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Effect of Laser Radiation Treatments on *in vitro* Growth Behavior, Antioxidant Activity and Chemical Constituents of *Sequoia sempervirens*.

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ABSTRACT

The present study was carried out at *in vitro* (Tissue culture and Germplasm Conservation Research Lab.) to investigate the effect of different laser rays on inducing growth behavior, some chemical constituents and antioxidant activity of *Sequoia sempervirens.* The *in vitro* shootlets were subjected to green (Argon laser), red (Helium neon laser) and blue (Helium cadmium laser) rays for exposure time 5 min. The obtained data pointed out that exposing the *in vitro* shootlets of *Sequoia sempervirens* to red laser radiation resulted in the best results in shooting behavior (number of both shootlets and leaves as well as shootlets length), the concentration of both chlorophylls *a,b* and proline content while, the shootlets exposed to blue laser irradiation led to the highest rooting percentage and number of roots . Using green laser irradiation resulted in the longest roots and augmented the endogenous content of carotenoids, cellulose and carbohydrate (%) as well as total phenolic, flavonoid, tannin, antioxidant activity (DPPH) and reducing power ability of *Sequoia sempervirens* plantlets in comparison with control.

Keywords: Shooting and Rooting, Antioxidant activity, Reducing power, Chemical constituents, Laser rays, *Sequoia sempervirens*.

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INTRODUCTION

Sequoia sempervirens (D. Don.) Endl. coast redwood, is an important conifer species as it is the tallest tree on earth with a high volume of standing biomass, in some stands exceeding 3500 metric tons/hectare.It belongs to the gymenosperma, class Coniferophytes, order Coniferales, sub-order Abietales, family Taxodiaceae [1]. Redwood possesses more decay- and fire-resistance and, thus, greater longevity than other tree species [2]. Chemical extracts of *Sequoia sempervirens* plants have several medicinal compounds extract, the methanol extracts of some parts of sequoia have many of compounds strongly inhibited colon, lung and breast tumors [3]. There are few *in vitro* studies on micropropagation of this coniferous plant using different sources of explants [4-7].As for previous studies indicated that sequoia is very hard to root and rooting percentage was low [8, 9].

Various physiological processes of growth and development in plants are modulated by internal and external factors because plants are particularly sensitive to external environmental factors. Previous studies have illustrated that laser radiation at suitable doses notably improves enzyme activities [10].

Lasers are divided into two types, pulsed wave lasers and continuous wave lasers [11]. The former, such as the neodymium: yttrium aluminum garnet (Nd: YAG) and XeCl lasers, is used mainly in medical therapy [12], whereas the latter, such as the He-Ne and CO_2 lasers, is used on crops [13, 14]. Many previous studies have shown that suitable doses of He-Ne and CO_2 lasers (continuous wave) have a positive effect in accelerating plant growth and metabolism [15, 16].[17] Found that low-intensity laser radiation stimulates morphogenetic processes in tissue cultures of wild grasses, such as rhizogenesis and the formation of morphogenic calli and regenerated plants. [18] suggest that a general cell response induced by laser-light irradiation can be divided into two specific responses: the first one consists in a rapid stress effect resulting an increase in the amount of lipid peroxidation products, and the second and longer one are the secondary reactions related to the adaptive metabolic changes and apparently accompanied by the stimulation of morphogenetic processes. Laser activation of plants results in an increase of their bioenergetic potential, leading to higher activation at phytochrome, phytochrome and fermentative systems, as a stimulation of their biochemical and physiological processes [19]. Therefore there is a need to run a more thorough investigation focused on the biochemical and physiological processes taking place in treated plants.

The aim of this study was to investigate the effect of different types of laser rays on *in vitro* growth behavior, chemical composition,DPPH scavenging activity and reducing power of *Sequoia sempervirens* plantlets to provide beneficial information about how and why laser has physiological effect on plant and improvement its quantity and quality.

MATERIALS AND METHODS

This experiment was carried out at tissue culture and Germplasm Conservation Research Laboratory, Horticulture Research Institute, Agriculture Research Center (ARC), Department of Ornamental Plants and Woody Trees, Department of Plant Biochemistry, National Research Center (NRC), Egypt during years 2013 and 2014 to evaluate some

morphological, chemical composition changes and antioxidant activity in *in vitro Sequoia sempervirens* plantlets treated with various laser types to provide beneficial information about how and why laser has physiological effect on plant and improvement its production quantitatively and qualitatively.

