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Article

Optimization of the Optimum Condition of Mushroom Light Excitation (*Neonothopanus* sp) Bioluminescence

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Abstract. On the South Coast of West Sumatra was found a luminous mushroom, the *Neonothopanus sp*. The light-emitting characteristics of this mushroom are not yet known. For the use of this bioluminescence in various fields, information on the optimal light emission conditions of the mushroom is required. This study aims to determine the optimal conditions of light emission from mushrooms such as temperature and pH. In this study, the measurement of the light spectrum of mushrooms with variations in temperature and temperature was carried out. The measuring instrument used is a nanofotometer. The results showed that the maximum intensity occurred at a wavelength of 505 nm in the green area. The maximum intensity occurs at a temperature of 15oC and pH 8.

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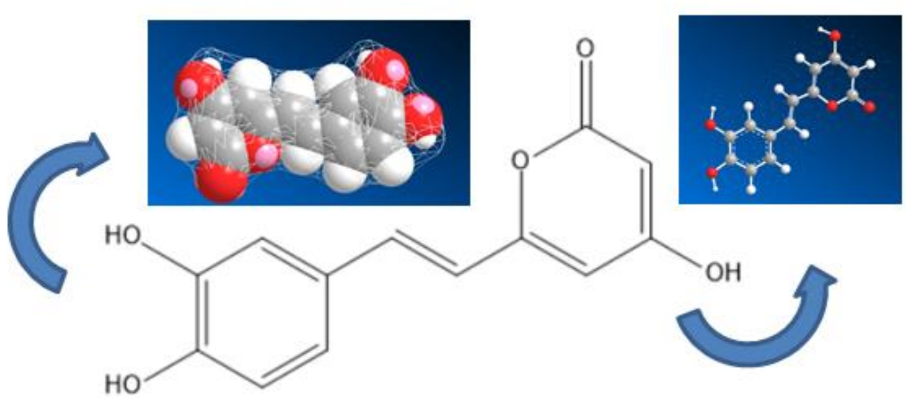
1. Introduction

Many organisms can live in organisms that can emit their own light, such as: bacteria, fungi, fireflies and fish. The emission of light from these organisms is called a biolumination that occurs in living things. Illumination is a biological process in which light is produced and emitted by living organisms resulting from chemical reactions in the organism's body [1][2]. Chemical reactions that occur in organisms to produce light involve luciferin (substrate), lucifcerase (enzyme) and molecular oxygen [3]. Luciferin compounds are organic molecules present in the body of organisms that can produce light and have heat-resistant substrates [4][5]. Luciferase functions as a catalyst for luciferin and oxygen compounds. The three main components in this bioluminescence process produce a complex reaction called a photoprotein [4]. When the chemical reaction between luciferin and luciferase is produced, the substances present in the process break down. It is produces energy called light emission [6][7].

Bioluminescence reactions vary widely among organisms but can be generally described as luciferase, the catalytic production of oxygen and luciferin which emits light when it returns to its ground state [8]. In addition, the principles of bioluminescence have been applied in many scientific fields such as agriculture, biology, ecology, and medicine [9][10]. Bioluminescent organisms use their light, which has various colors, periodic patterns, and intensities, for self-defense against predators, camouflage, specific intra communication, or attracting mates or prey [11].

The development of bioluminescence currently has been found naturally in various kinds of living things, such as fungi, bacteria, and aquatic organisms, terrestrial vertebrate animals, amphibians, and mammals [12]. The bioluminescence organisms of concern today are light fungi because of their use in various fields. The number of luminous mushrooms is increasing because many new species have been found in subtropical and tropical zones that have natural conditions suitable for where light fungi grow, such as in North and South America, Europe, Asia, Australia and Europe [13]. Fungi are non-chlorophyll microorganisms, in the form of hyphae or eukaryotic single cells, cell walls of chitin or cellulose that produce sexually or asexually. In the life of the world, fungi are a separate kingdom because the way they get their food Is different from other eukaryotic organisms, namely through absorption [14]. Mushrooms are one of the least studied life forms in the world and there are hundreds of species left that can be found in the form of bioluminescence.The discovery that 71 out of 100,000 species in the mushroom kingdom have been verified to emit natural light or what is called a bioluminescence [3]. Environmental conditions can affect the physical characteristics of bioluminescence in luminous fungi [1]. Bioluminescence fungi live on rotting wood or leaves. At night it can be observed when the bioluminescent fungi emit light [15].



