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Influence of Different Cow Manure Application Rates on the Growth, Yield and Indigo Blue Color of *Indigofera suffruticosa* Mill

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ABSTRACT

Background: Indigo is a key ingredient in the natural dyeing of fabrics. However, the conventional use of chemical fertilizers in indigo cultivation has led to an increase in production costs and soil degradation. Low-fertility soils further compromise the growth and yield of indigo plants. To address these challenges, employing manure is recognized as a sustainable approach for indigo cultivation. The use of manure enhances soil nutrient levels, improves soil structure and mitigates production costs. In this study, our objectives were to compare different rates of cow manure in relation to indigo growth, yield and the effect of cow manure rate on the value of indigo blue color.

Methods: The experiments were carried out at the Plant Science Field from June to October 2022. Employing a randomized complete block design (RCBD), the study featured four treatments with four replications each. The treatments included: Treatment 1: no fertilizer (control); Treatment 2: cow manure at a rate of 3,125 kg/ha; Treatment 3: cow manure at a rate of 6,250 kg/ha; and Treatment 4: cow manure at a rate of 9,375 kg/ha. Data collection included plant height, canopy width, number of branches, plant weight, indigo blue weight and indigo blue color value. The CIE color system (Commission International de l'Éclairage) determined the color value.

Result: The application of cow manure at a rate of 9,375 kg/ha resulted in the highest plant height and canopy width, with the corresponding number of branches of 150.73 cm, 46.45 cm and 31.73 cm, respectively. As compared to the other treatments, these values were statistically different. The color that was associated with the indigo finished product was dark blue and the yield; plant weight and indigo blue weight were recorded as 15,663.44 kg/ha and 2,540.44 kg/ha, respectively. The color value of indigo blue showed that the L* values of 24.94, a* -9.32, b* -2.44 and C* 9.63 were not significant but h* 194.66 was statistically significant.

Key words: Color, Growth, Indigo, Manure, Organic amendment, Yield.

INTRODUCTION

Indigo, belonging to the legume family (Leguminosae), known as the ″Queen of Color″ is utilized in the textile dyeing industry due to its distinct color features, including durability, non-flashiness, aesthetic appeal and distinguishing characteristics. The cultivation of indigo and the subsequent production of indigo blue dye for export occur in the Northeastern Thailand, particularly in Sakon Nakhon province. This area has a longstanding tradition of cultivating indigo plants and engaging in indigo dyeing practices (Panngom *et al.,* 2011; Teanglum, 2012). Since the majority of the population in Sakon Nakhon province is Tai Yo, Tai Yoy, Tai Kaloeng, Tai Zo, Tai Kha and Tai Lao, whose culture employs a dress dyed using black or blue as the base color and the color used to dye their fabrics is from indigo plants. Hence, this cotton-based textile colored indigo blue has received Geographical Indication (GI) status and has been chosen as a representative product of Sakon Nakhon Province. According to Srisamran (2015), the Sakon Nakhon Provincial Community Development Office has gathered revenue data pertaining to indigo-dyed fabric products. The findings indicate that these products contribute at least 48 million baht every year to the revenue of Sakon Nakhon Province. Furthermore, it is projected that in the year 2022, producers and entrepreneurs in this industry will generate an annual revenue of 239,110,635 baht. The demand for indigo-dyed fabrics is increasing both domestically and internationally, yet the current supply of indigo blue dye falls short of meeting market demands. A substantial amount of indigo is necessary, as research indicates that it takes 8 kg of indigo trees to produce 1 kg of indigo blue (Saithong, 2010; Wiwacharn *et al.,* 2015). Hence, in order to get a sufficient supply, it is necessary to

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increase the quantity of cultivated plants. Indigo plants are commonly cultivated with the application of inorganic fertilizers. Nevertheless, the utilization of many inorganic fertilizers has an impact on the cost of indigo production due to the expensive inorganic fertilizer. As the expenses associated with these inputs increase, the overall cost of production likewise increases. Furthermore, prolonged usage of these inputs leads to soil compaction, the depletion of beneficial microorganisms and nutrient deficiency. Additionally, overusing these chemicals has an impact on the development of nodules or rhizobium in the roots, which are crucial for fixing nitrogen in the air and supplying the plant with important nutrients and protein components for healthy growth and development (Kim *et al.,* 2003; Nohong *et al.,* 2019).

