Responses of He-Ne Laser Irradiation on Agronomical Characters and Cytological of *(Atropa Belladonna* L.).

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ABSTRACT

The goal of the present work was to investigate the influence of laser irradiation on *in vitro* and *in vivo* growth agro-morphological criteria, mitotic activities and the chromosomes on *Atropa belladonna* L. The influence of laser radiation on Atropa seeds was investigated using helium–neon (He–Ne) were exposed to levels of laser doses (0.0,10,15, 20, 25 and 30 J cm)$^{-2}$ using 7 m W He–Ne laser (632.8 nm),with a power density of 4.02 m W cm)^{2}.The most of morphological, floral parameters germination percentage, plant height (cm), no. of leaves per plant, no. of branches per plant, leaf Length, width of leaf, leaf area and root length were studied in, First, Second and third months. The results showed significant differences among the five doses of laser radiation on seed germination, seedling height, plant height, no. of Leaves per plant at 10,20 and 30 days. The best doses 25 Jcm^{-2} to have a stimulating effect on these traits. The cytogenetic effects of physical mutagens Laser beam (0.0,10,15, 20, 25 and 30 J cm)$^{-2}$were investigated on the root tip cells of *Atropa belladonna* L., Various types of chromosomal aberrations (multinucleate cells) were observed during the cytological analysis of the root tip cells of both treatments. On the other hand, the high doses caused significant reduction as comparing with the control for all studied traits.

Keywords: He–Ne laser, *in vitro, in vivo* Agro-morphological criteria, cytological, *Atropa belladonna* L., Mitotic activity, multinucleate cells.

INTRODUCTION:

Atropa belladonna is a perennial herb and belongs to the family Solanaceae, included under the threat category Rare in the Red Data Book of the People's Republic of Bulgaria and as Vulnerable in the Red list of Bulgarian vascular plants Genova and Komitska, (2009).

Laser rays belong to unionizing radiation. Laser is an abbreviation "Light Amplification by stimulation of radiation". It is identified by the emitted wavelength and the power. Laser was used widely as pre-seed treatments to increase seed germination and seedlings growth Zong-BoQiu *et al.,* (2008).A simple comparison of the FGP of control and the irradiated seeds was found to be the only germination index which indicated the influence of He-Ne laser irradiation on germination. It is generally accepted that germination process is sensitive to irradiation with various wavelengths of visible and infra-red light. Field experiments indicated that germination of buried seeds may be triggered by millisecond-exposures to sunlight and a five-seconds-long exposure to weak moonlight can saturate the germination of photosensitized seeds Hartmann and Mollwo, 2000; Hartmann *et al*., 2005).A large number of experimental studies carried out over the last years suggest that even an exposure of dry, dormant seeds to He-Ne laser irradiation (632.8 nm), also termed as laser stimulation, may trigger several biological reactions. Laser-inducted changes in electrochemical, biochemical and optical properties of seeds are well documented, and a majority of the ascribed biological effects of laser stimulations have been attributed to germination process and growth analysis Wu *et al*., 2007).

Dormancy breaking and germination stimulating laser-based pre-treatments have essentially been focused on the cereal grains and vegetable seeds and experimental evidence suggests that there is significant positive effects of laser irradiation in improving the quality of sowing material, like mustard seeds Anghel*et al*., (2000) and maize Herandez *et al*., (2006).

The effect of He–Ne laser irradiation on cell wall reconstruction mediating by cell wall polysaccharides and DNA fragmentation in tall fescue seedlings and evaluated the role of cell wall reconstruction and DNA damage repair in the induction of enhanced adaption capacity to saline conditions by the laser irradiation and further explored the physicochemical mechanism of the protective effects of He–Ne laser illumination on plants under unfavorable growth conditions Gao *et al*., (2016).

A regression was found in the mitotic index along with the increasing doses of the X-rays and Laser beams. Similar results were obtained after the treatment of different physical mutagens on *Lathyrus sativus* Kumar and Tripathy (2006), Shukla *et al.,* (2009).

This study aims to examine germination under the influence of gibberellic acid and the interaction between He–Ne laser irradiation and gibberellic acid on the comparative effect of He–Ne laser irradiated seeds of *Atropa belladonna* L. on *in vitro* and *in vivo* agro-morphological criteria and cytological characterization.

MATERIAL AND METHODS:

The present investigation was carried out at the Agric. Botany- Department, Genetics Lap., Faculty of Agriculture, Al-Azhar University Cairo, Egypt, and Botany Department, National Research Centre, El- Dokki,Giza, Egypt.

Collection of Plant material:

The plant material is *Atropa belladonna* L. seeds which kindly obtained from the Experimental station of Medicinal plants, Faculty of Pharmacy, Cairo University, Egypt.

Irradiation:

Each experimental group consisted of 100 seeds spread over 15 mm circular area. The treatment groups were applied with the single exposures of reassigned laser doses (0.0,10,15, 20, 25 and 30 J cm^{-2}) using 7 m W He–Ne laser (632.8 nm), with a power density of 4.02 m W cm)2.at National Institute of Laser Enhanced Sciences (NILES), Cairo University.

***In vivo* growth characteristics analysis**:

Field experiment was carried out at the greenhouse of Agric. Botany- Department, Genetics Lap., Faculty of Agriculture, Al-Azhar University Cairo, Egypt.

Planting:

Irradiated seeds were sown directly in multi pot transplant trays, filled with a mixture of peat moss and sand and arranged in a complete randomized block experimental design with three replications. Random samples from M1 generation plants were taken to study the effect of He–Ne laser irradiated on the following morphological characters, germination percentage, plant height (cm), no. of leaves per plant, no. of branches, Length of leaf, width of leaf, leaf area(cm^2) and root length, at 10,20and30 days. He–Ne laser irradiation caused a significant effect on seed germination, growth parameters, enzyme activity, and thermodynamic properties of the *Triticum aestivum* plant. Jamil *et al.,* (2013).

Tissue culture experiment:

Seed Surface sterilization:

The seeds were thoroughly washed one time using sterile distilled water. Thereafter under aseptic conditions using laminar air-flow cabinet, they were firstly immersed in 70% ethanol solution for 2 minutes, and then were soaked by immersion in clorox (sodium hypochlorite (v/v)) at different concentrations (Table 1) plus 2 drops of Tween 20. Finally it is washed three times using sterile-distilled water. Serialized seeds were cultured on MS medium without any additional plant growth regulators and incubated for germination under controlled conditions. The 30 days old seedlings (Fig.1) were used for explants preparation Abdelaziz *et al.,* (2017).

