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*Research Article*

# Effect of diode laser rays in stimulating anthocyanin pigment levels in radish (*Raphanus sativus* L.) plant tissues

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**Abstract**
Plant tissue culture is the method to produce many pigments, such as anthocyanin, regarding the importance of light in plant growth, especially when using laser rays. The present study aimed to establish the effect of laser rays on seed germination, initiation of callus, and the measurement of the amounts of anthocyanin pigment and protein for the growth of radish (*Raphanus sativus* L.). Sterilizing seeds were cultured on a surface of MS medium, then seedlings stems were exposed to laser rays for 0, 4, 8, and 12 minutes each alone and planted on MS medium supplemented with 0.5 mg/l of NAA and 1.0 mg/l of BA, the total protein of seedlings was estimated from all kinds of seedlings exposed to the rays. The anthocyanin pigment content was estimated and read Spectroscopically at a wavelength of 528 nm. The results refer to the different rates and periods of radish seed germination dependent on the exposure time to the laser rays. They also showed the ability of seedlings to initiate callus from hypocotyl stems, which was the fastest exposed for 12 minutes after 7 days at a rate of 100 %. The seeds which were previously exposed to diode laser rays at different times recorded a superiority in stimulating anthocyanin pigment content, which amounted to 1195.2 µg/g for stems and 333.8 µg/g for leaves over the rest of the treatments and the control treatment. This proved to be a modern method to develop forms of physical stimulation as a bio elicitor for the growth of radish tissue cells.

**Keywords**: Anthocyanin pigment level, Diode laser, Radish, *Raphanus sativus* L., Plant tissues

## INTRODUCTION

Plant tissue culture technology has gained unlimited importance due to the possibilities it provides for researchers and scholars in this field and what distinguishes it from others, as it has given the possibility of preserving plant species, producing medicinal drugs and pharmaceutical materials, as well as giving it alternative methods for the traditional methods of producing plant species that require a long period for multiplication vegetative and in a small surface area. In general, it means cutting and sterilizing any explant, placing it on an approved industrial food medium under sterile conditions, and then developing it under and then developing it under conditions of controlled light, heat and humidity (Al-Sumaidaie, 2015; Khalil and Yahya, 2021). It is necessary for success this technology to deliver suitable environmental situations for the plant portion cultured in vitro, similar to those environments where very beneficial factors are light and determine many morphological and anatomical properties and that have a great effect on the plant's physiological parameters such as seed germination, photosynthesis, chlorophyll biosynthesis, the behavior of organs, a number of growth and development interactions are affected by the existence and intensity of light (Talebi *et al.,* 2013; Kazemzadeh *et al.,* 2015). Also, light is related to the activation of enzymes and hormones that affect the growth and elongation of roots, and cell division (Cope *et al.,* 2013). Laser magnification of light by encouraging radiate is limited by the produced wavelength (Kazemzadeh *et al.,* 2015). It is an abbreviation of the word light amplification by stimulated emission of radiation and means the amplification of light by stimulated emission of radiation (Fathy *et al.,* 2012). It has different advantages from natural light. The work of the laser depends on the phenomenon of stimulated emission, for which Einstein laid the theoretical foundations in 1926 (Wayne *et al.,* 2016). Lasers were used in various ways, including industrial, medical and agricultural, and improvements were made to these techniques and their upgrading. The dose, time, type of laser and the type of plant part used all determine the effect, as it was initiated when the red light stimulated enzymes related to the creation of gibberellic acid (GA3), which has a role in cell division (Cope *et al.,* 2013), whereas blue light stimulates enzymes that synthesize the cytokinin, which encourages cell division (Kazemzadeh *et al.,* 2018). The light photons emerging from the laser rays affect the synthesis of specific compounds, such as proteins, carbohydrates, DNA, enzymes and many secondary metabolites (Samuliene *et al.,* 2010). One study found that exposing the seeds of the sunflower *Helianthus annuus* L. to red laser rays at a capacity of 50 milliwatts/cm and a wavelength of 650 nm for times 5,10,15 and 20 minutes stimulated them to germinate the seeds by 20 minutes of exposure at 100 % and also to stimulate the average of seedling callus fresh weights with increasing times of exposure to callus(Ghanem *et al.,* 2015). The radish plant (*Raphanus sativus L.*) belongs to the Brassicaceae family. It is a root vegetable whose roots, which range in color between white and black, are eaten and has many health and medical benefits (Lavanya *et al.,* 2014). It contains the natural anthocyanin pigment, which is the cause of the colors of flowers and fruits of the plant species (Khoo *et al.,* 2017). Anthocyanins are natural pigments that are present in all plant species. They belong to the water-soluble flavonoids group. Flowers and fruits acquire their colors ranging from red to violet. The true color of anthocyanins depends on the substituted groups attached to the basic ring. When methyl groups are present, anthocyanins color red, or when they are linked along with other types of phenolic compounds that cause the blue color (Santos-Buelga *et al*., 2014) this pigment is synthesized in the cytosol and is localized in the vacuoles of plant cells after their construction is complete (Fang *et al.,* 2019). Anthocyanins have an important role in the medical and industrial fields, as they have anti-oxidant activity and the ability to protect plant cells from UV damage and used as natural food colorings due to their low toxicity and anticancer activity as antioxidants (Sivamaruth*, et al.,* 2018). The present study aimed to establish the effect of laser rays duration on the seed's germination, seedlings' growth, development of callus, and the measurement of the amount of anthocyanin pigment and protein in the tissues for the growth of radish (*Raphanus sativus* L.).