The use of organic fertilizer, like manure, is a viable approach for cultivating indigo with the aim of achieving favorable outcomes in terms of yield, quality and growth. This practice offers the potential to decrease production costs, improve soil nourishment and promote environmental sustainability. Cow manure is often used as a valuable organic fertilizer. It is a type of animal waste that comes from cows. It comprises undigested feed, water and microorganisms and contains nitrogen, phosphorus and potassium nutrients (Dhananjay and Nalin, 2024). According to Whalen *et al.* (2000), the addition of cow manure as soil amendments has the potential to enhance pH levels and boost the availability of phosphorus (P) and potassium (K) for plants in acidic soils in the short term. Jimoh *et al.,* (2019) reported that cow manure has 15.6 N (g/kg), 6.9 P (g/kg) and 7.3 K (g/kg). Research has been conducted on the use of manure in growing a variety of crops, including lettuce, cucumber, broccoli, holy basil, kale and white mugwort (Masarirambi *et al.,* 2010; Ghasem *et al.,* 2014; Jigme *et al.,* 2015; Miadnok and Promatar, 2019; Kunlanit *et al.,* 2021; Lesing and Anugoolprasert, 2016). Kumudeswar and Chayanika (2016) showed results revealed that all the timings of fresh cow manure application significantly increased grain and straw yields of scented rice over the control. Cow manure applied in four splits as 2.5 t/ha each at 7, 15, 30 and 45 DAP in scented rice produced the highest seed and yields of linseed and organic fertilizer had beneficial residual effects on crop production, which in turn on improving the growth of barley plants and consequently positively affected yield (Arebu, 2022). Also, Almaz *et al.,* (2017), report that the substitution of 50% of inorganic fertilizer with poultry manure is recommended for a reduction in the use of chemical fertilizers for mono-cropping and intercropping of maize and soybean. Chinthapalli *et al.,* (2015) found that the length of shoot increased in the case of faba bean seedlings from organic fertilizers (cow manure) over inorganic fertilizer and the highest seed yield (2,714 kg/ha) of pigeonpea (Ramesh *et al.,* 2006). Cow manure was found to increase the growth of peanuts and mungbeans (Musdalifah *et al.,* 2021; Mahabub *et al.,* 2016). Moreover, growing indigo by adding 6,250 kg/ha of animal manure yields more than chemical fertilizers (Srisamran *et al.,* 2015). It was found that the use of animal manure for growing crops. This results in higher yields than the use of chemical fertilizers, as well as improving the quality of the soil and can be used as an alternative for organic farming.

The color of indigo blue is an important quality attribute for indigo blue dye fabrics. A color model has been widely applied in crop production, including seed color, leaf color and fruit color (Jasso-Chaverria *et al.,* 2005; Anuwong *et al.,* 2020; Zhang, *et al.,* 2022). The color difference has been classified with a CIE system that can be computed as the relative distance between two reference points with a color space and display its values for each coordinate L*, a*, b*, C* and h*. For indigo research, it was no reports on measuring the color value of indigo. Therefore, this research has studied the effect of the rate of cow manure for the growth, yield and color value of indigo blue.

MATERIALS AND METHODS

Experimental site

The experiment was conducted at the Plant Science Experimental Field, Faculty of Agricultural Technology, Sakon Nakhon Rajabhat University, Sakon Nakhon Province, Thailand, from June to October 2022, where the average rainfall was 178.45 mm and the temperature ranged from 27.4°C. The soil texture was loamy sand, pH 5.72 and N, P, K were 0.13, 18.3 and 29.3 g/kg, respectively (Sriraj *et al.,* 2022).

Experimental design and treatments

The experiment followed a randomized complete block design (RCBD) consisting of four replications and four treatments. These treatments involved varying rates of cow manure application for indigo planting, namely: 0 kg/ha (control), T1-3, 125 kg/ha, T2-6, 250 kg/ha and T3-9, 375/ha. The cow manure rate was a reference from the research of Srisamran *et al.* (2015).

