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INFLUENCE OF LED RED-LIGHT INTENSITY ON PHYCOCYANIN ACCUMULATION IN THE CYANOBACTERIUM *NOSTOC COMMUNE* VAUCHER

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ABSTRACT

To assess the effects of light intensity on the phycocyanin of Nitrogen-fixing cyanobacteria *Nostoc commune* Vaucher, which was isolated from Maetang District, Chiangmai Province, Thailand. The algae was cultivated in laboratory under different red light LED (LD) intensity of, 10, 20, 30, 100 and 120 µmol m^{-2} s^{-1}. The medium using BG 11 modified without N source. Results Biomass as fresh weight and dry weight, %Dry weights, protein, and Phycocyanin contents were fluctuation in difference light intensity. The highest biomass as fresh weight have shown in LD 30 with the value of 9.24 $g.L^{-1}$ and the lowest was at LD10 with the value of 2.56 $g.L^{-1}$ and as dry weight was maximum at LD120 with 0.21 $g.L^{-1}$ and minimum was 0.07 $g.L^{-1}$ at LD10. %Dry weights was found the maximum value at LD120 with 3.51% and lowest at LD 30 with the value of 1.8%. Protein content have shown the highest value at LD30 with 1.8%. Protein was high present at LD10 with the value of 1.00±0.12 $mg.L^{-1}$ and low value at LD100 with 0.79±0.09 $mg.L^{-1}$. The Red light intensity of 30 µmol m^{-2} s^{-1} was resulted in phycocyanin higher values than other intensities with the value of 0.32±0.09 $mg.L^{-1}$ and lowest concentration at LD120 with the value of 0.17±0.01 $mg.L^{-1}$. Conclusion LED Red light at 30 µmol m^{-2} s^{-1} were optimal for phycocyanin accumulation in *Nostoc commune* Vaucher.

Keywords: Light intensity, Phycocyanin, *Nostoc commune* Vaucher

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INTRODUCTION

Nowadays, light emitting diodes (LED) has been increasingly used as a source of artificial light in controlled environmental system due to its energy-efficiency. LED light quality and intensity have an effect to the growth of plant in semi-closed system. (Sakhonwasri et al. 2017).

Blue-green algae occupy an anomalous position in the biological world. They are treated by botanists as a division (or class) of algae because they are photoautotrophs that use water as an electron donor and contain the two photopigments (chlorophyll a and β-carotene) that are the chemical hallmarks of plant photosynthesis (Stanier et al.,1971). *Nostoc commune* is an blue green algae and edible fresh water cyanobacterium and its a nitrogen fixing blue green algae of the Division Cyanophyta . *Nostoc commune* contain rich a, amino acids, fatty acids, flavonoids polysaccharide, vitamins, and many kinds of minerals (Diao et al.,2014, Li and Liu,2003) It has been widely used as food in northeast of Thailand. The control and optimization of light intensity and light wavelength is regarded as one of the most important parameters for the culture of photosynthetic microorganisms (Ugwu et al., 2008). For last decade cyanobacteria have been receiving increasing interest due to their potential to produce a diverse range of chemicals and biologically active compounds, such as vitamins, carotenoid pigments, proteins, lipids and polysaccharides (Zhang et al., 1999). For exploration of these potentials of cyanobacteria it should be cultivated in commercial way. Globally researchers are trying to produce microalgae/ cyanobacteria commercially (Belay 1997; Ben-Amotz 2004). Yet very little or primary information is available on detailed design criteria and innovation, location selection, scaling considerations, or constrains involved in large scale cultivation. *Nostoc commune* is blue-algae which human use for organic food source and contains high proteins with well-balanced amino acids. It is also rich in carbohydrates, vitamins, minerals, phenolic, pigments (chlorophyll, carotenoids and phycobilins) and poly-unsaturated fatty acids (Miranda et al. 1998; Anupama 2000). *Nostoc commune* algae could be used for high value food. This algae is a nitrogen fixing ,so it's have high nutritional value, such as 20.84 gram / 100 g of protein, 0.43 grams of vitamin B 1, 1.54 grams of calcium, 0.37 iron, and 21.40 micrograms / 100g of vitamin A. and 17 essential amino acids. *Nostoc* is an edible blue green algae used for health food and herbal medicine due to its nutritional values and antioxidant properties (Yi and Zuiun. 2014). *Nostoc* has been used as a source of proteins, vitamin and unsaturated fatty acids for human and animals (Gao. 1998). However, wild type N. *commune* has been decreasing in quantity as a result of ever-growing market demand and environmental pollution. Therefore, artificial culture of N. *commune* is important