## MATERIALS AND METHODS

### Exposing the seeds of the radish (*Raphanus sativus L.*) to laser rays

A group of radish (*R. sativus* L.) seeds, which were obtained from local markets in Mosul city, were exposed to red laser rays using a semiconductor laser device (Uk-scientific Ltd) with a wavelength of 650 nm and a capacity of 50 milliwatts/cm  for periods (0, 4, 8, 12) minutes and at a distance of 15 cm from the radiation source (Ghanem, 2017).

The treated seeds were sterilized separately by immersing a minute in 70% ethyl alcohol, then immersed in 3% NaOCl solution for 15 minutes (Mohammed, 2020), then washed with 3 times/ minute of sterile distilled water and dried on sterile filter paper, the seeds were planted at a rate of 3-4 per treatment on a surface of MS solid medium (Murashige and Skoog, 1962 ). All samples were kept in the growing room in the dark at a temperature of 24±2°C, and after the rise of radicle and plumule, they were incubated to light for 16: 8 (light to dark) hours with an intensity of 1500 lux.

### Callus induction from hypocotyl stems for radish seedlings previously exposed to laser rays

Excised hypocotyl stems from seedlings of radish, previously exposed to laser rays at (0, 4, 8, 12) minutes each alone, after 15 days of germination, and planted on MS solid medium supplemented with 0.5 mg/l of NAA and 1.0 mg/l of BA (Mohammed, 2020). All samples were kept in the culture chamber under seedling-growing conditions.

### Determination of total protein amounts in different tissues of the radish plant

The test of (Lowry *et al*., 1951) was used to estimate the amount of total protein in the stems and leaves of seedlings of the radish plant whose seeds were exposed to diode laser radiation for times (0, 4, 8, 12) minutes at the age of 15 days and from the callus of the hypocotyl stems which induction from all kinds of seedlings exposed to the rays at the age 20 and 40 days by crushing one gram of each sample separately. Then, the protein amount was determined using the standard curve prepared from graded concentrations ranging from 10-100 µg/ml of BSA bovine serum.

### Estimation of anthocyanin pigment in seedlings and callus of radish plant

The anthocyanin pigment content was estimated from all samples mentioned above by taking half a gram of each and placing them separately in glass tubes with a capacity of 15.0 ml. The callus was crushed using sterile forceps into small pieces, and then 5.0 ml of methanol solution containing 1% HCL was added to it. At a temperature of 4C°, then shaken the tubes by vortex and centrifuged at 15000 rpm / 20 min at the same previous temperature, the scintillation was withdrawn, and it was read spectroscopically at a wavelength of 528 nm. The amount of anthocyanins was calculated according to the equation:

E11% cm= {680 at 528 nm} (Mori, *et al*., 1994) Eq.1

The concentration of anthocyanins is expressed in µg $gm^{-1}$ of callus weight.

