



## Physiological Response of *Lepidium Sativum* L. to Different Light Intensity Stresses

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### ABSTRACT

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*light intensity stress, chlorophyll concentration, free radicals, Lepidium sativum, plant metabolism*

Study carried out in Department of Biology Faculty of Education, University of Samarra to evaluation response of *Lepidium sativum* different light stress. Objective of this study is to demonstrate the direct effect of light stress on the levels of total chlorophyll pigment, and free radical, that effects on the physiological processes in plants. Cress plant *Lepidium sativum* was exposed to different levels of light intensity 10, 100, 370 $\mu$ E during the growth process in order to measure the change in the concentrations of the total chlorophyll pigment represented by chlorophyll a and b and measurement of H<sub>2</sub>O<sub>2</sub>. The result showed that the control group was grown at a light intensity of 54 $\mu$ E, the cress plant was exposed to Each light intensity mentioned above is divided into four time periods (2, 6, 12, 24 hours), we also showed a significant increase in total chlorophyll concentrations at 370 $\mu$ E with a 2-hour exposure duration, where the pigment concentration reached 0.65 mg total/g tw. Also indicated that reducing light intensity generally leads to a decrease in pigment concentration, except for a light intensity of 10 $\mu$ E for the durations of 24 and 12 hours, which showed an increase in total chlorophyll concentration, reaching 0.51mg total/g tw and 0.44mg total/g tw, respectively. While the values of free radicals recorded a significant increase with the increase in the period and duration of exposure compared to the control treatment. From the study it was concluded Light intensity stress experienced by plants directly affects the concentration of plant pigments, particularly chlorophyll, which is a product of secondary metabolism.

## 1. INTRODUCTION

The intricate process of photosynthesis transforms solar energy into chemical energy in plant. The productivity of crops relies heavily on their photosynthetic rates, which are influenced by photosynthetically active radiation (PAR) in higher plants [1]. At the level of chloroplasts, immediate responses include state transitions, non-photochemical quenching, and chlorophyll fluorescence, while long-term responses alter gene expression mediated by signal cascades and networks, these approaches enhance the plant's ability to capture light, enhance photosynthetic efficiency, shield the plant from the harmful effects of excessive light, and facilitate flexible adaptation to enhance plant fitness in changing natural environments [2]. It is noteworthy that based on the intensity of light they received during growth, radish seedlings underwent changes not only in the composition of pigments and quinones in the thylakoid membrane but also in the chloroplast's ultrastructure. Under strong light, radish sun chloroplasts bore a striking resemblance to those found in the sun leaves of beech trees, while those developed in dim light exhibited a typical shade chloroplast structure [3]. The findings indicated that the most intense light stress led to a reduction in maximum fluorescence, variable fluorescence, quantum yield of PSII photochemistry, chlorophyll (Chl), and photochemical quenching, while increasing non-photochemical quenching, minimum fluorescence (F<sub>0</sub>), and

anthocyanin levels [4]. Many studies have indicated that light intensity significantly impacts chlorophyll biosynthesis, leading to higher pigment levels under limited illumination. Conversely, cellular growth thrives best under higher light intensities. Hence, this study explores a two-step cultivation process for *S. platensis*, using urea and KNO<sub>3</sub> as nitrogen sources. The process involves initially exposing the culture to high light intensity to promote cellular growth, followed by reducing light intensity to enhance chlorophyll biomass production [5]. Throughout the day, plants are exposed to varying levels of light intensity, sometimes reaching very high levels, causing an imbalance in the photosynthesis process. This triggers reactions and stress responses in plants, prompting them to employ protective mechanisms against excessive energy from intense light. One such mechanism is non-photochemical energy quenching (NPQ), which rapidly dissipates excess light energy by converting it into heat and releasing it through plant tissues, primarily leaves [6]. Different types and intensities of abiotic stress and duration of exposure to abiotic stress have been studied in order to modify the plant's internal homeostasis. Reactive oxygen species (ROS), especially H<sub>2</sub>O<sub>2</sub>, signal molecules that initiate intracellular and systemic signaling, promote oxidative stress, and induce signaling associated with cell death [7-9]. Garden cress (*Lepidium sativum*) is a small, one-year-old herbaceous plant from the cruciferous family, with height of 50cm. It is rich in minerals and vitamins such as C and A, which are very

useful in food, health and treating some diseases. This plant has exceptional properties as an edible and medicinal plant given the effects of Light Intensity on plants, particularly their positive impacts on leaf vegetables, the main purpose of this study is to examine and compare different combinations of light intensity on levels of total chlorophyll pigment, and free radical in cress plant as an important leaf vegetable and medicinal plant relative to the control treatment. This is done in order to introduce the most ideal growing condition of cress in terms of lighting ratios of Light Intensity compare which natural sunlight is used.

