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# TOLERANCE LIMITS OF LAVENDER PLANT (*LAVANDULA ANGUSTIFOLIA* MILL) TISSUE CULTURES IN RESPONSE TO ABIOTIC STRESS

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**Abstract**

This study investigated the effects of gamma irradiation doses and mannitol levels of lavender (Lavandula angustifolia Mill.) cultured on solid MS medium. The effects of different growth regulators concentrations on lavender explant growth were also studied. For the first experiment, shoots of Lavandula angustifolia Mill were sterilized with different Sodium hypochlorite concentrations (15%, 20%, 25%, and 30%) for 10 min. Growing the lavender explants on MS medium sterilized with 25% Cl produced the highest survival percentage (88.59%). The results indicated that maximum numbers of shoots and leaves of lavender plants were produced on MS medium enriched with 2 mg/L BA, which also produced the maximum callus induction. Growing the lavender explants on MS supplemented with 1.0 BA gave the longest shoots (4.40 cm/ explant). Adding 2 mg/l IBA to MS medium enhanced root growth and significantly increased the average number of roots (3.40 roots/explant) and root length (2.33 cm/explant). Exposing lavender explants to gamma irradiation at high doses (8 krad) decreased lavender growth to the lowest values. A low dose of gamma rays (2 krad) significantly increased the average growth parameters to the highest values when compared with the control. Moreover, increasing mannitol levels induced an adverse effect on the lavender growth control. That decrease was proportional to the level of mannitol, but the total indoles and phenols and reduced sugar increased to their maximum tolerance for drought stress. The highest non-reducing sugar content (12.7 mg) was produced with 20 g/L mannitol when compared with the control. Gamma rays induced changes in the lavender chemical composition in this study.

**Key words:** *Lavandula angustifolia* Mill; Sodium hypochlorite; MS medium; Mannitol levels.

## Introduction

*Lavendula angustifolia* Mill, also called true lavender, belongs to the Lamiaceae or Labiate family, is grown as an evergreen shrub in the Mediterranean region and commercially cultivated in Spain, Australia, and China (Brailko *et al*., 2017; Woronuk *et al*., 2011). It is a Mediterranean perennial species, known and used since ancient times (Pohrib & Nistor, 2010).

The extracted volatile components from the oils of *L. angustifolia* extract have been used as a treatment for rheumatic diseases due to their antibacterial properties (López *et al*., 2017).

Lavender species are used for their pharmaceutical proprieties (antimicrobial, sedative, anti-inflammatory, and antiseptic (Cantor, 2018).

Plant tissue cultures provide researchers with unique materials that accelerate the development of new breeding cultivars and facilitate studies on off-type regenerates (Orłowska & Bednarek, 2020).

*In vitro* propagation is used successfully for producing many medicinal and aromatic species with limited reproductive capacity (Pence, 2010).

Jadczak *et al*., (2020) studied growing *L. angustifolia* plants *In vitro*, and also placed a single-node shoot and apical shoot and apical meristem on the Murashige and Skoog media containing 2 mg kinetin and 0.2 mg indole-3-acetic acid (Dominika & Danuta, 2018).

Drought is the most harmful environmental abiotic stress factor for plants (Woo *et al*., 2016). Plants respond to stressful conditions by reducing their growth (Mittal *et al*., 2018: Rao *et al*., 2015), inhibiting photosynthesis (Ahanger *et al*., 2017) and accumulating different osmolytes (Nahar *et al*., 2016). Growth inhibition is a fast plant response exposed to drought stress (Morosan *et al*., 2017) who reported that *L. angustifolia* was relatively resistant to water and salt stresses, and more tolerant than Phaseolus. While Du & Rennenberg (2018) and García-Caparrós *et al*., (2019) report a reduction of growth in *L. angustifolia* under water deficits. Soluble sugars play useful functional roles in abiotic stress responses (Gil *et al*., 2013).

Gamma radiation changes the physiological and biochemical processes in plants and improves the productivity of many plants. Using the gamma radiation technique is useful in plant breeding programs for many crops in stressful conditions (Borzouei, 2013). Chronic gamma irradiation is believed to induce a wide mutation spectrum with minimum radiation damage (Ahmad *et al*., 2018).

