabolites).
The energy source comes from sunlight, especially from the photosynthetic part of the active spectrum [3]. Light intensity is critical within the generation of indigo precursors. When plants beneath more prominent light regimes were exchanged to lesser light conditions, at that point indigo production declined [4]. The study found that 50% light intensity was the optimal light intensity for *Indigofera tinctoria* root length [5]. Area, specific leaf area and *Indigofera tinctoria* plant height were highest at 25% intensity [6]. Few research have been conducted on the combination of light intensity treatment with shading time intervals of *Indigofera tinctoria*. The novelty of this study is that it combines both light intensity and shading time intervals in *Indigofera tinctoria* in tropical climates. This research aims to examine the effect of shading time intervals and light intensity on the growth of *Indigofera tinctoria* L.

2. Materials and methods

The research was conducted in Hamlet II, Puron Village, Bulu District, Sukoharjo Regency, Central Java, Indonesia, with a geographical position of 1100 51'49.44 '' BT and 70 48 '54.3 ''LS. The study used a completely randomized block design with a split-plot design consisting of 2 treatment factors, namely: the shading time interval as the main plot with five levels, namely 1-4 weeks after planting, 1-8 weeks after planting, 1-12 weeks after planting, 8-12 Weeks After Planting and 4-12 Weeks After Planting. Light intensity as a sub plot with 3 levels, namely the light intensity of 50% (37900 lux), 25% (20200 lux) and 10% (7849 lux). Light intensity was observed at 12 pm with a light intensity of 100%, namely 78400 lux.

The tools used in this research were luxmeter and paranet as light intensity applications. The variables observed included: the number of nodia observed at 12 weeks after planting, the net assimilation rate of the vegetative phase, which was observed at T2 = 8 WAP and T1 = 4 WAP, the observed leaf area at 8 WAP, fresh weight, and root biomass at 12 WAP. The calculation of the net assimilation rate is carried out based on dry weight with plant leaf area per unit time using the formula [7]:

$$\text{Net Assimilation Rate} = \frac{1}{A} \times \frac{\Delta W}{\Delta t} \times \frac{\log A_2 - \log A_1}{A_2 - A_1} \times \frac{\log W_2 - \log W_1}{t_2 - t_1}$$

(1)

Note:
- A2 = area of plant leaves in t2 observation
- A1 = area of plant leaves in t1 observation
- W2 = plant biomass at observation t2
- W1 = plant biomass in observation t1

Leaf area using the Gravimetric method [8] with the formula: $LD = \frac{W_r}{W_t} L_k$, where $W_r =$ weight of leaf replica paper, $W_t =$ total paperweight, and $L_k =$ total leaf area. The research data were analyzed using variance based on the F test with a test level of $\alpha$ 5% (95% confidence level). If the significant effect was carried out further analysis using Duncan's Multiple Range Test (DMRT).

3. Results and discussion

3.1. Number of nodia

The results showed that the combination of the treatment intervals of shade time and light intensity significantly affected the number of nodia (Table 1). The combination of 1-4 WAP shading time intervals with 50% light intensity showed the highest nodia, namely 45.67 nodia. The longer the shading time interval with the lower the light intensity causes the growth of the nodus to decrease. This is because light plays a role in forming and distributing photosynthetic assimilation [9]. Long-time interception of
low light intensity can increase ethylene production, response to primary auxin, and proteins with similarities to AUX22 and 1-aminocyclopropane-1-carboxylic acid synthase ACS6 genes [10]. Light intensity affected the number of nodia (Table 1). The number of nodia decreases with decreasing light intensity. The results of this study are in accordance with [6] that light intensity affects the morphology and physiology of Indigofera tinctoria.