Plant materials

Shoots (5-10 mm) of *Sequoia sempervirens* collected from adult tree in Giza Zoo were used as explants source (stem node) for micropropagation.

Culture medium

Half strength of basal salts of MS- medium supplemented with 0.5 mg/l of 6-benzylamino-purine (BAP) and enriched with sucrose 25g/L and solidified with 0.7% agar. The media were adjusted to pH 5.7 ± 0.1, then autoclaved at 121°C and 1.2kg/ cm^2 for 15 min.

Culture conditions

The cultures were incubated in growth chamber at 24 ± 1°C under florescent lamps with light intensity of 3k lux at 16 hr. photoperiods.

Laser rays

Helium neon laser "He-Ne", Argon laser "Ar" and Helium cadmium laser "He-Cd" was used for shootlets (2cm) of *Sequoia sempervirens in vitro* for 5min.

The wavelengths of lasers rays were 632.8, 514.5 and 441.6 nm respectively. The power densities for "He-Cd" and organ laser were adjusted nearly 250 mw/cm2 and 100-120 mw/cm2 for He-Ne laser. Laser irradiation treatments emitted from the standards device was generally characterized in terms of power [in units of watts (w) and milli watts (Mw)]. The power levels vary from laser to laser [20]. The energy in joules is defined as the power multiply with time intervals during which it's estimated according the following equation:

Energy (Joules) = power (w) x time (second)

Each treatment consists of 25 replicates, seven- eight weeks after exposure treatments, the following data were recorded:

Shooting behavior

- Number of formed shootlets per explant.
- Shootlet length (mm)
- Number of leaves per shootlet.

Rooting behavior

- Percentage of roots formation (%)

- Number of roots /shootlet
- Root length (mm)

Extraction and determinations

Photosynthetic pigments

Photosynthetic pigments (chlorophyll *a* and *b*) as well as carotenoids were determined in shootlets tissues as mg/100g fresh weight, according to the procedure achieved by [21].

Cellulose, Proline and Carbohydrate

Cellulose percentage was determined according to [22]. Carbohydrate percentage was determined according to [23]. Proline content was determined in dry leaves by using the methods of [24].

Extract preparation

About one gram of *Sequoia sempervirens* plantlets powdered samples was accurately weighed and shacked with 200 ml of 85% methanol for 24 hr. using shaking incubator at room temperature. Solids were separated by centrifugation and filtration extracts were performed in triplicate for each individual sample then extracts were evaporated and subjected for determinations.

Total Phenolic content

The total phenolic content of methanol extracts of *Sequoia sempervirens* irradiated plantlets was determined according to the method described by [25]. Aliquots of the extracts were taken in a test tube and made up to the volume of 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the tubes were placed in the dark for 40 min and the absorbance was recorded at 725 nm against the reagent blank. The amount of total phenolic was calculated as Gallic acid equivalents from a calibration curve.

Total Flavonoid

Total flavonoid was estimated using the method of [26]. To 0.5 ml of methanol extracts of irradiated *Sequoia sempervirens* plantlets, 0.5 ml of 2% $AlCl_3$ solutions was added. After1 hr. at room temperature filtered, and then the absorbance was measured at 420 nm. Total flavonoid contents were calculated as quercetin equivalent from a calibration curve.

Determination of Tannins

Tannins of the *Sequoia sempervirens* plantlets treatments were determined using the modified vanillin hydrochloric acid (MV-HCl) as reported by [27]. Samples of 1 gram

dried irradiated plantlets were extracted with 1% concentrated hydrochloric acid in methanol. The mixture then were shaken for 24 hours and let to settle. A 5 ml of vanillin-HCl reagent (50:50 mixtures of 4% vanillin / 8% HCl in methanol) was quickly added to 1 ml extract. The developed color was measured at 500 nm using spectrophotometer. The standard curve of catechin was obtained and tannins were calculated.