Investigations have shown that relatively low doses of irradiation can be useful for cell proliferation, cell growth, and stress resistance (Ling *et al*., 2008). Sarduie-Nasab *et al*., (2013) reported that irradiation with high doses of gamma rays caused adverse effects on important components of plant cells.

Increasing gamma irradiation from 25 to 200 Gy increased all studied growth parameters. These results are consistent with Akshathal *et al*., (2013). The induction of plant growth may be attributed to RNA activation and protein synthesis during the early stages of germination (Aly, 2010).

In this regard, Sarduie-Nasab *et al*., (2013) reported that high doses of gamma irradiation reduce emergence index, stem height, and width of barley when compared with control plants. Moreover, Preuss & Britt (2003) stated that a high dose of gamma radiation contributed to cell cycle arrest during the G2 M phase, causing a decreased growth rate during cell division and varying damage to the entire genome. The reduction in fresh and

dry weights of shoots might be attributed to the decrease in shoot moisture content resulting from radiation stress (Majeed *et al*., 2010).

Total phenolic compounds content in *Lavandula angustifolia* has been determined by using the Folin-Ciocalteu assay, which is widely used to determine the content of polyphenols in plants grown *In vitro* (Sánchez-Rangel *et al*., 2013). Tian *et al*., (2018) determined the content of polyphenols in *Atropa belladonna* tissue treated with $Mn_2O_3$ NPs. The effect of gamma-irradiation on increasing the phenolic content was noticed in soybean plants treated with c-irradiation at levels ranging from 50 to 150 Gy compounds (Variyar *et al*., 2004).

Increasing total phenol can be attributed to activity by phenylalanine ammonia lyase (PAL), one of the synthesis enzymes of phenolic compounds (Gitz, D., 3rd, *et al*., 2004). Irradiation can increase PAL activity (Tomás-Barberán & Espín, 2001), resulting in phenolic accumulation in plant tissues.

Taheri *et al*., (2014) stated that a radiation dose up to 20 Gy can induce the accumulation of bioactive compounds, including phenolic and flavonoid ones leading to the improvement of scavenging activity in *Curcuma alismatifolia* leaves. It was found that mutagenesis is beneficial for breeding new varieties and for characterizing their genetic basis. They also indicated that mutations induced by gamma-ray radiation provide an overview of the feature of genetic mutations (Tan *et al*., 2019).

This study investigates the effect of growth regulators on the production of lavender (*Lavandula angustifolia* Mill.) plants *In vitro* under different levels of mannitol stress and gamma irradiation doses. The study also investigates the effect of different concentrations of mannitol and gamma doses on the chemical composition of *In vitro* plantlets of lavender (*Lavandula angustifolia* Mill.).

## Materials and Methods

**Experimental design:** This study was conducted at the biotechnology laboratory, Faculty of Science, Taif University, during 2019–2020. The shoots (0.5 cm) of lavender (*Lavandula angustifolia* Mill.) were collected and washed exhaustively with tap water and sterilized by soaking in different concentrations of commercial bleach (Clorox 52.5 g/l sodium hypochlorite) (15, 20, 25, and 30%) for 10 minutes. The shoots were then washed with four rinses of sterile distilled water.

The explants were cultured on jars containing full-strength MS medium (Murashige, T. and F. Skoog, 1962), with different BA concentrations (0, 1, 2, and 4 mg/L) to select the best concentrate to increase shooting and plant growth. The culture medium was supplemented with agar (7 g/L) and sucrose (30 g/L), and the pH was adjusted to 5.7-5.8 before the agar's addition. Jars were sterilized at 121°C for 20 min, and then the explants were cultured on the medium and kept under the following conditions. Temp: 21±2°C. Photoperiod: 16 hr light/8 hr darkness. A total of 15 explants were used per treatment and the experiment was repeated twice. Data included the morphological growth that occurred on the explants. The number of shoots, shoot length, number of leaves, number of roots, root lengths, and callus induction were collected after four weeks.