Table 1. Number of 12 MST nodia at a combination of shading time interval and light intensity.

| Shade time interval (Weeks After Planting) | Light Intensity (%) | Average |
|-------------------------------------------|---------------------|---------|
|                                           | 50                  | 25      | 10      |
| 1-4                                       | 45.67 f             | 35.67 ef| 26.00 cde| 31.67 d |
| 1-8                                       | 24.33 cde           | 16.00 abc| 11.67 ab | 14.89 a |
| 1-12                                      | 25.33 cde           | 22.33 bcd| 10.00 a  | 17.00 a |
| 8-12                                      | 33.00 de            | 25.00 cde| 22.00 bcd| 25.33 c |
| 4-12                                      | 24.67 cde           | 23.00 bcd| 17.33 abc| 21.56 b |
| Average                                   | 27.47 c             | 22.00 b  | 16.80 a  |

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

3.2. Net assimilation rate in the vegetative phase (g.cm².day⁻¹)
The results showed that the shading time interval had a significant effect on the net assimilation rate of the vegetative phase (Table 2). The highest net assimilation rate at 8-12 MST shading time was 0.0028. This is because the net assimilation rate of the vegetative phase is measured when the plants are 1-8 WAP so that the 8-12 MST shade treatment still gets full light intensity (100%). The net assimilation rate will increase with increasing light intensity [11]. The results showed that the net assimilation rate of the vegetative phase was negatively correlated with leaf area (Table 6). The high light intensity can increase gas exchange and stomatal density, light saturation point, light compensation point and is positively correlated with net assimilation rate [12].

Table 2. Net assimilation rate of vegetative phase at combination of shading time interval and light intensity (g.cm².day⁻¹).

| Shade time interval (Weeks After Planting) | Light intensity (%) | Average |
|-------------------------------------------|---------------------|---------|
|                                           | 50                  | 25      | 10      |
| 1-4                                       | 0.0012430           | 0.0022408| 0.0015183| 0.0016675 a|
| 1-8                                       | 0.0023389           | 0.0020393| 0.0018627| 0.0020803 a|
| 1-12                                      | 0.0019861           | 0.0021165| 0.0015726| 0.0018917 a|
| 8-12                                      | 0.0028517           | 0.0030677| 0.0027278| 0.0028824 b|
| 4-12                                      | 0.0013080           | 0.0014484| 0.0015147| 0.0014237 a|
| Average                                   | 0.0019456           | 0.0021825| 0.0018392|

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

3.3. Leaf area (cm²)
The results showed that the combination of shade time interval treatment with light intensity significantly affected the leaf area of Indigofera tinctoria (Table 3). The longer the shading time interval with lower light intensity causes an increase in leaf area. This is because at a low light intensity, the cell membrane permeability decreases, the accumulation of chlorophyll is low, and the photosynthetic index is inhibited [13]. The results showed that leaf area was negatively correlated with the number of nodia.
Table 3. Leaf area at a combination of shade time intervals and light intensity (cm²).

| Shade time interval (Weeks After Planting) | Light intensity (%) | Average |
|------------------------------------------|---------------------|---------|
|                                          | 50                  | 25      | 10      |
| 1-4                                      | 13.81 abc           | 17.14 abc | 20.95 cd | 17.30 b |
| 1-8                                      | 12.38 a             | 12.95 cd | 26.19 de | 19.84 b |
| 1-12                                     | 16.19 abc           | 24.76 de | 29.52 e  | 23.49 c |
| 8-12                                     | 10.47 a             | 12.85 abc| 13.81 abc| 12.38 a |
| 4-12                                     | 14.28 abc           | 17.14 abc| 20.00 bcd| 17.14 b |
| Average                                  | 13.42 a             | 18.57 b  | 22.09 c  |        |

Note: Numbers followed by letters and columns showed no significant differences based on the DMRT level of 5%.

3.4. Root fresh weight

The results showed that the shading time interval significantly affected root fresh weight (Table 4). Shading time 1-4 MST showed the highest fresh weight, namely 115.22 g. The longer the shading time, the lower the fresh weight of the roots. Root fresh weight decreased 35% at 1-8 MST shading intervals and 52% at 1-12 MST shading intervals compared to 1-4 MST shading intervals. Low light intensity encourages legume plant roots to be longer, but the fresh weight and fresh weight of root biomass are low [5]. This is because light intensity affects microbial activity in the soil [15]. In addition, low light intensity at the bottom limits photosynthesis and leaf aging as an important source of assimilation for crown and root growth [16].

