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Effects of Cultivation Factors on Indigo Dye Yield in Indigofera Plants

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Abstract

This research investigated the local wisdom of Sakon Nakhon province, Thailand, on cultivating Indigofera plants for optimal natural indigo dye yield. Focusing on *Indigofera suffruticosa* Mill. and *Indigofera tinctoria* L., the study explored the impact of pod development stages and harvesting time on dye yield. The findings showed that harvesting at the brown pod stage (18 - 20 weeks) and early morning (6:00 a.m.) yielded the most dye. Additionally, the study compared the Indigofera plants cultivation under 100 % and 60 % sunlight exposure, demonstrating that full sunlight significantly increased dye yield. Moreover, *Indigofera suffruticosa* Mill. gave significantly more indigo dye yield than *Indigofera tinctoria* L. ($p < 0.05$). This investigation, therefore, underscores the value of incorporating local knowledge into optimal cultivation practices for enhanced indigo production. However, further research should delve into additional factors, such as soil composition, fertilizer regimes and the genetics of the Indigofera plant.

Keywords: Natural indigo dye, Indigo dye yield, Indigo paste, Indigo blue, Indigofera plants, *Indigofera suffruticosa* Mill., *Indigofera tinctoria* L.

Introduction

Indigofera, a genus within the Fabaceae family, boasts a rich and extensive history of natural indigo dye production. Its vibrant blue pigment, obtained through the meticulous extraction of indican from the leaves of specific species, carries a legacy stretching back to the dawn of civilization [1-3]. Comprising over 750 documented species thriving across diverse geographical regions, Indigofera has played a pivotal role in shaping the art of textile dyeing, a practice deeply intertwined with cultural and historical significance. The journey of this remarkable plant began with its utilization in small-scale handicraft traditions, evolved through the global trade routes, and continues to be a natural and sustainable textile [1,4,5].

Indigo blue is the most important pigment that is synthesized by Indigofera plants through the specific metabolism pathway involving enzymatic reactions [6,7]. This process begins with the biosynthesis of indican, a colorless glucosylated indole derivative, within the leaves [7,8]. Upon harvest and soaking in water, indican is released from the leaves and undergoes enzymatic hydrolysis catalyzed by β-glucosidase. This reaction liberates the aglycone indoxyl, which is a colorless precursor [9,10]. Under alkaline conditions achieved by adding calcium oxide (CaO), which reacts with water to generate calcium hydroxide ($Ca(OH)_2$), 2 indoxyl molecules spontaneously dimerize in the presence of oxygen [5], leading to the formation of insoluble indigo blue. Moreover, calcium hydroxide also reacts with carbon dioxide in the environment to form calcium carbonate ($CaCO_3$), which precipitates together with indigo blue particles. After removing the excess liquid, the concentrated indigo pigment is collected and processed into the familiar deep blue dye used for dyeing fabrics [4,11].

Indigofera plants are the primary source material for natural indigo dye production, necessitating large-scale cultivation through specialized practices. These practices, deeply rooted in the history and culture of specific regions, represent a fusion of tradition, scientific knowledge and sustainable principles. Meticulously selected Indigofera cultivars thrive in warm climates with dedicated care encompassing irrigation, fertilization and pest control protocols [2,12,14]. Upon reaching peak indican content within their leaves, these plants undergo a transformative process culminating in the production of the coveted natural indigo dye [15,16].

Sakon Nakhon province, globally renowned for its vibrant indigo-dyed textiles, maintains a prominent position as a major producer within Thailand [17,18]. The indigo dye production chain in Sakon Nakhon province starts from the initial stages to the final product. Natural indigo dye is the main raw material in the production of natural indigo dyed textiles [17,19]. The indigo dyeing process in Sakon Nakhon province

is intricately connected to its essential raw material: Natural indigo dye derived from the Indigofera plant, with a focus on the extensively cultivated species *Indigofera suffruticosa* Mill. and *Indigofera tinctoria* L. [13,20,21].