(*E*)-6-(3,4-dihydroxystyryl)-4-hidroxy-2*H*-pyran-2-one

Figure 1. Structure of trans-Hispidin (Chemoffice 2020)

Luminous mushrooms can only emit light for a certain period of time during their life cycle and the light emitted by the mushroom will start to dim until finally extinguished [16]. The physical characteristics of bioluminescence in fungi can be applied in various fields, such as biosensors, and biomedicine [17]. In the biosensor field, bioluminescence in fungi is used to monitor environmental pollution and extraction of heavy metals. In the biomedical field it is used in cancer treatment [18]. In Figure 2. Is the chemical mechanism of illumination in fungi.

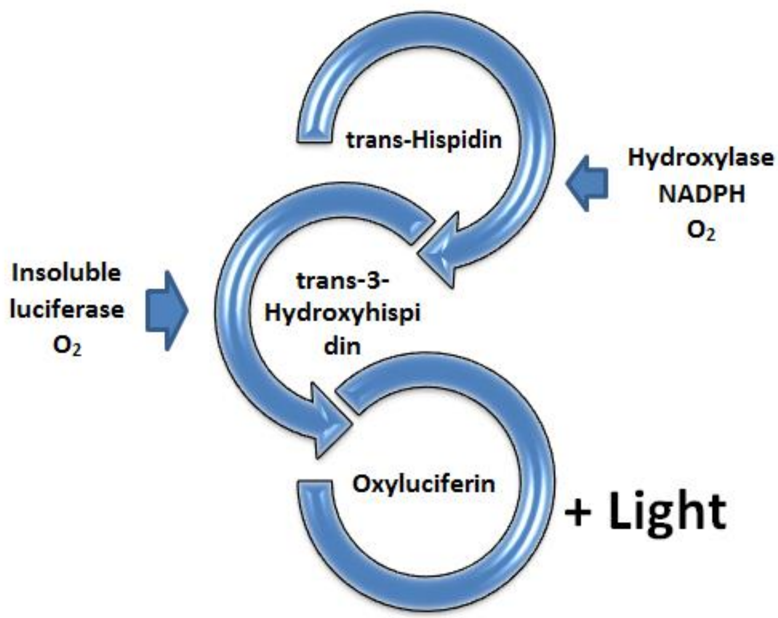
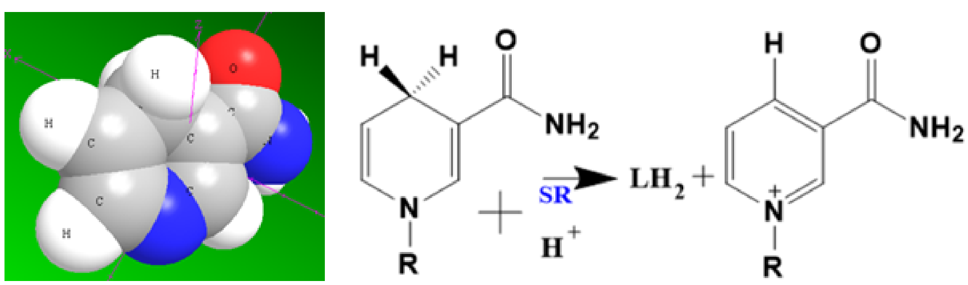


Figure 2. Chemical Mechanism of Fungal Illumination[19]

The chemical mechanism of illumination in fungi can produce light due to chemical reactions that occur in this mechanism as can be seen in Figure 2.



(a) (b) SR = Soluble Reductase

Figure 3. The molecular structure of NADPH was visualized by Chemoffice Software (**Cambridge Soft 2010; 2020) (a),** and Chemical Reactions of Bioluminescence Fungi (b) [23]

Figure 3 describes the bioluminescence reaction in fungi, in which luciferin is added with energy, namely NADH and oxygen, and then catalyzed by the enzyme luciferase and produces oxyluciferin and light [19][20]. Light emission from bioluminescence fungi is proportional to the light emission produced by fireflies. The light emission from bioluminescence mushrooms is proportional to the light emission produced by fireflies. Luminous mushrooms generally emit green light [21].

