EXTENDED ESSAY
BIOLOGY

The effect of the colour of light on the growth of fruiting bodies in *Pleurotus ostreatus*

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ABSTRACT

The aim of this experiment is to investigate the effect of the colour of light on the growth of fruiting bodies in *Pleurotus ostreatus*. The most significant role that light plays for mushrooms is in the phototropic responses and in the formation of reproductive structures. To test the hypothesis, 4 cultivation media (substrates) were prepared and inoculated with *Pleurotus ostreatus* spawn. Once the mycelium colonisation was completed and primordia appeared on the top layers of the substrates, the samples were exposed to light of different colours: blue, green, red, yellow for fruiting induction. Lengths of stipes and diameters of pilei of 15 fruiting bodies, chosen randomly, were measured in each sample for 7 days. The lengths of stipes and the diameters of pilei were the greatest in the sample exposed to blue light. Irradiation with red light resulted in the smallest values for both measurements. The lengths of stipes and the diameters of pilei in the sample grown under yellow light were similar to those of subjected to green light. The yellow light exposure, nevertheless, promoted slightly greater growth compared to the green one. The partial inhibition of pilei development, construed as disagreement with the range of values from the literature, could be justified by high CO_2 concentration in the cultivation place. These findings were taken into consideration when assessing the significance of the differences between the results. The outcomes of the study could contribute to the development of a new, more efficient method to cultivate oyster mushrooms for industrial production.

Word count: 251

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1. RESEARCH QUESTION

How does the colour of light inducing the formation of *Pleurotus ostreatus* fruiting bodies affect their growth?

2. INTRODUCTION

Last summer I visited my uncle for a couple of days. Because the property was located in a rural area, he had the conditions to boost his experience in amateur agriculture. It came to me as a surprise to learn he cultivated oyster mushrooms in his garage. Far from being a specialist, my uncle harvested the mushrooms for his own use exclusively. What intrigued me the most was the technique he applied. Neither temperature-humidity chambers nor special light installations were present. It made me wonder under what conditions he would obtain a richer harvest. Although oyster mushrooms are of great economic importance, relatively few attempts have been made to enhance their cultivation and yield performance commercially. It directly encouraged me to pursue the topic of my extended essay in more depth. I have decided to investigate the factor affecting growth of *Pleurotus ostreatus* – the colour of light inducing the development of fruiting bodies. The results of the study might reveal that the treatment with certain colours of light could be used as a more efficient method to cultivate oyster mushrooms for industrial production.

2.1 KEY TERMS

- **"Fruiting body** – a macroscopic spore-producing structure, especially of a fungus" ("American Heritage Dictionary" 2000).
- **"Fungus –** a multicellular organism which obtains its food using extracellular digestion and has cell walls made of chitin" (Damon, McGonegal, and others 2007).
- **"Incubation (spawn running / mycelial growth)** – the phase in the development of a fungus between the time of inoculation and the time the substrate is completely permeated with mycelium" (Change and Miles 2004).
- **"Inoculation –** the process of introducing microorganisms into a culture medium" ("American Heritage Dictionary" 2000).
- **"Light** – electromagnetic radiation of wavelengths to which the human eye is sensitive ($\lambda \approx 400$ to 700 nm)" (Aphalo 2006).
    - The approximate wavelengths of the selected lights (Chambers 2011):
        - Blue light – 475 nm;
        - Green light – 510 nm;
        - Yellow light – 570 nm;
        - Red light – 650 nm.
- **"Mushroom –** a macrofungus with a distinctive fruiting body which can be either epigeous or hypogeous and large enough to be seen with the naked eye and to be picked by hand" (Chang and Miles 2004).
- **"Mycelium –** a vegetative part of a fungus; comprises a system of threads and cordlike strands that branch out through the soil, compost, wood log or other lignocellulosic material on which the fungus is growing" (Chang and Miles 2004).
- **"Pileus** – the upper cap-shaped part of a mushroom or similar spore-producing body" ("Collins English Dictionary" 2003).
- **"Primordium –** a cell or organ in its initial stage of development; a pinhead" (Roberts 2006; Change and Miles 2004).
- **"Spawn –** a medium impregnated with mycelium and used as inoculum in propagation for mushroom production" (Change and Miles 2004).
- **"Stipe –** a stalk especially of fungal fruiting bodies" ("Biology-Online Dictionary" 2005).
- **"Substrate** – a lignocellulosic material that supports the growth, development, and fruiting of mushroom mycelium" (Chang and Miles 2004).

2.2 CHARACTERISTICS OF PLEUROTUS OSTREATUS

Figure 1 *Pleurotus ostreatus* by Bohumil Vancura (1981).

Figure 2 *Pleurotus ostreatus* by J. Carlos Nieto (1988).

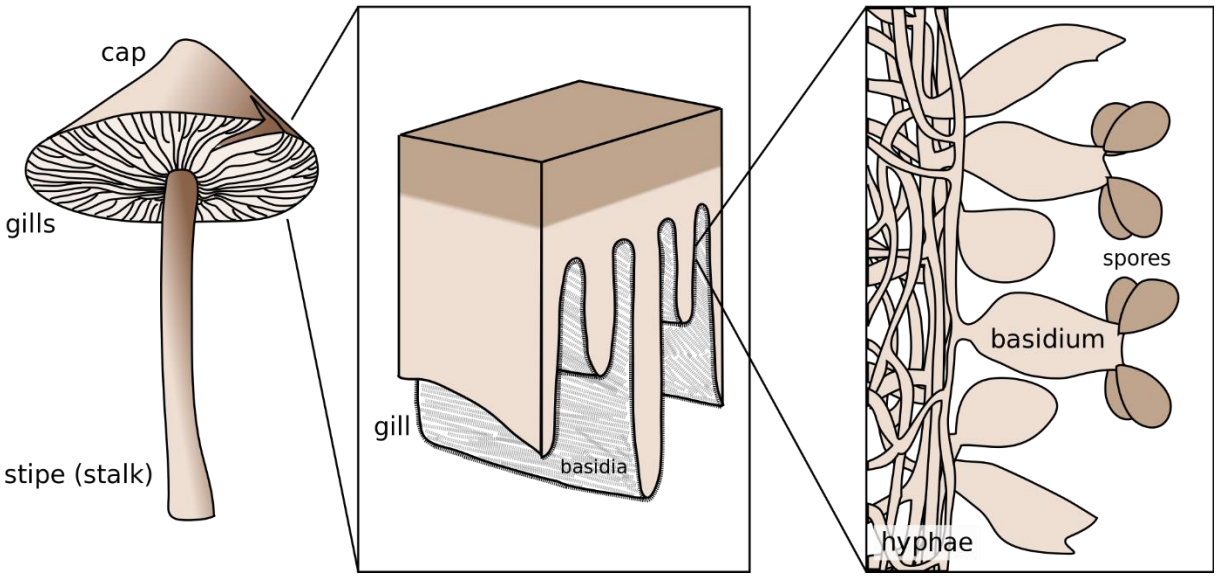


Figure 3 The Basidiomycota bear their sexual spores externally on club-shaped structures called basidia (Woller 2007).

Taxa	Oyster mushroom
Kingdom	Fungi
Phylum	Basidiomycota
Class	Agaricomycetes
Order	Agaricales
Family	Pleurotaceae
Genus	*Pleurotus*
Species	*ostreatus*

Table 1 Scientific classification of *Pleurotus ostreatus* (Woller 2007).

The *Pleurotus ostreatus* is named the oyster mushroom as the pileus is shell-like, spatulate, and the stipe eccentric or lateral. The species is used as food, for medical purposes and it plays an important role as a commercial edible mushroom. One reason is that oyster mushrooms are the easiest and least expensive to grow of all cultivated edible mushrooms. There is an opportunity for cultivation under different climatic conditions too (Chang and Miles 2004).

Oyster mushrooms are saprotrophs, meaning "organisms that live on or in non-living organic matter, secreting digestive enzymes into it and absorbing the products of digestion" (Damon, McGonegal, and others 2007). "*Pleurotus ostreatus* secretes the enzymes through tips of root-like extensions called hyphae. The enzymes hydrolyse cellulose leaving a space filled with nutrients the hyphae can absorb. The hyphae branch and form a thicker mass – mycelium, which maximizes the surface area through which feeding can occur" (Woller 2007).

"They grow naturally in temperate zones on rotting trees such as oak, elm, maple, bass, poplar, holly, and laburnum" (Chang and Miles 2004). There are many methods for the cultivation of oyster mushrooms. Some are cultivated by using wood logs, some are cultivated on culture media contained in jars or plastic bags, and others are cultivated by placing the culture substrates on the surface of the ridge, bed frame. The substrates used for cultivation include "plant wastes, such as sawdust, paddy straw, bagasse, cornstalks, waste cotton, stalks and leaves of bananas" (Chang and Miles 2004).

The oyster mushrooms are diversely coloured, including dark blue, white, brownish, yellow. The pileus is shell and tongue-shaped. The colour intensity can alter due to changes in environmental factors, e.g. light and temperature. The colour is darker in conditions of more light and cold weather, and it is lighter in weak light and hot weather. "The size can vary from 5 to 30 cm in diameter. The gills are whitish or grey. The stipe is usually eccentric or lateral" (Chang and Miles 2004). Its size can vary from 2-5 (length) × 1-2 cm (thickness) (Grzywacz 1988).

"The life cycle of *Pleurotus* consists of the vegetative stage and the reproductive growth stage. Some kinds of stimuli are needed to shift from mycelial growth to the fruiting body formation phase. It includes abrupt changes in temperature, humidity, gas concentration, light and nutrient reserves, and physical stimuli" (Kong 2004).

Although the mycelium of *Pleurotus ostreatus* can tolerate a high concentration of CO_2, the fruiting body of the oyster mushroom cannot endure high CO_2. When the CO_2 concentration is higher than 600 ppm, the stipe elongates and the growth of the caps is prevented (Chang and Miles 2004). The optimal temperature for the development of fruiting bodies ranges from 15 to 20℃ and the optimal humidity – 80 to 95% (Gupta 2013).

2.3 EFFECT OF LIGHT

The most significant role light plays for fungi is in the phototropic responses of reproductive structures and in their formation. The positioning of the stipe and pileus has been shown to be controlled by phototropic responses. “Phototropism is a growth response causing a bending toward or away from light” (Chang and Miles 2004). Not only is light required for the normal expansion of the pileus, but phototropism plays also a prominent role in spore dissemination (Chang and Miles 2004).

The light requirements are different for various stages of growth. Mycelium growth does not need light, and cultivation of the oyster mushroom in darkness is better than in a bright place. The formation of primordia and the growth of fruiting bodies requires light, however. The former requires light of 200 lux intensity for about 12 hours a day. The growth of the fruiting body requires light of 50 – 500 lux intensity (Chang and Miles 2004). The fact that light “induces the development of fruiting bodies including stipe elongation and cap formation” has been confirmed in ordinary mushroom cultivations (Miyazaki, Masuno, and others 2011).

To induce the formation of fruiting bodies in *Pleurotus ostreatus* after mycelia growth, Light Emitting Diodes (LEDs) are recommended. “LEDs have longer life and greater energy efficiency than fluorescent lamps, and they do not emit heat rays” (Jang, Lee, and others 2013).

3. HYPOTHESIS

Considering the light stimuli essential to initiate fruiting body formation, sensory factors such as photoreceptors are believed to be necessary (Miyazaki, Masuno, and others 2011). A study suggests (Light-stimulative effects on the cultivation of edible mushrooms by using blue LED) that a blue-light photoreceptor was identified as a resident protein containing a photo-reactive domain responding to light stimuli essential for fruiting development. Furthermore, the results of another investigation (Jang, Lee, and others 2013) reported that "in mushrooms cultivated under 340~520 nm light there was an increase in the yield of fruit body because of activation of ATP synthase". An additional factor to be taken into account is the natural habitat of *Pleurotus ostreatus*. The forest environment entails varying degrees of light penetration. Tree foliage acts as a selective filter and blue light, estimatedly, predominates in the vicinity of the forest floor, so mushrooms respond particularly to blue light (Gupta 2013). Therefore, I assume the lengths of stipes and the diameters of pilei will be greatest in the fruiting bodies of the sample subject to blue light. The remaining sizes will be decreasing in the following order (according to the colour of light inducing the formation of fruiting bodies):
green > yellow > red.