Generations of farmers have honed their expertise in Indigofera cultivation, making crucial decisions about planting seasons, optimal weather conditions, ideal harvesting times and the appropriate growth stage to maximize indigo dye yield [2,4]. However, it is important to study local wisdom through the principles of science and technology. This integration will enhance our comprehension of traditional knowledge, concurrently paving the way for future advancements in Indigofera cultivation. Therefore, this research aimed to investigate the factors influencing natural indigo dye yield within Indigofera plants, particularly *Indigofera suffruticosa* Mill. and *Indigofera tinctoria* L., the prominent species cultivated in Sakon Nakhon province. The comprehensive utilization of this invaluable local wisdom in a multi-disciplinary framework plays a role in advancing the production of natural indigo dye.

Materials and methods

Chemical and instrument

All chemicals used were of analytical grade. Indigo blue was purchased from Acros Organics (Belgium). Sulfuric acid (98 % w/w) was obtained from Caro Erba (Italy). Calcium hydroxide was bought from Loba Chemie (India). An UV-Vis spectrophotometer used for determination indigo blue content in indigo paste was model Go Direct SpectroVis Plus from Vernier Science Education (USA).

Indigofera plants cultivation

This research determined the indigo blue content of 2 indigo plant varieties, *Indigofera tinctoria* L. and *Indigofera suffruticosa* Mill., using a randomized complete block design (RCBD). The experiment was performed during the typical planting season (May to September 2023 or 30 weeks). The treatments of the experiment were, T_1 = *Indigofera suffruticosa* Mill. and T_2 = *Indigofera tinctoria* L. Each treatment had 4 plots (n = 4). Each treatment consists of 4 plots organized in a block of the experimental design. In each plot, 60 Indigofera plants are cultivated, arranged in 4 rows, with each row containing 15 plants. These prepared plots were specifically designated to investigate factors influencing harvesting, with a particular emphasis on the pod stage and harvesting timing.

The Indigofera seeds underwent a 24 h warm water soaking process before planting to enhance germination. Afterward, the harvested Indigofera seeds were placed in moss peat within a seedling tray for a duration of 3 weeks to cultivate seedlings. For the preparation of the cultivation plot used in this research, it began with plowing and leaving the soil for 1 week during its resting period. While the soil was resting, weed control and soil loosening were performed to improve soil texture. The plot was then fertilized with cow manure at a rate of 2 kg/plot. In each treatment, 3-week-old Indigofera seedlings were transplanted into individual plots, with rows spaced 30 cm apart and plants spaced 60 cm apart within each row. The plots received cow manure fertilization at a rate of 2 kg/plot at 15, 30 and 60 days after transplanting, and they were watered daily.

The effect of the pod development stage on the Indigofera plants harvesting

The traditional practice of harvesting Indigofera plants in Sakon Nakhon province has utilizes the visual inspection of plant characteristics. Distinct features are observed in plants ready for harvest during the pod development stage, which is known for producing the best indigo blue. Therefore, the local knowledge was validated by employing a RCBD to study the effect of pod development stage of Indigofera plants harvesting for the yield of indigo dye. The interesting pod stage of Indigofera plant in this experiment were flowering (A), young pod (B) and brown pod (C) stages.

The treatments of this experiment were T_1A = flowering stage of *Indigofera tinctoria* L., T_2A = flowering stage of *Indigofera suffruticosa* Mill., T_1B = young pod stage of *Indigofera tinctoria* L., T_2B = young pod stage of *Indigofera suffruticosa* Mill., T_1C = brown pod stage of *Indigofera tinctoria* L. and T_2C = brown pod stage of *Indigofera suffruticosa* Mill. The sampling involved selecting Indigofera plants from each treatment (n = 4) in individual plots. These samples were meticulously separated into leaves and stems, and the weight of the leaves was measured for the indigo paste production process. Subsequently, the indigo paste samples were analyzed blue content for facilitating a comparison of the optimal timing for harvesting the Indigofera plants.