The addition of NADPH to the extract triggers light emission which increases sharply in intensity to reach a peak value and then gradually decreases with time reaching a relatively stable level. From this point on, the luminescence slowly weakened over the hours. Repeated addition of NADPH to the test did not affect light emission [22].

Aristotles in 382 BC, for the first time observed a luminous mushroom that emits greenish light and the wavelengths of these mushrooms range from 520 nm to 530 nm [23]. Mycena lucentipes fungi are found on damp wood surfaces and on rotting wood [24] [25]. Mycena lucentipes emit light with a wavelength of 320 nm [24]. The beam of light on the fungus Mycena lucentipes is used to attract insects to spread the spores of the fungus so that the fungus can breed [25].

The mushroom Omphalotus nidiformis has a brownish color and grows on the base of eucalyptus trees in Australian forest areas [26]. The resulting wavelengths from the glow of the mushroom Omphalotus nidiformis range from 300 nm to 650 nm [27].

Basidiomycete's species of the genus Armillaria. From these observations, not all of Armillaria's body can emit light, which can emit light only the head and trunk of the mushroom [28]. The mushroom Neonothopanus Nimbi found on the dead wood surface of Neonothopanus Nimbi emits light with a wavelength of 480 nm-700 nm [29]. Research on the illuminate mushroom Neonothopanus Nimbi, the mushroom was found in the forest area of Thailand. From the results of the study, the wavelength emitted by the mushroom was 480 nm [23]. Further research was carried out by Zahra and Ratnawulan (2019) on the physical characteristics of the luminous mushroom emission of Mycenae noctilucen species. The results obtained a maximum wavelength of 510 nm with green visible light [30].

The phenomenon of glowing mushroom illumination also occurs in the luminous mushroom Nenothopanus sp which is found in the forests of the South Coast of West Sumatra. Information regarding the physical characteristics as well as the optimum pH and temperature for the light emission of the fluorescent mushroom Nenothopanus sp is not yet known.

2. Experimental Section

In this study, the instrument used was the Nanofotometer N50. The sample in this study is a luminous mushroom species *Neonothopanus sp* taken in the coastal area of the South Coast of West Sumatra. This research was conducted by invitro. In this study, measurements of the wavelength and light intensity of the fluorescent mushroom *Neonothopanus sp*, the optimum temperature and pH were measured.

Sample preparation

In this study, the sample used was the mushroom species *Neonothopanus sp* which was taken in a humid area, namely the forest area of Pesisir Selatan, West Sumatra. This luminous mushroom is extracted from its substrate, namely dead bamboo wood. Then put in a closed container with air holes. Figure 4 is a sample of luminous mushrooms. At night, the light produced by this luminous mushroom is green.

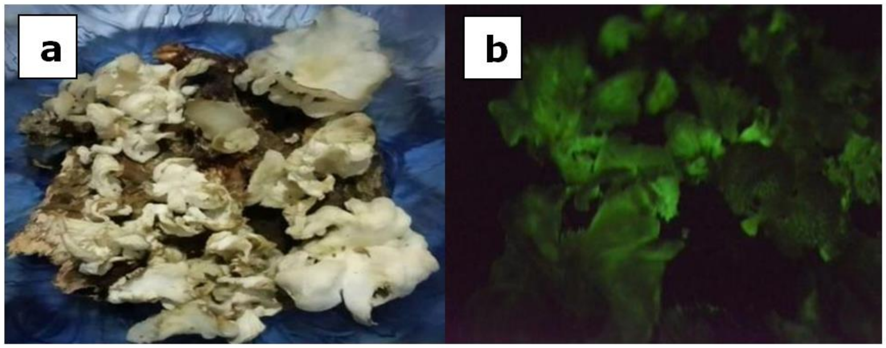


Figure 4. Sample Luminous Fungi *Neonothopanus* sp (a) and green light at the night

Characterization

For the measurement of light intensity at temperature variations, a centrifuge and thermo shaker is used to adjust each temperature variation. Temperature measurement can be done by inserting a curette that has been filled with extract and NADH into the thermo shaker then adjusting the temperature according to the variations. Furthermore, measuring the wavelength and relative intensity of the luminous mushrooms using a Nanofotometer N50.