Antioxidant Activity (DPPH Assay)

The free radical scavenging activity using the 1.1-diphenyl-2-picryl- hydrazil (DPPH) reagent was determined according to [28].0.5 ml of the methanolic extracts of *Sequoia sempervirens* irradiated plantlets were added to 1.5 ml of freshly prepared methanol DPPH solution (20 µg ml) and stirred. The decolorizing processes was recorded after 5 min of reaction at 517 nm and compared with a blank control.

Antioxidant activity = [(control absorbance - sample absorbance) / control absorbance] × 100%

Reducing Power Assay

The reducing power were determined according to [29].To 2.5 ml methanolic extracts of *Sequoia sempervirens* irradiated plantlets were mixed with phosphate buffer (2.5 ml, 0.2M pH 6.6) and potassium ferricyanide [K_3 Fe $(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture to stop the reaction, which was then centrifuged at 3000 rpm for10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Statistical analysis

The data obtained were subjected to standard analysis of variance procedure whereas values of LSD were obtained at 0.05% as reported by [30].

RESULTS AND DISCUSSION

***In vitro* Shooting and rooting behaviors**

The *in vitro* shooting behavior of *Sequoia sempervirens* after the treatment of plantlets for 5min. with green [Argon (Ar)], red [Helium neon (He-Ne)] and blue [Cadmium (Cd)] laser is shown in Table (1). Data indicated that, the maximum shootlets number per explants, shootlet length and number of leaves per shootlet (5.86, 10.53 and 27.77, respectively) were obtained with red laser treatment. The exposure to all laser irradiations types used (green, red and blue lasers) was the preferred for shooting behavior as compared to control treatments. The data go in line with those of [31], on *Isatis indogotica* who mentioned that, the growth and development of seedlings were accelerated because of He-Ne laser pretreatment of seeds, as it cause long-term alterations in biochemical and physiological characters of the seedlings. [32] indicated that exposing the *in vitro* plantlets

of *Balanites aegyptiaca* to red laser radiation for time exposure 4 or 8 min. resulted in the best results in shooting behavior (number of both shootlets and leaves) while, using green laser radiation for 1 min. resulted in the longest shootlets in *Cotoneaster horizontalis,* increasing the exposure time to 3 min. led to the highest number of leaves/ shootlet.

Table 1: Effect of different types of laser rays on *in vitro* shooting and rooting behavior of *Sequoia sempervirens* plants.

Characters / Laser type	Number of shootlets	Shootlet length (mm)	Number of leaves	Rooting percentage (%)	Number of roots /shootlet	Length of roots (mm)
Control	1.66	3.58	15.33	6.67	0.33	1.17
Green	4.33	8.43	13.66	13.33	0.67	5.17
Red	5.86	10.53	27.77	33.33	1.67	4.5
Blue	4.00	9.16	17.83	66.67	3.33	2.07
LSD 0.05%	1.43	1.74	2.94	15.86	0.69	3.44

Concerning rooting behavior of *in vitro Sequoia sempervirens* shootlets as effected by various types of laser irradiations, the data in table (1) showed that using cadmium ion laser (blue) for 5 min., resulted in the highest values of both rooting percentage and number of roots formed per shootlet (66.67 and 3.33%respectively) as compared to untreated plantlets which gave the lowest values. Whereas, argon ion laser (green) irradiation resulted in the longest roots (5.17mm). This finding was in agreement of that found by [32] on *Cotoneaster horizontalis* and pointed out that exposing the *in vitro* plantlets to both argon (green) or Cadmium (blue) laser irradiations for any time exposure (1,3 or 5 min.) caused the highest rooting percentage, number and length of roots as compared to untreated ones (control). Increasing the time exposure to 5 min. led to the best result in rooting behavior of *Contoneaster horizontalis.* In addition, [17] found that low –intensity laser radiation stimulates morphogenetic processes in tissue cultures of wild grasses, such as rhizogenesis and the formation of morphogenic calli and regenerated plants. Similar effect of laser rays on roots of sage plants was noticed by [33].The studies of [34] demonstrated the ability of green argon laser to induce effect of laser irradiation on organs that chiefly of light, electromagnetism, temperature and pressure effects. However, the low power laser, especially the laser of visible wavelength, was supposed to-emit little heat and pressure effect, therefore the influence mechanism of laser irradiation is most likely attributed to its light and electromagnetism effects [35].