The effect of harvesting time

This study investigated the effect of harvesting time on the yield of indigo dye from *Indigofera tinctoria* L. and *Indigofera suffruticosa* Mill. Three specific harvest times were compared: 6:00 a.m., 12:00 p.m. and 6:00 p.m. This experiment was studied in the brown pod stage (19 weeks) of the Indigofera plants. The sampling process included collecting Indigofera plants from each treatment (n = 4) in separate plots by harvesting at the specified times of interest. The harvested Indigofera plants were separated into leaves and stems. The leaves were weighed and processed into indigo paste. Afterward, the indigo paste was analyzed for its indigo blue content.

The effect of lighting on the indigo blue dye yield of Indigofera plants

The research on the influence of lighting conditions on indigo blue production was conducted using a RCBD. The experiment comprised 4 treatments: T1 = cultivating *Indigofera suffruticosa* Mill. with 60 % received sunlight, T2 = cultivating *Indigofera suffruticosa* Mill. under full sunlight, T3 = cultivating *Indigofera tinctoria* L. with 60 % received sunlight and T4 = cultivating *Indigofera tinctoria* L. under full sunlight. Each treatment had 4 plots (n = 4), which was planted in a separate plot with 10 indigo seedlings. The plants in the normal light group received full sunlight, while the plants in the low light group were shaded with a black net that allowed 60 % of the sunlight to pass through. All treatments received the same amount of cow manure and water. The indigo plants were harvested at the brown pod stage (19 weeks). The leaves were removed from the plants for producing indigo paste.

Preparation of indigo paste

Indigo paste is essential for indigo dyeing. It is made from properly aged Indigofera plant leaves that have a dark green color. The indigo paste production in this research was adapted from Kongkachuichay *et al.* and Purnama *et al*. [22,23]. The procedure involved soaking 250 g of Indigofera plant leaves in 1000 mL water for 18 h, gentle stirring every 6 h. After that, the leaves were filtered from the greenish-yellow indigo solution using a polyester filter. During the flocculation step, 20 g of $Ca(OH)_2$ was added to the greenish-yellow indigo solution. The mixture was stirred for 30 min to allow the dispersion of $Ca(OH)_2$ and air. During this time, O_2 reacted with indigo white to form indigo blue, and CO_2 reacted with $Ca(OH)_2$ to form $CaCO_3$. The mixture was then allowed to stand for 2 h to enable the indigo dye to precipitate out of the solution. The solution was removed by decanting it out of the beaker, and a highly moist indigo dye remained. The indigo dye was filtered through the polyester filter for 24 h to remove water, and the indigo paste product was obtained.

The calculation to determine the yield of indigo paste per Indigofera plant leaves (YIPL) can be obtained from Eq. (1), where W_{IPP} is the weight of indigo paste included polyester filter, W_{PEF} is the weight polyester filter, and W_{IL} is the weight Indigofera plant leaves.

$$\text{YIPL (g/kg fresh leaves)} = [(W_{IPP} - W_{PEF})/W_{IL}]\times 100 \quad (1)$$

Determination of indigo blue in indigo paste

The method of determining the indigo blue content in indigo paste using UV-Visible spectrophotometry was exemplified by our previous research [19]. This approach involves converting indigo blue into indigo carmine through a chemical reaction facilitated by concentrated sulfuric acid (98 % w/w). Indigo carmine is a stable form of indigo blue that easily dissolves in water. The working standard solutions were provided in the range of 0.00 - 16.00 mg/L. The sample preparation was performed by weighing 0.50 g indigo paste sample into a beaker, adding 6.0 mL concentrated H_2SO_4 (98 % w/w) and allowing it to stand for 10 min. The mixture was then transferred to a volumetric flask and adjusted to a final volume of 50.00 mL using a 5 % w/w H_2SO_4 solution. The sample solution was diluted with 5 % w/w H_2SO_4 solution prior to analyze by using a UV-Visible spectrophotometer at a wavelength of 610 nm. The indigo blue content in the indigo paste sample was then determined using a calibration curve for indigo blue.