4. VARIABLES

4.1 INDEPENDENT VARIABLE

- Colour of light inducing the formation of *Pleurotus ostreatus* fruiting bodies after mycelia growth (blue, green, red, yellow).

4.2 DEPENDENT VARIABLE

- Growth of *Pleurotus ostreatus* fruiting bodies expressed as:
    - length of stipes;
    - diameter of pilei.

4.3 CONTROLLED VARIABLES AND THE MEANS OF MONITORING

Table 2 Type of controlled variable and a means of monitoring it.

Controlled variable	Value / unit	Monitoring
Temperature of the air	20.0℃	A mercury thermometer (-10.0 to +50.0 ℃, ±0.5 ℃) is used to measure the temperature. Central heating and air-conditioning systems are adjusted. No windows are open.
Number of fruiting bodies investigated in each sample	15	To provide statistically processable data and reasonable conclusions 15 fruiting bodies, chosen randomly, are investigated in each sample.
Type of substrate	Mixture of agro-waste materials including cereal grains, sawdust, straw and other lignocellulosic wastes	The substrate comes from the same mushroom substrate supplier (Planto) and is not mixed with any other type.
Mass of substrate per sample	3 kg	Substrate bags are prepared and weighed by the supplier.
Type of spawn	*Pleurotus ostreatus* spawn	Spawn bags come from the same mushroom spawn supplier (Planto).
Mass of spawn per sample	100 g	Spawn bags are prepared and weighed by the supplier.
Volume and source of water used to water the samples	100.0 cm^3 of tap water (Supplier: Wodociągi Kieleckie Sp. z o. o.)	A graduated cylinder (100.0 cm^3 ±0.5 cm^3) is used to measure the volume of tap water for everyday watering. Both the volume of water and the same mineral ions concentration maintain constant abiotic factors.

Controlled variable	Value / unit	Monitoring
Frequency and time of watering the samples	Twice a day; 8:00 a.m. and 8:00 p.m.	Each sample receives 50.0 cm^3 of tap water at 8:00 a.m. and 50.0 cm^3 of tap water at 8:00 p.m. using a spray bottle.
Light intensity (power of LEDs; distance between the bulbs and the samples)	1 W; 15.00 cm	The light bulbs taken from the same distributor (elektrykon.pl) are arranged 15.00 cm above the samples. The distance is measured using a ruler (20.00 cm $\pm$0.05 cm). The cardboard installation is used to separate the samples.
Time of light exposure (after mycelia growth; incubation under dark conditions)	12 hours / 7 days	The exposure starts at 8:00 a.m. and ends at 8:00 p.m. to maintain the same abiotic factors.
Place of cultivation	Basement	The investigation is conducted in the same place.
Humidity	High (about 80-95%)	High humidity is achieved using a plastic vapour barrier. Everyday watering coupled with open water containers maintain the optimal level. Central heating and air-conditioning systems are adjusted. No windows are open.
Time of incubation	21 days	The substrates are inoculated with *Pleurotus ostreatus* spawn on the same day. The samples are left for 21 days for the mycelial growth.
Ruler used for measuring the sizes	200.0 mm $\pm$0.5 mm	The same ruler is used throughout the experiment.

Controlled variable	Value / unit	Monitoring
Time of taking measurements	8:00 p.m.	The sizes of *Pleurotus ostreatus* fruiting bodies are recorded at 8:00 p.m. every day.
Fresh air exchanges	Once a day; 8:00 a.m.	Fresh air exchanges, and in particular CO_2 removal, are performed by means of opening the basement doors and fanning for about 15 minutes each day.

5. MATERIALS

- Protective gloves
- Camera
- 100.0 cm^3 graduated cylinder ($\pm$0.5 cm^3)
- Mercury thermometer (-10.0 to +50.0℃, $\pm$0.5℃)
- 100 cm^3 spray bottle
- Scissors
- 20.00 cm ruler ($\pm$0.05 cm)
- 4 × 1 W light bulbs (LEDs) in different colours: blue, green, yellow, red
- 200.00 cm retractable metric tape measure ($\pm$0.05 cm)
- Pen
- Notebook
- Wire
- 4 × 100 g *Pleurotus ostreatus* spawn bags
- 4 × 3 kg mushroom substrate bags
- 4 × transparent heat-resistant plastic bags with dimensions (length × width): 50 cm × 30 cm
- Kettle
- Long spoon
- 4 × zip ties
- Tap water (approximately 47.2 l)
- Fan
- 4 × cardboard boxes with dimensions (length × width × depth): 45 cm × 30 cm × 40 cm
- Watch
- 2 × 15 l open water containers

6. METHOD

6.1 LED GROWTH CHAMBERS

LED growth chambers were constructed as diagrammed below. For this purpose 4 cardboard boxes with dimensions (length × width × depth): 45 cm × 30 cm × 40 cm were used.

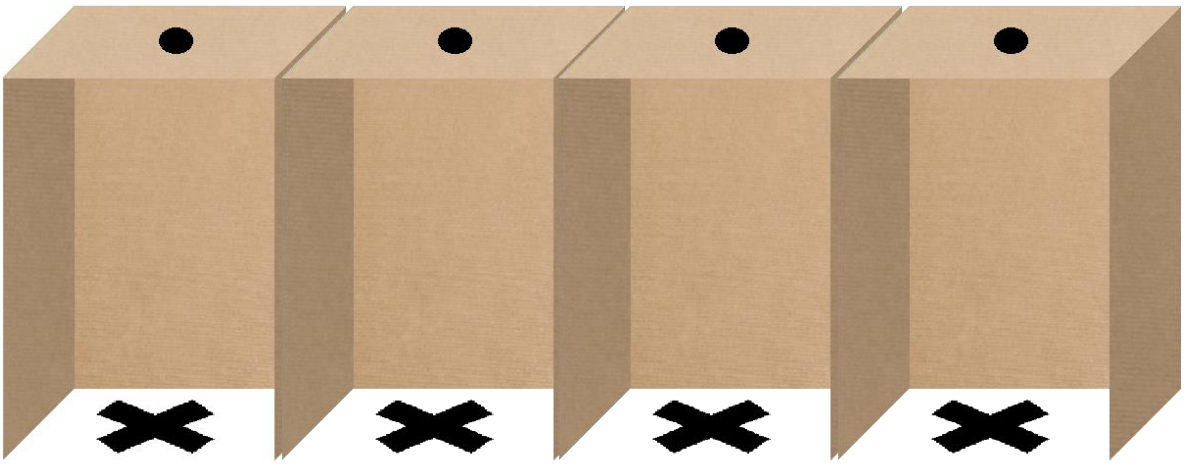


Figure 4 Cardboard arrangement – the framework for LED growth chambers.
The black circles at the top indicate LEDs placement (cut with scissors) and the black crosses indicate the location of the samples.

4 LEDs of 1 W power were applied: blue, green, yellow, red. To install LED lighting, the light bulbs were connected by a wire and placed in the boxes. The distance between the light bulbs and the samples equalled 15.00 cm (measured with a ruler, ±0.05 cm).
The following picture shows LEDs installation.

Picture 1 LEDs installation suitably designed for mushroom cultivation.

6.2 MUSHROOM CULTIVATION

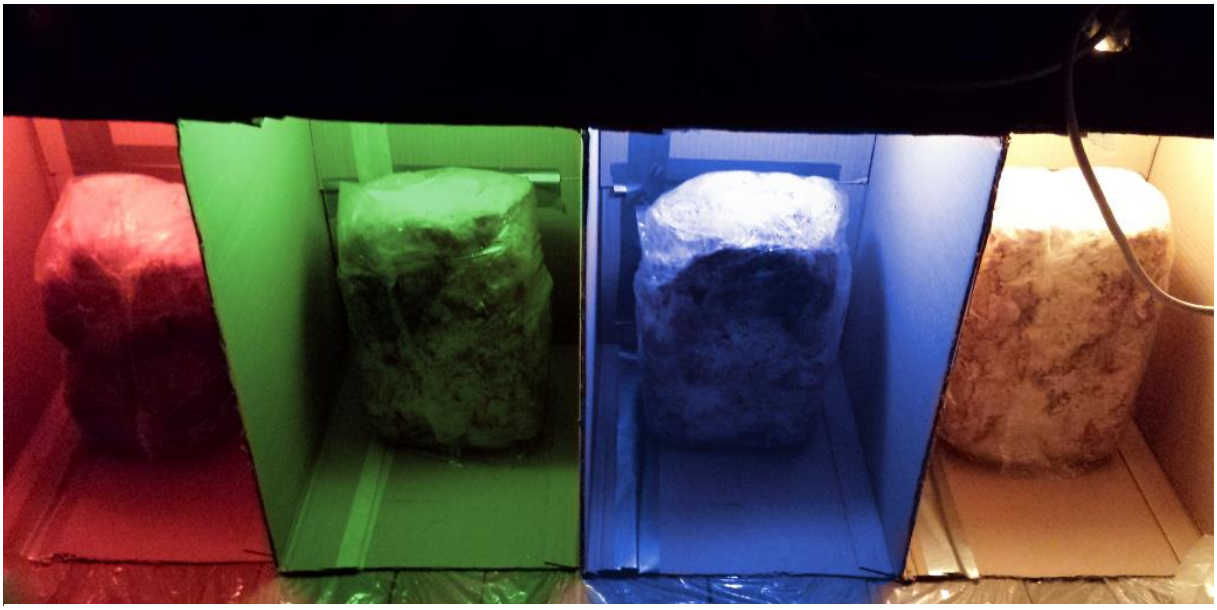
The method for preparation of the substrate was based on Oei's instructions (2005) and modified by the experimenter.

The content of each mushroom substrate package was transferred to the transparent heat-resistant plastic bags with dimensions (length × width): 50 cm × 30 cm before heat treatment. To kill competing microorganisms and dispose of soluble nutrients, pasteurisation by immersion in hot water was undertaken. After reaching the boiling point, the water was left for 3 minutes to make it slightly colder. Approximately 1.5 l of prepared hot water was poured into each heat-resistant plastic bag containing the substrate. The bags were tightened using zip ties in the upper parts (the hot water increased the volume) and left to cool down for 2 hours. The bags were then opened and the excess water was drained from the heat-treated substrates. In order to facilitate the drainage and prospective *Pleurotus ostreatus* development, the bags were perforated using scissors. The cross-like holes (2 cm × 2 cm) were arranged on both sides of each bag. 4 holes at intervals of 5 cm (horizontally and vertically) were made on each side.

After the substrates reached room temperature (measured with a mercury thermometer, -10.0 to +50.0℃, ±0.5℃), they were inoculated with *Pleurotus ostreatus* spawn. A small hole (of diameter 4 cm and extending almost to the bottom of the bag) in the middle of each substrate was made using a long spoon. The 100 g bags containing *Pleurotus ostreatus* spawn were opened and their contents were poured into the holes. The bags with inoculated substrates were re-plugged using zip ties and placed in a basement for the incubation phase (21 days).

The mycelial growth occurred at 20.0℃ (measured with the mercury thermometer, -10.0 to +50.0℃, ±0.5℃) in darkness. To obtain high humidity, the samples were watered twice a day – each received 50.0 cm^3 of tap water at 8:00 a.m. and 50.0 cm^3 of tap water at 8:00 p.m. using a spray bottle. To measure the volumes for everyday watering, a graduated cylinder (100.0 cm^3 ±0.5 cm^3) was used. The optimal humidity was maintained due to plastic coverage and open water containers (2 × 15 l) in the vicinity of the samples. Fresh air exchanges were provided by means of opening the basement doors and fanning for about 15 minutes every day at 8:00 a.m.

