In vitro photobiological studies on two woody tree species

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Abstract: The enforcement work was accomplishments *in vitro* (Tissue culture and Germplasm Conservation Research Lab.) to study the effect of different laser rays in inducing growth behavior and leaf anatomical structure on *Balanites aegyptiaca and Contoneoster horizontalis.* Exposing Planlets of *Balanites* plants developing in vitro to various types of lasers (helium-neon laser, cadmium neon laser and Argon neon laser) at 20 minutes exposure time each separately. The results showed increase in vegetative growth performance, photosynthetic pigments and anatomical characteristics. Exposure time 20 min. He-Ne were more effective it increased of root length, root number, chlorophyll b, carotenoids, the thickness of both lamina and midvine, the number of xylem rows and number of vessels and dimensions bundle. While, 20 min. exposure time Cd-Ne recorded highest values in shoot length, leaves number, chlorophyll a and leaf anatomical structure as compared to untreated plants. Exposing Planlets of *Cotoneoster horizontalis* plant in vitro to helium-neon laser, at 0, 2.5, 6.5 and 11.5 mints exposure time. The results showed increase in vegetative growth, photosynthetic pigments and anatomical characteristics. Treated plantlets with 2.5 min. exposure time He-Ne recorded highest increasing in shoot length, chlorophyll a, b, the thickness of lamina and midvine, the number of xylem rows, number of vessels and palisade thickness. 11.5 min. exposure time recorded increasing in leaves number, root length, root number, dimensions bundle and spongy thickness as compared to plantlets control plants.

Keywords: in vitro, laser rays, woody trees, leaf anatomy, photobiology

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1 Introduction

Balanites aegyptiaca is belonging to family *Balanitaceae*. This tree grows in many types of soils from sandy to heavy clay. It has a multi-purposes fodder, charcoal and timber tree, which is valuable in arid areas where they produce fruit in times of drought and its medical uses such as the treatment of diarrhea and alboisaroualemgs contagious and contagious fever. (Chothani and Vaghasiya, 2011 and Prashant et al., 2011). *Cotoneaster horizontalis* is a deciduous ornamental shrub and is a tree classified as a member of family (Rosaceae) it is famous by beauty of their fruits and flowers (Zeilinga, 1964). They needs lightly maintenance and use as a ground overlay, sand soil fixation and …act. These shrubs are scarcely in Egypt, (Khalifa and Loutify, 2006). So tissue culture technique very important to help us to produce mass production, disband quandary and produces progeny that are conformity to the mother plant (Bergmann and Moon, 1997; Zixiong et al., 2009).

Laser rays belong to unionizing radiation laser is an abbreviation of "light Amplification by Stimulation of Radiation". It is identified by the emitted wavelength and the power. Carruth (1987) reported that when tissue absorbs laser energy, the temperature rises. No changes in tissue structure are evident between 37^{o}C and 60^{o}C, however, above that temperature, tissue being to coagulate.

Absten (1992) reported that "the nature of the interaction between laser light and biological tissue can be described in terms of reflection, scattering,

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transmission and absorption. Laser beam is possible could affect on plants tissues by coagulation or vaporization in this concern, Berns et al., (1996) reported that the temperature rise in an irradiated tissue is proportional to light absorption in that tissue which in turn is determined by how effectively its constituent molecules absorb incident photons of a particular wave length.

Adely (1997) reported that when laser energy is higher than those required for photo-coagulation, tissue temperature can reach the boiling point of water and rapidly expanding water vapour will cause disruption “photo vaporization” before denaturation can cauterize the tissue.

A direct interaction between laser photons and molecules is responsible for “photochemical” effect (Jeff, 1992). The leaf is the key organ for photosynthesis and transpiration. Therefore, leaf morphology and cell distribution may be important in influencing physiological processes (Parkhurst, 1986). The leaf contains both the assimilating and conducting tissues, and either or both tissues could be affected by laser radiation. On gerbera plant Sami (2010) revealed that, laser radiation increased leaf growth parameters and also showed increments in number of vascular bundle, thickness of midvein, thickness of lamina and xylem rows.