as it can bring great social and economic benefits. (Yi Diao and Zujun Yang.2014). C-phycocyanin (C-PC) could be extracted from cyanobacteria such as *Spirulina platensis*, which has been widely used in commercial applications in the food and cosmetic industry as a natural blue dye (Romay et al., 2003), anti-inflammatory (Romay et al.,2003; Reddy et al., 2003; Bhat and Madyastha, 2001) and antioxidant (Estrada et al., 2001; Bhat and Madyastha, 2000). Some papers report C-PC extraction from cyanobacterium. Estrada et al., 2001) and also studied the optimization of extraction from dried biomass. The extraction using ultrasonic bath in the presence of glass pearls in the biomass proved to be more efficient method, 56% higher than using freezing and thawing, and presented a extraction yield of 43.75 $mg.g^{-1}$ and a C-phycocyanin concentration of 0.21 $mg.mL^{-1}$. (Moraes et al.2011)

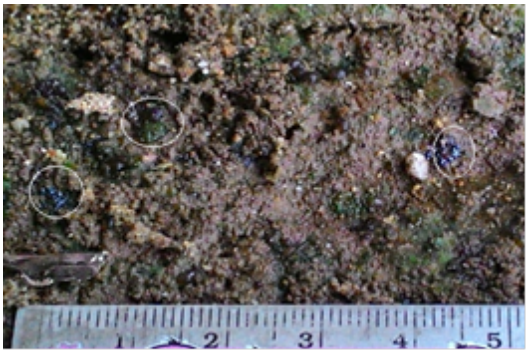
The previous study was comparing the light quantity between red light and white condition. The result was shown that the red light was exhibit the higher phycocyanin content than white light. In this study to find out the quality of red light intensity by adjust the treatment of light between the previous red light intensity. To find more the influence of red light intensity on phycocyanin accumulation

MATERIAL AND METHODS

A. Microorganism and culture medium:

The *Nostoc commune* stain was isolated from natural Mae Taeng clean and shallow river. Located in Chiang Mai province. The sample was carried out to Program in Agricultural Interdisciplinary Lab, Maejo University. The colony was washed with distilled water and follow with Ethyl alcohol 10% Clorox 5% and then washing with distilled water done in 3 times replicated. The pure colony was transfer to culture in BG 11 modified (Das Karabi et al. (2014), Slant agar media to growth under white light condition as the stock. This medium was also used to mass production. All the reagents used were of commercial grade. The collected algal samples and isolated the samples by pick cell technique and transferred in BG-11 modified medium in vials bottle at 28±2 °C under light intensity (2000 lux) and photoperiod of 12:12 hour for 2 mouths. Then transferred samples were spread on algal broth agar plates and incubated at the above mentioned condition. After the single colonies grown were picked out and transferred to BG-11 modified medium in 250 ml. conical flask and shaken manually for 2 to 3 times in a day. The isolated microalgae were identified with standard manual for algae (Desikachary, 1959) and confirmed species to Standard species from TISTR 8160 from Thailand Institute of Scientific and Technological Research (Fig.1).


A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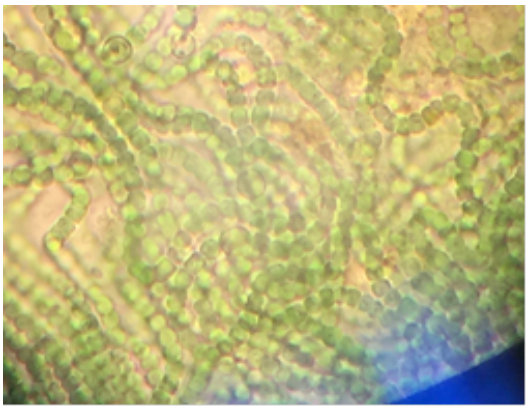
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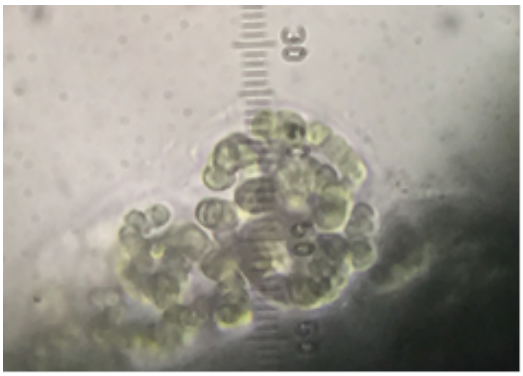