In vitro germination of Atropa belladonna L. seeds under effect of laser doses:

The treatment groups were applied with the single exposures of reassigned laser doses (0.0,10,15, 20, 25 and 30 J cm^{-2}) using 7 m W He–Ne laser (632.8 nm), with a power density of 4.02 m W cm)²at National Institute of Laser Enhanced Sciences (NILES), Cairo University. Seeds were planted aseptically in MS medium Murashige and Skoog (1962). containing 30g/L Sucrose and solidified with 7g/L agar. The PH was adjusted to 5.8. after which the medium was dispensed (40 ml each) in culture bottles and sterilized by auto calving at 121 ºC for 20 min. seed cultures were maintained in dark at 27± ºC for 11 days. Upon germination, seedlings were transferred under continuous light at 2,000-Lux intensity produced from cool white florescent tubes. Random samples from the generated M1 plants were taken to study the effect of laser doses on the following morphological characters germination percentage, plant height (cm), no. of leaves per plant, no. of branches, length of leaf, width of leaf, leaf area and root length at first, second and third months.

Cytological procedures:

The root tips (1-2cm in length) of control and treated materials were fixed in a freshly prepared carnoys fixative (3:1v/v absolute alcohol and glacial acetic acid, respectively) and kept in refrigerator for 24 hours. They were then stored under refrigeration in 70% ethyl alcohol as a preservative solution for the time being for cytological studies. Cytological

preparation were carried out using the Feulgen's squash technique as follows root tips of *Atropa belladonna* L**.** were washed thoroughly in distilled water and hydrolyzed in 1N HCl at 60 ºC for 6-8 minutes. The root tip were washed carefully with distilled water and stain with Feulgen's staine for 2 hours as kept in darkness Feulgen's reagent (Coleman,1938):

Materials required:

Basic fuchsin 0.5g,1N HCl 10 ml Potassium meta- bisulphate1.5 g, Activated charcoal 0.5g and Distilled water 100mls.

Preparations:

0.5 gram of basic fuchsin was dissolved in 100 ml boiling distilled water. The solution was left to cool to 60 ºC then it was filtered and left to cool to 30ºC. Thereafter,10ml of 1N HCl was added followed by 1.5-gram potassium meta – bisulphate. The solution was stirred well. After 24 hours the solution (transparent, straw colored) was ready for use. If the solution was colored,0.5 gram of activated charcoal was added, shacked, and kept overnight in refrigerator. Then the solution was filtered and used for staining the plant material.

Statistical analysis procedure:

The experiments were subjected to completely randomized design. Each treatment was replicated 3 times. Values from replicate determinations of each sample were averaged and represent as mean± standard deviation (SD). The data were analyzed statistically by analysis of variance (AN-O-VA), and the differences between the mean of sample were analyzed by least significant differences (LSD) test at a probability level of 1% and 5% Sneddecor and Cochran (1980).

RESULTS AND DISCUSSION

Seed Surface sterilization:

Data in Table (1) showed that the effect of 70 % ethanol (C_2H_5OH) for 2 minutes and clorax at different concentrations on seed surface sterilization. Table (1) and Figure (1) showed that there were significant differences of seed germination. The highest percentage was 90% while contamination free culture gave the highest percentage (95%) which achieved by using NaOCl 100% for 20 minutes. *Atropa belladonna* L. Mohamed *et al*.,(2018).seeds were washed with tap water, immersed in Clorox 50% v/v (2.5% sodium hypochlorite) for 20min and then rinsed with sterile distilled water under sterile conditions in a laminar airflow cabinet till free from chloride. Toaima *et al*., (2017). showed that, *A.belladonna* seeds were washed several times with commercial detergent and tap water and surface sterilized by immersion in 70 % ethanol for 10 sec., followed by three washes with sterile distilled water, then they were immersed in 50 % commercial Clorox solution (2.5% sodium hypochlorite) containing few drops of Tween 20 for 15 min. The seeds were subsequently rinsed five times with sterile distilled water to remove the residual sodium hypochlorite. The sterile seeds were sown on 350ml jars containing 25 ml of MS basal medium and 30 g/l sucrose, solidified with 2 g/l phytagel and incubated for 10 days in the dark for germination**.**

Influence of Gibberellic acid (GA3) concentrations on seed germination percentage of *Atropa belladonna* L.:

Atropa belladonna L. seeds are influenced at different times by 1.00 mg/l concentrations of gibberellic acid in table 2 and figure 2. Poorest germination was recorded. *In vitro* seed germination (%) at 12, 14, 16 and 18 days of sowing 1.00 mg/l gibberellic acid at different times. The concentrations of 1.00 mg/l duration of soaking at 16 hours significantly stimulated seed germination – 51.67%, 54.33%, 57.67% and 98.33%, respectively. The concentration of 1.00 mg/l at an exposition of 24 h affected the germinative process and produced an optimal effect. The maximum germination recorded was 89.5%. The established concentration of gibberellic acid is significantly lower compared to the reference data of 0.7 g/l Geyer (1987) and 0.3–2.5 g/l Shain, (1987).

Regarding the effect of GA3 on seed germination, an increase in germination percentage was observed by increasing GA3 concentration. In conformity with Ruminska *et al*., (1978), who reported that the seed soaking, preceding the sowing, in solutions of 500, 1000, 1500 and 2000 ppm of GA3 improved the germination ability of seven species of seeds. Particularly good effects were achieved with *Lavandula vera* and *Atropa belladonna* where not only germination ability was improved, but also accelerated and even shoots, accelerated and even shoots.

The laser irradiation positively and significantly affected the seed germination and time to 50% germination at lower doses of laser rays. The enhanced percentage of germination due to laser irradiation has a positive correlation with the induced internal energy level of seeds. A similar result of laser

biostimulation was reported by Chen *et al*., (2005b) they reported the positive effects of the spectral influence of laser irradiation on seed germination and suggested that the laser energy induces alterations in enzyme activities during germination and triggers the rate of cell division, which ultimately leads to an enhanced rate of growth and development. Hence, the impact of laser energy on seed germination shown in this study might be due to an increased rate of cell division during seed germination similarly.

Effect of He–Ne laser irradiated and GA3 concentrations on seed germination percentage of *Atropa belladonna*:

Seed germination (%) in vitro:

The data in Table (3) and figure (3) indicated that seed germination percentage increased by 25 Jcm^{-2}, but it did not reach the level of significance as compared with the control. On the contrary, there was a highly significant decrease in seed germination at 30 Jcm^{-2} maximum decreases in *Atropa* seed germination percentage in all studied laser doses, as compared to the control, was observed at 30 Jcm^{-2}.

There were highly significant differences between He–Ne laser doses and GA3 concentrations of 1mg/l and the interactions between them were also significant. Seed germination was increased by increasing He–Ne laser doses, until 25 Jcm^{-2} of laser doses, which resulted in a decrease in germination percentage in comparison to previous doses. These results confirmed the main concept of laser dose effects. Hence, the impact of laser energy on seed germination shown in this study might be due to an increased rate of cell division during seed germination Dziwulska (2005). Evenari, (1965) has suggested that He-Ne laser irradiation breaks the seed dormancy and shows a significant effect even on slow-germinating viable seeds. In general, the different levels of laser energy irradiation have induced the internal energy of seeds, which leads to an increase in the percentage of germination through enhanced activity of amylases and proteases involved in the physiology of seed germination, growth and development of seedlings.