## 2. MATERIALS AND METHODS

The experiment applied inside green hose, in Department of biology, University of Samarra, Samarra-Iraq, to study the effect of Light stresses on bioactivity indicators of cress *Lepidium sativum*. Plant stresses include four treatments (10, 100, 370 $\mu$ E and 54  $\mu$ E as control) with four period of time (2, 6, 12 and 24 h) to be 13 treatments is listed in Table 1 below depended on initial experiences and previous studies [10].

**Table 1.** Treatments in experiments of study

No.	Treatment Code	Light Level	Period Time
1	T0	54 $\mu$ E	Normal
2	T1	10 $\mu$ E	2h
3	T2	10 $\mu$ E	6h
4	T3	10 $\mu$ E	12h
5	T4	10 $\mu$ E	24h
6	T5	100 $\mu$ E	2h
7	T6	100 $\mu$ E	6h
8	T7	100 $\mu$ E	12h
9	T8	100 $\mu$ E	24h
10	T9	370 $\mu$ E	2h
11	T10	370 $\mu$ E	6h
12	T11	370 $\mu$ E	12h
13	T12	370 $\mu$ E	24h

The replicates and their combinations resulted in 12 treatments for each replicate, including the control treatment. Experimental units were distributed randomly to reduce experimental error, each treatment was coded with a symbol for ease of handling and distinction from T0 to T12.

### 2.1 Measurement of chlorophyll

To get the most accurate calculation for chlorophyll measurement, 20mg tissue samples were ground in liquid nitrogen and homogenized in 1ml of 80% acetone. By using vortex for almost 5 mints, samples suspension was mixed and followed by incubation for 1h in darkness.

The samples centrifuged at 13000 rpm for 15 min and the supernatants were collected in new tubes to use for measurement. Chlorophyll content in aqueous acetone was quantified spectrophotometrically by measuring the absorbance of the extract at 646.6 nm and 663.6 nm. The total chlorophyll content (chlorophyll a and b) in each sample was calculated according to Hazrati et al. [7].

- Total chlorophyll [ $\mu$ g/mg]= $(17.76(A_{646.6})-7.34(A_{663.6}))$  / fresh weight.
- Chl a [ $\mu$ g/mg]= $(12.25(A_{663.6})-2.55(A_{646.6}))$  / fresh weight.
- Chl b [ $\mu$ g/mg]= $(20.31(A_{646.6})-4.91(A_{663.6}))$  / fresh weight.

Cold methanol /HCl/ water (90:1:1, vol) was used as buffer to extract Anthocyanins with the same procedure as that for the chlorophyll measurements as described by Huang et al. [11].

Anthocyanins concentration was estimated as the following:

- Anthocyanins ( $\mu$ mol ml<sup>-1</sup>)= $A_{529}-(0.288 A_{650})$ .
- Chl a ( $\mu$ mol ml<sup>-1</sup>)= $0.01373 A_{663}-0.000897 A_{537}-0.003046 A_{647}$ .
- Chl b ( $\mu$ mol ml<sup>-1</sup>)= $0.02405 A_{647}-0.004305 A_{537}-0.005507 A_{663}$ .

While the total carotenoids in the tissues, founded using the following equation:

$$\text{Carotenoids } (\mu\text{mol ml}^{-1}) = (A_{470} - (17.1 \times (\text{Chl a} + \text{Chl b}) - 9.479 \times \text{xanthocyanin})) / 119$$

### 2.2 Measurement of H<sub>2</sub>O<sub>2</sub>

The hydrogen peroxide content in the leaf samples from both treated and control groups was determined using the method described by Porra [12]. Approximately 0.1g of frozen leaf material was homogenized on ice with 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was then centrifuged at 15,000g for 15 minutes at 4°C. The resulting supernatant was transferred to a new tube, and 0.5 ml of this supernatant was mixed with 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0) and 1 ml of 1 M KI. The mixture was gently mixed, and its absorbance was measured at 390 nm.