Data analysis

The data analysis was carried out using SPSS version 19.0 (SPSS Inc.). For comparing groups within the completely randomized design, the 1-way analysis of variance (1-way ANOVA) was used, followed by the Duncan's multiple comparison test.

Results and discussion

Determination of indigo blue in indigo paste

The quantitation of the indigo blue content in indigo paste was carried out by using UV-Visible spectrophotometry. This method entails the conversion of indigo blue into indigo carmine through a chemical reaction aided by concentrated sulfuric acid. Indigo carmine represents a stable variant of indigo blue that readily dissolves in water. The calibration graph illustrates the correlation between the absorbance value and the quantity of indigo blue (**Figure 1**). The linear equation is y = 0.0505× + 0.0062, and it exhibited a good linearity (r^2) of 0.9996. Therefore, this calibration curve was used for precise indigo blue quantity calculations.

The calculation of indigo blue content in indigo paste samples (IBIP) can be determined using Eq. (1), where CC represents the concentration of IB in the sample solution calculated from the curve (mg/L), DF is the dilution factor, SV is the sample solution volume (mL), SW is the weight of the indigo paste sample, and 1000 is a constant for converting mg to g. The yield of indigo blue from Indigofera leaves (YIBL) using the indigo paste production process was calculated with Eq. (3) [19]. These equations calculated the yield of indigo blue for each cultivation factor of Indigofera plants. They could enhance our understanding of natural indigo dye production.

$$\text{IBIP (g IB/kg IP)} = [(CC \times DF \times SV)/(SW \times 1000)] \times 100 \quad (2)$$

$$\text{YIBL (g/kg fresh leaves)} = (\text{YIPL} \times \text{IBIP})/1000 \text{ g of IP} \quad (3)$$

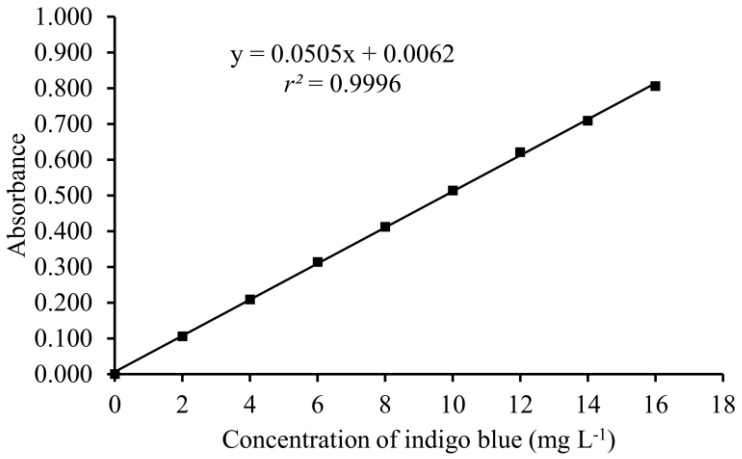

Figure 1 Calibration curve of indigo blue standard solution.

The effect pod development stage of Indigofera plants on the indigo dye yield

The Indigofera plant cultivation in Sakon Nakhon province follows a biannual cycle. The initial planting phase, spanning from April to June, is followed by harvesting from August to October, lasting approximately 3 - 4 months. The 2nd planting phase occurs between July and September, with harvesting from November to December, extending over approximately 4 - 5 months. Additionally, there is a method of cultivating indigo as rootstock, where the plant regrows from the remaining root after cutting the indigo plants in the 1st round. The age of indigo plants varies each season. Therefore, harvesting is determined by observing physical characteristics such as flowering and pod development, rather than relying on the age of the plants [13].

Figure 2 illustrates the characterization of pod development stages in *Indigofera suffruticosa* Mill. and *Indigofera tinctoria* L. It was observed that the flowering stage occurs between 12 to 16 weeks of plant age, the young pod stage falls within the range of 16 to 18 weeks, and the brown pod stage spans from 18 to 20 weeks. Now, the pods begin to exhibit a brown color but are not fully mature. Allowing the plants to age beyond this stage leads to the mature pod stage, characterized by yellowing leaves that eventually fall off. This stage is unsuitable for harvesting for dye production [13,20]. Therefore, these specific pod development stages were selected for harvesting to study the effect of timing on the yield of indigo dye. Additionally, the Indigofera plant ages for harvesting at the timing of the flowering, young pod and brown pod stages were 13, 17 and 19 weeks, respectively.