Seed germination (%) in vivo:

Table 4 and figure 4 show there were highly significant differences between He–Ne laser doses and GA3 concentrations of 1 mg/l and the interactions between them were also significant. Seed germination was increased by increasing He–Ne laser doses, until 25 Jcm^{-2} of laser doses, which resulted in a decrease in germination percentage by laser doses of 30 Jcm^{-2}, in comparison to previous doses. These results confirmed the main concept of laser dose effects. Another explanation for this is the increase in enzyme activity by accelerating the cellular reactions involved in seed germination Danie, (1996).

In vitro growth parameters analysis:

Data in table (5) and figure (5) were found to be significant ($P<0.001$) compared with the un-irradiated control group. The influence of different laser doses on growth parameters of *in vitro Atropa belladonna* L. seedlings is indicated in Table 4. increased the morphological characters, such as, plant height (cm), no. of leaves per plant, no. of branches, length of leaf, width of leaf, leaf area and root length at first, second and third months. Following single exposure to different laser doses are shown in Table (5) and figure (5).

The laser treatment at 25 J cm^{-2}, shows the maximum response in terms of all morphological characters. Single laser exposure at 25 J cm^{-2} increased the plant height (cm), no. of leaves per plant, no. of branches, length of leaf, width of leaf, leaf area ($P<0.001$) and ($P<0.05$) and root length compared with the un-irradiated control set. Even though all morphological characters were found to be higher in 25 J cm^{-2} compared with other laser doses and unirradiated control, the difference was not found to be statistically significant. In the case of all morphological, single exposure of laser at 20 J cm^{-2} caused an increase ($P<0.001$) compared with the un-irradiated group (Control), (Fig. 3). The laser irradiation induced significant improvement in seed germination and subsequent growth of seedlings. Our results are in conformity with Chen *et al*., (2005b), who have reported the significant improvement in biomass and leaf area of the seedlings of Isatis. Similarly, a 7.6% and 5.7% increase in phytomass was noted with laser irradiation in tomato and cucumber respectively, by Petkova and Cholakov (2002). Cholakov and Petkova (2002) have reported higher quality seedlings with laser irradiation. Furthermore, Govil*et al*., (2006) reported the maximum increase in shoot and root length and dry weight of the seedlings from laser irradiated (30 min) green gram seeds. The laser also influenced the 63% gain in dry mass of maize seedlings, Hernandez *et al*., (2006).and enhanced emergence, early flowering and maturity, plant height than the control plants of faba bean Podleony and Podleoena, (2004).

He–Ne laser irradiation caused a significant effect on seed germination, growth parameters, enzyme activity, and thermodynamic properties of the *Triticum aestivum* plant Jamil *et al*., (2013). Similarly, continuous laser He–Ne wave with low power had an effect on the thermodynamics of seed, the rate of germination, and the activity of enzymes during the germination process in sunflower Perveen *et al.,* (2010).

In vivo growth parameters analysis:

Data in table (6) and figure (6) were found to be significant (P<0.001) compared with the un-irradiated control group. The influence of different laser doses on growth parameters of *in vivo Atropa belladonna* L. seedlings increased the morphological characters, plant height (cm), no. of leaves per plant, no. of branches, length of leaf, width of leaf, leaf area and root length at first, second and third months. Following single exposure, different laser doses are shown in Table (6).

The laser treatment at 25 J cm)2 shows the maximum response in terms of all morphological characters. Single laser exposure at 25 J $cm)^{-2}$ increased the plant height (cm), no. of leaves per plant, no. of branches, length of leaf, width of leaf, leaf area (P<0.001) and (P<0.05) and root length compared with un-irradiated control, set up the Laser was discovered in the past century and has been applied to society from its conception until today. Among its applications is its use in agriculture as a biostimulator device. The laser light at low intensity produces biostimulation when used on seeds and seedling plants Chen *et al*., (2005). The basis of the laser stimulation mechanism in any physiological plant stage is the synergy between the polarized monochromatic laser beams and the photoreceptors (Bielozierskich and Zolotariewa 1981; Koper *et al*., 1996). There are many facts that indicate the biostimulating action of laser radiation on various organs and tissues in plants Anisimov *et al*., (1997). These results were in agreement with Sahar *et al*., (2014), and Ali *et al*., (2014). The cell elongation resulted from laser treatments increased gibberellic acid, which increased the cell vacuoles Mahmoud and Brahem (2000). The highest number of leaves per shoot appeared with a red laser for 5 min as compared to control. These results were confirmed by researchers such as Rania *et al*., (2015). Using He-Ne laser beam stimulation related to higher activity of some enzymes in treated biological material Dobrowolski *et al*., (1987). All laser radiation treatments had no significant effect on the rooting percentage of *Eustoma grandiflorum* plants as compared to control. Some studies similar to our study, like Hanna and Babelewski(2014) mentioned that laser radiation did not affect the percentage of rooted cutting. This was due to the first type and concentration of auxin. The highest number of roots was obtained with a blue laser for 5 min. However, the minimum of root/shoot let was observed with a red laser for 25 min as compared to control. These results were confirmed by Rimal *et al*., (2014). Regarding the effect of laser radiation on the length of roots as affected by different types of laser radiation and various time exposures, data showed that the longest roots resulted from irradiation of shoots with green laser for 10 min as compared to control. Our study was confirmed by Metwally *et al*., (2013). In this investigation, results in the acclimatization stage also showed that the best results for the number of branches per plant were obtained from the green laser for long time exposure (25 min) and the red laser for short time exposure (5 min). These results are similar to Osman *et al*., (2009) and Aguilar *et al*., (2015), who reported that laser radiation could cause enhancement of enzyme activity. Also,it may be the endogenous content of GA3 and its role in cell elongation, where GA3 may cause cell elongation by induction of enzymes that weaken the cell wall Macleod and Millar (1962). Nabil *et al*., (2015). They reported the positive effects of the seeds treated with three doses of gamma radiation (5, 10 and 15 Kr), and arranged in a randomized complete block design with three replicates). The results showed significant differences among the three doses of gamma radiation on seed germination ,seedling height, plant height, no. of Leaves per plant at 10,20 and 30 days. The lowest dose (5 Kr) seemed to have a stimulating effect on these traits. On the other hand the high doses caused significant reduction as comparing with the control for all studied traits.