Indigo planting

Indigo seeds were initially sown in a seedling tray filled with peat moss as the growing medium. After 30 days of growth, the indigo seedlings were transplanted into the plot. A plot (2.5×3.0 m) was designed for 4 plots of each treatment, bringing about a total of 16 plots. We arranged the indigo seedlings in four planting rows, ensuring a spacing of 30×60 cm between each plant and row, totaling 36 plants per plot. Prior to planting, cow manure was added according to the specified treatments. The cow manure properties were pH, EC, OM, N, P, K and C/N ration of 8.14, 1.26 ds/m^{-1}, 3.41%, 0.17%, 0.92%, 1.49% and 11.56, respectively.

Data collection

Data collection occurred at 15-day intervals post-transplanting, focusing on plant height and the width of the canopy. Throughout the 120-day period, indigo was harvested by cutting the whole plant (the aboveground

part), key metrics such as the number of branches per plant, plant weight, weight of indigo blue and the color value of indigo blue were recorded by the CIELAB color system. The CIELAB color system contains the lightness dimension, represented by L*, which ranges from 0 (pure black) to 100 (diffuse white) and the chromatic a* axis extends from green (-a*) to red (+a*) and the chromatic b* axis extends from blue (-b*) to yellow (+b*). The CIELAB system has been improved by converging a* and b* values to hue and chroma values by assigning two color terms, hue (h*) and chroma (C*). The value of chroma C* is the distance from the lightness axis (L*) and starts at 0 in the center. The hue angle starts at the +a* axis and is expressed in degrees (0° is red, 90° is yellow, 180° is green and 270° is blue) (Nitayaphat, 2015).

Weight of indigo blue

The harvested indigo plant was placed in a plastic bucket and immersed in water, allowing it to soak for 24 hours. Afterwards, the indigo plant was removed, followed by adding 12.5% (w/w) of red lime to the water, that it bought from grocery shop in Muang, Sakon Nakhon province and the indigo-water mixture was mechanically stirred for 10 minutes. The mixture was then allowed to stand for an additional 24 hours to facilitate precipitation. Following this period, the upper part of the indigo water was decanted, leaving the indigo sediment. The sediment was filtered through cloth for 24 hours, resulting in the extraction of indigo blue for weighting.

Color of indigo blue

The Indigo blue was photographed with a digital camera and 50 grams of indigo blue were used to measure indigo color values. A colorimetric machine was utilized to measure indigo color values, employing the CIELAB color system. This system encompasses one 'channel' for lightness (L*) and two channels for color (a* and b*). In its three-dimensional representation, the chromatic a* axis spans from green (-a*) to red (+a*), while the chromatic b* axis ranges from blue (-b*) to yellow (+b*). L* stands for the lightness dimension, which ranges from 0 (pure black) to 100 (diffuse white). At the intersection of the a* and b* axes, specifically at the L* value of 50, we find pure, balanced, neutral gray. The C* value signifies the degree of blue-green and h* represents a color angle in degrees. These angles range from 0 degrees for red, 90 degrees for yellow, 180 degrees for green and 270 degrees for blue.

Data analysis

The data were statistically analyzed using the ANOVA technique. Subsequent mean separation was conducted through Duncan's new multiple range test (DMRT) using SAS program version 9.1.

RESULTS AND DISCUSSION

Plant height

At 30-60 days of indigo growth, there were no statistically significant differences in plant height among the four treatments. However, during the 75-120-day period, a significant difference ($p \leq 0.05$) was observed across the treatments. Specifically, the application of cow manure at a rate of 9.375 kg/ha resulted in the tallest plants, reaching 150.73 cm. This was followed by cow manure at rates of 6.250 kg/ha, 3.125 kg/ha and the control, with corresponding heights of 148.50 cm, 144.93 cm and 143.35 cm, respectively (Fig 1a). The findings suggest a positive correlation between plant height and increasing cow manure levels, aligning with previous research on mungbean growth patterns (Mahabub *et al.,* 2016). Cow manure, known for its higher soil organic carbon and available N and K, also demonstrated enhanced dehydrogenase and phosphatase activity in the soil (Ramesh *et al.,* 2006). These enzymes will catalyze the transformation of organic matter in the soil. This causes the release of nutrients, due to improved nutrient availability and the indication of increased soil biological activity. As a result, the plant has rapid growth. Similar trends were noted in studies involving pea and faba beans, which exhibited optimal growth with the application of 15,000 kg/ha of cow manure (Chinthapalli *et al.,* 2015). Furthermore, Musdalifah *et al.* (2021) found that the treatment of cow manure at 15,000 kg/ha yielded the highest average peanut height at 42 days after planting (DAP).