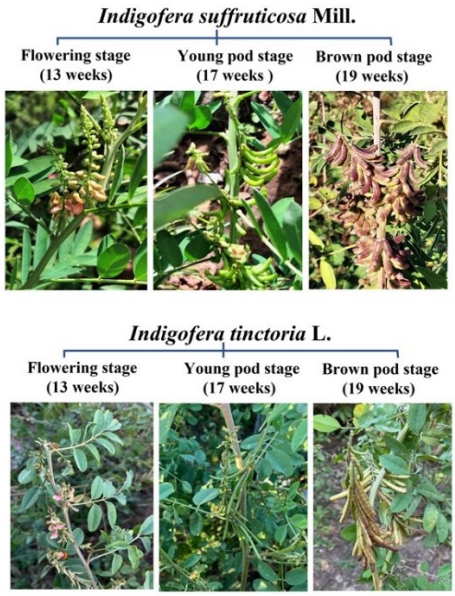

Figure 2 The characterization of pod development stages in *Indigofera suffruticosa* Mill. and *Indigofera tinctoria* L.

The effect of the indigo pod development stage on the indigo blue yield of indigo plants is presented in **Table 1**. The YIPL produced from Indigofera leaves collected at all 3 stages was not significantly different ($p > 0.05$). Meanwhile, the Indigofera leaves collected at all 3 stages exhibited a significant difference of YIPL and YIBL ($p < 0.05$). Additionally, *Indigofera suffruticosa* Mill. demonstrated a higher YIBL than *Indigofera tinctoria* L. at all indigo pod development stages. Therefore, it is recommended to harvest indigo plants at the brown pod stage to obtain the maximum indigo blue yield, which is most suitable for indigo paste production.

This phenomenon could be discussed that the growth stages of Indigofera pods significantly affect the synthesis of indican in these plants, with various factors at play. Enzymes crucial for indican synthesis, like indoxyl oxidase and UDP-glucosyltransferase (UGT), are most abundant in the leaves, followed by stems and roots [7,24,25]. The increased efficiency of UGT enzymes in mature plants is attributed to enhanced enzyme stability [26]. As a result, the mature pod stage is a period of optimal enzyme activity, leading to higher indican production. Furthermore, during the mature pod stage, the leaves of Indigofera plants are more resistant to degradation compared to other stages. This heightened resistance suggests a more effective extraction of indican from the leaves during the extraction process, resulting in a more vibrant indigo color. It indicates that leaves of Indigofera plants in the mature pod stage have higher indican levels, possibly due to elevated levels of structural proteins in the leaves [27,28].

Furthermore, the leaves harvested from *Indigofera tinctoria* during the mature pod stage exhibit a more vibrant indigo blue color compared to those harvested at other growth stages [4]. This increased intensity is likely linked to higher indican levels in the leaves, influenced by hormones like auxins and cytokinins [28,29]. These hormones play crucial roles in regulating plant growth, development and the production of secondary metabolites such as indican. Hormone levels change throughout the developmental stages of pod, with a decrease in auxin levels during the mature stage leading to reduced plant growth [29]. Conversely, an increase in cytokinin levels promotes growth, cell expansion, accumulation of lignin in leaf tissues, enhancement of photosynthesis-related protein levels and inhibition of leaf aging and abscission [27,30].

The cultivation of Indigofera plants in the community for natural indigo dye production relies not on recording the age of the plants but on observing their physical characteristics as an appropriate indicator for harvesting time. It has been observed that Indigofera plants exhibit varying growth and maturity across different seasons. Local wisdom often guides the selection of harvesting when the pods have a light brown color. This research demonstrates that this period corresponds to the time when the plants yield the highest indigo dye content. The findings validate that local wisdom plays a crucial role in determining the optimal time for cultivating and harvesting Indigofera, ensuring the production of indigo dye of the highest quality. Integrating traditional knowledge with scientific understanding represents a significant advancement in this field.