Photosynthetic pigments

The results of laser light influence on parameters of photosynthetic pigments in *Sequoia sempervirens* shootlets for all the examined types are presented in Table (2). Red [Helium neon (He-Ne)] laser light treatment significantly increased both chlorophyll *a* and *b* to the highest values (549.26 and 292.35 mg/100gF.W. respectively) as compared to the control and exposure the in vitro plantlets to argon ion laser (green) irradiation resulted in the highest content of carotenoids (168.61mg/100gF.W.). Similarly [31] exposed the seeds of *Isatis indogotica* to He–Ne laser irradiation for 5 min. Laser pretreatment also resulted in a significant increase in the concentration of chlorophyll *a*, chlorophyll *b* and total chlorophyll. This may attributed to short time irradiation with laser light has an influence on the course of metabolic processes in cells, as well as on their photosynthetic activity. This

indicates that the cell is able to absorb, transform and use the energy of laser light photons [36].

Table 2: Effect of different types of laser rays on photosynthetic pigments of *Sequoia sempervirens* plantlets (mg/100g).

Determinations / Laser type	Chl.a	Chl.b	Carotenoids
Control	122.5±22.72	83.86±12.71	72.53±8.24
Green	357.7±5.77	250.98±42.18	168.61±2.43
Red	549.26±39.04	292.35±17.31	137.42±9.40
Blue	401.78±29.73	227.15±11.99	122.37±3.71
LSD at 0.05%	51.20	45.96	12.49

All values are the mean of three replicates (±Standard division)

Cellulose, total carbohydrates and proline content

The values of cellulose and carbohydrate (%) of *Sequoia sempervirens* plantlets as shown in Table (3) showed that the treatment with argon ion laser (green) irradiation resulted in remarked increase in both cellulose and carbohydrate percent (41.66 and 11.28%, respectively) while, the highest value of proline (5.73 µmoles/g D.w.) was obtained from the He–Ne (red) laser irradiation as compared to control and other treatments. These results suggest that for different types of radiation with laser had the greatest effect on cellulose, carbohydrate and proline contents. There are some reports showing that pretreatment by laser irradiation increased the quality and quantity of produced plants. According to [37] pre sowing stimulation of seeds of alfalfa with laser light caused a decrease in the content of crude fibre. [38] Showed that, both fennel and coriander chemical content of carbohydrate increased with all laser used treatments than the contents of control (untreated fruits). In higher plants, proline is synthesized in cytosol either from L-glutamic acid or from L-ornithine. On the other hand, proline is metabolized in the mitochondria to L-glutamic acid via proline dehydrogenase [39]. The glutamic kinase requires ATP for the reactions. For regulatory enzymes requiring ATP, the energy gradient, ATP/ADP thus plays an important regulatory role [40]. Some researches show a physical-chemical difference in the ATP molecule after irradiation with lasers beam [41]. Therefore one of the reasons for an increase of the proline content by irradiation, can be the effect of laser to activity of ATP molecules, the content of ATP was used for glutamic kinase at mitochondria.

Table 3: Effect of different types of laser rays on endogenous concentration of cellulose, total carbohydrate, proline, total phenolic, flavonoid and tannin content of *Sequoia sempervirens* plantlets.

Determinations / Laser type	Cellulose (%)	Total Carbohydrate (%)	Proline µm /gD.W.	Total Phenolic (mg gallic /g D.W.)	Total Flavonoid mg Querstine /gD.W.	Tannins (mg catechin/g D.W.)
control	10±2.5	4.77±0.4	5.173±0.07	6.27±0.272	1.306±0.01	0.56±0.041
Green	41.66±3.82	11.28±1.06	2.54±0.037	22.70±1.04	3.673±0.49	1.14±0.123
Red	6.33±1.25	9.48±2.19	5.73±0.079	17.00±0.49	2.946±0.27	0.95±0.03
Blue	20.83±2.88	9.38±1.22	2.92±0.109	10.20±0.2	1.533±0.05	0.77±0.042
LSD at 0.05%	5.22	2.59	0.15	1.129	0.528	0.132

All values are the mean of three replicates (±Standard division).