Also, Marcos et al. (2010) reported that, blue light regulates several sides of plant function and may encourages some growth characteristics and biomass backlog. Additionally, some anatomical modifications, such as increased leaf thickness and reduction in epidermal cell length and xylem vessel diameter are the result of light quality.

Laser rays have attained much interest at different parts of the world for improving growth and quality of plants. In this concern, Laser treatments can modify important components of plant cell and have been reported to affect differentially, the morphology, shooting and rooting behavior, anatomy, biochemistry and physiology of plant depending on the source and time of laser exposure. In this respect, Lobna et al (2014) on Sequoia plant reported that, exposing the in vitro shootlets of sequoia to red laser resulted the best results in shooting behavior and concentrations of chlorophyll a, b and protein content.

Also, Helium neon (He-Ne) laser rays improved Caster bean growth and decreased osmotic potential followed by increasing relative water content and help plants to complete its life cycle in comparison with untreated plants Metwally et al. (2014).

This work aimed to throw more interest on *in vitro* growth and anatomical parameters of *Balanites aegyptiaca* and *Cotoneaster horizontalis* plantlets under some types of laser, to provide more information about laser physiological and morphological effect on plants.

2 Materials and Methods

These experiments were carried out at tissue culture and Germplasm Conservation Research Laboratory, Horticulture Research Institute, Agriculture Research Centre (ARC), and Department of Ornamental Plants and Woody Trees, National Research Centre (NRC), Egypt during years 2016 to evaluate some morphological and anatomical changes in *in vitro Balanites aegyptiaca* and *Cotoneaster horizontalis* plantlets treated with various laser types for different time exposures to provide beneficial information about the physiological effect on plant and improvement its production quantitatively and qualitatively.

2.1 Plant materials

Shoots (10-15 mm) of heglieg (*Balanites aegyptisca*) collected from adult tree in Giza Zoo were used as explant source (stem node) for micropropagation. The explants used for *Cotoneaster horizontalis* were taken from shrub at Orman Garden, Giza, Egypt.

2.2 Culture medium and conditions

MS- medium supplemented with 0.2 mg L^{-1} of 6-benzylamino-purine (BAP) and enriched with sucrose 25 g L^{-1} and solidified with 0.7% agar. The media were adjusted to pH 5.7+–0.1, then autoclaved at 121°C and 1.2 kg cm^{-2} for 15 min. The cultures were incubated in growth chamber at 24°C±1°C under florescent lamps with light intensity of 3k lux at 16 h photoperiods.

2.3 Laser rays treatments:

Helium neon “He-Ne”, Argon “Ar” and Helium cadmium “He-Cd” laser were used for shootlets (2cm) of

Balanites aegyptiaca and cotoneaster horizontalis in vitro.

The wavelengths of lasers rays were 620, 530 and 460 nm respectively. The power densities for Cadmium neon “Cd-Ne” and Argon (Ar) laser were adjusted nearly 250 Mw cm^{-2} and 100-120 Mw cm^{-2} for helium neon (He-Ne) laser.

The experiment was divided into two division according to the type of plant, one for *Balanites aegyptiaca* plantlets and second for *cotoneaster herizontalis* plantlets. *Balanites aegyptiaca* plantlets were treated by three laser types helium neon (He-Ne), Argon (Ar) and Cadmium neon (He-Cd) with exposure time 20 min. While, *cotoneaster herizontalis* plantlets just was treated by He-Ne laser at (2.5, 6.5 and 11.5 min. exposure time), 12 weeks after exposure treatments the following data were recorded:

I- Shooting parameters:

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

II- Rooting parameters:

- Percentage of roots formation (%)
- Number of roots /shootlet
- Root length (mm)

III-Anatomical study:

At multiplication stage, samples were taken from third leaf on the *in vitro* plantlets. The preparation of leaf section was carried out according to the methods described by Johansen (1940) and Corgen and Widmayer (1971). Leaf section was mounted in Canda balsam then examined microscoply and microphotography. The following parameters were recorded:

- Upper epidermis and palisade thickness (μ)
- Spongy thickness (μ)
- Number of bundles.
- Dimension of bundles (length –wide) (μ)
- Thickness of midvien (μ)
- Thickness of lamina (μ)
- Number of xylem rows.
- Number of vessels

2.4 Layout of the experiments

The experiments were designed in completely randomized design and the test of LSD was used for comparison among means according to Steel and Torrie (1980).