Table 1 The effect Indigofera fruit stages on the indigo blue production.

Indigofera species	Indigofera fruit stage	YIPL (g IP/kg fresh leaves)	IBIP (g IB/kg IP)	YIBL (g IB/kg fresh leaves)
Indigofera suffruticosa Mill.	Flowering	201.65 ± 9.92	7.85 ± 0.13[a]	1.71 ± 0.020[b]
	Young pods	202.68 ± 11.57	8.68 ± 0.18[b]	2.21 ± 0.049[c]
	Brown pods	201.65 ± 9.92	16.01 ± 0.27[d]	3.60 ± 0.063[e]
Indigofera tinctoria L.	Flowering	206.25 ± 10.15	8.57 ± 0.10[b]	1.57 ± 0.025[a]
	Young pods	209.29 ± 14.39	11.07 ± 0.25[c]	1.74 ± 0.036[b]
	Brown pods	204.92 ± 12.07	18.02 ± 0.32[e]	3.20 ± 0.054[d]
F-value		0.209	1155	1136
p-value		0.95	0.00	0.00
CV (%)		3.82	37.12	37.10

The letters a, b, c and d are used to indicate significant differences in the column at $p < 0.05$.

The effect of harvesting time

The effect of harvest times on the yield and indigo blue content in the Indigofera plants is presented in **Table 2**. It was observed no statistically significant differences in the YIPL at all harvest times (6:00 a.m., 12:00 p.m. and 6:00 p.m.). However, the IBIP and YIBL exhibited significant differences with different harvesting time ($p < 0.05$). Moreover, harvesting the Indigofera leaves at 6:00 a.m. gave the highest IBIP and YIBL of both Indigofera species. These findings could be discussed that Indigofera leaves fermentation for indigo dye extraction involves immediate processing after harvesting. Choosing the optimal harvest time is crucial, with early mornings (around 6:00 a.m.) often preferred over other times (noon and 6:00 p.m.). This preference is driven by several factors, primarily the enzyme β-glucosidase, which breaks down indican into indoxyl and glucose. Early morning temperatures are lower, promoting optimal enzyme activity. The β-glucosidase in Indigofera plants functions optimally between 30 - 50 °C and becomes inactive at 55 - 70 °C [31,33].

Additionally, early mornings offer cooler temperatures and higher humidity compared to other times of the day, further preserving the quality of harvested leaves [11]. Lower temperatures reduce water loss, maintaining leaf freshness and efficiency, while higher humidity prevents wilting, which can negatively impact β-glucosidase activity [4,7]. These findings affirm the local wisdom, suggesting that harvesting Indigofera plants in the early morning yields the highest indigo dye production.

This research aligns with the traditional method of harvesting indigo leaves early in the morning, a practice aimed at maintaining leaf freshness, a critical factor for obtaining high-quality indigo dye. As temperatures rise during the day, leaves may undergo wilting, impacting the activity of the β-glucosidase present in the leaves. Therefore, precise control of the timing of leaf harvesting is essential for managing the production process and achieving a high-quality and efficient indigo dye product. Furthermore, the study underscores the importance of local wisdom in optimizing cultivation practices, contributing to the sustainable and effective production of indigo dye.

Table 2 The effect of harvesting time of Indigofera plants on indigo dye yield of the Indigofera plants.