Once the mycelium colonisation was completed and primordia appeared, the bags were exposed to light of different colours (1 W LEDs – blue, green, red, yellow) for fruiting induction (7 days). The exposure started at 8:00 a.m. and ended at 8:00 p.m. The conditions of the LED growth chambers remained unchanged; it was conducted at 20.0°C (measured with the mercury thermometer, -10.0 to +50.0°C, ±0.5°C) with high humidity and proper ventilation (100.0 cm^3 of tap water, plastic vapour barriers, open water containers; 15-minute fresh air exchanges). Lengths of stipes and diameters of pilei of fruiting bodies were measured during this period. 15 fruiting bodies, chosen randomly, were investigated in each sample. The collected data were useful to determine means, percentage increases, standard deviations, significance of differences, and eventually the most effective light conditions inducing the formation of *Pleurotus ostreatus* fruiting bodies. The calculations were done using a calculator and Excel spreadsheet.



Picture 2 Light exposure for fruiting induction.

7. DATA COLLECTION AND PROCESSING

The following table shows mean lengths of stipes and diameters of pilei in each day.

Sample	Mean Length of stipe & Diameter of pileus / mm (±0.5 mm)													
	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7	
Blue light	2.6	1.3	8.9	3.9	15.3	6.9	21.1	10.4	26.4	12.9	30.9	14.3	34.6	15.1
Green light	2.6	1.1	6.9	2.3	11.9	4.1	16.0	5.5	19.8	7.4	22.6	8.9	24.4	10.0
Red light	1.6	0.8	4.0	1.4	5.8	2.2	7.8	2.8	9.2	3.4	10.1	4.1	10.9	4.9
Yellow light	2.2	1.1	7.6	2.7	11.7	4.7	16.3	6.7	19.7	8.8	22.7	10.7	25.1	11.7

Table 3 Mean lengths of stipes and diameters of pilei in each day.

Example 1

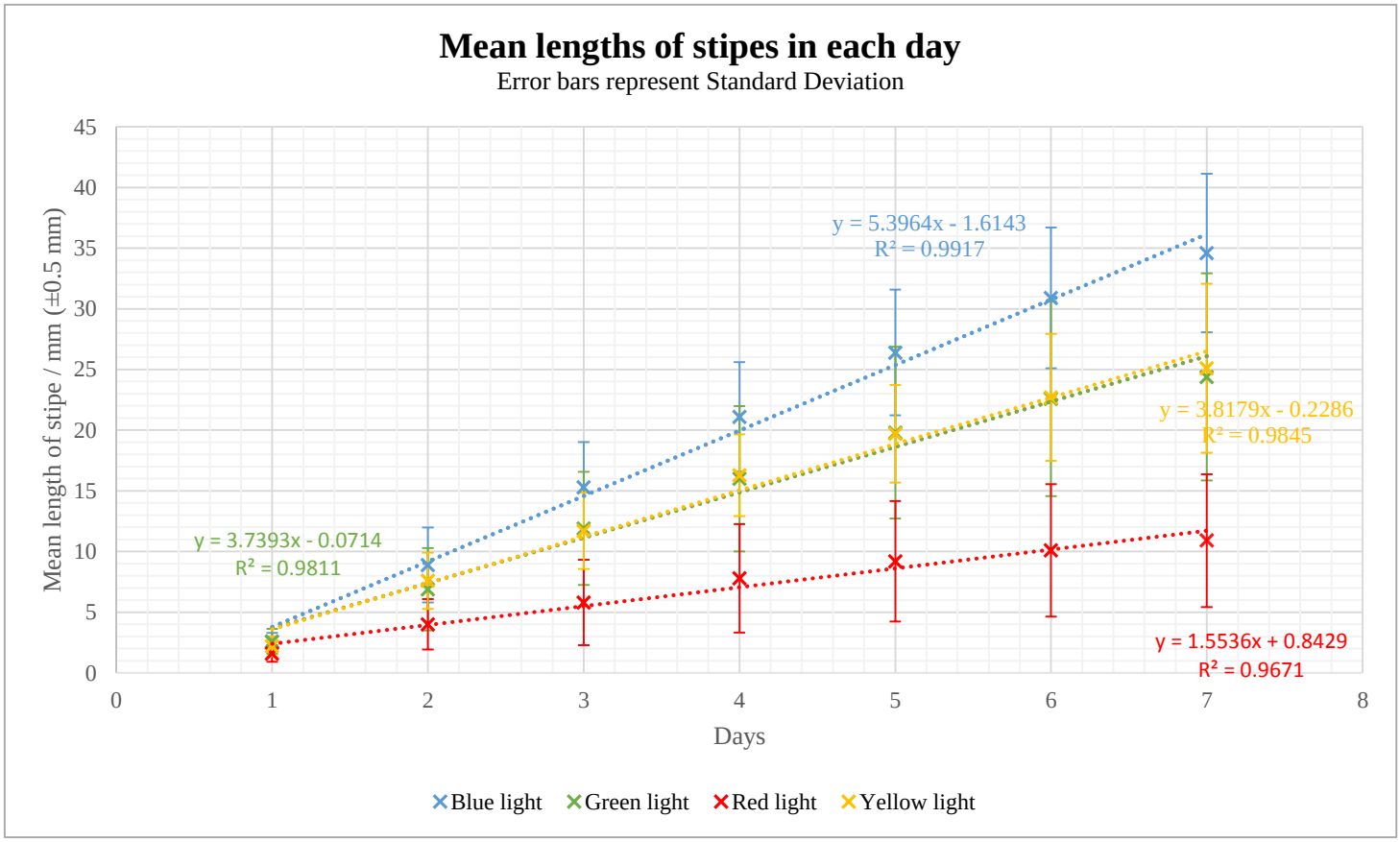
To calculate the mean length of stipe (Day 1; Blue light), the values (1-15) are added and then divided by 15.

Mean length of stipe (Day 1; Blue light) = $\frac{3.0+3.0+3.0+2.0+3.0+3.5+4.5+3.0+3.0+4.0+2.5+4.5+3.5+4.5+4.0}{15}$ = 3.4 (mm)

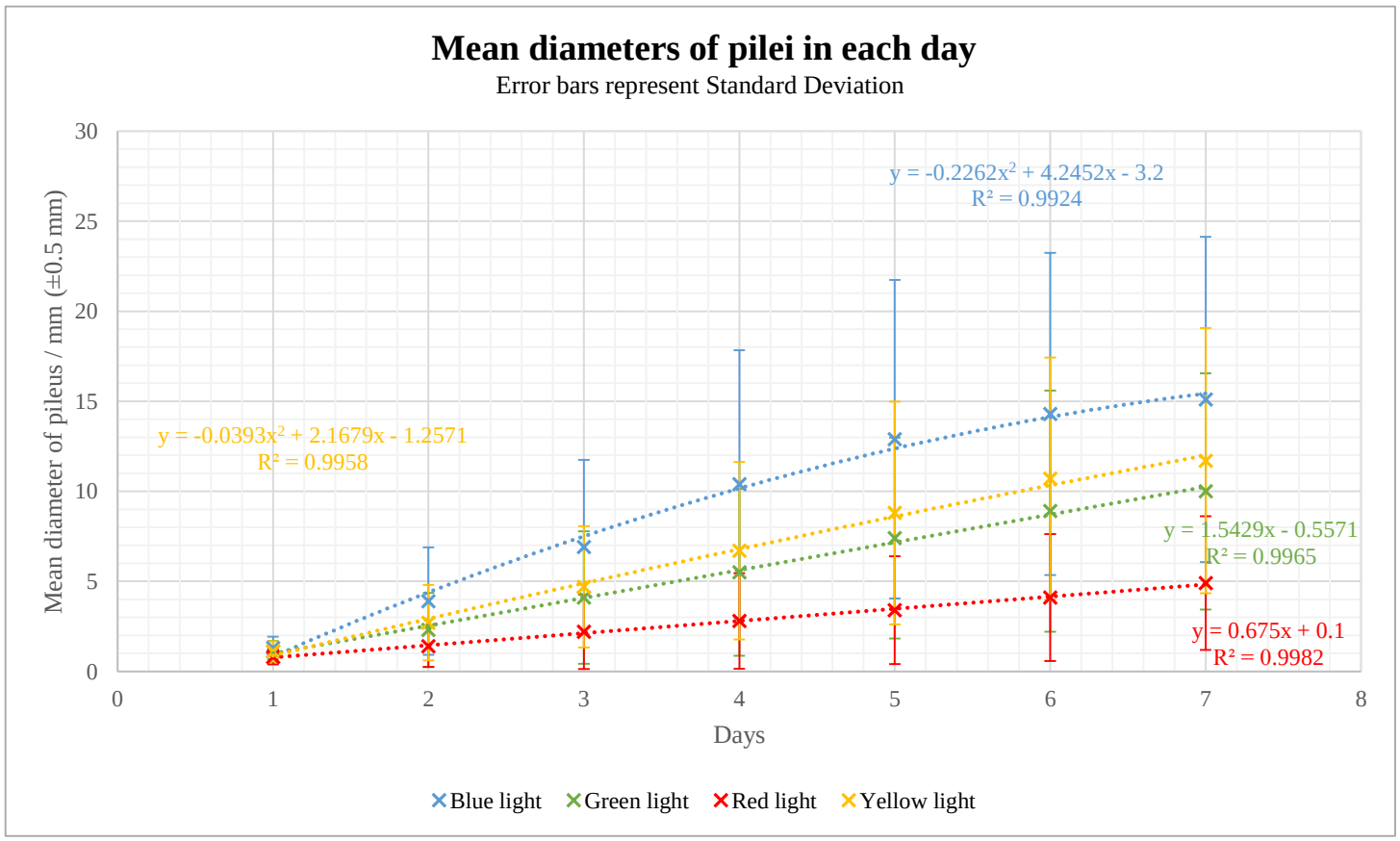
Propagation of uncertainty = $\frac{\pm0.5 + \pm0.5 + \pm0.5 + \pm0.5 + \pm0.5 + \pm0.5 + \pm0.5 + \pm0.5 + \pm0.5 + \pm0.5 + \pm0.5 + \pm0.5 + \pm0.5 + \pm0.5 + \pm0.5}{15}$

= ±0.5 (mm)

Mean diameters of pilei are calculated in the same manner.



Graph 1 Mean lengths of stipes (±1SD) in each day.