Width of the canopy

Between 30-75 days, no statistical differences were observed in the canopy width among the four treatments. However, at 90-105 days and 120 days, statistically significant differences were noted ($p \leq 0.01$ and $p \leq 0.05$, respectively). Specifically, the application of cow manure at a rate of 9.375 kg/ha resulted in the widest canopy, measuring 46.45 cm. This was followed by rates of 6.250 kg/ha, the control and 3,125 kg/ha, with canopy widths of 44.80 cm, 43.13 cm and 43.10 cm, respectively (Fig 1b). This might be because cow manure improved the physical-chemical soil properties and increased the level of soil nutrition, which improved the canopy width of indigo. This trend aligns with findings by Daba *et al.,* (2018), who reported an increase in carrot canopy diameter when using cow manure at a rate of 10,000 kg/ha compared to non-fertilizer control.

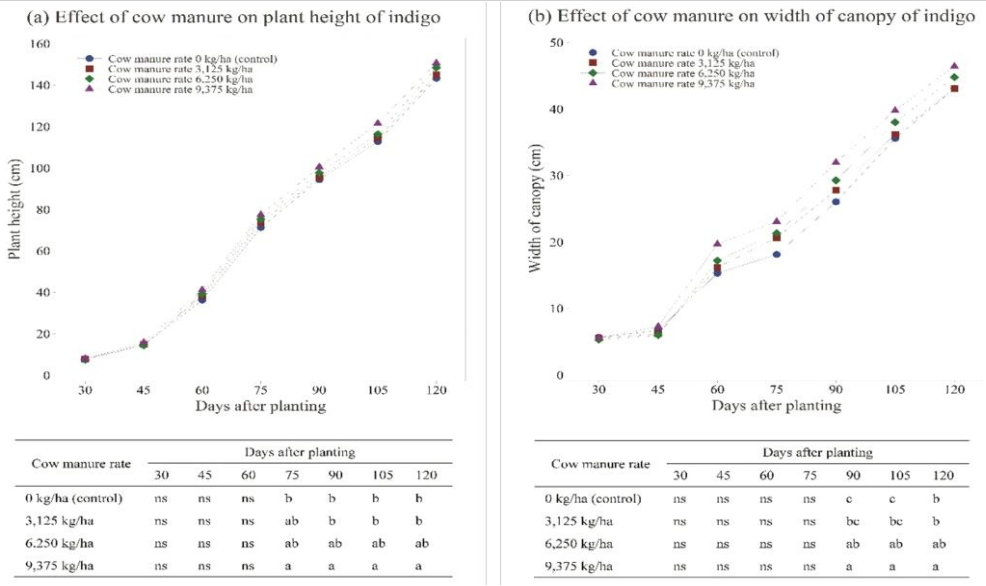
Number of branches, weight of the plant and weight of indigo blue

The number of branches in the indigo plants ranged from 29.13 to 31.73, with no statistical differences observed among all treatments. Regarding plant weight, the application of 9.375 kg/ha of cow manure yielded the highest weight at 4.84 kg/plot, translating to a maximum weight of 15,663.44 kg/ha and subsequently decreased at the rates of 6.250 kg/ha, 3.125 kg/ha and the control (without cow manure) with plant weights of 10,485.44 kg/ha, 9,660.18 kg/ha and 8,414.25 kg/ha, respectively. The differences observed were statistically significant ($p \leq 0.01$). In terms of indigo blue production, the highest yield was recorded at 2,540.44 kg/ha, primarily attributed to the application of