For the measurement of light intensity at pH variations, 1 M concentration of HCl (acid) and 0.1 M concentration of NaOH (base) are used to adjust each pH variation. Measuring pH can be done by adding HCL for acid variations, then adding NaOH for base variations into the curette that has been filled with luminous mushroom extract and NADH then adjusting the pH according to the variation. Furthermore, measuring the wavelength and relative intensity of the luminous mushrooms using a Nanofotometer N50. Furthermore, from the measurement results, the maximum wavelength and light intensity data are obtained with respect to temperature and pH variations.

3. Result and Discussion

From the research conducted on the glowing mushroom *Neonothopanus sp*, the data obtained were in the form of the relative intensity value of each change in wavelength at the optimized pH and temperature changes. From measurements made on the mushroom Neonothophanus sp also obtained maximum intensity data from the optimization of temperature variations at a wavelength of 505 nm as shown in Figure 5.

Figure 5 shows that the relative light intensity produced by the mushroom Neonothophanus sp based on temperature variations of 5ºC, 10ºC, 15ºC, 20ºC, 25ºC, 30ºC. At 5ºC the resulting relative intensity is 398.9 au, then at 10ºC the relative intensity rises to 417.8 au and 15ºC the relative intensity increases to a maximum point of 491.9 au, then at 20ºC the relative intensity drops back to 392.6 au at 25ºC the relative intensity decreases to 378.6 au and at 30ºC the resulting relative intensity is 371.7 au. Based on the data, it can be seen that temperature affects the relative intensity of light produced from the mushroom *Neonothopanus* sp.

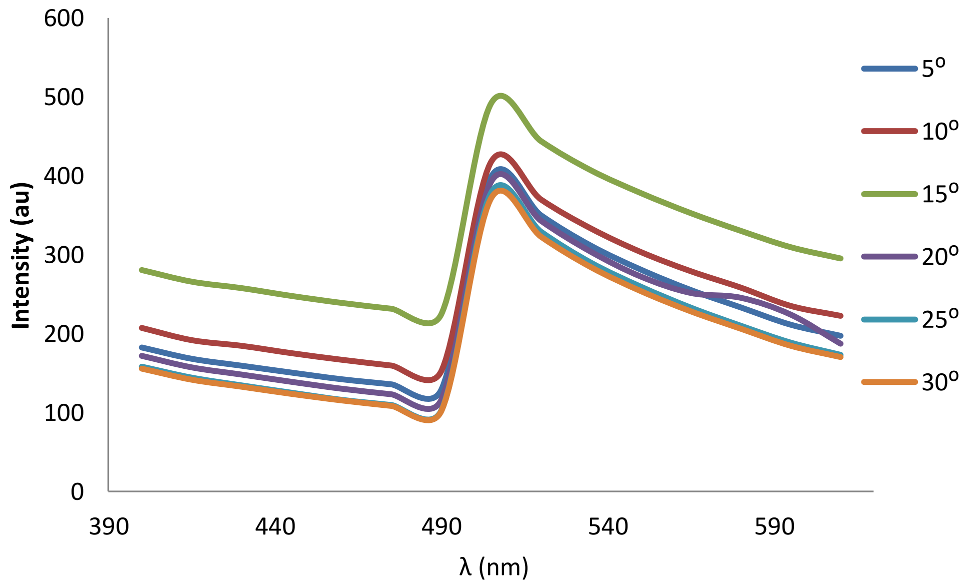


Figure 5. The light spectrum of mushroom *Neonothopanus* sp with temperature variations

From the measurements made on the mushroom Neonothophanus sp, data on the maximum relative intensity were also obtained from the optimization of pH variations at a wavelength of 505 nm as shown in Figure 5.