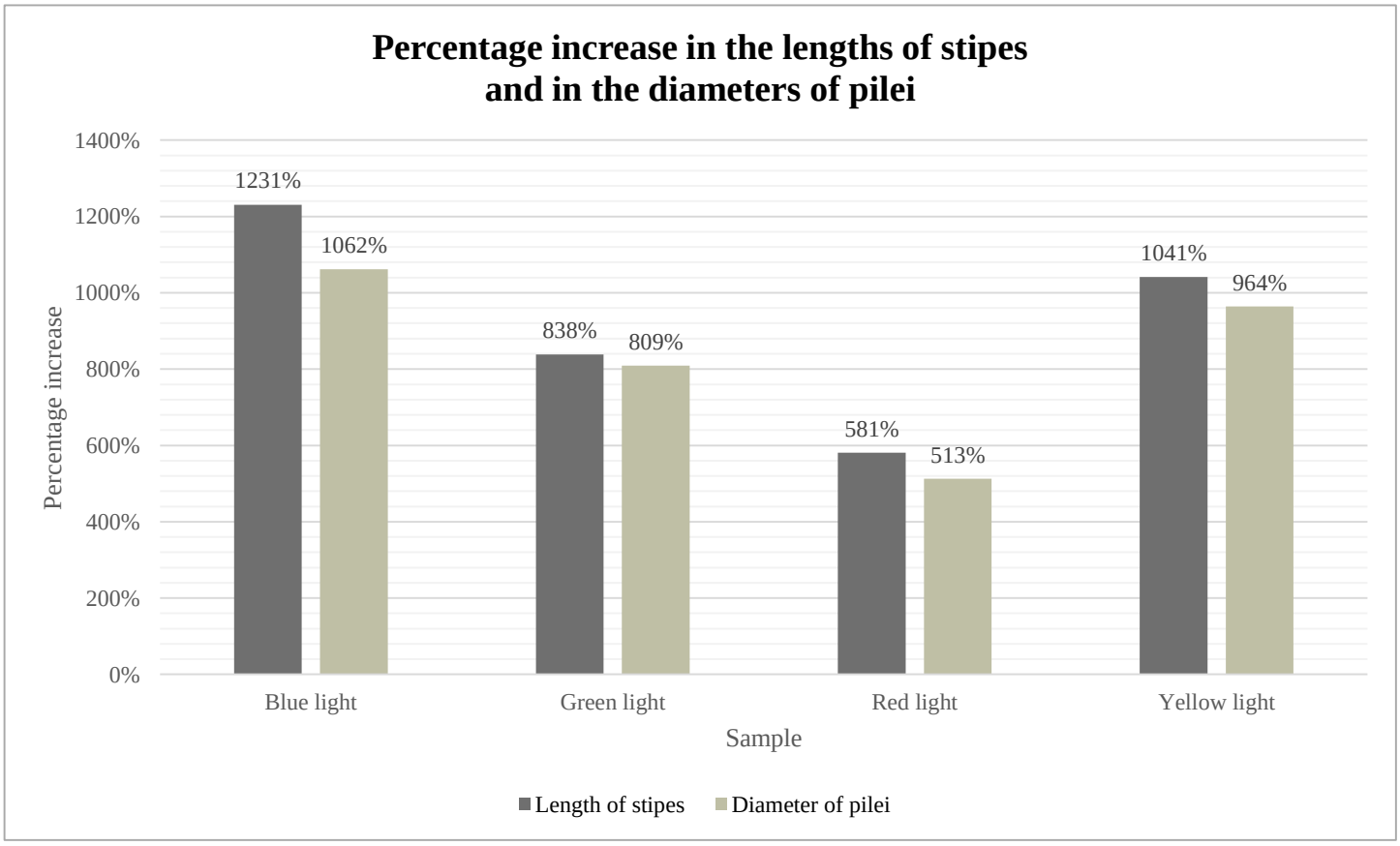
Graph 2 Mean diameters of pilei (±1SD) in each day.

To calculate percentage increase, the following formula is applied:

$$\boldsymbol{Percentage\ increase} = \frac{Final\ value\ (Day\ 7) - Starting\ value\ (Day\ 1)}{Starting\ value\ (Day\ 1)} \times 100\%$$

Sample	Percentage increase	
	Length of stipes	**Diameter of pilei**
Blue light	$\frac{34.6 - 2.6}{2.6} \times 100\% = \mathbf{1231}\%$	$\frac{15.1 - 1.3}{1.3} \times 100\% = \mathbf{1062}\%$
Green light	$\frac{24.4 - 2.6}{2.6} \times 100\% = \mathbf{838}\%$	$\frac{10.0 - 1.1}{1.1} \times 100\% = \mathbf{809}\%$
Red light	$\frac{10.9 - 1.6}{1.6} \times 100\% = \mathbf{581}\%$	$\frac{4.9 - 0.8}{0.8} \times 100\% = \mathbf{513}\%$
Yellow light	$\frac{25.1 - 2.2}{2.2} \times 100\% = \mathbf{1041}\%$	$\frac{11.7 - 1.1}{1.1} \times 100\% = \mathbf{964}\%$

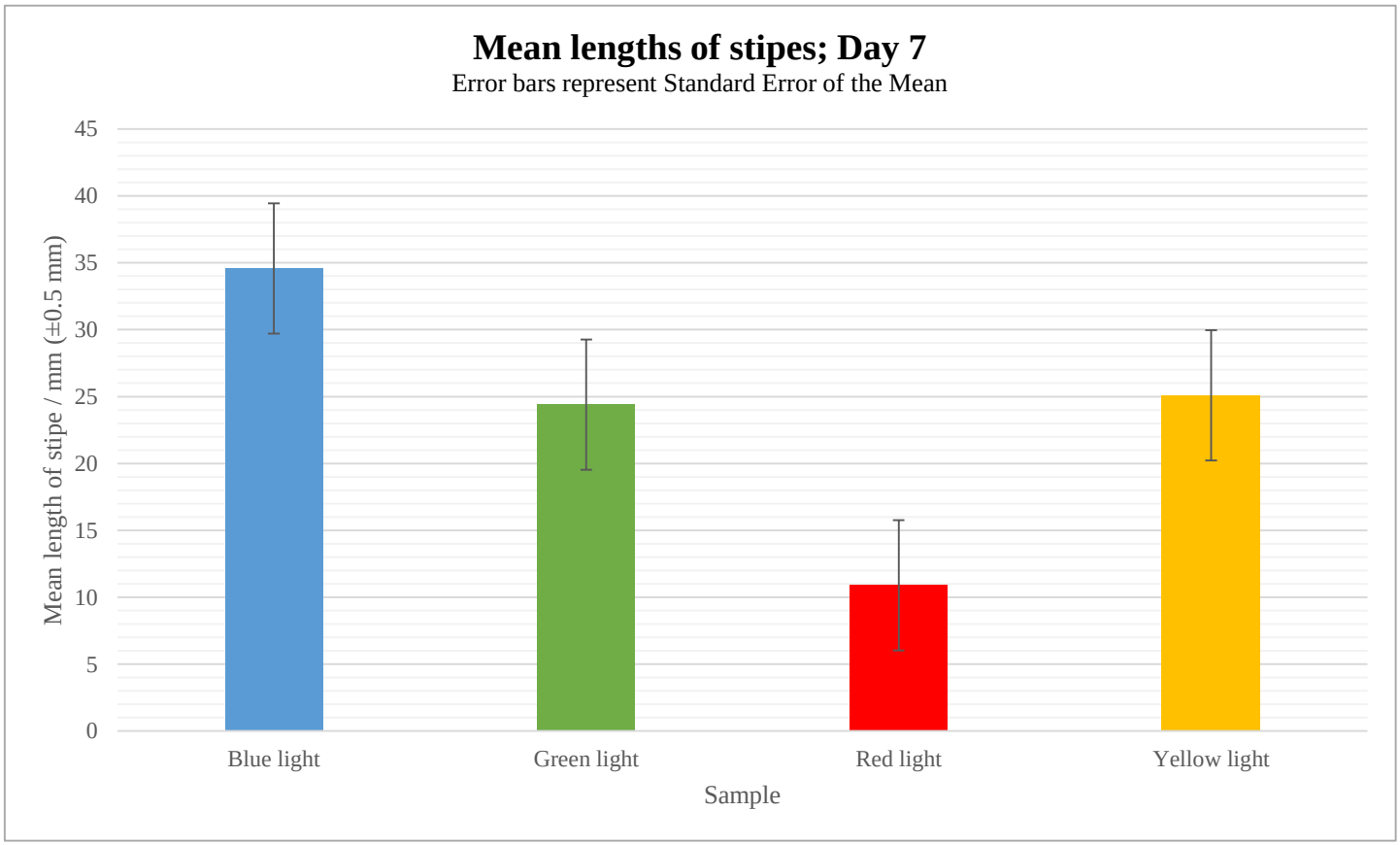
Table 4 Percentage increase in the lengths of stipes and in the diameters of pilei.



Graph 3 Percentage increase in the lengths of stipes and in the diameters of pilei.

Owing to the partial inhibition of pilei development (mean diameters < literature value), hereafter follows the data processing of lengths of stipes only.

The following graph shows mean lengths of stipes concerning the last stage of the investigation – Day 7.



Graph 4 Mean lengths of stipes (±1SEM) concerning Day 7.

To calculate standard deviation of the length of stipes concerning Day 7, the following formula is applied:

$$\boldsymbol{Standard\ deviation} = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}}$$

where:

x_i – individual data points

$\bar{x}$ – mean of x_i

n – number of data points

Σ – the sum of.

Sample	Standard deviation of the length of stipes / mm
Blue light	6.52
Green light	8.53
Red light	5.47
Yellow light	6.97

Table 5 Standard deviation of the length of stipes concerning Day 7.

Example 2

Standard deviation of the length of stipes (Day 7; Blue light) is calculated in the following manner:

Standard deviation =

$$= \sqrt{\frac{(35.0-34.6)^2+(36.0-34.6)^2+(14.5-34.6)^2+(42.5-34.6)^2+(36.0-34.6)^2+(37.5-34.6)^2+(38.0-34.6)^2+(33.5-34.6)^2+(32.0-34.6)^2+(29.5-34.6)^2+(36.5-34.6)^2+(39.0-34.6)^2+(41.0-34.6)^2+(32.0-34.6)^2+(35.5-34.6)^2}{15-1}} =$$

= 6.52 (mm)

In order to check whether the data follow normal distribution, bell-shaped graphs for each sample has been plotted and relegated to the appendix (pages: 39-42).

8. DATA ANALYSIS

Initially, mean lengths of stipes and diameters of pilei in each day were considered (Table 3). All the samples show a growth of *Pleurotus ostreatus* fruiting bodies. The growth increases at a relatively constant rate with time. Overall, the mean lengths of stipes were greater than the mean diameters of pilei in each sample. Blue light exposure promotes greatest growth in both measurements, whereas red light exposure appears to cause smallest growth. The mean of the sample grown under blue light is 23.7 mm higher in the length of stipe and 10.2 mm in the diameter of pileus than the fruiting bodies exposed to red light. Lengths of stipes and diameters of pilei of fruiting bodies subject to green light and yellow light are intermediate. Irradiation with yellow light leads to slightly greater fruiting bodies, however. It is 0.7 mm higher in the mean length of stipe and 1.7 mm in the mean diameter of pileus than the fruiting bodies grown under green light.

According to Graph 1, showing mean lengths of stipes in each day, a linear fit for all the data points is obtained and no anomalous results are observed. The high positive correlation is shown by means of the coefficient of determination (R^2). The values of R^2 decrease in the following order:

0.9917 (Blue light) > 0.9845 (Yellow light) > 0.9811 (Green light) > 0.9671 (Red light). The best-fit line for the sample exposed to green light is almost coincident with the best-fit line for the sample grown under yellow light.

A degree of similarity depicts Graph 2, which shows mean diameters of pilei in each day. The data are positively correlated and no outliers are distinguished. Nevertheless, the strength of the linear association follows an inverse order, namely:

0.9982 (Red light) > 0.9965 (Green light) > 0.9958 (Yellow light) > 0.9924 (Blue light). The best-fit line concerning the sample subject to blue light indicates highest rate of growth at the beginning of the study which tapers off slightly at the later stage. Additionally, the best-fit line for the sample irradiated with green light does not coincide with the best-fit line for the sample grown under yellow light.

As far as percentage increase is concerned (Table 4), the same trends are maintained for both the lengths of stipes and the diameters of pilei. Percentage increases of the sample exposed to blue light are greater than in other samples. The sample subject to red light reveals the lowest percentage increases. The percentage increase in the lengths of stipes of the fruiting bodies grown under yellow light is greater than for the sample irradiated with green light.

The difference equals 203%. Consequently, the difference between the two samples concerning the percentage increase in the diameters of pilei amounts to 155%.
Judging from Graph 3, it can be seen that the greatest difference between the percentage increases within the same sample exhibit fruiting bodies exposed to blue light (169%), whereas the smallest difference show fruiting bodies subject to green light (29%).

Owing to the partial inhibition of pilei development, further data processing centred around lengths of stipes only. In order to justify this decision, the mean diameters of pilei were compared with the literature value. It has been emphasised in the introduction that "the size of pilei can vary from 5 to 30 cm in diameter" (Chang and Miles 2004). However, the greatest mean diameter of pileus of all the investigated samples is equal to 15.1 mm (Blue light). The mean lengths of stipes remain in conformity with the literature value. These findings will be taken into consideration when assessing significance of the differences between the results.