cow manure at a rate of 9.375 kg/ha. Following this, cow manure at rates of 6.250 kg/ha, 3,125 kg/ha and the control (without cow manure) resulted in indigo blue weights of 1,585.75 kg/ha, 1,212.50 kg/ha and 1,156.94 kg/ha, respectively. A statistically significant difference ($p \leq 0.01$) was observed, as highlighted in Table 1. Consistent with these findings, Srisamran *et al.,* (2015) reported that an increase in manure rates (1,562.5, 3,125 and 6,250 kg/ha) resulted in a proportional increase in the weight of indigo trees. Specifically, the application of manure at the rate of 6,250 kg/ha demonstrated the highest indigo tree weight, reaching 13,500 kg/ha, along with the highest indigo blue weight of 1,750 kg/ha, exceeding the non-fertilizer control. Similar outcomes were documented in pea and faba bean studies by Chinthapalli *et al.,* (2015), where cow manure at 15,000 kg/ha yielded the highest fresh weight and dry weight biomass. Additionally, optimal productivity in mungbean was achieved using cow manure at 15,000 kg/ha (Musdalifah *et al.,* 2021). Conversely, the highest peanut yield was reported with the application of cow manure at 10.000 kg/ha (Mahabub *et al.,* 2016). In the case of indigo, if more than 9.375 kg/ha of cow manure is used, the yield and quality of indigo may increase accordingly because it was found that the appropriate rate of cow manure was 10.000 kg/ha and 15.000 kg/ha in peanut and mung bean, respectively. Additionally, cow manure will have a positive long-term effect, both in terms of reducing production costs and improving soil properties. If the soil has rich nutrients and good properties, it will promote better growth and yield of indigo than soil with low nutrients and poor properties and indigo can be produced as an organic planting.

Color of indigo blue

The color of indigo blue was blue when viewed with the eye in the photo (Fig 2). There is no difference in color between all treatments. And when used to measure color, the CIELAB



Cow manure rate	Days after planting						
	30	45	60	75	90	105	120
0 kg/ha (control)	ns	ns	ns	b	b	b	b
3,125 kg/ha	ns	ns	ns	ab	b	b	b
6,250 kg/ha	ns	ns	ns	ab	ab	ab	ab
9,375 kg/ha	ns	ns	ns	a	a	a	a

Cow manure rate	Days after planting						
	30	45	60	75	90	105	120
0 kg/ha (control)	ns	ns	ns	ns	c	c	b
3,125 kg/ha	ns	ns	ns	ns	bc	bc	b
6,250 kg/ha	ns	ns	ns	ns	ab	ab	ab
9,375 kg/ha	ns	ns	ns	ns	a	a	a

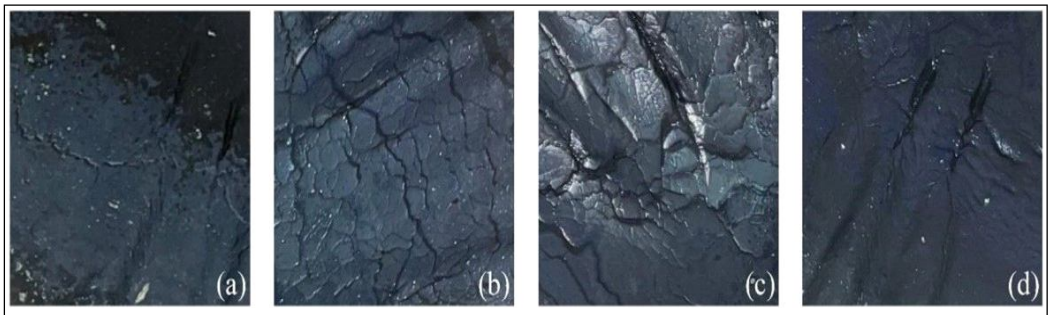
(a) plant height of indigo and (b) width of indigo canopy. The accompanying tables for each figure provide detailed comparisons of plant height and canopy width at various intervals post-planting. The same letters in the same column denote they are not significantly different by Duncan's New Multiple Range Test ($p \leq 0.05$).

Fig 1: Investigating the effect of cow manure.

Table 1: Effect of cow manure on number of branches, plant weight and indigo blue weight.