## RESULTS AND DISCUSSION

**Germination of radish seeds and seedling growth**

The results refer to the different rates and periods of radish seed germination dependent on the exposure time to the laser rays (0, 4, 8,12) minutes and after 15 days are given in Table, 1.

It was observed that the exposure time of 12 minutes from the diode laser rays exceeded the germination of seeds by 100 % after two days of culturing them on MS medium, followed by the exposure time of 8 minutes by 95% after 3 days. The exposure time of 4 minutes from the diode laser rays by 94% related to the control ( Not exposed to radioactivity) as its germination rate to 90% after 4 days. The study also found the role of laser rays in the growth of seedlings of those seeds (Table, 1). The maximum average lengths of the root and shoot system reached 32 and 10 cm, respectively, for those exposed to their seeds for 12 minutes, then followed by that treatment for 4, 8 minutes and the comparison treatment, and this was evident on the developing seedlings (Fig.1).

One of the major radiation properties was affecting the biotic factors of living cells such as proteins, carbohydrates, nucleic acids and lipids (Metwally, 2010). There are different results in the rates and speed of their germination due to exposing the radish seeds to a red diode laser showed and that encouraged the exposure times compared to the control sample, may be due to its capacity to the dormancy and thus rapidity their germination, also promote the essential enzymes for seed germination and in encouraging vegetable seeds and initiate the necessary phytochrome for that (Blume and Matthes, 2012; Yahya and Sultan, 2022).The phytochrome dye can absorb red light, stimulating vegetative growth, and Phytochrome proteins refer to light effects (Ghanem and Abboud, 2015).

**Quantity of anthocyanin pigment and total protein in seedlings of radish plant whose seeds exposed to diode laser radiation at different times**

The data of the anthocyanin pigment content determination in the tissues of stems and leaves of radish seedlings, the seeds of which were previously exposed to diode laser rays and at different times, displayed the repetition of the proportions of their contents from these rays, allowing to the altered the times of exposure after 15 days (Table,2), exposure time 12 minutes recorded a superiority in stimulating the formation of anthocyanin pigment, which amounted to 1195.2 µg/g for stems and 333.8 µg/g for leaves over the rest of the treatments and the control treatment, whose content in tissues in stems and leaves of seedlings reached 314.16 µg/g and 156.3 µg/g respectively.

**Table 1.** Effect of diode laser rays on the germination of radish seeds grown on solid MSO medium and the root and shoot system of its seedlings of radish after 15 days.

| Seeds exposed to laser rays (min) | Germination percentage (%) | Germination period (day) | Average root system lengths (cm) | Average shoot system lengths (cm) |
|---|---|---|---|---|
| 0 | 90 | 4 | 21 | 5 |
| 4 | 94 | 3 | 32 | 6 |
| 8 | 95 | 3 | 31 | 7 |
| 12 | 100 | 2 | 32 | 10 |

-Each reading characterizes an average of four replications

**Table 2.** Showing the amount of anthocyaanin pigment and total protein of stems and leaves of seedlings of radish exposed to diode laser rays at different times after 15 days

| Seeds exposed to laser rays (min) | Anthocyanin concentration (µg/g) | | Protein concentration (mg/g) | |
|---|---|---|---|---|
| | Stems | Leaves | Stems | Leaves |
| 0 | 314.16 | 156.3 | 340.1 | 268.6 |
| 4 | 818.7 | 255 | 482.5 | 295.5 |
| 8 | 928.2 | 304.6 | 521.5 | 314.1 |
| 12 | 1195.2 | 333.8 | 662.8 | 333.11 |