Indigofera species	Harvesting time	YIPL (g IP/kg fresh leaves)	IBIP (g IB/kg IP)	YIBL (g IB/kg fresh leaves)
Indigofera suffruticosa Mill.	6:00 a.m.	196.01 ± 4.90	9.57 ± 0.28[d]	1.86 ± 0.019[d]
	12:00 p.m.	206.68 ± 9.20	9.09 ± 0.03[c]	1.85 ± 0.036[d]
	6:00 p.m.	202.99 ± 12.37	8.82 ± 0.09[b]	1.76 ± 0.017[f]
Indigofera tinctoria L.	6:00 a.m.	201.29 ± 12.57	8.07 ± 0.14[b]	1.61 ± 0.028[e]
	12:00 p.m.	202.63 ± 9.35	7.64 ± 0.11[a]	1.53 ± 0.022[a]
	6:00 p.m.	200.92 ± 9.79	7.52 ± 0.20[a]	1.50 ± 0.041[a]
F-value		0.36	94.89	85.74
p-value		0.87	0.00	0.00
CV (%)		4.45	9.45	9.42

The letters a, b, c, d, e and f are used to indicate significant differences in the column at $p < 0.05$.

The effect of light exposure on indigo dye yield from the Indigofera plants

Light is an important factor in the growth and synthesis of indigo blue in Indigofera plants. **Table 3** shows the results of cultivating Indigofera plants under different light exposure conditions (60 % received and 100 % sunlight). The quantity of indigo paste remained statistically consistent, regardless of the light exposure condition. However, light exposure significantly influenced the IBIP and the YIBL ($p < 0.05$). Additionally, the results indicated that *Indigofera tinctoria* L. consistently produced higher levels of indigo blue compared to *Indigofera suffruticosa* Mill. across all sun light exposure conditions.

Light intensity regulation significantly influences the growth of *Indigofera tinctoria* L., impacting both the color quality extracted and the yield of biomass. This influence is closely tied to its effects on photosynthetic efficiency and the net assimilation rate. The net assimilation rate is a crucial metric reflecting the efficiency of light assimilation in plants [12,34]. Insufficient light conditions can lead to stem elongation and leaf expansion, optimizing light absorption.

This physiological response aims to increase leaf surface area for improved light absorption. Environments with lower light intensity may reduce leaf numbers, potentially compromising the plant's leaf production capacity. Plants adapt by increasing the specific leaf area at lower light levels, amplifying the leaf surface area for enhanced light absorption [12,34]. For instance, increasing the specific leaf area by 60 % under low-light conditions is a strategy that helps plants adapt to reduced light environments, enhancing their efficiency in light absorption and resulting in an expansion of the specific leaf area. Understanding this information will facilitate advancements in the cultivation and application of *Indigofera tinctoria* L. in the textile industry and the future production of natural indigo dye.

Table 3 The effect of lighting condition on indigo blue production in Indigofera plants.

Indigofera species	Lighting conditions	YIP (g IP/kg fresh leaves)	IBIP (g IB/kg IP)	YIB (g IB/kg fresh leaves)
Indigofera suffruticosa Mill.	60 % received sunlight	201.35 ± 5.41	8.72 ± 0.26^{a}	2.30 ± 0.04^{b}
	100 % received sunlight	201.29 ± 8.92	12.33 ± 0.14^{c}	2.84 ± 0.01^{d}
Indigofera tinctoria L.	60 % received sunlight	204.32 ± 7.11	11.48 ± 0.20^{b}	1.74 ± 0.05^{a}
	100 % received sunlight	202.25 ± 10.25	14.18 ± 0.04^{d}	2.47 ± 0.03^{c}
F-value		0.091	470.89	469.81
p-value		0.96	0.00	0.00
CV (%)		0.70	18.81	18.85

The letters a, b, c and d are used to indicate significant differences in the column at $p < 0.05$.

Conclusions

This study highlights key practices for maximizing indigo dye yield in Sakon Nakhon, Thailand. Harvesting Indigofera plants at the brown pod stage (18 - 20 weeks) in the early morning (6:00 a.m.) significantly enhances indigo production. The 100 % sunlight exposure during cultivation outperforms 60 % sunlight exposure, emphasizing the role of light in plant growth *Indigofera suffruticosa* Mill. consistently gave indigo dye yields more than *Indigofera tinctoria* L. The integration of local wisdom is crucial for effective indigo cultivation. Future research should explore additional factors, including soil composition, fertilizer application and advanced extraction techniques, to enhance sustainable indigo dye production.

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