### 2.3 Statistical analysis

The data were analysed statistically using the SAS program, and the arithmetic means of the coefficients were compared based on the Duncuns multiple range test at a probability level of 5%.

## 3. RESULTS AND DISCUSSION

### 3.1 Total chlorophyll

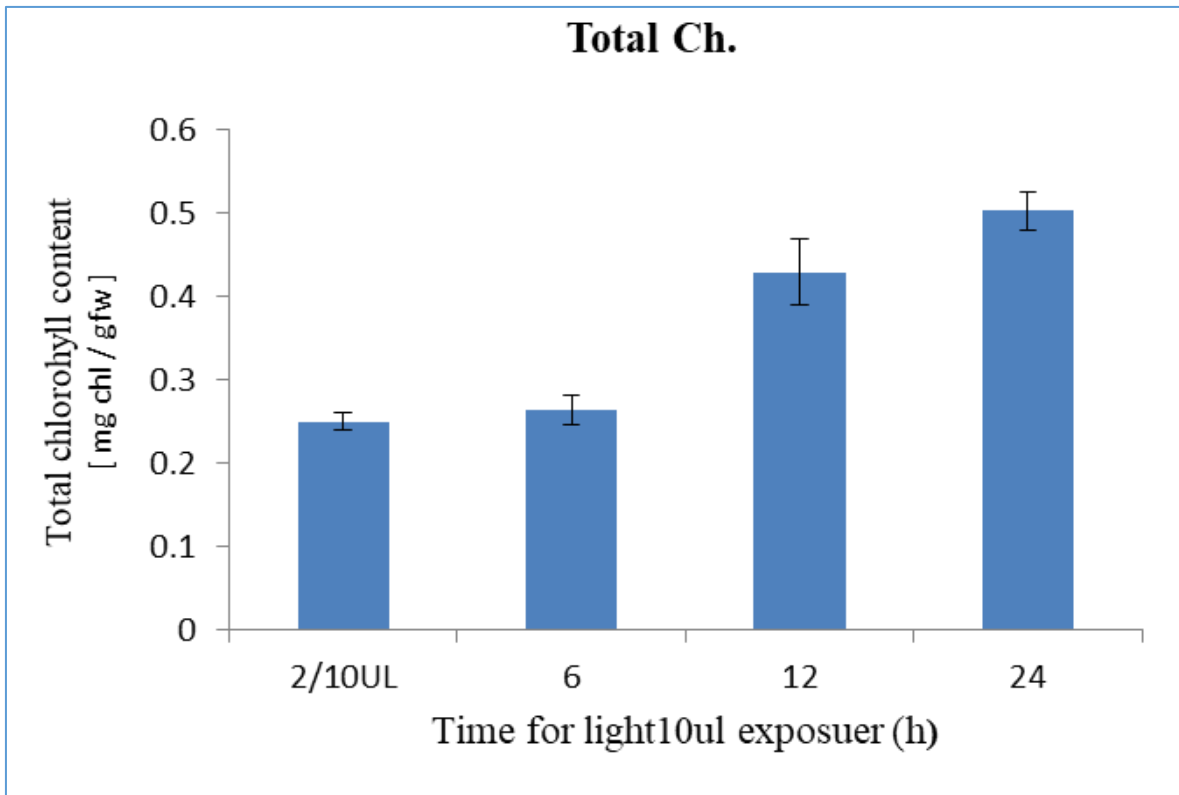
The results showed a clear increase in total Ch. concentrations of plants exposed to a light intensity level of 10  $\mu$ E for 24 hours of exposure which was 0.51mg total/g tw. However, we found that this level of light intensity does not sufficiently support the plant during short periods of exposure (2 hours) where it reached 0.25mg total/g tw compared to the control group, which was 54  $\mu$ E, this is as shown in the Figure 1.