**Irradiation experiments:** During the two months of the irradiation studies, the explants were exposed to gamma irradiation at doses of 0, 1, 2, 4, and 8 krad emitted from a cobalt-60 source in a Gamma Chamber 4000 to select the best gamma dose. The explants were transferred into MS medium containing IBA at concentrations of 0, 1, 2, and 3 mg/L with different concentrations of mannitol (0, 20, 40, and 80 g/L) to find the best IBA concentration. To encourage the best root elongation, the jars were placed in the growth chamber in the following conditions. Temp: 22±2°C day/night. Photoperiod: 16 hr light/8hr darkness. Fluorescent tubes, using Gro-Lux 20 Wm-2 as a source of light.

**Measurement of chemical contents:** Total phenols were determined using Folin–Ciocalteu (Singleton & Rossi, 1965). Indolic compounds were determined spectrophotometrically (Glickmann & Dessaux, 1975). Reducing sugar content was analyzed according to the procedure of Krivorotova & Sereikaite, (2014). Non-reducing sugars were calculated by subtracting the reducing sugars from the total sugars content.

### Statistical analysis

All experiments were conducted in triplicate (n = 3). The results were shown as mean ± standard error. The experimental data was analyzed using statistical software SPSS.

## Results

**Sterilization:** Concerning the response of survival percentage to concentrations of commercial bleach (Chlorox NaOCl g/l) and the duration of the sterilization process (Fig. 1), the results clearly indicated that the highest survival percentage (88.59%) was recorded with 25% Chlorox for 10 minutes., whereas the lowest (28.66%) was recorded with 30% Chlorox for 10 minutes. The experiment also showed that the presence of the detergent was a must.

**Effect of BA on production of lavender plant (*Lavandula angustifolia* Mill.) *In vitro*:** As shown in Table (1), lavender explants (*Lavandula angustifolia* Mill.) cultured on MS medium containing BA at 2 mg/l, formed significantly more shoots and leaves than the control and other BA concentrations; they produced 4.3 shoots and 4.2 leaves, respectively. Increasing levels of BA to 4mg /l reduced the length of shoots to the lowest values (2.9 cm). Adding 1 mg/l BA to the culture media formed a significantly longer average shoot length (4.4 cm) than the other treatments. The largest increase in callus growth was recorded with the increase of BA to 2mg/l and reached 3.4 gm.

**Effect of gamma irradiation on growth of lavender plant (*Lavandula angustifolia* Mill.) *In vitro*:** As shown in (Figs. 2 and 3), exposing the lavender explants

(*Lavandula angustifolia* Mill.) to gamma irradiation at a dose of 2 krad stimulated the shoot numbers, shoot length, and leaf numbers. Compared with the control and other gamma irradiation doses, the shoot number, shoot length, and leaf number averages were 2.7, 3.3, and 3.4, respectively. On the other hand, a gamma dose of 8 krad reduced the number of shoots, shoot length, and leaf number to the lowest values 1.3, 1.4, and 1.3, respectively. Callus formation was best with a gamma dose of 4 krad and reached the highest value (2.5 gm) compared with the control and other gamma treatments. Raising the level of gamma irradiation to 1 krad showed low callus formation.

**Effect of IBA on production of lavender plant (*Lavandula angustifolia* Mill.) *in vitro*:** Explants cultured on the MS medium supplemented with IBA at concentrate 2 mg/l produced the largest averages for shoot number, shoot length, root number and root length and reached 3.6, 4.2, 3.4, and 2.3, respectively. The data in Table (2) clearly indicated that regardless of IBA treatments, there was a significant difference in the formation of roots among treatments. The MS medium containing 0 or 4 mg/l IBA formed significantly lower roots per explant than other treatments. The average root numbers were 1.3 and 1.4 cm, respectively.