By taking into account sample size and considering how far apart two Standard Error of the Men (SEM) error bars are, Cumming (2007) determined some rules for deciding when a difference is significant or not:

- "If two SEM error bars overlap, then the P value is greater than 0.05 (5%), so the difference is not statistically significant.
- If two SEM error bars do not overlap, the P value could be less than 0.05, or it could be greater than 0.05."

According to Graph 4, which shows mean lengths of stipes ($\pm$1SEM) concerning the last stage of the investigation – Day 7, the difference in the mean length of stipe between the sample subject to green light and the sample exposed to yellow light should not be statistically significant because the two SEM error bars overlap. The significance of the differences between the remaining results cannot be assessed, however. The error bars do not overlap or just touch. The error bars has been set to represent SEM instead of SD in this graph because "no conclusions can be drawn knowing whether SD error bars overlap or not" (Motulsky 2014).

Subsequently, standard deviations of the lengths of stipes concerning Day 7 were calculated (Table 5). The data points concerning the sample irradiated with green light are spread widely around the mean and there are many extremes – the standard deviation is the greatest. The data points of the sample subject to red light are more clustered together and there are fewer

extremes – the standard deviation is the smallest. The two SD values differ by 3.06 mm. The SD of the sample grown under yellow light is greater than the SD of the sample exposed to blue light by 0.45 mm.

All of the samples show Gaussian distribution (Graphs 5-8) – "about 68% of all values lie within ± 1 SD from the mean and about 95% lie within ± 2 SD" (Damon, McGonegal, and others 2007). Thus a t-test can be carried out.

"A difference is considered statistically significant if the probability of it being due to random variation (P) is 5% or less" (Allott 2012).

The following samples will be compared in terms of testing the difference between mean lengths of stipes concerning Day 7:

- Blue light and Green light;
- Blue light and Red light;
- Blue light and Yellow light;
- Green light and Red light;
- Green light and Yellow light;
- Red light and Yellow light.

Sample Blue light and Sample Green light

1. Null hypothesis (H_0):
   There is no significant difference between the mean lengths of stipes.
2. Alternative hypothesis (H_1):
   There is a significant difference between the mean lengths of stipes.
3. The degree of freedom is 28 (15 + 15 – 2 = 28).
4. The value of t, calculated using Excel spreadsheet, equals 3.6666.
5. The critical value of t, found using the table of t values, amounts to 2.05.
   The chosen level of significance (P) is 0.05 (5%).
6. The calculated value of t is greater than the critical value, showing that the difference between the mean lengths of stipes is significant.

The remaining comparisons are relegated to the appendix (pages: 43-44).

The following table summarises the statistical conclusions based on the t-test.

Compared samples	Statistical conclusion	t value
Blue light and Green light	The difference is significant.	3.6666
Blue light and Red light	The difference is significant.	10.7648
Blue light and Yellow light	The difference is significant.	3.8411
Green light and Red light	The difference is significant.	5.1595
Green light and Yellow light	The difference is not significant.	0.2462
Red light and Yellow light	The difference is significant.	6.2081

Table 6 Statistical conclusions and calculated values of t based on the t-test comparing the difference between mean lengths of stipes concerning Day 7. Critical value for t = 2.05 (P = 0.05).

9. CONCLUSION

The aim of this experiment was to investigate the effect of the colour of light on the growth of fruiting bodies in *Pleurotus ostreatus*. Taking everything into account, it is highly probable that the colour of light affects the growth of oyster mushrooms. The lengths of stipes and the diameters of pilei were greatest in the sample exposed to blue light. Irradiation with red light resulted in the smallest values for both measurements. The lengths of stipes and the diameters of pilei in the sample grown under yellow light were similar to those of subjected to green light. The yellow light exposure, nevertheless, promoted slightly greater growth compared to the green one.

These findings are in conformity with the literature to some extent. It was reported that irradiation with blue LED enabled efficient fruiting body formation. A blue-light photoreceptor was identified as a resident protein containing a photo-reactive domain responding to light stimuli essential for fruiting development. Irradiation with red LED had no apparent effect on the vegetative mycelium growth (Miyazaki, Masuno, and others 2011).
Other studies (Jang, Lee, and others 2013) are also in agreement as to the blue LEDs which "produced fruit body with better features than the other treatments". The same studies concluded that "in mushrooms cultivated under 340~520 nm light there was an increase in the yield of fruit body because of activation of ATP synthase". Judging from the wavelengths of light, the exposure to green light should have promoted greater growth in comparison with the yellow light. While these assumptions has been rejected, the residue of the hypothesis is supported.

The partial inhibition of pilei development can be clarified by comparison with Kong's findings (2004). The researcher stated that "high CO_2 concentration (approximately 1000 ppm) causes mushrooms to produce longs stipes with tiny pilei". Due to this unexpected outcome, a detailed statistical analysis could not be completed and original research question could not be fully answered.

The results of the study might contribute to the development of a new method to cultivate oyster mushrooms for industrial production.

10. EVALUATION

Mushroom cultivation methods require that growers possess knowledge of principles and techniques to mimic interactions favouring growing processes in the natural environment. Isolating one factor that constitutes an independent variable in these complex events remains elusive. It is, therefore, unreasonable to have complete confidence in the strength of the conclusion.

One of the major factors influencing the growth of *Pleurotus ostreatus* fruiting bodies is CO_2 concentration. Although the procedure included fresh air exchanges aimed at lowering the ambient CO_2 content, the exact concentration was not controlled. From the final results it can be inferred that the CO_2 level was too high. It should be measured by a CO_2 sensor. It could be further reduced by more frequent ventilation, the example being three times a day. Alternatively, Petri dishes containing KOH could be placed in the vicinity of the samples to absorb CO_2 (Niederpruem 1963).

Furthermore, the light intensity should be considered in more detail. This is due to the fact that the formation of primordia and the growth of fruiting bodies require light of specified intensities, measured in lux. Application of a lux meter exemplifies appropriate measures.

The partial inhibition of pilei development and further inconsistencies could stem from the degree of workplace cleanliness, particularly during preparation of the substrates. Despite complying with standard cleaning methods, protection against contaminants could be insufficient. An antibacterial cleaning soap and a surface disinfectant spray could be implemented.

“One of the features of *Pleurotus ostreatus* is that it requires a low temperature treatment to initiate successful primordia formation” (Kong 2004). The procedure of the investigation should incorporate this step after inoculation – the temperature in the cultivation place ought to be decreased by 10℃ for 3 days. This could be accomplished by using a portable air conditioner.

Additionally, performing several t-tests entails the problem of multiple comparisons. As more samples are included in the study, the likelihood of observing one or more significant P values by chance increases. The data should not be analysed with t-tests, but rather with one-way ANOVA, which would compare all the samples at once (Motulsky 2014).

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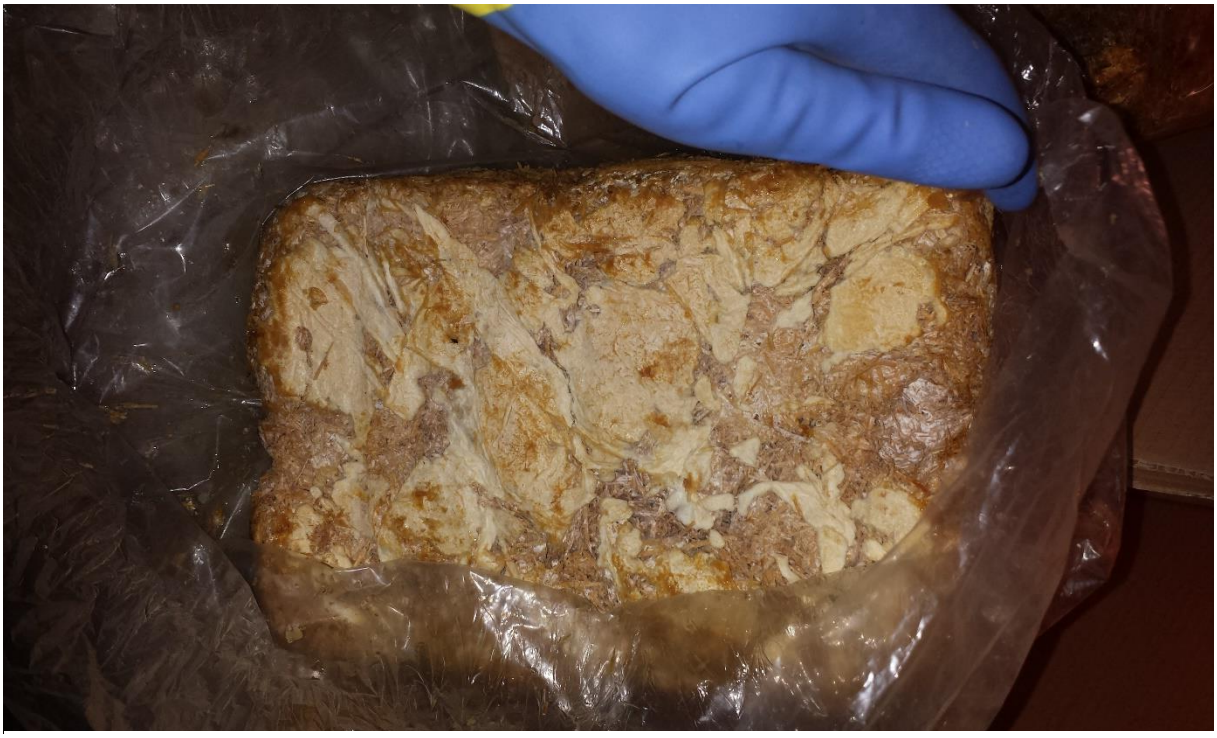
12. APPENDIX

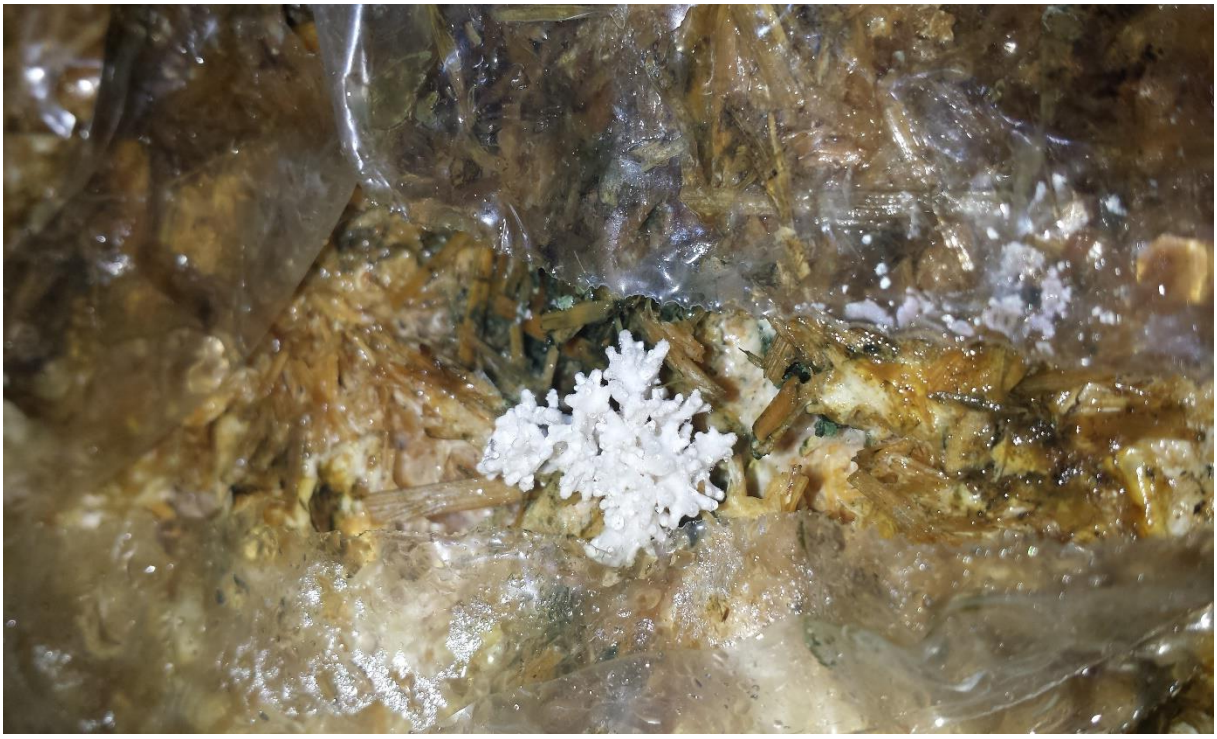
12.1 QUALITATIVE DATA

Table 7 Observations made during the investigation.