3 Results

Data presented in Table 1 shows that application of He-Ne and Cd-Ne treatments on *Balanites aegyptiaca* trees showed significant differences on growth parameters of treated plants and control plants. The highest increments in shoot length and leaves number reached 19.30 and 65.32% respectively over the untreated plants. When *Balanites sp* plants treated with Cd-Ne laser (Blue). These results are analogy with the finding of, Lobna et al (2014) on Sequoia plant. On the other hand application of He-Ne laser (red) were more effect and favorable to increasing shoot number, root length and root number. The increments reached 43.44%, 284.61% and 200% respectively over the control plants.

Table 1 In vitro Effect of different types of laser rays on shooting and rooting behavior of *Balanites aegyptiaca* plantlets plants (Means of two seasons 2015 & 2016)

Chac. / Treat.	Shoots No.	Shoot length (cn)	Leaves No.	Root length (cn)	Roots No.
Cont.	1.22	3.73	3.23	0.91	1
He-Ne 20 min.	1.75	3.16	5.15	3.5	3
Cd-Ne20 min.	1.59	4.45	5.34	1.46	1..66
Ar 20 min.	-	-	-	-	-
LSD at 0.5%	0.162	1.10	0.712	0.303	0.665

Note: He-Ne = (helium neon), Cd-Ne = (Cadmium neon), Ar = (Argon laser).

3.1 Photosynthetic pigments

It can be observed from Table 2 that, the lowest concentration of chlorophyll a, b as well as carotenoids were recorded for untreated plantlets.

Table 2 In vitro Effect of different types of laser rays on photosynthetic pigments of *Balanites aegyptiaca* plantlets (Means of two seasons 2015& 2016)

Chrac. / Treat.	Chl a, mg g^{-1}	Chl b, mg g^{-1}	Carotenoids, mg g^{-1}
Cont.	121.03	82.1	61.06
He-Ne 20min.	345.46	237.5	155.2
Cd-Ne 20 min	430.54	185	123.6
Ar 20 min	-	-	-
LSD at 0.5%	9.78	5.24	3.07

Note: He-Ne = (helium neon), Cd-Ne = (Cadmium neon), Ar =(Argon laser).

In spite of that treatment of Cd-Ne laser showed the highest content of chlorophyll a. The same table also showed the application of He-Ne laser was favorable for induce the highest values of chlorophyll b and carotenoids. The increments over the control reached 189.28 and 154.17% respectively over the control plants.

Results in Table 3 revealed all the different treatments He-Ne, Cd-Ne, and Ar increased Thickness of midvein (μm), Thickness of Lamina (μm), number of xylem rows and number of vessels compared with the control plantlets. The highest values of the previous mention parameters were obtained by He-Ne treatments at exposure time 20 min. The increments reached 66.66%, 88.23%, 55.55% and 78.57% respectively.

The highest number of vascular bundles was recorded for Argon treatment, while length of vascular bundle, showed approximately the same equal value when exposed to He-Ne, Cd-Ne and Ar. Clearly increase in wide of vascular bundle, upper epidermis as well as spongy thickness were recorded for He-Ne treatment, the values reached 27,6 and 30 (μm) as compared with 13, 3 and 12 (μm) respectively for the control plantlets.

Table 3 Effect of different types of laser rays on leaf anatomical structure parameters *Balanites aegyptiaca* (Means of two seasons 2015& 2016)

Treat. \ Charac.	Thickness of midvein, μm	Thickness of Lamina, μm	No. of xylem rows	No. of vessels	No. of vascular bundle	Length of vascular bundle, μm	Wide of vascular bundle, μm	Upper epidermis and palisade thickness, μm	Spongy thickness, μm
Control	36	17	9	28	5	21	13	3	12
He-Ne 20 min.	60	32	14	50	6	31	27	6	30
Cd-Ne 20 min.	46	20	11	37	8	29	13	3.6	15
Ar 20 mn.	51	20	12	28	10	31	21	3.1	18

Note: He-Ne = (helium neon), Cd-Ne = (Cadmium neon), Ar =(Argon laser).