Table 4. Fresh weight of roots at the combination of shade time interval and light intensity (g).

| Shade time interval (Weeks After Planting) | Light intensity (%) | Average |
|------------------------------------------|---------------------|---------|
|                                          | 50                  | 25      | 10      |
| 1-4                                      | 137.00 c            | 115.67 bc | 93.00 abc | 115.22 b |
| 1-8                                      | 47.00 ab            | 85.33 abc| 89.67 ab  | 74.00 a  |
| 1-12                                     | 67.33 abc           | 57.00 ab | 39.00 a   | 54.44 a  |
| 8-12                                     | 99.00 abc           | 35.67 a  | 42.33 ab  | 59.00 a  |
| 4-12                                     | 67.67 abc           | 60.67 ab | 35.00 a   | 54.44 a  |
| Average                                  | 83.60               | 70.87    | 59.80    |

Note: Numbers followed by letters and columns showed no significant differences based on the DMRT level of 5%.

3.5. Root biomass

The results showed that the combination of the treatment intervals of shade time and light intensity significantly affected root biomass (Table 5). The longer the shading time interval and the lower the light intensity, the reduced root biomass. This is because light plays a role in cell division and differentiation in the meristem area. The growth of plant stems at low light intensity increases, while root growth is reduced as a result of changes in hormone transport and decreased proliferation of meristematic cells [17]. The results showed that light intensity had a significant effect on root biomass (Table 5). Based on these results, it was because *Indigofera tinctoria* was a legume family. The roots of legume plants can symbiosis with mycorrhizal and rhizobium bacteria [5]. Mycorrhizae and rhizobium
can synergize with each other at high and low light intensity. This study indicated that root fresh weight had a positive correlation with root biomass and crown number (Table 6). This is because plants have an aerenchyma network consisting of interconnected gas channels that facilitate the rapid transport of O$_2$ from the leaves to the underground tissue [18], aerenchyma O$_2$ supply supports aerobic metabolism in the root apical meristem [19].

**Table 5.** Root biomass at combination of shade time interval and light intensity.

| Shade time interval (Weeks After Planting) | Light intensity (%) | Average |
|-------------------------------------------|---------------------|---------|
|                                           | 50                  | 25      | 10      |
| 1-4                                       | 60.10 d             | 52.78 cd| 39.27 abcd| 50.72 b |
| 1-8                                       | 45.18 bcd           | 35.70 abcd| 19.03 ab  | 33.31 a  |
| 1-12                                      | 31.27 abc           | 27.65 abc| 19.54 ab  | 26.15 a  |
| 8-12                                      | 48.68 bcd           | 20.97 abc| 25.77 abc | 31.81 a  |
| 4-12                                      | 33.12 abc           | 29.68 abc| 14.93 a   | 25.91 a  |
| Average                                   | 43.67 b             | 33.36 ab | 23.71 a  |

Note: Numbers followed by letters and columns showed no significant differences based on the DMRT level of 5%.

**Table 6.** Correlation between the number of nodia, net assimilation rate, leaf area, root fresh weight and root biomass.

| Number of nodia | Leaf Area | net assimilation rate | Root fresh weight | Root biomass |
|-----------------|-----------|-----------------------|-------------------|--------------|
|                | -.314**   | -.050                 | .362*             | .535**       |
| Leaf Area       | -.314**   | 1                     | -.397**           | -.043        | -.260       |
| net assimilation rate | -.050       | -.397**               | 1                 | -.151        | -.126       |
| Root fresh weight | .362*         | -.043                 | -.151             | 1            | .706**      |
| Root biomass    | .535**     | -.260                 | -.126             | 1            |

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

4. Conclusion

The growth and production of secondary metabolites of *Indigofera tinctoria* are strongly influenced by climate change, especially light factors. The combination of the shading time interval and light intensity affected the number of nodes, leaf area, and root biomass. The combination of light intensity treatment of 50% and the shaded interval of 1-4 MST showed the highest number of nodia and biomass. The lower the light intensity with the longer shading intervals, the longer the leaf area increases but the growth of nodus and roots decreases.

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