Cytological examination:

Figure 7 shows that the most common visible multinucleated cell was the unorientation plate (B, C, D, E and F). Moreover, their relative to providing the facilities for laser beam frequencies were dose-dependent. (a multinucleate cell with the capacity for indeterminate growth in size), Similar results were obtained while studying the effects of age and X-rays on *Allium* Nicholas (1942) and on hexaploid wheat using X-rays, laser rays and u.v. radations Kannan *et*

al., (2007).The He-Ne laser plays a positive role in stimulating plant growth and metabolism and protects against biotic and abiotic damage caused by various adverse environmental factors Chen *et al*, (2005), Dziwulska *et al*., (2004). It also increases tolerance, mainly by improving the rate of seed germination, as well as stimulating the elongation growth of plants and their biomass. Qiu *et al*., (2010), Qiu *et al*., (2013). Babiychuk *et al*., (1992) reported that the chromosome number in the species was reported to be 2n=72. The positive effect of laser radiation on plant growth and development can be caused by the 'excitement' of the bio-energetic structure by the formation of cells with excess energy and an increase in bio-energy levels in organisms Vasilevski, (2003). The increase in the metabolic activity of seeds is due to the absorption of more energy from the environment Chen *et al*., (2005b). Irradiating the seeds before sowing with the He-Ne laser increases the emergence, accelerates flowering and maturation as well as the growth of plants, e.g., *Lupinus albus* L., *Helianthus annus* L., *Vicia faba* L. Podlesny and Podlesna (2004), Perveen *et al*., (2011).

CONCLUSIONS

It was conducted in the present research that He– Ne laser treatment of seeds achieves biostimulation in many aspects of agro-morphological criteria of *Atropa belladonna* L. In general, most agro-morphological criteria increased, especially with seed group pre-laser treatments, were exposed to levels of laser dose of 25 Jcm^{-2} using a 7 m W He–Ne laser (632.8 nm), with a power density of 4.02 m W cm^{-2}. Various types of chromosomal aberrations (multinucleate cells) were observed during the cytological analysis of the root tip cells of both the treatments compared with control. Laser mutagenesis is an easy and new tool for breading.

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Table 1: Relationship between some disinfectant treatments and contamination free culture (%) and germination (%) of *Atropa belladonna* L.

Ethyl alcohol C_2H_5OH 70%	Clorax Concentration (v/v)	Contamination free culture (%)	Germination (%)
2 minutes	10% (20 minutes)	10	10
2 minutes	20% (20 minutes)	30	15
2 minutes	30% (20 minutes)	50	35
2 minutes	40% (20 minutes)	50	35
2 minutes	50% (20 minutes)	60	30
2 minutes	60%(20 minutes)	60	55
2 minutes	70%(20 minutes)	75	40
2 minutes	80%(20 minutes)	80	45
2 minutes	90%(20 minutes)	85	70
2 minutes	100%(20 minutes)	95	90

Table 2: *In vitro* seed germination (%) at the 12,14,16 and 18thdays of sowingonGA3 soaking of *Atropa belladonna* L. seeds.

Seed germination *in vitro* %				
Duration of soaking Treatments	After 12 days	After 14 days	After 16 days	After 18 days
Control	1.67%	2.67%	2.67%	5.33%
8hour	7.33%	9.67%	10.33%	18.33%
12 hour	13.33%	14.67%	14.67%	19.00%
16 hour	51.67%	54.33%	57.67%	98.33%
20 hour	19.00%	21.33%	27.67%	24.67%
Sig.	HS	HS	HS	HS
P-Value	0.000	0.000	0.000	0.000
L.S.D 5%	4.61%	4.68%	4.93%	6.71%
L.S.D 1%	6.55%	6.66%	7.01%	9.55%

Columns with similar letters are not significantly different according to LSD. non –significant (NS) significant (S) at P < 0.05, HS = significant(S) at P < 0. 01.

Table 3: Influence of interaction between - He–Ne laser irradiated and GA3 on seed germination % in of *Atropa belladonna* L. plants.

Seed germination (%) *in vitro*				
Doses	After8 days	After10 days	After12 days	After14 days
Control	0.00%	0.00%	0.00%	0.00%
Control+1mlg/L GA3	0.00%	2.20%	2.20%	3.60%
10J cm^{-2}+ 1mlg/L GA3	14.20%	32.00%	35.60%	40.00%
15 J cm^{-2} + 1mlg/L GA3	24.20%	30.20%	32.40%	44.00%
20 J cm^{-2} + 1mlg/L GA3	27.80%	33.20%	45.20%	62.00%
25 J cm^{-2} + 1mlg/L GA3	45.00%	51.40%	76.20%	95.20%
30 J cm^{-2} + 1mlg/L GA3	9.40%	10.80%	11.40%	12.80%
Sig.	HS	HS	HS	HS
P-value	0.000	0.000	0.000	0.000
L.S.D 5%	8.46	10.5	10.3	11.9
L.S.D 1%	11.4	14.2	13.8	15.9

Columns with similar letters are not significantly different according to LSD. non–significant (NS) significant (S) at P < 0.05, HS = significant(S) at P < 0. 01.

Table 4: Effect of interaction between laser- treated and GA3 on seed germination% *in vivo* of *Atropa belladonna.*

Seed germination (%) *in vivo*				
Doses	After8 days	After10 days	After12 days	After14 days
Control	0.00%	0.00%	0.00%	0.00%
Control+1mlg/L GA3	0.00%	0.00%	6.67%	6.67%
10J cm^{-2}+ 1mlg/L GA3	13.33%	23.33%	39.00%	43.00%
15 J cm^{-2} + 1mlg/L GA3	22.00%	32.00%	39.33%	52.33%
20 J cm^{-2} + 1mlg/L GA3	27.33%	41.00%	55.33%	77.00%
25 J cm^{-2} + 1mlg/L GA3	36.33%	57.00%	81.33%	97.33%
30 J cm^{-2} + 1mlg/L GA3	13.00%	13.67%	14.33%	15.33%
Sig.	HS	HS	HS	HS
P-value	0.000	0.000	0.000	0.000
L.S.D 5%	7.45	8.17	13.17	9.44
L.S.D 1%	10.33	11.34	18.25	13.09

Columns with similar letters are not significantly different according to LSD. non – significant (NS) significant (S) at P < 0.05, HS = significant(S) at P < 0. 01.

Table 5: Influence of laser dose on growth parameters of the *in vitro* seedlings of *Atropa belladonna* L.