-Each reading represents four repetitions

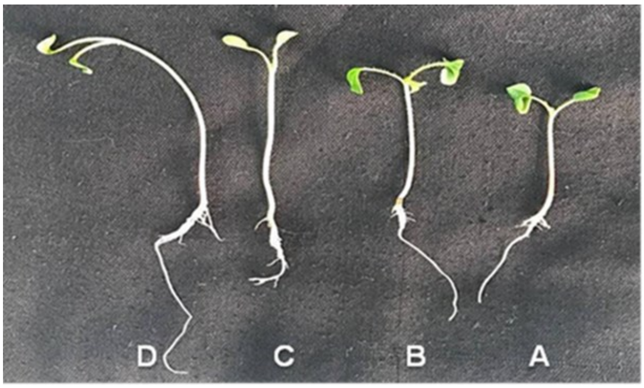


**Fig. 1.** *Growth of seedlings of radish on solid MS medium and its seeds previously exposed to diode laser rays at the following times (A=0, B=4, C=8, D=12) minutes*

Table 2 shows the protein concentration in seedlings of radish plants whose seeds were earlier treated to diode laser rays at different times after 15 days. The highest content appeared in seedlings exposed to laser rays for 12 minutes, and it was 662.8 mg/g in stems and 333.1 mg/g in leaves. The lowest percentage of protein was in the comparison seedlings, as it was 340.1 mg/gm in the stems and 268.6 mg/gm in the leaves. The red light has a role in stimulating the biosynthesis of the gibberellin hormone (GA3) complex ring, which mainly causes the seedling's growth and stimulates the biosynthesis of biomolecules such as proteins and nucleic acids (Kamiya *et al*., 1999).

**Callus induction from hypocotyl stems for radish seedlings**

The stems of seedlings showed a difference in their callus-inducing ability based on the duration of exposure of their seeds to laser rays and a solid Murashige and Skoog medium complemented with 0.5 g/l of Naphthaleneacetic acid (NAA) and 1.0 g/l of Benzyladenine (BA). The results showed the ability of seedlings of all kinds to induction callus from hypocotyl stems, which was the fastest exposed for 12 minutes after 7 days at a rate of 100% (compared with the rest of the seedlings, which was after 6 days and at a rate ranging between (90%- 92%)and its growth on solid medium(MS) supplemented with 0.5 mg/l NAA and 1.0 mg/l BA, which required re-implantation after 20 days(Table. 3).

When measuring its growth after 40 days, its average fresh weight was 3.8 g and the callus was notable by its rigid surface. The green color reflected it (Fig. 2-D), overtaking the rest of the 4 and 8-minute treatments that encouraged callus development after 10 days. The average fresh weight of the callus induced after 40 days was 1.9 and 2.7 g, separately (Fig. 2 B, C) compared to that of the callus on MS medium (the comparison) alone was 1.6 g after 40 days. The callus showed dark green in color and brittle quality (Fig. 2 A). The balance between NAA and BA had an active role in initiating the seedling's stem callus (Shilpa and Kaur, 2017). The positive role of these rays causes a rise in the callus fresh weights initiated from the seedlings stems, whose seeds were primarily exposed to rays when compared with the control sample by stimulating continuous cells divisions and the growth according to the different times of exposure. This is similar to the callus fresh weight increase of the sunflower plant whose parts were exposed to red laser rays (Ghanem and Abboud, 2019).

**Quantities of anthocyanin pigment and protein in callus of radish**

The results of estimating the content of anthocyanin pigment in the tissues of the hypocotyl stem callus of radish seedlings plant whose seeds were prior exposed to diode laser rays explain a variation according to the different exposure times after 20,40 days (Table. 4).

The mainly induced callus from seedlings grown from seeds exposed to radiation in 12 minutes detailed advantage in pigment concentration by 1280.1 µg/g over the rest of the other treatments, and the total protein content was 1460.1 mg/g. The lowest concentration of anthocyanin pigment from callus induced mainly from seedlings exposed at the time of exposure 4 minutes was recorded at 462.1 µg/g after 40 days. Also, the lowest content of protein was 880.1 mg/g, whereas the average of anthocyanin pigment and protein amounts in the control sample (callus resulting from seedlings not treated with diode laser) were 491.2 µg/g and 798.9 mg/g, respectively. The rise in the callus fresh weights was supplemented by an increase in the content of the cell anthocyanin pigment and total protein, with the same callus induced generally of seedlings whose seeds were not exposed to rays, which confirms the vital activity of the tissues generated from the exposed seeds and the accompanying physical effect on them with their different stages of growth and the possibility of building many vital compounds important to the plant and this may explain the ray encouragement to nucleic acids, DNA and RNA and proteins (Dong, *et al*., 2019)