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Total phenolic, flavonoid and tannins content

Data illustrated in (Table 3) showed that, the level of endogenous total soluble phenolic, flavonoid and tannins content in *Sequoia sempervirens* plantlets were significantly affected by laser irradiance treatments as compared to the control. The *in vitro* plantlets treated with argon ion laser (green) irradiation resulted in the highest values (22.70 mg, 3.67 mg, and 1.14 mg respectively) while control (untreated plantlets) showed the lowest values. Such results are in agreement with the findings of [42] who pointed out that total soluble phenol in seed coats of *Acacia farnesiana* were significantly affected by laser irradiance treatments as compared to the control, regardless of the effect of exposure time treatments. [43] suggest that a general cell response induced by laser light irradiation can be divided into two specific responses: the first one consists in a rapid stress effect resulting in an increase in the amount of lipid peroxidation products, and the second and longer one are the secondary reactions related to the adaptive metabolic changes and apparently accompanied by the stimulation of morphogenetic processes. The 532 nm laser affected the photosynthesis efficiency of soybean seedlings and could increase the isoflavone content [44].

Similar studies [45] investigated that the primary response of plant tissues to radiations is an increase in the content of lipid peroxidation products of peroxide oxidation. Their data demonstrated that the laser light stimulates morphogenetic processes in plant tissues at later stages, as well and believing that this stimulation may be conditioned metabolic changes caused by the change of content of a number of compounds formed as a result of the primary photoreactions .Such compounds might also include products of peroxide oxidation with an increase in their amounts as response to the impact of laser radiation.

DPPH Free Radical Scavenging Activity

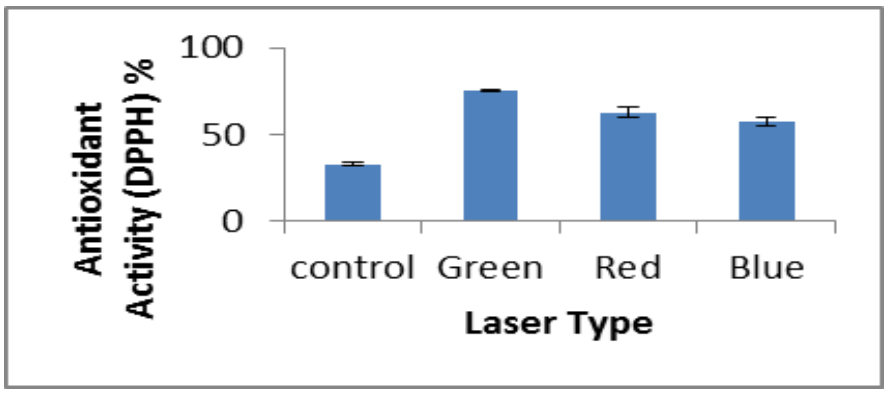

Figure 1: Effect of different types of laser rays on Radical Scavenging activity (DPPH) of *Sequoia sempervirens* plantlets. Data are shown as the mean of three replicates ± S.D. of all treatments (LSD 0.05% 3.81).

The stable radical DPPH has been used widely for the determination of primary antioxidant activity. The assay is based on the reduction of DPPH radicals in methanol which causes an absorbance drop at 517nm. As shown in Fig. (1), the antioxidant activity of *Sequoia sempervirens* plantlets exhibited a significant increase in radical scavenging activity

with all laser types. The argon ion laser (green) irradiation resulted in the highest DPPH radical scavenging activity (75.01%) whereas the He–Ne (red) laser and the Cadmium (blue) laser irradiations recorded (62.50 and 57.2% respectively). The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability [46]. The study showed that all types of lazer irradiation positively affect the radical scavenging activity of *Sequoia sempervirens* plantlets.