D

Fig.1. The study site (A) and *Nostoc commune* algae from Mae Taeng River (B, C) and Standard *Nostoc commune* species TISTR 8160(D)

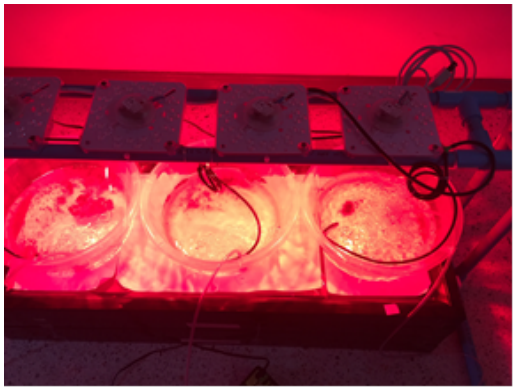


Fig.2. Culture of *Nostoc commune* in LED Red-light condition

B. Cultivation condition

C. The cyanobacterium *Nostoc commune* was grown 3 litre media in 5 litre plastic bowl under 5 levels of irradiance, 10. 20, 30, 100 and 120 µmol m^{-2} s^{-1}, with illumination provided by Red light LED lamps. The irradiance was measured on the surface of the media using light meter. The pH was measured using a 7.5 pH meter. Temperature was adjusted the air condition to 25 $\pm$ 2 $^{\circ}$C (Manigandam.2014). CO^2 was used to increase the source for photosynthesis and the source for mass production. (Manigandam, 2014) and pH sensor was to adjust pH of the optimum algae growth condition under CO_2 adjustment. Coincide with controlling the light source by use LED Red-light as the irradiance source. Light 12:12 Turn off switch was used to control the growth.

D. Biomass measurements

21 days after culturing of *Nostoc commune*. The samples were yield to find biomass as the term of fresh weight dry weight and %dry weight. Collected though the 100 µm. mesh net weight the fresh sample and then to keep dry by heat in oven-dried of 60 $^{\circ}$C for 1 hour or dry weights were determined when the specimens were at constant weights after continuous drying, and the dry matter content was calculated as a ratio of dry to fresh weight, expressed as percentage. The biomass and % dry weight were used to calculated the pure phycocyanin content of *Nostoc commune* Vaucher

E. Determination of total protein

Protein Estimation by Lowry's Method (Lowry et al., 1951) One ml. of algae cell suspension from 5 different LED Red-light condition harvested and was washed twice and suspended in sterile water. 0.1N NaOH was added and the cell suspensions were heated at 100°C to lyses cells. The hydrolysate was cooled to room temperature and 1 ml. of freshly mixed complex forming reagent (2.8598 g. NaOH, 14.3084 g. Na_2CO_3 for 500 ml. 1.4232 g. $CuSO_4.5H_2O$ for

100 ml. and 2% Sodium potassium tartrate for 100 ml.) was added. The solution was allowed to stand at room temperature for 10 minutes. One ml of folin reagent was added and vortexed and the mixer were allowed to stand at room temperature for 30-60 minutes. The absorbance was read at 750 nm. Standard curve of absorbance was plotted as a function of initial protein concentration and use it to determine the unknown protein concentration.

F. Estimative of C-phycocyanin determination

Phycocyanin was extracted from the dry biomass of algae by using Low temperature method, this is a spectrophotometry method adapted to extracted and quantify a relatively pure C-Phycocyanin fraction (Boussiba and Richmond. 1979). By weigh accurately 40 mg. *Nostoc commune* powder into a 10 ml. centrifuge tube, than add 10 ml. of 100 mM phosphate buffer (100 mM phosphate buffer contains 10.64g. K_2HPO_4 and 5.29g. KH_2PO_4 per liter, pH7). Next vortex to mix well. Store the solution in refrigerator overnight and vortex to mix well again. Take the sample to centrifuge at 100 °C at 3500 RPM and the supernatant used to verify the extraction yield. Read absorbency of each replicate at 620 nm. Using phosphate buffer as blank. Average absorbency readings for dilution replicates. Calculated percent C-Phycocyanin as follow equation.