Data presented in Table 4 show that the growth parameters of cotoneaster plantlets expressed in term of shoot, leaves and root number as well as shoot and root length were increased significantly in most cases by increasing He-Ne exposure time up to 6.5 min. The highest increments in root length were at exposure time 11.5 min. followed in descending order in root number by 233% at exposure time 11.5 min. However the increment in leaves number reached 82.81% at the same time of exposure.

Table 4 In vitro Effect of time exposure of helium neon laser on shooting and rooting behavior of *Cotoneaster herizontalis* plantlets (Means of two seasons 2015 & 2016)

Treat. \ Chac..	Shoot Number	Shoot length (cn)	Leaves Number	Root length (cn)	Root Number
Cont .	1	2.4	9.66	0.13	1
He-Ne 2.5 min.	1	3.83	12.58	0.23	1.33
He-Ne 6.5 min.	1.75	3.76	9.46	0.2	1.66
He-Ne 11.5 mim.	1.33	3.32	17.66	0.66	3.33
LSD at 0.5%	0.24	0.29	1.15	0.22	1.1

Note: He-Ne = (helium neon laser).

3.2 Photosynthetic pigments

Concerning, shooting behavior of in vitro cotoneaster plants shootlets as effected by helium neon laser, the data in Table 5 showed that using (He-Ne) laser, in general recorded significant increase in morphological and photosynthetic pigments in compared with untreated plants.

Table 5 show that He-Ne laser treatments increased significantly Chlorophyll a, chlorophyll b and carotenoids at He-Ne at 2.5 min. exposure time of cotoneaster plantlets compared with check plantlets. Raising He-Ne exposure time upto 11.5 min. decreased gradually photosynthetic pigments. The lowest values were obtained by the highest exposure time 11.5 min. (He-Ne) laser.

Table 5 In vitro Effect of exposure time of helium neon laser on photosynthetic pigments of *Cotoneaster herizontalis* plantlets (Means of two seasons 2015 & 2016)

Treat. \ Chrac.	Chl a, mg g^{-1}	Chl b, mg g^{-1}	Carotenoids, mg g^{-1}
Cont.	62.26	24	41.15
He-Ne 2.5 min.	100.47	77. 92	70.32
He-Ne 6.5 min.	81.38	63.91	32..94
He-Ne 11.5 min.	65.41	47.71	24.85
LSD at 0.5%	1.92	1.87	1.34

Note: He-Ne = (helium neon laser).