Reducing Power Ability:

The reducing power assay using Fe (III) reduction as an indicator of electron donating showed the presence of antioxidants in the extracts reduced the Fe (III) to Fe (II) by donating an electron. Amount of Fe (II) complex can be then be monitored by measuring the formation of Perl's Prussian blue (Fe4 [-Fe (CN) 6]^3) at 700 nm [47]. Figure (2) indicated that laser types exhibited a significant effect on *Sequoia sempervirens* plantlets reducing power activity. The *in vitro* plantlets treated with argon ion laser (green) irradiation recorded the highest reducing power (273% of control) followed by the He–Ne (red) laser irradiation (193.65% of control), while the Cadmium (blue) laser irradiations exhibited (139.68% of control). Different studies have indicated that the electron donation capacity reflects the reducing power of bioactive compounds is associated with antioxidant activity. The increase in reducing power ability may be attributed to the increase in total phenolic, flavonoid and tannins contents in response to lazer radiation.

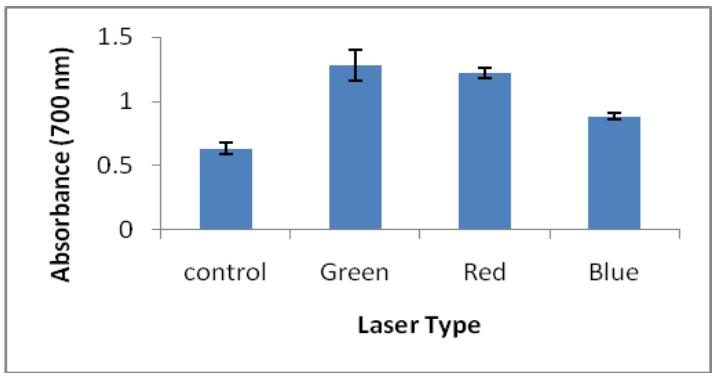

Figure 2: Effect of different types of laser rays on Reducing Power ability of *Sequoia sempervirens* plantlets. Data are shown as the mean of three replicates ± S.D. of all treatments (LSD 0.05% 0.103).

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REFERENCES

[1] Dallimore W, Bruce JA. A Handbook of Coniferae and Gikcgoaceae. 4th edition. Martin`s Press, New York, 1967, pp: 729.

[2] Sawyer J O, Sillett S C, Libby W J, Dawson T E, Popenoe J H, Largent D L, Van Pelt R, Veirs S D Jr, Noss R. F, Thornburgh D A, Tredici P D. Redwood trees, communities, and ecosystems. The Redwood Forest Island Press, Washington DC. 2000; 81–118