Derivation of pure C-Phycocyanin:

$$\%\text{pure CPC} = \frac{A620 \times (10) \times (100)}{7.3 \times (\text{sample}) \times (\%\text{dry wt.})}$$

(Where 7.3 is Extraction coefficient of CPC at 620 nm. And 10 is total volume, 100 represents 100%)

G. Analytical procedures and Statistical analysis

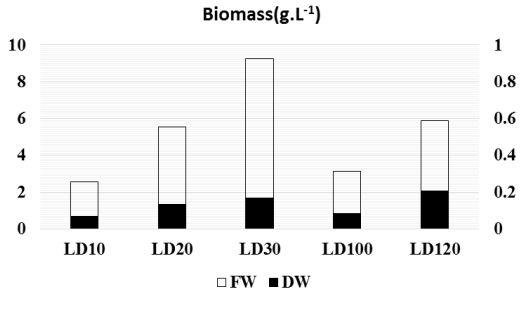
To validate the results reproducibility each treatments was done in triplicate. Statically of compare mean between treatments was done. All analyses were performed considering a level of 95% of confidence ($p<0.05$). The difference relation of biomass, protein and phycocyanin between different light intensities

RESULT AND DISCUSSION

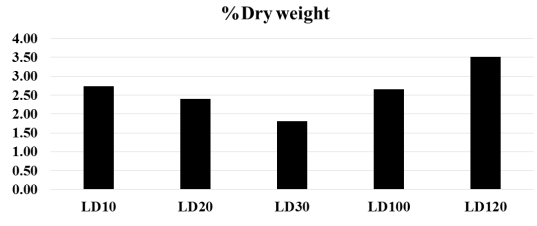
1) *Nostoc commune* Biomass

The results biomass as fresh weight have shown the highest at LD30 µmol m^{-2} s^{-1} with the value of 9.24 $g.L^{-1}$ and the lowest value was 2.56 $g.L^{-1}$ at LD10. biomass as dry weight have shown the highest at LD120 µmol m^{-2} s^{-1} with the value of 0.21 $g.L^{-1}$ and the lowest value was 0.07 $g.L^{-1}$ at LD10. (Fig.3A).

The %dry weight was shown the maximum value at LD120 with 3.51% and the minimum value at LD30 with 1.8 %. (Fig.3B).



A



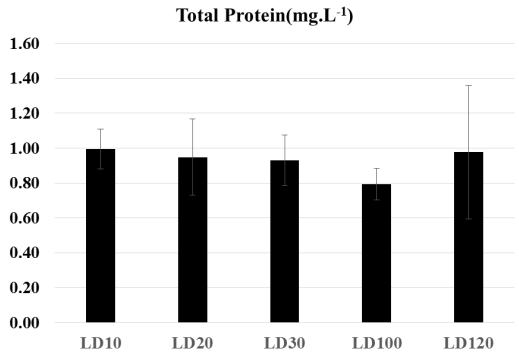
B

LD= LED Red-light at 10, 20, 30, 100 and 120 µmol m^{-2} s^{-1}

A =Biomass B=%Dry weight

Fig.3. Biomass and %dry weight of *Nostoc commune* at difference light intensity

2) Total protein content



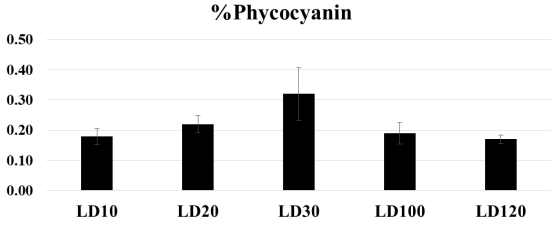
LD= LED Red-light at 10, 20, 30, 100 and 120 µmol m^{-2} s^{-1}

Fig.4. Total Protein content of *Nostoc commune* at difference light intensity

The results of total protein have shown the highest concentration at LD10 µmol m^{-2} s^{-1} with the value of 1.00±0.12 mg.L^{-1} and the lowest value was 0.79±0.09 g.L^{-1} at LD100. (Fig.4).

3) C-Phycocyanin production

The pure C-phycocyanin concentration was present higher than others at LD30 with the value of 0.32±0.09 % and most lower at LD120 with the value of 0.17±0.01%. The phycocyanin content was significant difference ($p>0.05$) between LD20 and LD120 condition (Fig 5).



LD= LED Red-light at 10, 20, 30, 100 and 120 µmol m^{-2} s^{-1}

Fig.5. C-Phycocyanin content of *Nostoc commune* at difference light intensity

CONCLUSION

The Smart culture for *Nostoc commune* in closed system, under red light controlling in this research have shown the results of the idea to plant factory or make a large scale of *Nostoc commune* production and food safety and C-phycocyanin seem to be increase in the optimum of the red light LED intensity. In this study red light LED intensity 30 µmol m^{-2} s^{-1} have an idea for plant of this algae for the purpose of increasing C-phycocyanin concen- tration and also the high yield of freshweight.

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