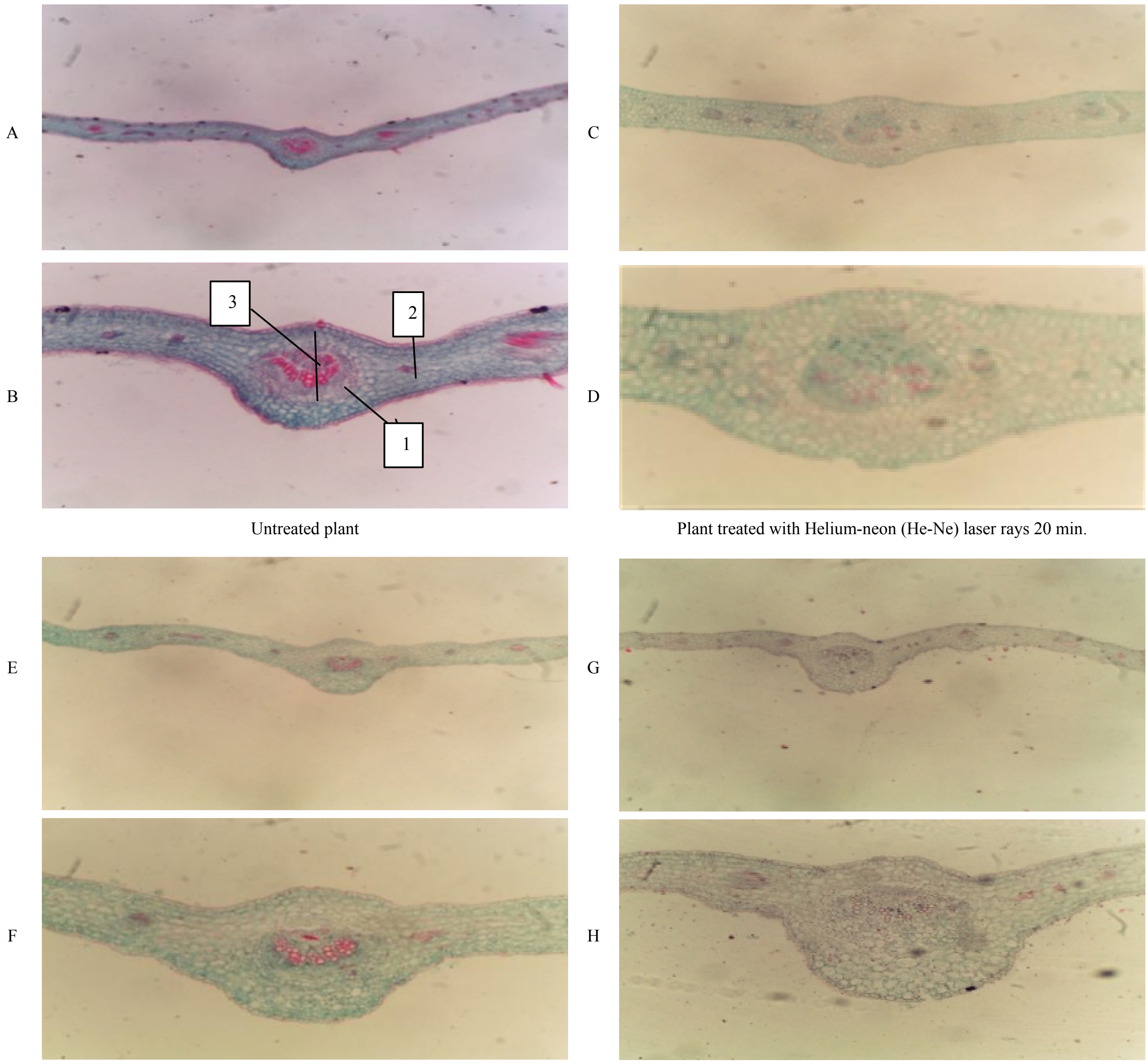
It is evident from Table 6 that in general plantlets exposed to He-Ne laser with different exposure time super induced increases in leaf anatomical structure compared with the control plants. For 2.5 min. exposure time highest values were recorded for thickness of midvein, thickness of lamina, number of xylem rows, number of vessels and upper epidermis the values reached 110.75, 16, 75 and 22.5 respectively. While He-Ne laser

6.5 min. exerted the highest increments in length of vascular, as well as spongy thickness, the increment reached 64% and 176.66% respectively over the control plantlets.

Table 6 In vitro Effect of exposure time of helium neon laser on leaf anatomical structure parameter of *Cotoneaster herizontalis* plantlets (Means of two seasons 2015& 2016)

Treat. \ Charac.	Thickness of midvein, μm	Thickness of Lamina, μm	No. of xylem rows	No. of vessels	No. of vascular bundle	Length of vascular bundle, μm	Wide of vascular bundle, μm	Upper epidermis and palisade thickness, μm	Spongy thickness, μm
Cont.	67	41	10	35	3	50	44	12.5	15
He-Ne 2.5min.	110	75	16	75	5	80	32	22.5	39
He-Ne 6.5min.	95	60	14	70	6	82	35	20	41.5
He-Ne 11.5min.	90	50	15	50	6	75	30	18	40

Note: He-Ne= (helium neon laser).



Untreated plant

Plant treated with Helium-neon (He-Ne) laser rays 20 min.