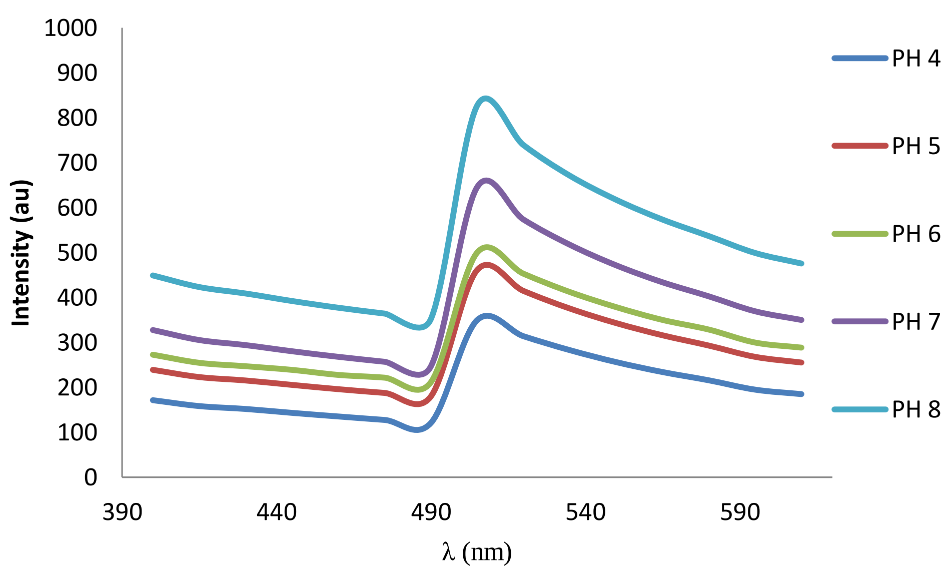


Figure 6. The light spectrum of mushroom *Neonothopanus* sp with pH variations

From Figure 6, it can be seen that the relative light intensity of *Neonothopanus sp* is based on variations in pH 4, 5, 6, 7, 8, 9. At pH 4 the relative intensity produced is 349.4 au then at pH 5 the relative intensity increases to 460 au and at pH 6 the relative intensity increased to 499.6 au. Furthermore, at pH 7 the resulting relative intensity increased to 645.2 au and pH 8 the relative intensity rose to a maximum of 826.7 au then at pH 9 the resulting relative intensity began to decrease to 547.5 au. Based on the data, it can be seen that pH affects Relative intensity of light produced from the mushroom *Neonothopanus sp*. Based on data from measurements of relative light intensity that have been carried out on the mushroom *Neonothopanus sp*, light emission with maximum relative intensity can occur at optimum conditions.

The luminous mushroom Nenothopanus sp can emit light with maximum relative intensity at optimum conditions at pH 8 and temperature 15°C. Furthermore, in utilizing the light intensity of the mushroom *Neonothopanus sp*, it can be done at the optimum conditions that have been obtained. If not at the optimum conditions, the intensity of light emission from the mushroom *Neonothopanus sp* will decrease.

Figures 5 and 6 show that the light intensity of Neonothophanus sp is influenced by the temperature and pH of the environment. Temperature and pH that are too high or low can cause denaturation, namely a decrease in light intensity in mushrooms. The temperature that is too low causes the luciferase enzyme not excited enough at the energy level it should be so that the energy emitted is also not optimal. Conversely, very high temperatures cause damage to the luciferase enzyme and interfere with the intensity of light emitted [19, 29]. The same thing happens when the environmental pH conditions are too low (acidic) or too high (alkaline). In low or high pH conditions, it will interfere with the activity of the luciferase enzyme so that the intensity of the light produced will decreas.

4. Conclusion

Based on the research that researchers have done, it can be denied that: Luminous mushrooms can emit maximum light at a temperature of 15°C with a pH of 8. The maximum intensity produced by luminous is at a wavelength of 505 nm. The wavelength of 505 nm includes the wavelength of visible light emitted by the green luminous mushroom *Neonothopanus sp*. The wavelength obtained is in accordance with the theory, namely the wavelengths of the *Neonothopanus* species range from 480 nm to 700 nm.

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