However, the results found that this level of light intensity does not sufficiently support the plant during long periods of exposure 24 hours where its reached 0.35mg total/g tw, this is as shown in Figure 2. Sims and Gamon [13] found similar results, demonstrating that variations in chlorophyll levels are an early sign of light stress experienced by plants during their growth, which significantly impacts overall productivity and biological activity.

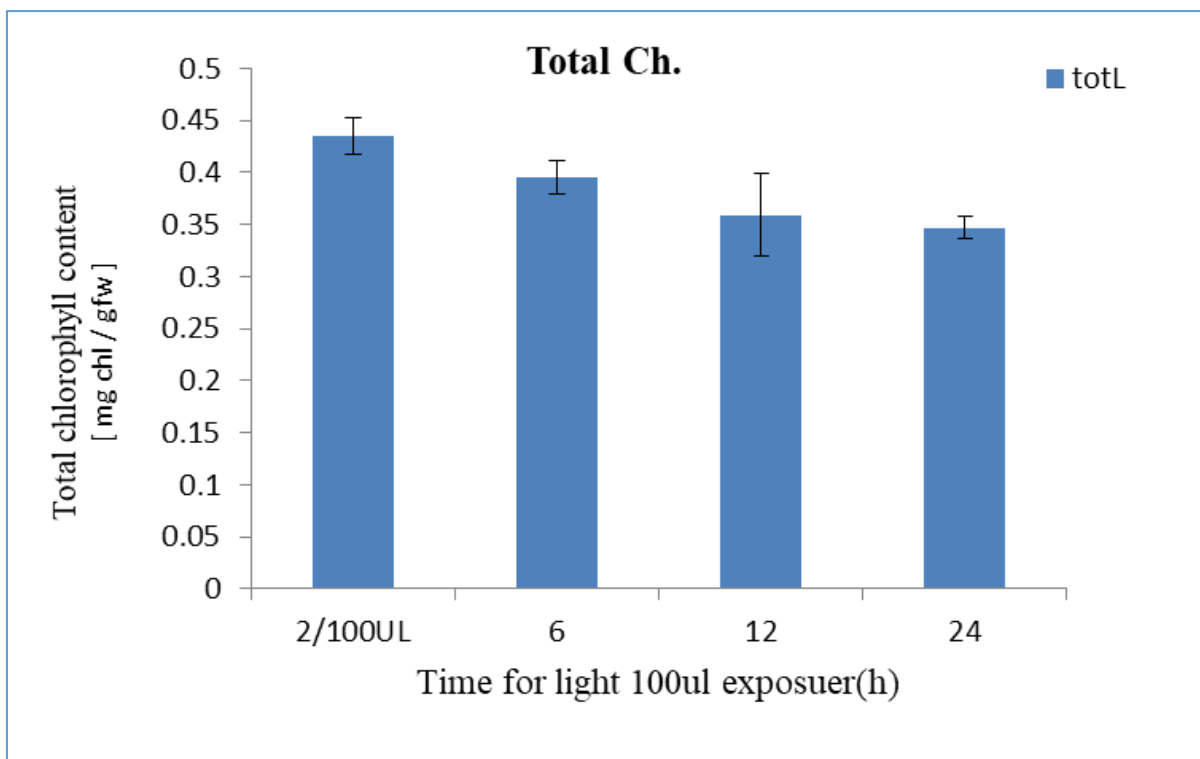
The results we obtained showed a high affinity in total Ch.

concentrations of plants exposed to a light intensity level of 370  $\mu\text{E}$  for the applicable light levels, which were 2, 6, 12 and

24 hours of exposure which was 0.65, 0.6, 0.58 and 0.57 respectively as shown in Figure 3.