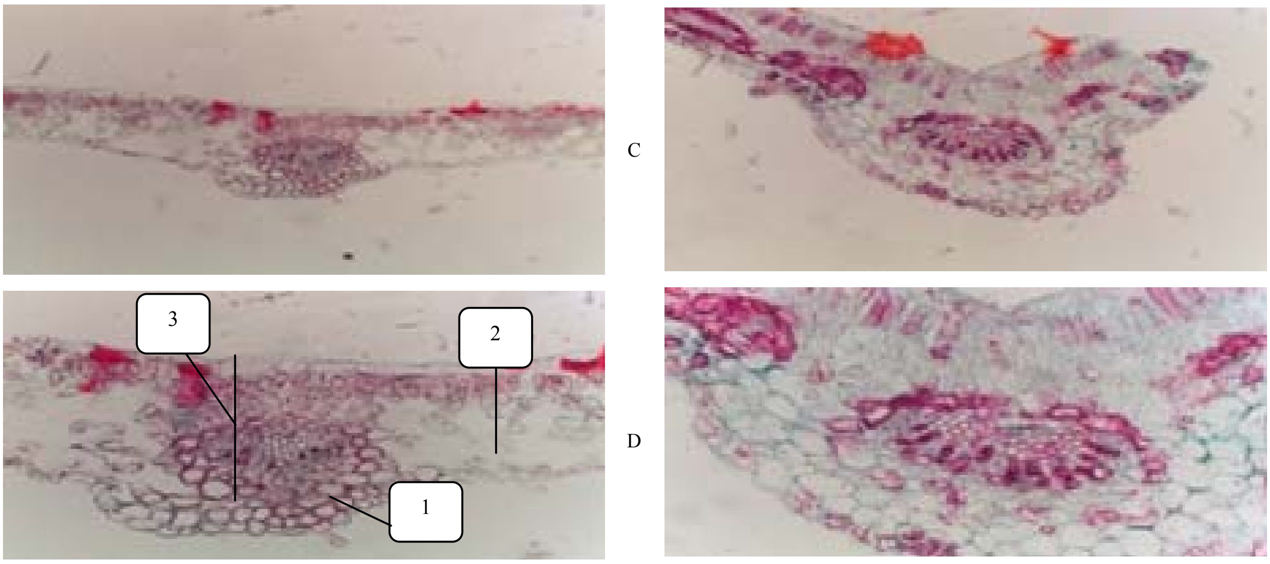
Plant treated with Cd-Ne (cd) laser rays 20 min. time exposure

Plantlets treated with Argon (Ar2) laser rays 20 min. time exposure

Note: 1 = vascular bundle, 2 = Thickness of lamina, 3 = Thickness of medvien.

Figure 1 A, C, E, G: Light Microphotograph showing transverse section through the blade of the third in vitro leaf developed on the main stem of *Balanites aegyptiaca* plantlets affected with He-Ne laser rays. (x=10) (Bar=0.1 mL). B, D, F, H: Light Microphotograph showing transverse sections through the blade of the third in vitro leaf developed on the main stem of *Balanites aegyptiaca* plantlets affected with He-Ne laser ray. The section shows vascular bundle (vessels and number of xylem rows(x40) (Bar=0.05 mL)