**Table 3.** Growth of callus of radish seedlings whose seeds were exposed to diode laser rays at different times and grown on MS medium added with 0.5 mg/l NAA and 1.0 mg/l BA after 20, 40 days

| Callus of seedlings treated with laser rays (min) | Fresh weight (gm) | |
|---|---|---|
| | 20 days | 40 days |
| 0 | 1.2 | 1.6 |
| 4 | 1.3 | 1.9 |
| 8 | 1.9 | 2.7 |
| 12 | 2.9 | 3.8 |

-Each reading represents four repetitions

**Table 4.** Growth of callus stems of radish seedlings grown on MS solid medium added with 0.5 mg/l NAA and 1.0 mg/l BA after 20 and 40 days and its contents of anthocyanins and protein.

| Callus of seedlings treated with laser beams (min) | 20 days | | 40 days | |
|---|---|---|---|---|
| | Anthocyanin concentration (µg/g) | Protein concentration (mg/g) | Anthocyanin concentration (µg/g) | Protein concentration (mg/g) |
| 0 | 412.1 | 421.2 | 491.2 | 798.9 |
| 4 | 399.2 | 591.9 | 462.1 | 880.1 |
| 8 | 627.7 | 712.9 | 990.1 | 1218.1 |
| 12 | 821.4 | 980.9 | 1280.1 | 1460.1 |

-Each reading represents an average of four repetitions

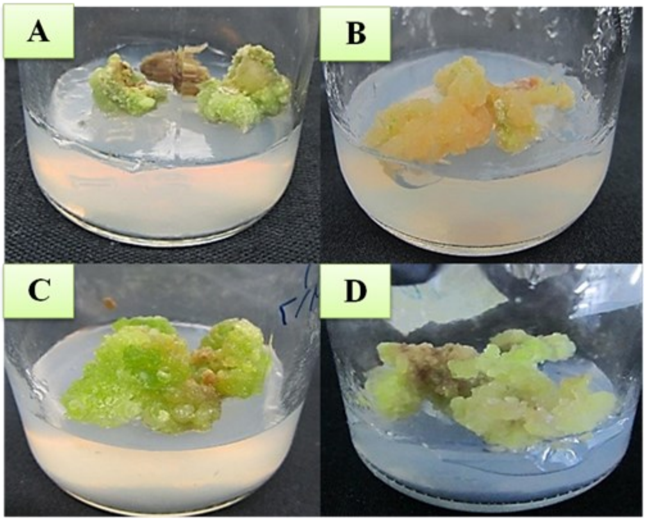


**Fig. 2.** *Callus of hypocotyl stems of radish seedlings exposed to diode laser rays at diverse times (A=0, B=4, C=8, D=12) min on MS solid medium added with 0.5 mg/l NAA and 1. 0 mg/l BA after 40 days*

which give the increasing in the expression of genes which responsible for the formation and accumulating anthocyanins, also red laser light can encourage photoreceptors (phytochrome) that controls the activation of HY5 and PIF5 enzymes to rise the expression of constructing anthocyanin genes (Bao-xing *et al*., 2019).

## Conclusion

The positive role of the diode laser rays caused a rise in the radish callus fresh weights initiated from the seedling's stems. Also, the ray encouraged to nucleic acids (DNA, RNA) and protein synthesis, which raises the expression of genes responsible for producing and accumulating anthocyanin pigments. Thus, the current study proved the success of one of the physical agents using a type of laser radiation at specific levels for the growth, development and level of important metabolites of radish plant tissue cells. It represents a modern path in the field of plant tissue culture, which is linked between physical factors and the development of the cultivated explant, and opens new future scopes for its application to other types of laser beams at different levels as a catalyst for the growth and products of important economic and medical plants .

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## Conflict of interest

The authors declare that they have no conflict of interest.

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