**Figure 1.** Total chlorophyll content with light intensity at 10  $\mu\text{E}$  and different period time from 2-24h



**Figure 2.** Total chlorophyll content with light intensity at 100  $\mu\text{E}$  and different period time from 2-24h

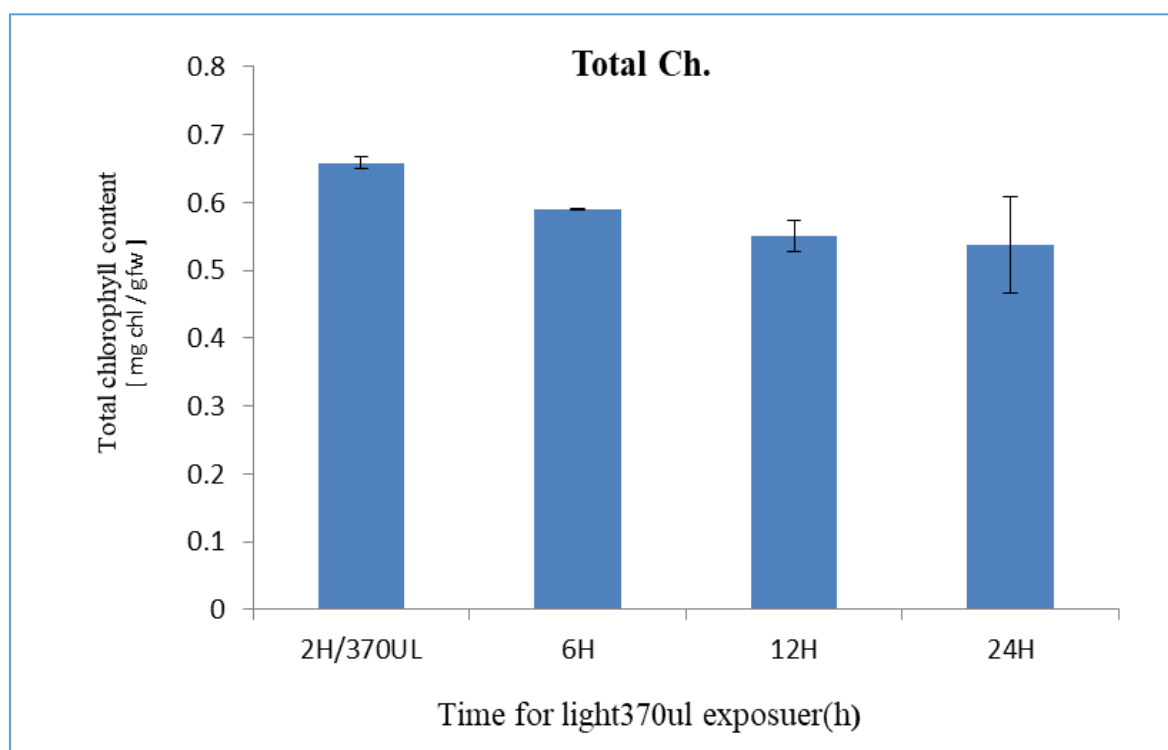
In case of 100  $\mu\text{E}$  exposure the results showed a clear increase in total Ch. concentrations of plants exposed 2 hours of exposure which was 0.48mg total/g tw. However, we found that this level of light intensity does not sufficiently support

the plant during long periods of exposure 24 hours where its reached 0.35mg total/g tw, this is as shown in Figure 3. Faseela et al. [14] found similar results, demonstrating that variations in chlorophyll levels are an early sign of light stress

experienced by plants during their growth, which significantly impacts overall productivity and biological activity. These results indicate that at this level of light intensity, significant changes in the levels of total chlorophyll do not occur in the plant. In Figure 4, it can be clearly observed that the results obtained with a light intensity of 370 are the highest when compared to the control group and the other groups of light intensity levels, these results were consistent with what reached by Wu et al. [15].

Much research has confirmed the effect of light stress on various photosynthesis processes. This is perhaps due to damage to the enzymatic systems at high levels of photo-oxidation or severe lack of light, which directly affects the photosystem, which leads to a reduction in the leaf content of green pigments and other auxiliary pigments, which leads to a decrease in the efficiency rate of photosynthesis. Through the

lack of preparation of the necessary elements for this, along with the weak fixation of carbon dioxide gas in the photosynthesis pathways through the stomata, the number, size, and frequency of which are affected by the weakness of the leaf and the lack of the number of green pigments. Also under different stress, the plant may upregulate antioxidant enzyme activities such as superoxide dismutase and catalase to mitigate oxidative damage. Additionally, increased levels of secondary metabolites like phenolic and flavonoids may be observed, which act as photo protective agents against excess light. The results of the current study were in agreement with the results of studies [16, 17]. The light intensity and exposure period directly affected the chlorophyll content, as the content and effectiveness increased by increasing the light intensity and effectiveness, and thus the construction efficiency and dry matter accumulation.