Initial appearance of substrates	The substrates are in the form of agro-waste materials. They are made of cereal grains, sawdust, straw and other lignocellulosic wastes. The mixture is yellow-brown in colour.
Initial appearance of *Pleurotus ostreatus* spawn	The bags are filled with grain seeds thoroughly coated with a whitish powder-like substance.
Appearance of inoculated substrates	Mycelium grows out from the spawn – visible as a white substance which permeates the inoculum sites. After a few days, the substrates are entirely colonised with mycelia. The resulting substrates are light creamy yellow in colour. There are yellowish patches on their surfaces.
Observations made during fruiting body development	Primordia appears on the top layers of the substrates. They are in the form of small white pinhead-like structures arranged in clusters. They develop into fruiting bodies due to light exposure. Their pilei are convex, pale brown, beige or greyish blue with smooth surface. Then they flatten out or become concave (up-rolled at the margins). Gills are white to pale yellow. Stems are elongated, white and downy.

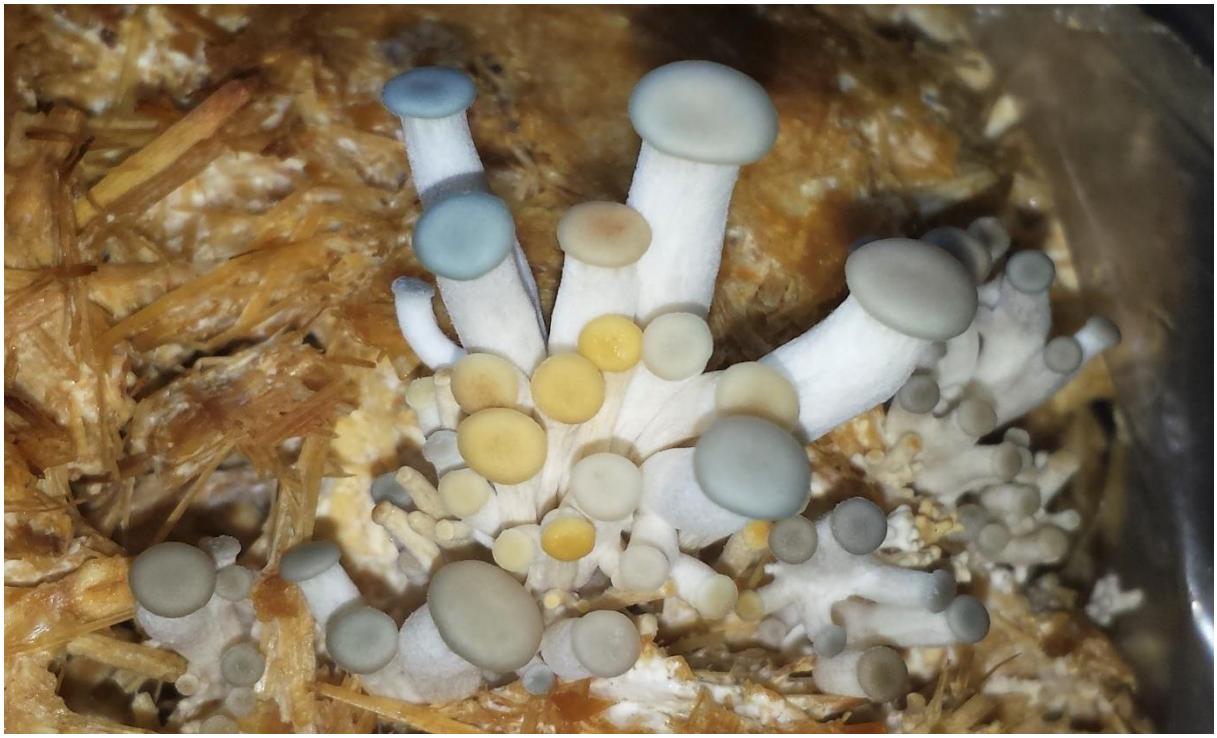


Picture 3 Colonised substrate with yellowish patches on its surface.

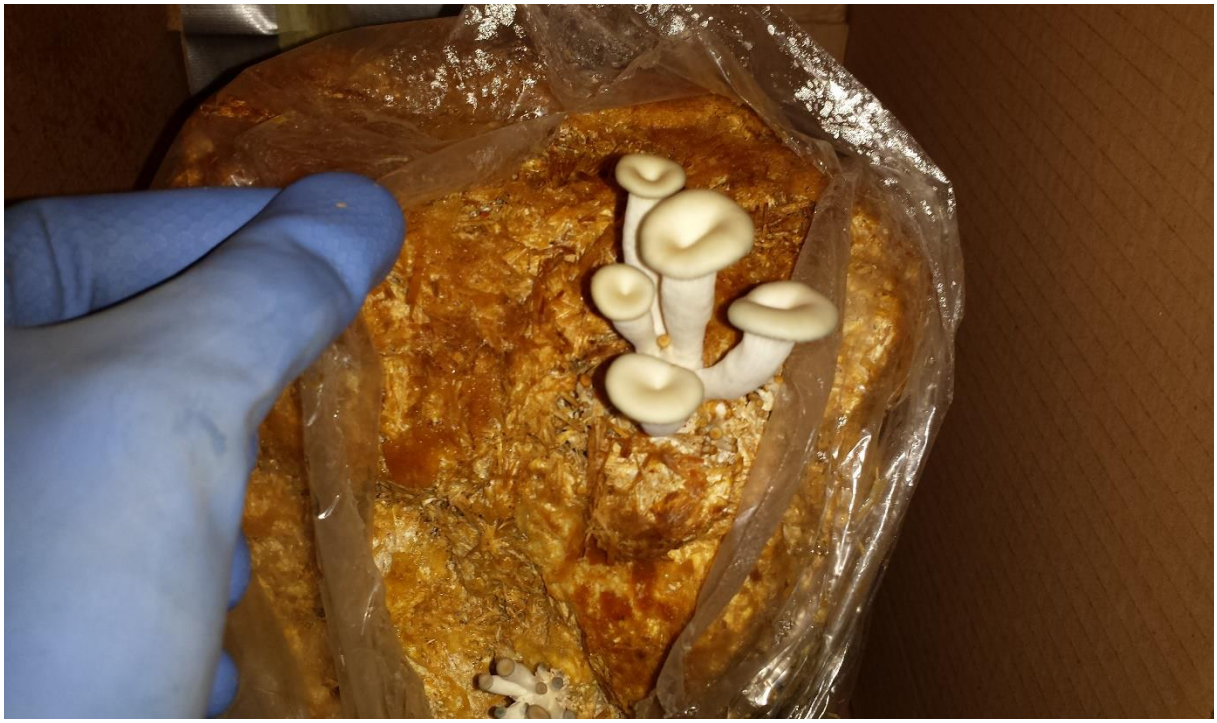


Picture 4 Primordia formation.

Picture 5 White pinhead-like structures arranged in clusters.



Picture 6 Developing fruiting bodies.



Picture 7 Further fruiting bodies development.

Picture 8 Partial inhibition of pilei development.

12.2 QUANTITATIVE DATA

Table 8 Lengths of stipes and diameters of pilei; Blue light. Values are obtained from three trials.

Sample		Length of stipe & Diameter of pileus / mm (±0.5 mm)													
		Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7	
Blue light	**1.**	2.5	0.5	5.0	1.0	12.5	3.0	18.0	5.0	24.5	10.5	31.0	12.5	35.0	14.0
	2.	2.5	1.0	7.0	2.0	14.0	2.5	19.5	5.0	26.0	7.0	32.5	11.0	36.0	11.5
	3.	3.0	1.0	6.5	2.5	9.5	4.5	13.0	5.0	13.5	5.0	14.5	5.0	14.5	5.0
	4.	1.5	0.5	5.5	1.0	19.5	2.5	24.0	4.0	30.0	8.0	36.5	9.0	42.5	9.5
	5.	3.0	1.5	10.0	3.0	19.5	5.5	27.0	7.5	29.5	9.0	32.0	9.0	36.0	9.0
	6.	3.0	1.0	8.5	4.0	14.0	7.5	21.5	8.0	29.0	9.0	35.0	10.5	37.5	12.0
	7.	2.5	2.0	13.0	4.5	18.5	11.0	23.5	13.0	27.0	14.5	32.5	15.0	38.0	15.0
	8.	1.5	1.5	8.0	4.5	15.0	6.5	21.5	13.5	28.0	14.0	33.0	15.0	33.5	15.0
	9.	2.0	1.0	6.5	2.0	12.0	6.0	17.5	7.5	26.0	9.0	29.5	12.0	32.0	14.5
	10.	4.0	2.0	9.0	11.0	11.5	16.5	18.0	28.0	21.0	36.0	24.5	38.5	29.5	39.5
	11.	2.5	0.5	5.0	1.0	12.0	3.0	19.0	6.5	24.0	9.5	31.0	11.0	36.5	12.0
	12.	3.5	1.5	10.5	3.5	18.0	7.0	25.5	12.0	33.0	14.5	37.5	16.0	39.0	17.0
	13.	2.0	1.0	11.0	5.0	15.0	5.0	20.5	7.5	27.0	8.0	34.0	10.5	41.0	11.0
	14.	3.0	2.0	12.5	4.0	15.0	5.0	17.5	7.0	22.0	8.5	25.5	8.5	32.0	9.0
	15.	3.0	2.5	15.0	10.0	23.0	18.5	31.0	26.5	35.0	31.0	35.0	31.5	35.5	32.0

Table 9 Lengths of stipes and diameters of pilei; Green light. Values are obtained from three trials.