Cow manure rate	No. of branch/ plant	Weight of plant (kg/plot)	Weight of plant (kg/ha)	Weight of indigo blue (kg/plot)	Weight of indigo blue (kg/ha)
0 kg/ha (control)	29.13	2.60^{c}	8,414.25^{c}	0.36^{c}	1,156.94^{b}
3,125 kg/ha	30.85	2.99^{bc}	9,660.18^{bc}	0.38^{bc}	1,212.50^{b}
6,250 kg/ha	31.03	3.24^{b}	10,485.44^{b}	0.49^{b}	1,585.75^{b}
9,375 kg/ha	31.73	4.84^{a}	15,663.44^{a}	0.79^{a}	2,540.44^{a}
p value	0.06	0.00	0.00	0.00	0.00
F test	ns	**	**	**	**
CV (%)	3.75	9.44	9.44	15.60	15.85

Mean with a column followed by the same letter are not significantly different by DMRT.
ns: Non-significant at $p>0.05$ and **Significant at $p \leq 0.01$.



(a) cow manure rate 0 kg/ha, (b) cow manure rate 3,125 kg/ha, (c) cow manure rate 6,250 kg/ha and (d) cow manure rate 9,375 kg/ha.

Fig 2: Influence of cow manure on the color of indigo blue.

Table 2: Effect of cow manure on the color value of indigo blue.

Cow manure rate	L*	a*	b*	C*	h*	Color
0 kg/ha (control)	24.92	-9.25	-2.42	9.56	194.65^{ab}	dark blue
3,125 kg/ha	24.84	-9.14	-2.38	9.45	194.59^{b}	dark blue
6,250 kg/ha	24.94	-9.33	-2.45	9.64	194.70^{a}	dark blue
9,375 kg/ha	24.94	-9.32	-2.44	9.63	194.66^{a}	dark blue
p value	0.71	0.53	0.29	0.51	0.02	
F test	ns	ns	ns	ns	*	
CV (%)	0.56	-2.09	-2.11	2.08	0.02	

Mean with a column followed by the same letter are not significantly different by DMRT.
ns: Non-significant at $p>0.05$ and *Significant at $p\leq0.05$.

color values of indigo revealed distinctions among treatments, specifically the control (without cow manure) and cow manure at rates of 3,125, 6,250 and 9,375 kg/ha. The results of the experiment showed that there was no statistical difference in the color value of indigo blue of cow manure compared to the control. It was shown that cow manure has no effect on the color value but it has a direct effect on the growth and yield of indigo. Specifically, the rate of cow manure was 9,375 kg/ha. As a result, the plant height, the width of the canopy, the fresh weight of the plant and the weight of the indigo blue were the highest and there was a statistical difference with other cow manure rates. The L* values, measuring brightness, were observed to be 24.92, 24.84, 24.94 and 24.94, respectively. These values indicate low brightness, suggesting that the indigo blue is dark across all treatments, with no statistical differences noted. Examining the a* value, which signifies the direction of color, it was observed that indigo blue from all treatments exhibited color on the green axis. Similarly, for the *b value, indigo blue was situated along the blue axis. Importantly, no statistical differences were detected in these color components, suggesting consistent color characteristics across the treatments. In terms of C*, no statistical differences were observed, indicating consistent color saturation in the blue-green spectrum for all treatments. Examining h*, which denotes the color angle, revealed a distinctive greenish-blue hue for indigo treated with 6,250 and 9,375 kg/ha of cow manure, registering h* values of 194.66 and 194.70 degrees, respectively. In contrast, the h* values for 3,125 kg/ha and the control were 194.59 and 194.65 degrees, respectively, showcasing a statistically significant difference ($p\leq0.05$) (Table 2). The color of indigo blue significantly influences the final appearance of indigo-dyed fabrics. In a study by Kaewklaikhajornsiri and Sikkha (2012), indigo dyeing with a controlled formula resulted in L*, a*, b*, C* and h* values of 27.45, -3.20, -15.46, 15.78 and 258.30, respectively. Additionally, research by Kawahito *et al.* (2002) highlighted a brightness range of 17.35 to 21.84 for cloth dyed with natural indigo.

CONCLUSION

In summary, the optimal rate of cow manure for indigo growth was determined to be 9,375 kg/ha. This resulted in the highest overall growth and yield, including significant enhancements in plant height, canopy width, number of branches, plant weight and indigo blue yield. Notably, the color of the indigo blue achieved at this rate exhibited the deepest and most saturated blue hue.

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Conflict of interest

This manuscript has not conflict of interest. It no financial support or benefits have been received by myself, by any author.

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