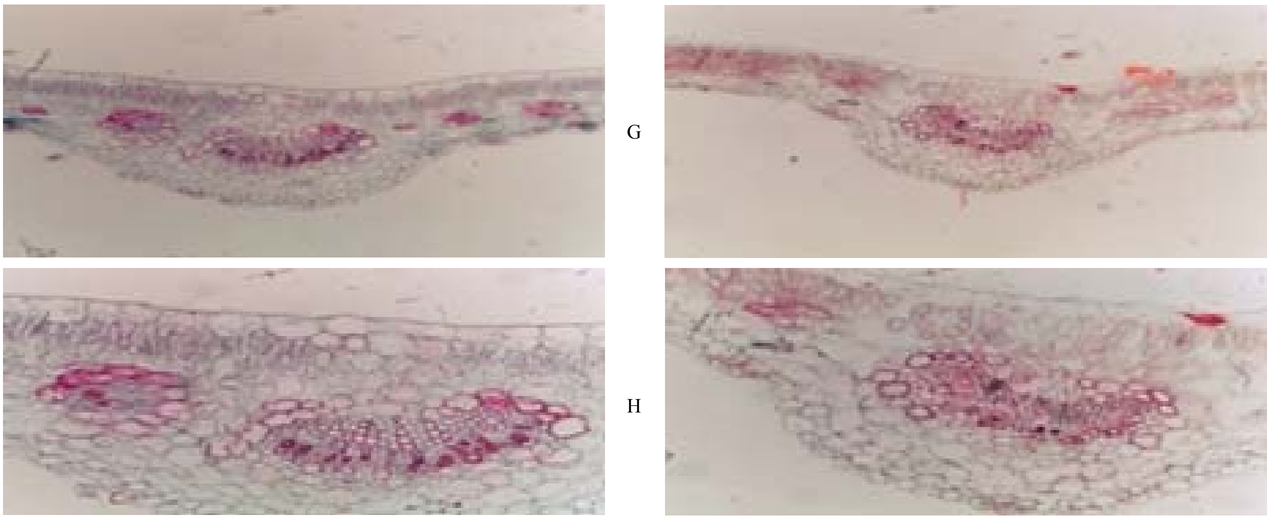
A C B D



Untreated plantlets

Plantlets treated with Helium-neon (He-Ne1) laser rays 2.5 min time exposure.

E G F H



Plantlets treated with Helium-neon (He-Ne2) laser rays 6.5 min time exposure

Plantlets treated with Helium-neon (He-Ne3) laser rays 11.5 min time exposure

Note: 1 = vascular bundle, 2 = Thickness of lamina, 3 = Thickness of medvie.

Figure 2 A, C, E, G: Light Microphotograph showing transverse section through the blade of the third in vitro leaf developed on the main stem of *Cotoneaster horizontalis* plantlets affected with He-Ne laser rays.(x = 10) (Bar = 0.1 mL). B, D, F, H: Light Microphotograph showing transverse sections through the blade of the third in vitro leaf developed on the main stem of *Cotoneaster horizontalis* plantlets affected with He-Ne laser ray. The section shows vascular bundle (vessels and number of xylem rows(x40) (Bar=0.05 mL).

4 Discussion

The response of *Balanites aegyptiaca* and *cotoneaster herizontalis* plantlets to laser exposure He-Ne,Cd-Ne and/or Ar laser under this study were clear Table 1 except argon treatment which could not complete its life cycle. The results showed increase in all growth parameters compared with the control plantlets. These results may be due to the effect of laser treatments on biosynthesis of plant constituents.

The obtained results in the current study are in agood agreement with finding of Atansova (1983) on carnation and. In this connection Markov at al. (1987) treated gladiolus corms with He-Ne laser, the treatment improved plant growth and development. Also Batov and Kitin (1993) on *Robinia pseudoaccia* reported that, laser treatment not only improved rooting percentage but also increased the number of adventitious root hairs formed. These results confirmed by the results of Sami (2010) on gerbera, who found that He-Ne laser, Argon exposure increased indigenous gibberellins GA-like substances concentration of gerbera which led to increase in most growth parameters. The increments in photosynthetic due to laser exposure was confined by the finding of Sebanak et al. (1989), Chen et al. (2005), Wessam (2005), and Metwally et al., (2014) on *Ricinis communis*.

The response of *Balanites aegyptiaca* plantlets to laser treatments He-Ne, Cd-Ne and/or Ar laser was clear on anatomical structure specially argon on number of vessels. These results are harmony with the finding of Sami (2010) on gerbera.

Our results on *cotoneaster herizontalis* plantlets exposed to different exposure time of laser treatments showed important factors in this respect. Exposure time (6.5 min) of He-Ne laser was the most efficient to increase growth parameters expressed in 1.75, 3.76, 9.46, 0.20 and 1.66...

Also, this time exposure enhanced photosynthetic pigments. However our results showed that the exposure time (2.5 min) was with highest effect on anatomical modification in *cotoneaster herizontalis* plantlets and the lowest modification were recorded by increasing exposure time up to 6.5 min.

In this respect Kamiya et al. (1999), Metwally et al. (2014) on Caster bean and Lobna et al. (2014) on *Sequoi sempervirens* mentioned that the complex cycle of gibberellic formation is promoted by red light He-Ne laser. This induction of GA induced formation of protolytic enzymes that would be expected to release tryptophan precursor of IAA (Van and overbeek, 1966). So it means that laser enhanced GA formation and encourage the release of IAA which had promotive effect on root growth, nutrient and water uptake and this reflected in plant growth (Kuraishi et al., 1963).

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