	In vitro parameters							
	Characters	Plant Height	No. of branches	No. of Leaves	Leaf length	Leaf width	Leaf area	Root length
First month	Control	4.33 ±0.25 d	0.00 ± 0.00	4.67 ±0.34 c	3.09± 0.27 e	2.25± 0.21 cd	5.31± 0.75 de	3.17 ±0.11 c
	10 Jcm^{-2}	5.17± 0.31 c	0.00 ± 0.00	5.00 ±0.26 c	4.17± 0.28 d	2.75 ±0.22 c	8.75 ±1.11 d	4.84 ±0.38b
	15 Jcm^{-2}	5.67 ±0.25 c	0.00 ± 0.00	5.33 ±0.21 c	5.09± 0.16 c	3.67 ±0.25 b	13.99± 1.06 c	4.84 ±0.31 b
	20 J cm^{-2}	7.42 ±0.20 b	0.00 ± 0.00	7.67± 0.56 b	6.67 ±0.25 b	3.75 ±0.11 b	18.63 ±0.74 b	5.92 0.16 a
	25 J cm^{-2}	9.66 ±0.17 a	0.00 ± 0.00	11.00 ±0.36 a	8.66 ±0.25 a	5.08 ±0.30 a	35.56± 2.36 a	6.33 ±0.17 a
	30 J cm^{-2}	3.33 ±0.40 e	0.00 ± 0.00	3.33 ±0.21 d	3.09± 0.20 e	1.75 ±0.17 d	4.16 ±0.71 e	2.25± 0.18 d
	F value	70.30	0.00	64.03	85.96	30.66	85.80	45.06
	P value	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Sig.	HS	NS	HS	HS	HS	HS	HS
Second month	Control	5.09± 0.30 c	1.34 ±0.21 d	4.84± 0.31 c	3.50± 0.26 d	2.17± 0.21 d	5.88± 1.02 d	3.59± 0.16 d
	10 J cm^{-2}	5.00± 0.37 c	2.50 ±0.23 c	5.50± 0.35 c	4.67 ±0.17 c	3.08 ±0.16 c	10.78± 0.64 c	5.17 ±0.36 c
	15 J cm^{-2}	5.84 ±0.25 c	2.17 ±0.17 c	5.67± 0.22 c	5.16 ±0.17 c	3.75 ±0.17 b	14.19± 0.87 c	5.00 ±0.26 c
	20 J cm^{-2}	8.08 ±0.33 b	3.67 ±0.21 b	8.00± 0.45 b	7.08 ±0.49 b	4.91 ±0.16 a	26.31± 2.43 b	6.67 ±0.28 b
	25 J cm^{-2}	10.91 ±0.27 a	5.34± 0.33 a	11.34± 0.34 a	9.00 ±0.18 a	4.84 ±0.25 a	33.80± 2.50 a	7.76± 0.25 a
	30 J cm^{-2}	3.33 ±0.40 d	0.00 ±0.00 e	3.33± 0.21 d	2.91 ±0.27 d	2.25 ±0.53 d	4.97 ±0.74 d	2.41 ±0.20 e
	F value	70.21	73.92	78.73	66.88	38.78	54.54	57.18
	P value	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Sig.	HS	HS	HS	HS	HS	HS	HS
Third month	Control	6.00± 0.18c	1.84± 0.17d	4.91± 0.33d	4.00± 0.29de	2.34 ±0.31cd	7.28± 1.36de	3.84± 0.17d
	10 J cm^{-2}	5.42± 0.30c	2.84± 0.17c	6.00 ±0.26c	4.75± 0.17d	2.84± 0.25c	8.99 ±1.70d	5.67± 0.22c
	15 J cm^{-2}	6.25± 0.17c	3.00± 0.26c	6.34± 0.34c	6.08± 0.08c	4.42± 0.24b	20.12± 1.01c	5.75 ±0.17c
	20 J cm^{-2}	9.34± 0.34b	4.84± 0.17b	8.59± 0.35b	7.50± 0.41b	5.42± 0.16a	30.56 ±2.14b	6.91± 0.16b
	25 J cm^{-2}	12.25± 0.36a	5.67± 0.34a	11.67± 0.21a	9.17± 0.17a	5.25± 0.25a	36.03± 1.57a	8.41± 0.20a
	30 J cm^{-2}	3.33± 0.40d	0.00± 0.00e	3.50 ±0.18e	3.25± 0.34 e	1.75 ±0.17d	4.31± 0.66 e	2.66± 0.21e
	F value	109.28	95.85	104.06	70.78	44.95	78.89	122.45
	P value	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Sig.	HS	HS	HS	HS	HS	HS	HS

Columns with similar letters are not significantly different according to LSD. non – significant (NS) significant (S) at $P < 0.05$, HS = significant(S) at $P < 0.01$.

Table 6: Influence of laser dose on growth parameters of the *in vivo* seedlings of *Atropa belladonna* L.

	In vivo parameters							
	Characters	Plant Height	No. of branches	No. of Leaves	Leaf length	Leaf width	Leaf area	Root length
First month	Control	6.00 ±0.51c	0.00 ± 0.00	5.16±0.31d	4.25±0.36de	3.17±0.25c	10.35±1.42d	4.33±0.28c
	10 Jcm^{-2}	9.16±0.71b	0.00 ± 0.00	7.66± 0.71c	5.16±0.21d	3.66±0.21c	14.28±1.16d	4.92±0.30c
	15 Jcm^{-2}	6.75± 0.17c	0.00 ± 0.00	7.00±0.26c	7.91±0.16c	6.33±0.16b	37.59±1.15c	4.50±0.18c
	20 J cm^{-2}	9.08±0.16b	0.00 ± 0.00	9.33±0.34b	11.58 ±0.71b	6.66±0.21b	51.64±7.74b	5.75±0.25b
	25 J cm^{-2}	16.16±0.72a	0.00 ± 0.00	13.83±0.31a	16.33±0.57a	7.91±0.23a	97.15±5.32a	7.25±0.17a
	30 J cm^{-2}	3.16±0.34d	0.00 ± 0.00	3.16±0.17e	3.58±0.33e	2.33±0.16d	5.51±0.87d	3.16±0.11d
	F value	81.25	0.0000	89.87	130.41	115.71	77.35	38.22
	P value	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Sig.	HS	NS	HS	HS	HS	HS	HS
Second month	Control	8.33±0.357 d	2.17±0.166 d	5.67±0.33 d	5.00±0.39 de	3.34±0.17 d	12.69±1.53 e	4.92±0.16 d
	10 J cm^{-2}	11.09±0.800 bc	3.50±0.223 c	8.17±0.60 c	5.75±0.28 d	4.84±0.62 c	21.22±3.18 d	6.25±0.22 c
	15 J cm^{-2}	10.00±0.633 cd	3.67±0.211 c	8.50±0.34 c	8.42±0.16 c	6.92±0.20 b	43.62±1.25 c	6.67±0.25 bc
	20 J cm^{-2}	12.67±0.459 b	6.17±0.477 b	10.33±0.21 b	12.17±0.75 b	7.09±0.20 b	64.46±3.66 b	7.34±0.28 b
	25 J cm^{-2}	17.92±1.045 a	8.67±0.422 a	15.5±0.23 a	16.92±0.51 a	9.25±0.25 a	117.07±3.86 a	11.91±0.42 a
	30 J cm^{-2}	3.33±0.333 e	0.00±0.000 e	3.33±0.21 e	3.75±0.25 e	2.84±0.11 d	7.97±0.75 e	3.25±0.11 e
	F value	54.1	105.1	143.59	133.94	65.53	293	130.51
	P value	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Sig.	HS	HS	HS	HS	HS	HS	HS
Third month	Control	8.84± 0.42 c	3.16± 0.17 d	6.00 ±0.45 d	5.91 ±0.33 d	3.83± 0.33 c	17.19 ±1.66 de	6.50 ±0.13 d
	10 J cm^{-2}	11.92 ±0.67 b	4.50 ±0.34 c	8.66 ±1.86 c	6.91 ±0.16 d	4.00± 0.26 c	19.56 ±1.85 d	8.50 ±0.18 bc
	15 J cm^{-2}	11.84 ±0.63 b	4.16 ±0.17 c	9.66 ±0.51 bc	9.34± 0.33 c	7.25± 0.17 b	50.87± 2.57 c	8.00 ±0.26 c
	20 J cm^{-2}	12.34± 1.05 b	8.00 ±0.37 b	10.67± 1.50 b	12.59± 0.64 b	7.41 ±0.09 b	70.25± 4.07 b	9.08 ±0.16 b
	25 J cm^{-2}	20.42 ±0.84 a	10.84± 0.48 a	16.16 ±0.75 a	17.50 ±0.47 a	11.08± 0.24 a	145.73 ± 6.44 a	14.09± 0.23 a
	30 J cm^{-2}	3.41± 0.33 d	0.00 ±0.00 e	3.50 ±0.55 e	4.00 ±0.23 e	3.25± 0.17 d	8.59 ±0.83 e	4.09 ± 0.28 e
	F value	63.19	162.58	83.78	161.53	252.28	222.53	244.47
	P value	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Sig.	HS	HS	HS	HS	HS	HS	HS