[3] Kwang HS, Hyun O, Sung KC, Dong CH, Byoung M K. Bioorg Med Chem Lett 2005; 15: 2019-2021.
[4] Boulay. In: Green CE, Somers DA, Hackett WP & Biesboer DD. Plant Tissue Cell Cult 1987; 367–382.
[5] Fouret Y, Larrieu C, Arnaud Y. Ann Rech Sylv 1988; 55–82.
[6] Thorpe T A, Harry IS, Kumar PP. Technology and Application Kluwer Acad Publ, Dordrecht 1991; 331–336.
[7] Sul IW, Korban SS. J Hort Sci Biotech 1997; 73: 822–827.
[8] Ball EAL, Morris D M, Rydelius GA. Round Table Conference. *Invitro* Multiplication of Woody Species. Gembloux Belgium 1978; 181-226.
[9] Bekkaoui F, Arnoud Y, Larrieu C, Miginiac E. Ann Afocel 1983; 5-25.
[10] Qi Z, Cai SW and Wang XL. Chin J Northwest University 2000; 30: 45–48.
[11] Markolf H N Laser and Matter. Springer-Verlag, Berlin 1996; 8–15.
[12] Terry AC, Stark WJ, Maumence HE , Fagadau W. Am J Ophthalol 1983;96: 716–720.
[13] Voronkov LA, Tsepenyuk DY. Biol Nauk 1988; 11: 24–27.
[14] Cai SW, Qi Z, Ma X L. Chin J Lasers 2000; 27: 284–288.
[15] Paleg LG, Aspinall DD. Nature 1970; 5275: 970–973.
[16] Govil SR, Agrawal DC, Rail KP, Thakur S N. Indian J Plant Physiol 1991; 1: 72–76.
[17] Salyaev RK, Dudareva LV, Lankevich SV, Sumtsova VM. Biochem Biophysics 2001; 379(6): 279-280.
[18] Salyaev R K, Dudareva LV, Lankevich SV, Makarenko SP, Sumtsova VM, Rudikovaskaya EG. Biol Sci 2007; 412: 87-88.
[19] Vasilevski G, Bosev D, Bozev Z ,Vasilevski N. Byophisical methods as a factor in decreasing of the soil contammination. Int. Workshop Assesment of the Quality of Contaminated Soils and Sites in Central and Eastern European Countries (CEEC) and New Independent States (NIS) 2001; 30.
[20] Alawacy AA. Laser and Its Applications. Arab Scientific Publishers 1988; 255.
[21] Saric MR, Kastrori R, Curic T, Cupina C, Geric I. Univerzit ET Novon Sadu. Praktikum Izfiziologize Biljaka 1967; 215.
[22] Crempton E W, Maynord L A.F. Nutr 1938; 15:383-395.
[23] Smith FM, Dubois M, Gilles KS, Hamilton D.K, Rebers PA. Annal Chem 1956; 28: 350-356.
[24] Bates L S, Waldren R P, Teare I D. Plant Soil 1973; 205-207.
[25] Makkar H P S, Becker K, Abel H, Pawelzik E. Journal Sci Food Agri 1997; 75: 511-520.
[26] Ordonez A A L, Gomez J D, Vattuone M A, lsla M I. Food Chem 2006; 97: 452-458.
[27] Maxson E D, Rooney L W. Crop Sci 1972; 12: 253.
[28] Brand-Williams W, Cuvelier M E, Berset C. Lebensmittel Wissenschaften und Technologi 1995; 28: 25-30.
[29] Ebrahimzadih M A, Nabavi S F, Nabavi S M, Eslami B. Grasas Aceites 2010; 61: 30-36.
[30] Snedecor G W, Cochran W G. Statistical Methods, 7th Edition. Ames Iowa Lowa State University Press 1980.
[31] Yi-Ping C, Ming Y, Xun-Ling W. Plant Sci 2005; 168: 601-606.
[32] Hwida A M, Metwally S A, Lobna S T. J App Sci Res 2012; 8(4): 2386-2396**.**
[33] Wessam M S E A. National Institute of laser 2005
[34] Berns MW, Olson RS, Rounds DE. Nature 1996; 221: 74-75.
[35] Xiang Y. Human Sci and Tech. Press, Changsha 1995; 124-127.
[36] Szyrmer J, Klimont K. Biul. IHAR, (in Polish) 1999; 210:165-168.

[37] Cwintal M, Dziwulska-Hunek A, Wilczek M. Int Agrophys 2010; 24: 15-19.
[38] Yasser A, Osman H, Kareem M, El Tobgy K, El Sayed, EL Sherbini A. J App Sci Res 2009; 5(3): 244-252.
[39] Di Martino C, Pizzuto R. Planta 2006; 223: 1123–1133.
[40] Stefl M, Vasakova L. Czechoslovak Chemical 1982; 47: 360–369.
[41] Amat A, Rigau J, Nicolau R, Aalders M, Fenoll MR, Gemert MV, Tomas J Photochem Photobiol Chem 2004; 168: 59–65.
[42] Soliman AS, Harith MA. Acta Horticult 2010; 854: 41-50.
[43] Salyaev RK, Dudareva LV, Lankevich SV, Sumtsova VM. Biochem Biophys 2001; 379(6): 279-280.
[44] Tian J, Jin LH, Li J M, Shen B J, Wang C Y, Lu X, Zhao X L. Laser Phys Biophoton 2009.
[45] Salyaev RK, Dudareva L, Lankevich S, Makarenko S,Sumtsova V, Rudikovska E doklad Biol Sci 2007; 412: 87-88.
[46] Baumann J, Wurn G, Bruchlausen FV. Deutsche Pharmakolo-gische Gesellschaft Abstracts of the 20th spring meeting, Naunyn-Schmiedebergs Abstract No: R27 cited in Arch Pharmacol 1979; 307: R1-R77.
[47] Hawas UW, Abou El-Kassem LT, Awad H M, Taie HAA. Curr Chem Biol 2013; 7:188-195.