**Figure 3.** Total chlorophyll content with light intensity at 370  $\mu$ E and different period time from 2-24h

### 3.2 Effects of light quantity on H<sub>2</sub>O<sub>2</sub> content

Result in Table 2 and Figure 4 showed different values of free radical at treatments under study that recorded an increase in free radical rates with increasing exposure time and intensity of light compared with control treatment that gave lowest rates reached 75.53, 76.67, 49.47 and 49.47  $\mu$  mol / mg fw at 2h, 6h, 12h and 24h respectively. While low light intensity values of free radicals recorded with lowest duration of exposure at different period time. The mean values recorded at 2 hours were 709.56, 910.33, and 955.64  $\mu$ mol/mg FW at light intensities of 10  $\mu$ E, 100  $\mu$ E, and 370  $\mu$ E, respectively. As noted, there was an increase in free radical values with prolonged exposure to light intensity over a period of 24 hours, yielding values of 1824.24  $\mu$ mol/mg FW, 1818.18  $\mu$ mol/mg FW, and 1763.63  $\mu$ mol/mg FW at 10  $\mu$ E, 100  $\mu$ E, and 370  $\mu$ E, respectively.

Light intensity plays an important role in its effects on free

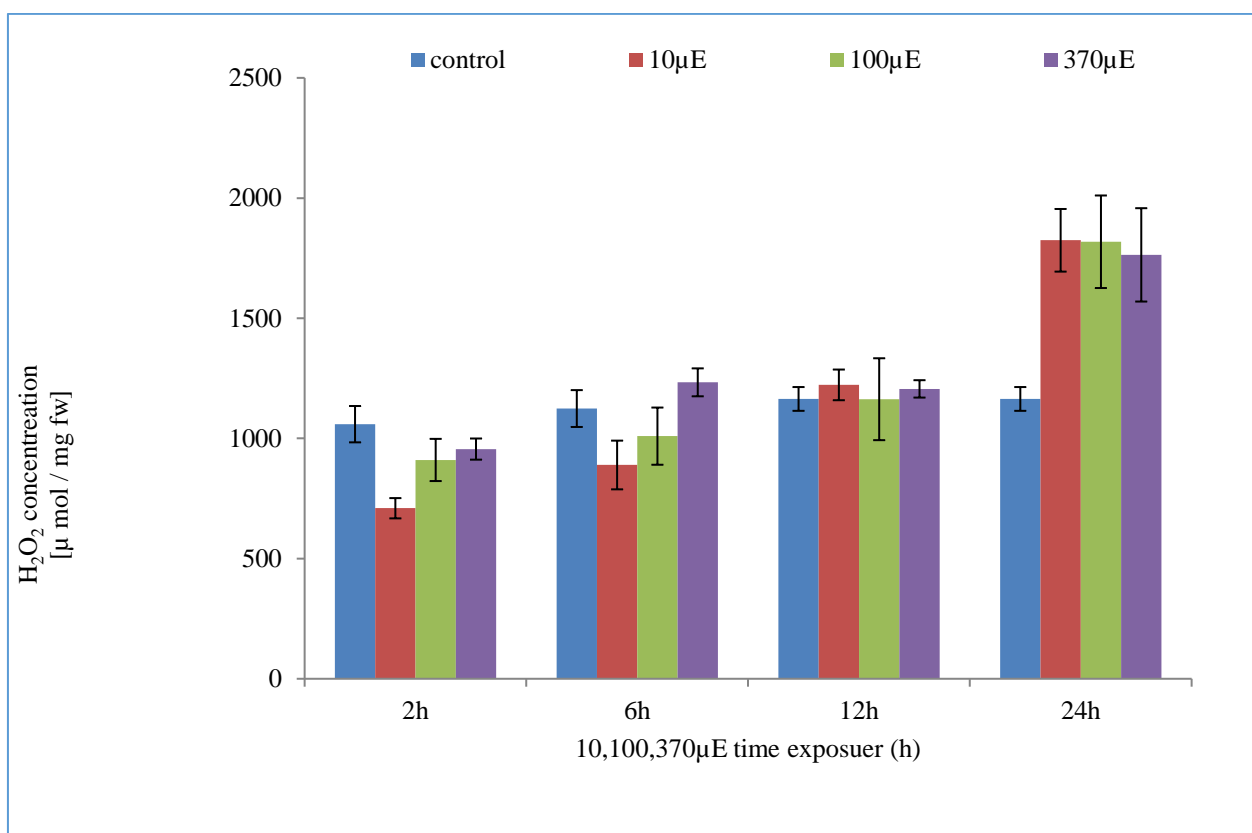
radical content in plants. Free radicals are highly oxidative efficient molecules that form in plant cells as a result of normal metabolic processes or as a result of environmental stresses such as light stress. High light intensity increases the process of photosynthesis in plants, which increases energy production and thus may increase the generation of free radicals as a by-product of metabolic processes [18].