Columns with similar letters are not significantly different according to LSD. non – significant (NS) significant (S) at P < 0.05, HS = significant(S) at P < 0. 01.

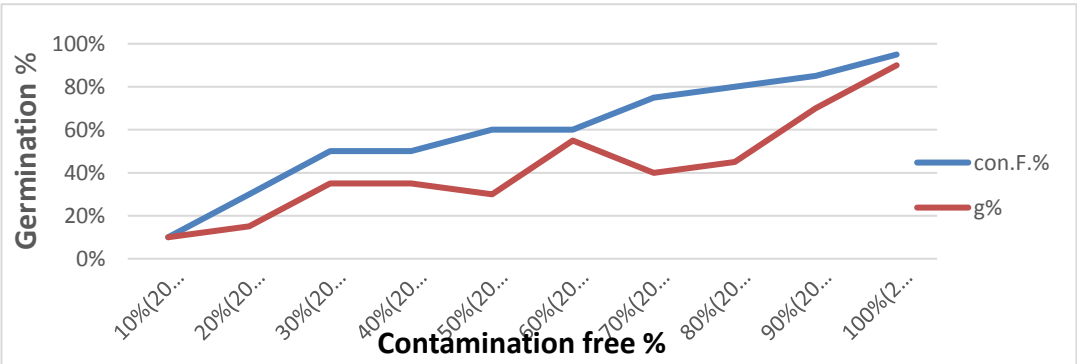


Figure1: Relationship between some disinfectant treatments and contamination free culture (%) and germination (%) of (*Atropa belladonna)*

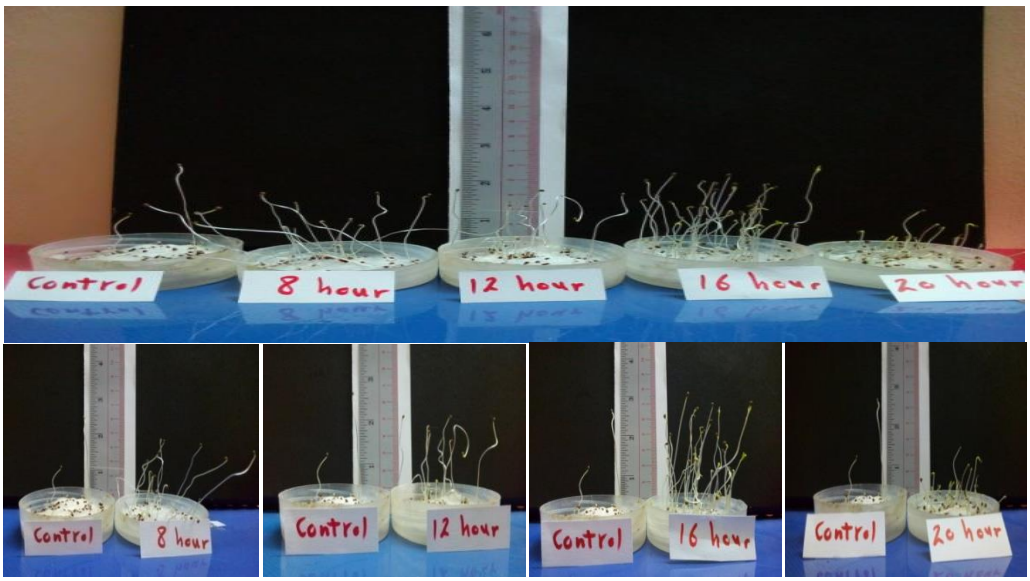


Figure 2: *In vitro* seed germination (%) at the 12,14,16 and 18th day after sowing on GA3 soaking of *Atropa belladonna* L.seeds.

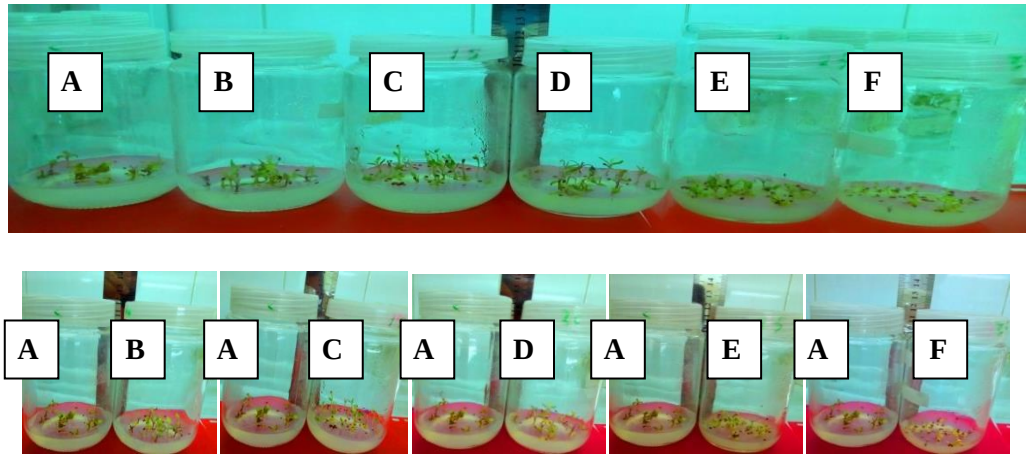


Figure 3: Influence of interaction between He–Ne laser irradiated and GA3 concentrations on seed germination % in of *Atropa* plants. A: Control, B: 10 Jcm^{-2}, C: 15 Jcm^{-2}, D: 20 Jcm^{-2}, E: 25 Jcm^{-2},F: 30 Jcm^{-2}