Sample		Length of stipe & Diameter of pileus / mm (±0.5 mm)													
		Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7	
Green light	**1.**	2.0	0.5	5.5	1.0	11.0	3.0	14.5	4.5	18.5	5.5	21.0	7.0	23.0	9.0
	2.	3.5	0.5	5.0	1.0	12.5	3.5	18.0	5.0	24.5	10.5	31.0	12.5	35.5	13.0
	3.	1.5	1.0	3.5	1.0	6.0	2.5	7.5	3.0	8.0	4.0	8.5	4.0	9.5	5.5
	4.	1.5	1.0	4.5	2.0	10.0	3.5	13.5	5.0	19.5	5.5	22.0	7.0	23.5	8.0
	5.	2.0	1.5	4.0	3.5	6.0	3.5	9.5	3.5	14.0	4.0	17.5	4.5	20.5	5.0
	6.	2.0	0.5	2.0	0.5	4.5	1.5	5.0	2.0	7.5	3.0	9.5	3.5	11.0	4.5
	7.	4.5	1.0	12.0	2.5	18.5	4.0	23.0	5.0	28.5	7.5	30.5	7.5	32.0	8.0
	8.	3.5	2.0	11.0	3.0	16.0	3.0	19.5	4.5	23.5	6.0	24.0	6.0	25.5	7.5
	9.	4.0	2.5	14.0	9.0	22.0	17.0	29.0	21.5	33.0	26.0	34.0	31.0	34.5	31.5
	10.	3.0	0.5	8.5	1.0	13.0	2.5	16.5	2.5	18.0	5.0	19.5	7.5	20.5	8.5
	11.	3.5	1.0	6.5	2.5	12.0	3.5	18.0	4.0	20.0	5.5	20.5	7.0	22.5	10.0
	12.	2.0	1.0	6.5	2.5	9.0	4.0	13.0	4.5	13.5	5.0	14.5	5.0	14.5	5.0
	13.	3.0	1.5	6.5	2.0	12.0	5.5	17.5	7.0	25.0	9.0	29.5	12.0	31.5	13.5
	14.	2.5	1.0	5.0	1.0	13.0	3.0	19.0	6.5	24.0	9.5	33.0	11.0	36.5	11.0
	15.	1.0	0.5	9.5	1.5	13.0	2.0	16.0	3.5	19.5	5.0	23.5	8.0	25.5	9.5

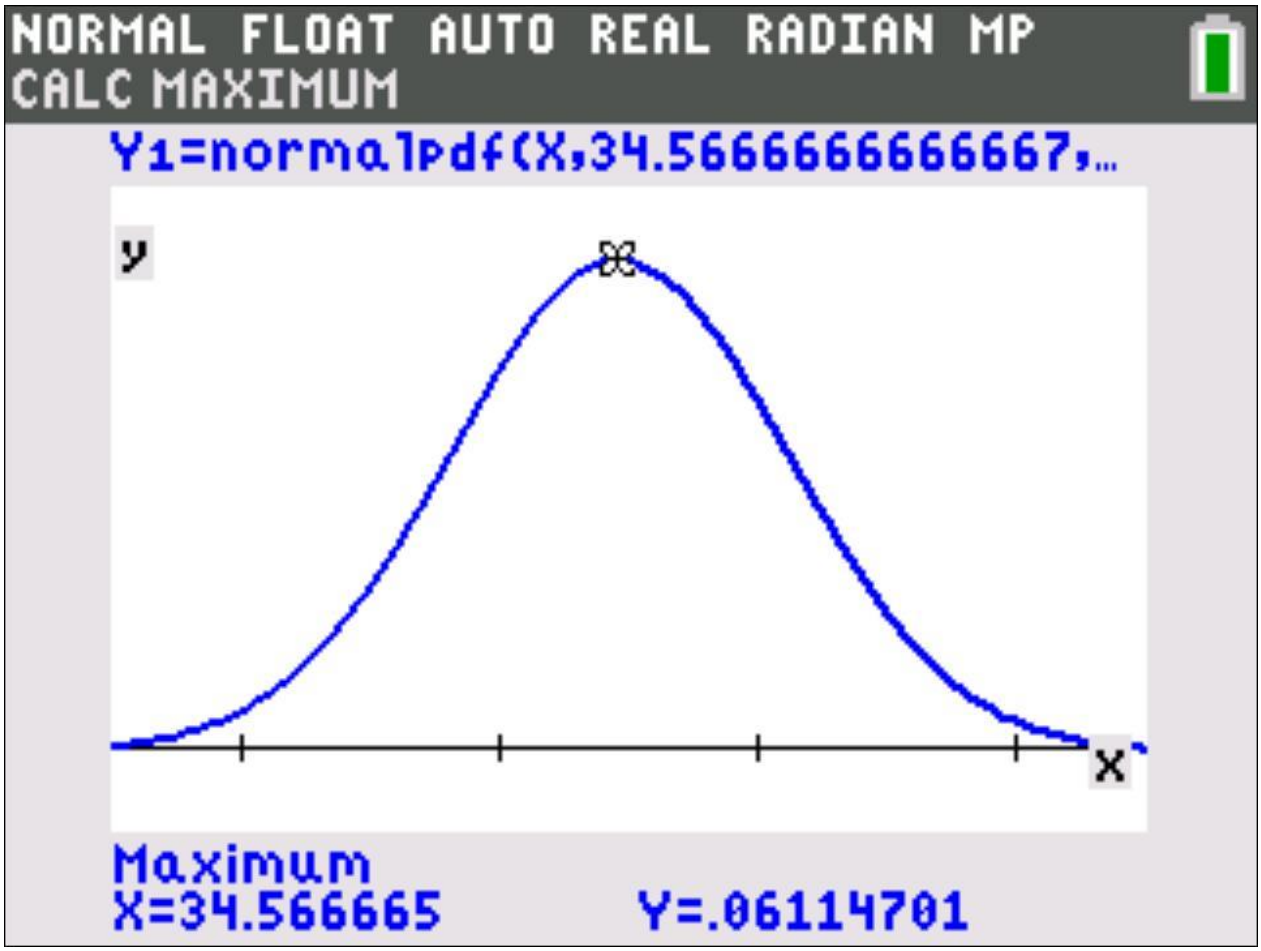
Table 10 Lengths of stipes and diameters of pilei; Red light. Values are obtained from three trials.

Sample		Length of stipe & Diameter of pileus / mm (±0.5 mm)													
		Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7	
Red light	**1.**	1.0	0.5	3.0	0.5	3.5	0.5	4.0	1.0	5.0	1.0	5.0	1.5	6.0	2.5
	2.	2.0	0.5	6.5	2.0	11.5	3.0	13.5	4.5	18.0	5.0	20.0	7.0	21.0	9.0
	3.	2.0	1.0	2.5	1.0	2.5	1.0	5.0	2.0	8.0	3.5	9.5	4.0	11.0	5.5
	4.	1.5	0.5	4.0	1.5	5.0	2.0	6.5	2.0	7.5	2.5	9.5	3.0	10.5	3.5
	5.	1.0	0.5	2.0	0.5	2.0	1.0	3.0	1.0	3.0	1.5	3.5	1.5	4.0	1.5
	6.	3.5	2.0	8.0	5.0	11.5	9.0	15.0	11.5	16.5	13.0	17.0	15.0	17.5	15.5
	7.	1.0	0.5	2.0	1.0	4.5	2.0	8.0	2.5	9.0	3.0	9.0	3.0	10.0	4.0
	8.	1.0	0.5	1.0	0.5	2.0	1.0	3.5	1.0	4.5	1.0	5.0	1.5	5.5	1.5
	9.	2.0	1.0	5.5	2.5	7.0	4.0	13.0	4.5	14.0	5.0	14.5	5.0	15.0	5.5
	10.	1.0	0.5	3.5	1.0	5.0	2.0	5.5	2.0	7.5	2.5	9.0	3.5	10.0	4.0
	11.	1.5	1.0	4.5	1.5	7.0	2.0	9.0	2.5	10.5	2.5	11.0	3.0	12.0	4.5
	12.	2.0	0.5	7.5	1.0	12.5	2.0	16.0	2.5	17.0	5.0	20.0	7.0	20.5	8.5
	13.	1.5	1.0	2.5	1.0	3.0	1.0	4.0	1.5	4.5	1.5	4.5	1.5	5.0	2.0
	14.	2.0	1.0	3.5	1.0	6.0	2.0	6.0	2.5	7.5	2.5	9.0	3.5	9.5	4.5
	15.	1.0	0.5	3.5	0.5	3.5	1.0	4.5	1.0	5.0	1.5	5.5	1.5	6.0	2.0

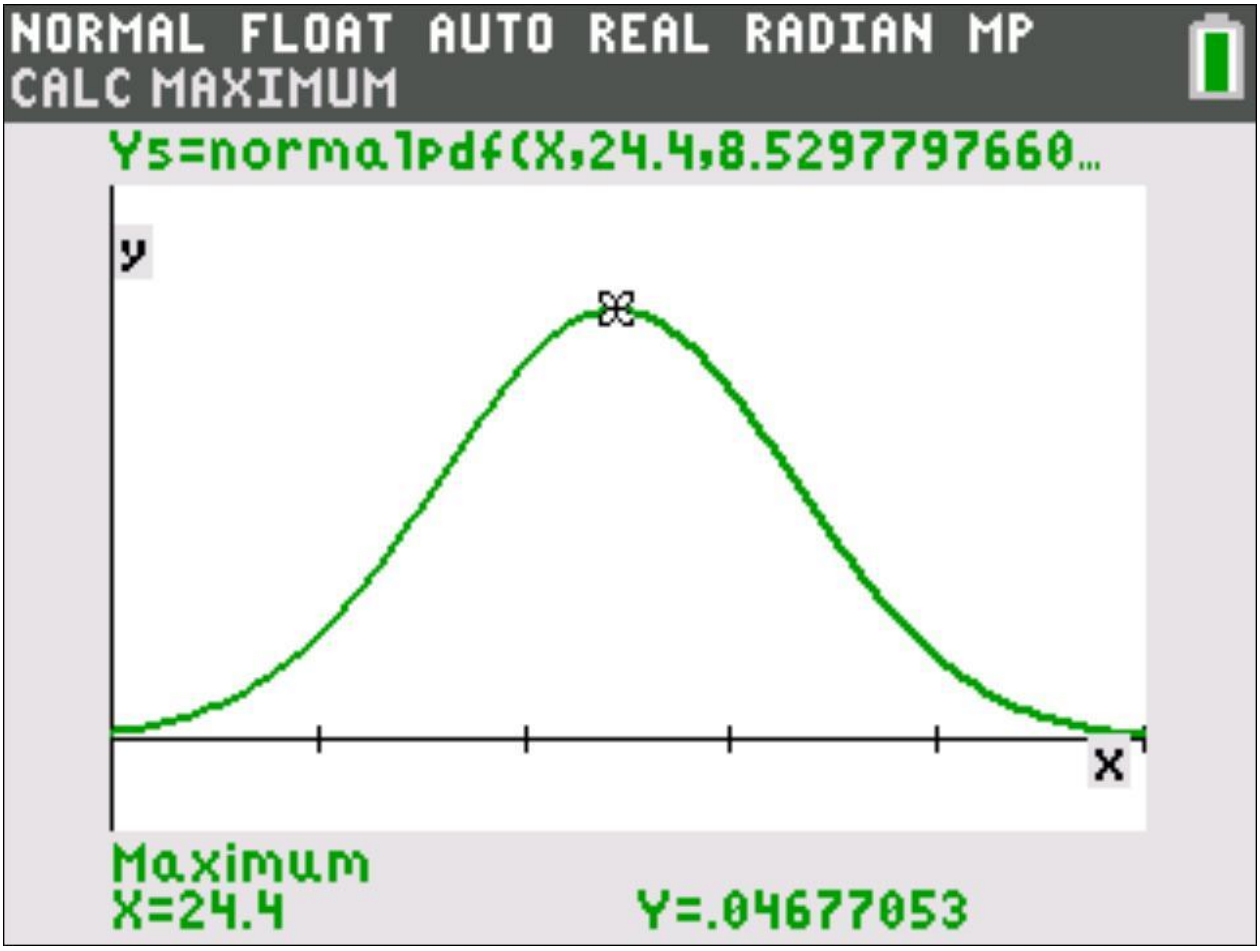
Table 11 Lengths of stipes and diameters of pilei; Yellow light. Values are obtained from three trials.