**Table 2.** Total content of H<sub>2</sub>O<sub>2</sub>  $\mu$  mol / mg fw in leave with different light intensity from 100-370  $\mu$ E and different period time from 2-24h

Light Intensity	Time of Duration			
	2h	6h	12h	24
Control				
$\mu$ E54	75.53612	76.67625	49.47806	49.4780
10 $\mu$ E	709.5605	889.393	1222.727	1824.242
100 $\mu$ E	910.3306	1009.366	1163.131	1818.182
370 $\mu$ E	955.6474	1233.333	1206.061	1763.636

Many studies have shown that plants exposed to high light intensity may increase the production of free radicals. Free radicals are heterogeneous molecules containing unbound atoms that can react with plant cells and cause oxidative damage [19]. It is thought that increased light intensity may increase the production of active oxygen and free radicals in plants as a result of harmful light processes such as photo oxidation in chloroplasts. This may sometimes exacerbate oxidative damage within plant cells. Free radicals are part of the plant response to environmental stresses and can contribute to the regulation of plant growth and development including the plant's response to light and other environmental conditions. Many Researchers has shown that plants exposed to high light intensities often exhibit increased oxidative stress markers. For example, studies have documented higher levels of hydrogen peroxide and malondialdehyde (a marker for lipid peroxidation) in plants under excessive light conditions. Plants have evolved mechanisms to cope with varying light conditions. Studies have highlighted the upregulation of antioxidant enzymes under high light conditions, which help mitigate the damage caused by increased free radicals. For

instance, the activity of SOD and CAT typically increases in response to high light intensity to neutralize excess ROS. Also, some studies have focused on plant adaptation to different light conditions. For example, certain plant species have developed specific light-harvesting complexes and protective pigments like carotenoids to mitigate the effects of high light intensity and reduce oxidative damage [20, 21]. Many researcher found quantity of light can significantly affect hydrogen peroxide ( $H_2O_2$ ) levels in plants and photosynthetic organisms. Photosynthesis and ROS Production. The results are also indicative higher light levels can enhance the production of reactive oxygen species (ROS), including  $H_2O_2$ , due to increased electron transport in chloroplasts. In addition, excess light can lead to photo inhibition, where the photosynthetic machinery becomes damaged. This stress can result in increased  $H_2O_2$  accumulation as a byproduct of impaired photosynthesis. Plants often respond to increased light intensity and  $H_2O_2$  accumulation by activating antioxidant systems, such as catalase and peroxidase, to mitigate oxidative stress. Different wavelengths of light can also affect  $H_2O_2$  levels [22-24].



**Figure 4.** Concentration of  $H_2O_2$  with three light intensity (100 to 370  $\mu E$ ) and different period time from 2-24h

#### 4. CONCLUSIONS

Light intensity stress experienced by plants directly affects on the concentration of plant pigments, and product of secondary metabolism. increased light intensity levels resulted in higher total concentration of chlorophyll. Furthermore, treating plants with varying light intensity levels produced different free radicals ( $H_2O_2$ ) concentration outcomes, reinforcing our assumption of the direct impact of light intensity on overall plant metabolism.

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