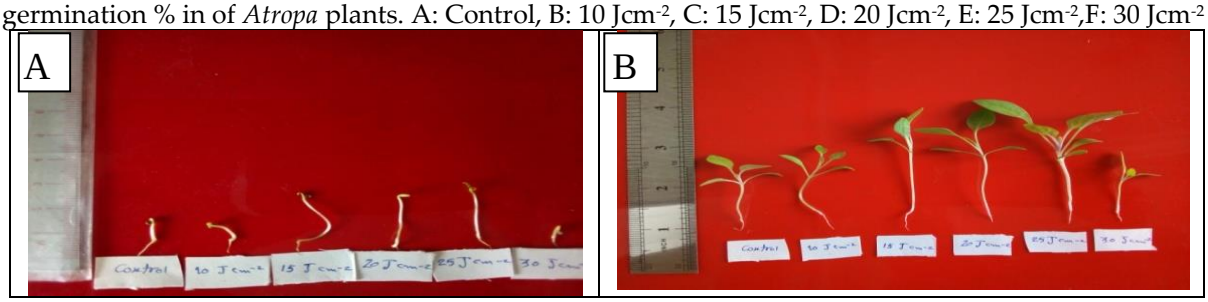
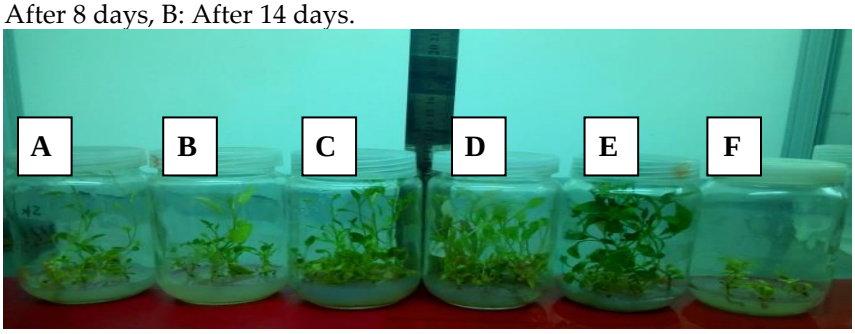


Figure 4: Effect of Interaction between Laser- treated and GA3 on seed germination% in *vivo* of Atropa *belladonna L. A:* After 8 days, B: After 14 days.



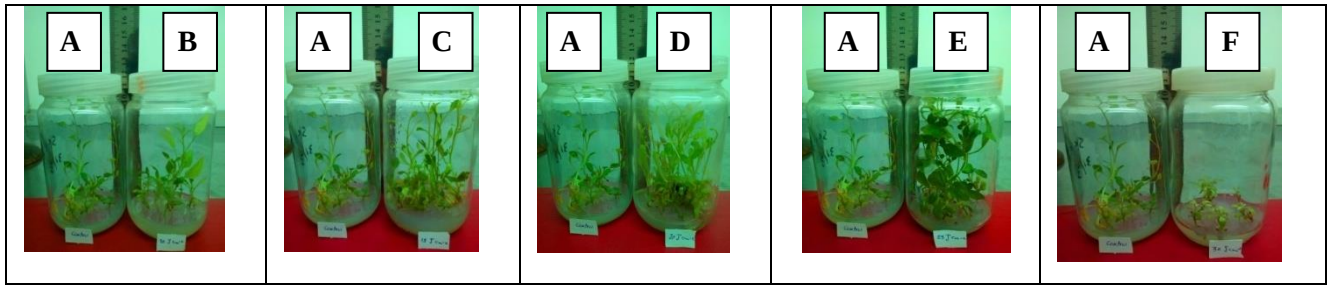


Figure 5: Influence of laser dose on growth parameters of the *in vitro* seedlings of *Atropa belladonna* L. A: Control, B: 10 Jcm^{-2}, C: 15 Jcm^{-2}, D: 20 Jcm^{-2},E: 25 Jcm^{-2},F: 30 Jcm^{-2}.

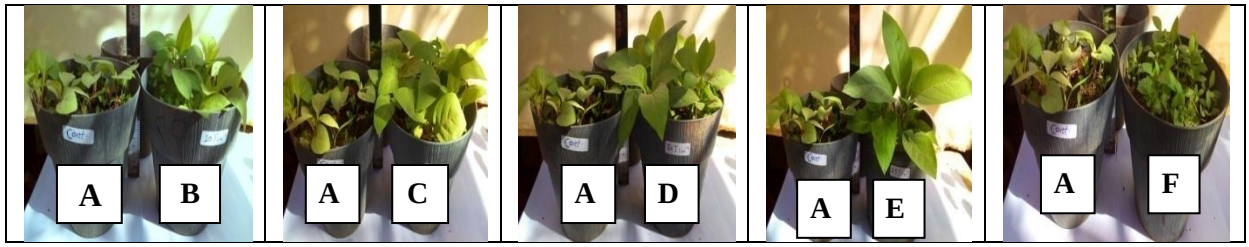
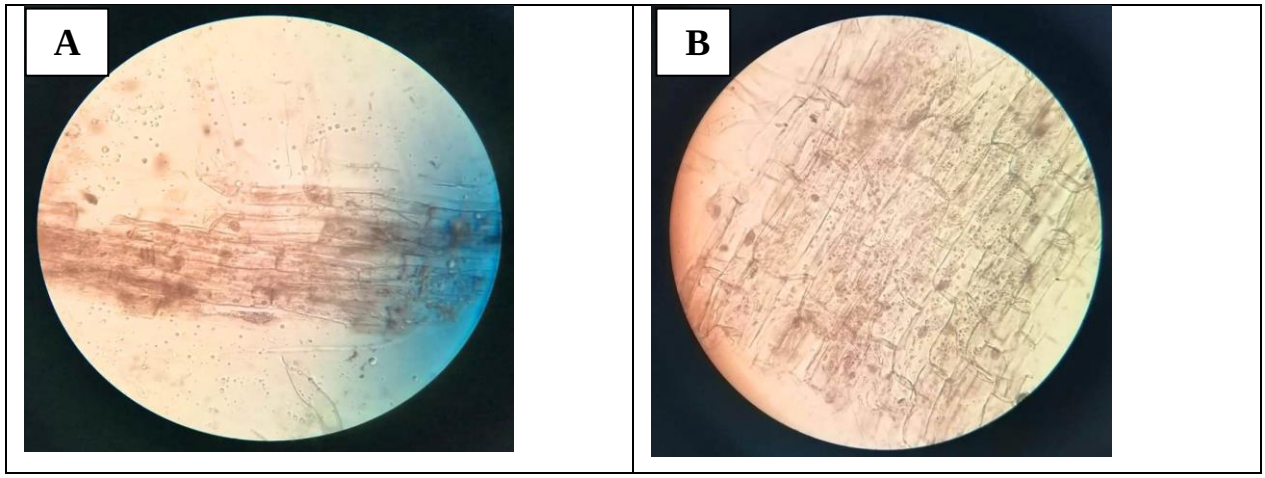


Figure 6: Influence of laser dose on growth parameters of the *in vivo* of *Atropa belladonna* L. A: Control, B: 10 Jcm^{-2}, C: 15 Jcm^{-2}, D: 20 Jcm^{-2},E: 25 Jcm^{-2},F: 30 Jcm^{-2}.