Sample		Length of stipe & Diameter of pileus / mm (±0.5 mm)													
		Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7	
Yellow light	**1.**	3.5	2.0	12.0	4.5	17.5	10.0	23.0	12.5	27.5	14.0	31.5	14.5	38.0	15.5
	2.	4.0	2.0	12.5	3.0	15.5	5.0	18.5	6.5	22.5	8.5	26.0	9.0	31.5	9.5
	3.	2.0	1.0	8.5	3.0	15.5	6.5	20.0	8.0	22.5	9.0	23.5	9.0	25.0	9.5
	4.	1.5	1.0	4.0	1.5	7.5	2.0	10.0	2.5	10.5	3.0	12.0	4.0	12.0	4.5
	5.	1.0	0.5	6.5	2.5	10.5	4.0	13.0	5.0	19.5	7.5	23.5	9.0	26.5	10.0
	6.	1.5	1.0	7.0	2.5	11.0	5.0	16.5	5.5	20.0	6.0	21.5	8.0	24.0	9.0
	7.	2.5	1.0	5.5	1.5	13.0	3.0	18.0	5.0	24.0	9.5	30.5	12.5	35.5	13.0
	8.	3.0	2.0	7.5	9.5	11.0	14.0	16.5	21.5	20.5	28.0	26.0	32.5	29.5	36.0
	9.	1.5	1.0	8.0	3.5	15.0	5.0	19.5	7.0	21.5	8.0	23.0	10.5	24.0	11.0
	10.	2.5	0.5	9.5	1.0	12.0	2.5	17.5	3.0	19.0	4.5	20.0	8.0	20.5	8.5
	11.	1.5	0.5	6.5	1.5	10.0	4.0	13.5	9.5	16.0	13.0	17.5	14.0	18.5	15.0
	12.	2.0	0.5	6.5	1.5	11.0	1.5	16.5	2.0	18.5	3.5	24.0	5.5	25.0	7.5
	13.	2.0	1.0	5.5	2.0	7.5	4.0	12.5	4.5	14.5	5.0	14.5	5.5	15.0	5.5
	14.	2.5	1.5	6.5	2.0	7.0	2.0	13.5	5.0	19.0	7.5	24.0	10.0	26.5	11.5
	15.	2.0	0.5	7.5	1.5	12.0	2.0	16.0	3.5	19.5	5.0	23.5	8.0	25.0	10.0

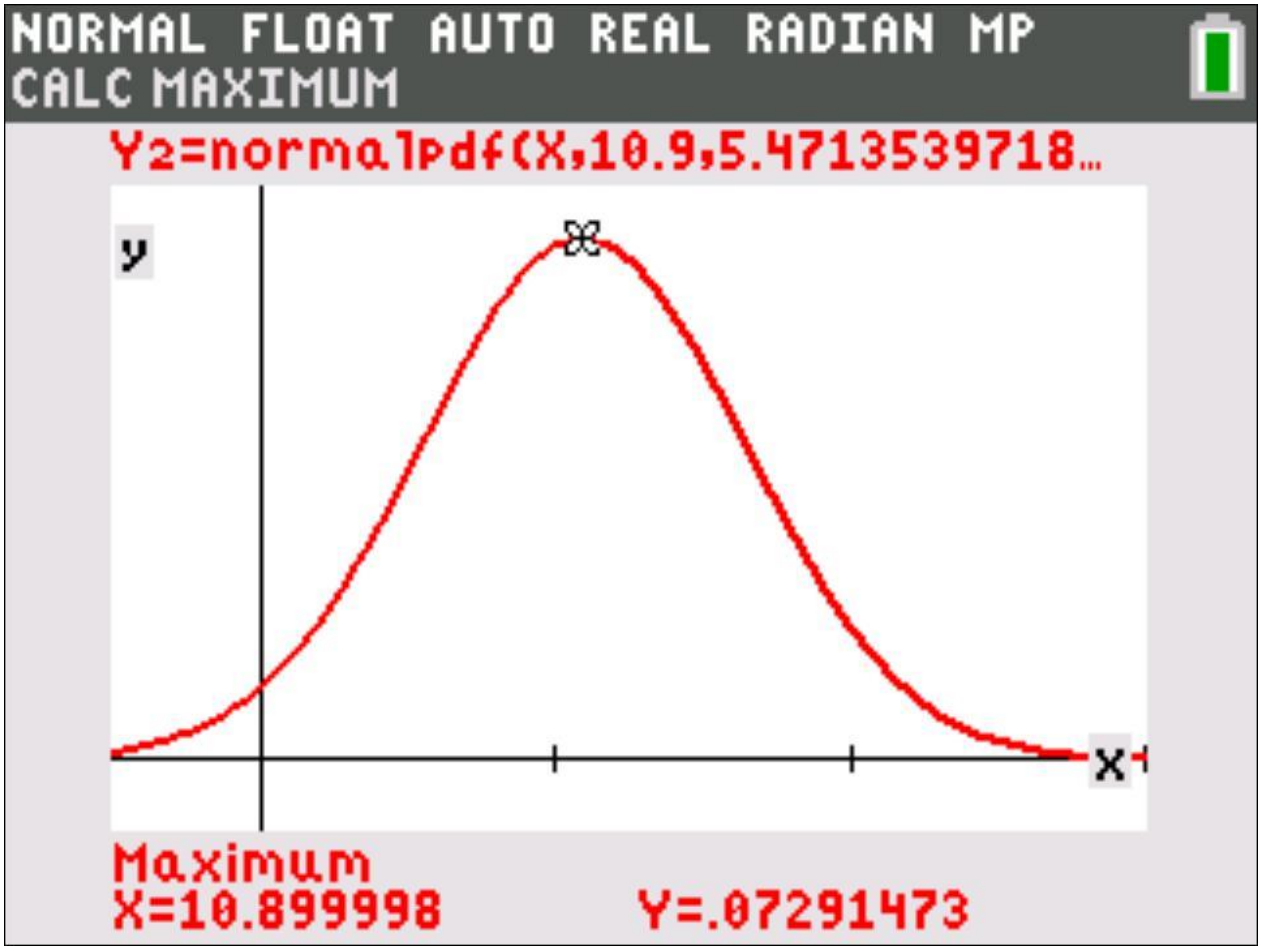
12.3 NORMAL DISTRIBUTION CURVES



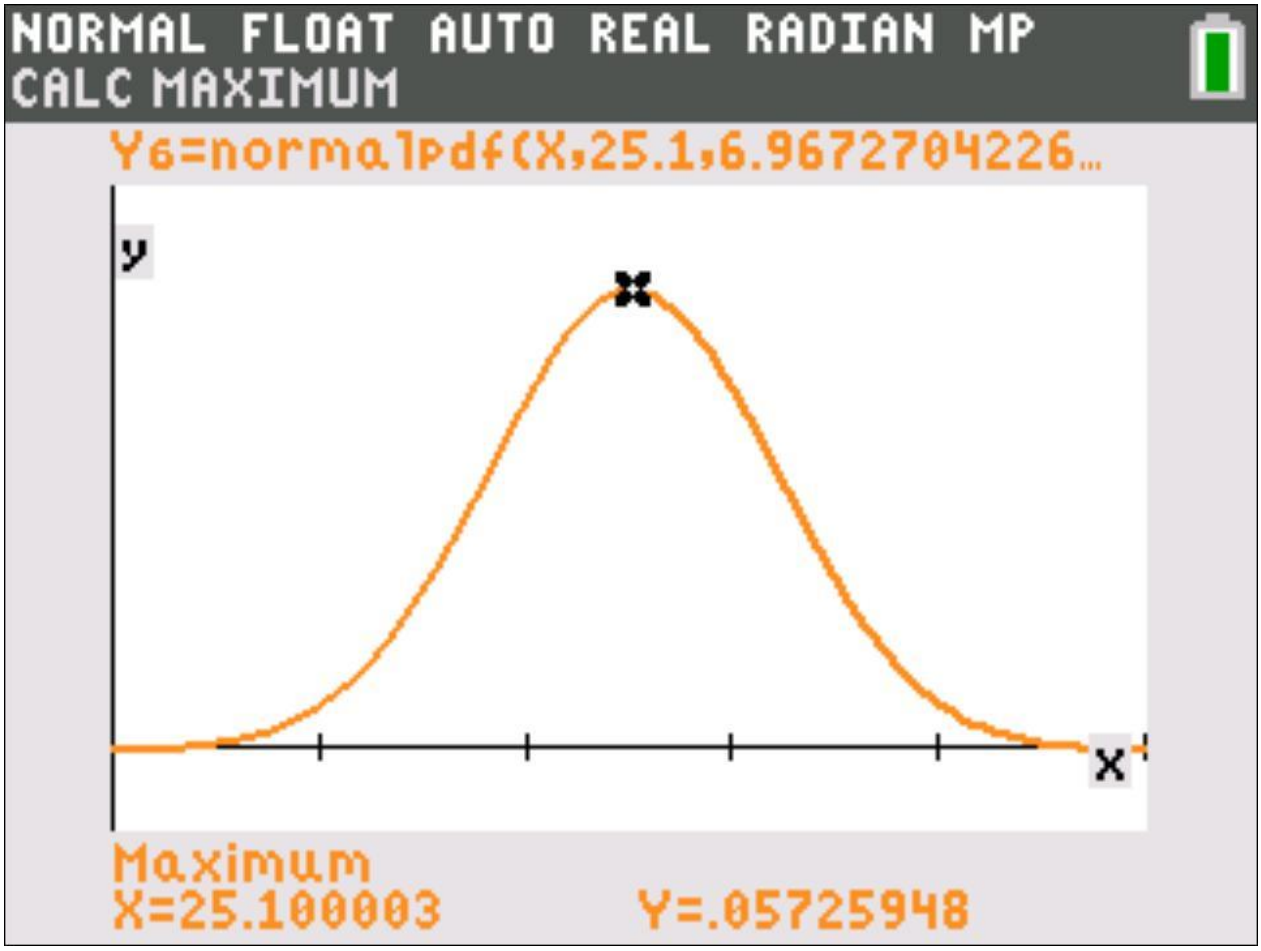
Graph 5 Normal distribution of lengths of stipes concerning Day 7; Blue light, drawn with the help of a GDC, Ti-84. The x-axis shows the lengths of stipes / mm (±0.5 mm) and the y-axis shows the frequency.



Graph 6 Normal distribution of lengths of stipes concerning Day 7; Green light, drawn with the help of a GDC, Ti-84. The x-axis shows the lengths of stipes / mm (±0.5 mm) and the y-axis shows the frequency.



Graph 7 Normal distribution of lengths of stipes concerning Day 7; Red light, drawn with the help of a GDC, Ti-84.
The x-axis shows the lengths of stipes / mm (±0.5 mm) and the y-axis shows the frequency.



Graph 8 Normal distribution of lengths of stipes concerning Day 7; Yellow light, drawn with the help of a GDC, Ti-84. The x-axis shows the lengths of stipes / mm (±0.5 mm) and the y-axis shows the frequency.

12.4 T-TEST

Sample Blue light and Sample Red light

1. H_0: There is no significant difference between the mean lengths of stipes.
2. H_1: There is a significant difference between the mean lengths of stipes.
3. df = 28
4. t = 10.7648
5. Critical value for t = 2.05 (P = 0.05)
6. The calculated value of t is greater than the critical value, showing that the difference between the mean lengths of stipes is significant.

Sample Blue light and Sample Yellow light

1. H_0: There is no significant difference between the mean lengths of stipes.
2. H_1: There is a significant difference between the mean lengths of stipes.
3. df = 28
4. t = 3.8411
5. Critical value for t = 2.05 (P = 0.05)
6. The calculated value of t is greater than the critical value, showing that the difference between the mean lengths of stipes is significant.

Sample Green light and Sample Red light

1. H_0: There is no significant difference between the mean lengths of stipes.
2. H_1: There is a significant difference between the mean lengths of stipes.
3. df = 28
4. t = 5.1595
5. Critical value for t = 2.05 (P = 0.05)
6. The calculated value of t is greater than the critical value, showing that the difference between the mean lengths of stipes is significant.

Sample Green light and Sample Yellow light

1. H_0: There is no significant difference between the mean lengths of stipes.
2. H_1: There is a significant difference between the mean lengths of stipes.
3. df = 28
4. t = 0.2462
5. Critical value for t = 2.05 (P = 0.05)
6. The calculated value of t is smaller than the critical value, showing that the difference between the mean lengths of stipes is not significant.

Sample Red light and Sample Yellow light

1. H_0: There is no significant difference between the mean lengths of stipes.
2. H_1: There is a significant difference between the mean lengths of stipes.
3. df = 28
4. t = 6.2081
5. Critical value for t = 2.05 (P = 0.05)
6. The calculated value of t is greater than the critical value, showing that the difference between the mean lengths of stipes is significant.