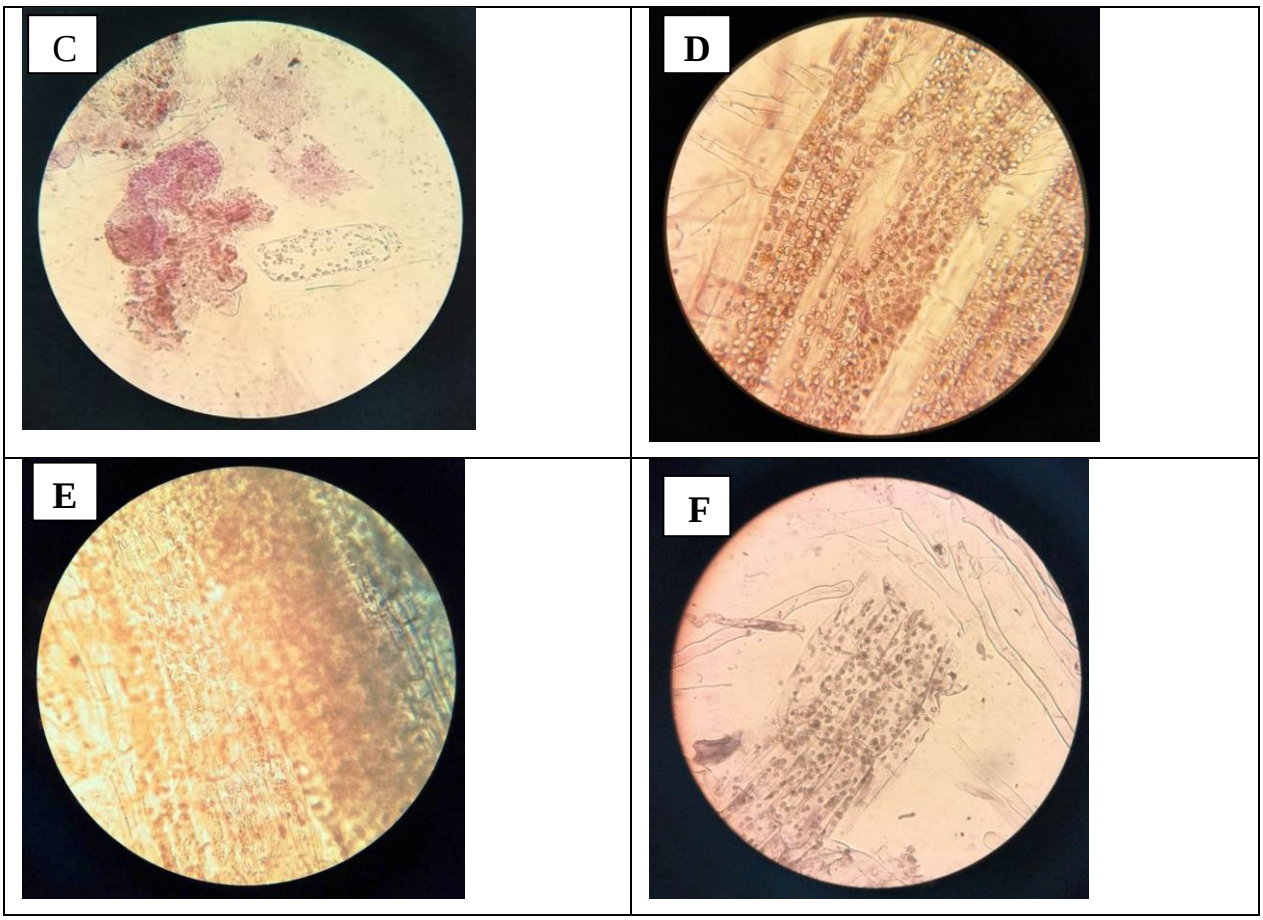


Figure 7: Mitotic chromosomal aberrations induced by different doses of laser irradiation treatments of *Atropa belladonna* L. root tips. Plate (A)Control: Normal chromosomes; Plate(B)Multinucleated cell induced by 10 Jcm^{-2}; Plate (C) Multinucleated cell induced by 15 Jcm^{-2}; Plate (D) Multinucleated cell induced by 20 Jcm^{-2}; Plate (E) Multinucleated cell induced by 25 Jcm^{-2}; Plate (F) Multinucleated cell induced by 30 Jcm^{-2}

استجابة الصفات الخضرية والسيتولوجية للتشعيع بأشعة الليزر لنبات الأتروبا بيلادونا

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الملخص العربى

يهدف البحث إلى دراسة تأثير أشعة الليزر على نسبة إنبات البذور وبعض الصفات الخضرية معمليا (باستخدام مزارع الأنسجة)،الحقلية وكذلك الانقسام الميتوزى والكروموسومات لنبات (الاتروبا بيلادونا) وذلك فى الجيل الأول الإشعاعي حيث تم تشعيع بذور نبات الاتروبا بأشعة الليزر هليوم نيون بجرعات 30,25,20,15,10,0 جول /سم$^{-2}$,وأظهرت النتائج تأثير معنوي على نسبة إنبات البذور , وارتفاع النبات (سم) ,عدد الأوراق لكل نبات عدد الأفرع, طول الورقة, عرض الورقة, مساحة الورقة وطول الجذر على فترات زمنية مختلفة وهى شهر, شهرين و ثلاثة أشهر من الزراعة. لنبات الأتروبا تحت الدراسة. وقد أظهرت الجرعة 25جول /سم$^{-2}$ إلى زيادة معنوية فى الصفات المدروسة لنبات الأتروبا تحت الدراسة بينما أوضحت الدراسة انخفاض معنوي لتلك الصفات بزيادة الجرعات الإشعاعية الى 30 جول /سم$^{-2}$, وذلك مقارنة بالكنترول. كذلك تم دراسة تأثير أشعة الليزر على الكروموسومات والانقسام الميتوزى تحت تأثير نفس الجرعات السابقة عند فحص القمة النامية للجذور فوجد أن أشعة الليزر بالجرعات السابقة تسبب حدوث الخلايا ذات الانوية المتعددة وأعطت الجرعة 25 جول /سم$^{-2}$ أعلى معدل من الخلايا ذات الانوية المتعددة الذي كان يصاحبها زيادة فى حجم الأعضاء المختلفة للنبات وأظهرت الجرعة 30 جول/سم$^{-2}$تأثيرها سلبي على الخلايا مما أدى الى انخفاض فى حجم الأعضاء النباتية المختلفة محل الدراسة. وتعتبر الدراسة ذات أهمية تطبيقية في انتخاب أصناف ذات حساسية إشعاعية مما يسهل الحصول على طفرات متميزة من خلال مزارع الأنسجة باستخدام أشعة الليزر.

الكلمات الاسترشادية:ليزر هليوم نيون, معمليا, حقليا, الصفات الخضرية, السيتولوجية, نبات الاتروبا بيلادونا, نشاط الانقسام الميتوزى, خلايا متعددة الانوية.