**Table 1. The optimum dose of BA for the current experiment upon shoots, leaves, and callus parameters.**

| BA conc. (mg/L) | Shoot | | Leaf number | Callus |
|---|---|---|---|---|
| | Number | Length | | |
| 0 | 2.77±0.09 c | 3.20±0.21 b c | 2.27±0.23 c | 1.93±0.03 c |
| 1 | 3.50±0.06 b | 4.40±0.06 a | 3.27±0.20 b | 2.65±0.06 b |
| 2 | 4.30±0.06 a | 3.87±0.15 b | 4.29±0.06 a | 3.44±0.11 a |
| 4 | 2.47±0.07 c | 2.93±0.09 c | 4.10±0.06 a | 2.18±0.01 b |
| LSD | 0.1 | 0.19 | 0.23 | 0.09 |

Data are expressed in mean ± SE. Data in the same column annotated with the same number have statistically significant ($p \leq 0.05$) differences. Least significant difference (LSD)

**Table 2. The optimum dose of IBP for the current experiment upon shoot, and root parameters.**

| IBA conc. (mg/L) | Shoot | | Root | |
|---|---|---|---|---|
| | Number | Length | Number | Length |
| 0 | 2.77 ± 0.09 b | 3.40 ± 0.12 b | 1.57 ± 0.15 c | 1.33 ± 0.03 b |
| 1 | 3.27 ± 0.09 a | 3.70 ± 0.06 b | 1.87 ± 0.03 c | 2.13 ± 0.09 a |
| 2 | 3.63 ± 0.03 a | 4.27 ± 0.18 a | 3.40 ± 0.06 a | 2.33 ± 0.09 a |
| 4 | 2.73 ± 0.09 b | 3.00 ± 0.06 c | 2.30 ± 0.06 b | 1.40 ± 0.06 b |
| LSD | 0.11 | 0.16 | 0.12 | 0.1 |

Data are expressed in mean ± SE. Data in the same column annotated with the same number have statistically significant ($p \leq 0.05$) difference. Least significant difference (LSD)

**Effect of mannitol levels on growth of irradiated lavender explant (*Lavandula angustifolia* Mill.) *In vitro*:** The results of (Figs. 4 and 5) explain the effect of different mannitol concentrations on lavender tissue culture. The data revealed that growing the explants in MS medium containing mannitol at any concentrations decreased all growth parameters compared with control explants, which produced the best growth. The data also revealed that supplementation of the culture MS medium with 20 gm/L mannitol encouraged significant though slight increases in the shoots. The data also showed that the decreased proliferation and growth were treated with different mannitol levels than the control and the decrease was proportional with the mannitol level.

**Effect of mannitol levels on chemical contents of irradiated lavender explant (*Lavandula angustifolia* Mill.) *In vitro*: (**Figs. 6 and 7) indicate that the highest level of mannitol concentrations (80 g/l) produced the greatest values of total indoles and total phenols, 8.9 and 9.3 mg/100gm, respectively. The data also shows that the non-reducing sugars and reducing sugar levels increased significantly with 20 g/L mannitol and produced 12.7 and 10.1 mg when compared with other treatments. These results indicate a direct relationship between increasing the mannitol concentrations and the total indoles, phenols, and reduced sugar accumulations in the cultured plantlets. These results explain the mechanism by which the plants tolerated higher levels of mannitol in the medium. The increase of some chemical contents of the lavender plant encouraged the cultured plantlets to tolerant drought.

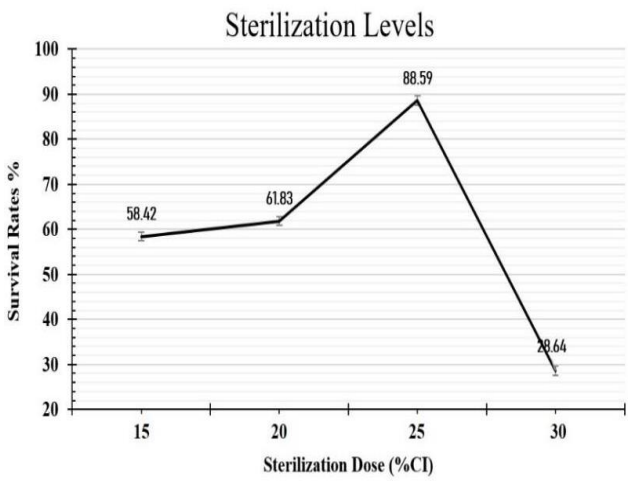


Fig. 1. Sterilization optimization curve showing the optimum dose was 25% CI.

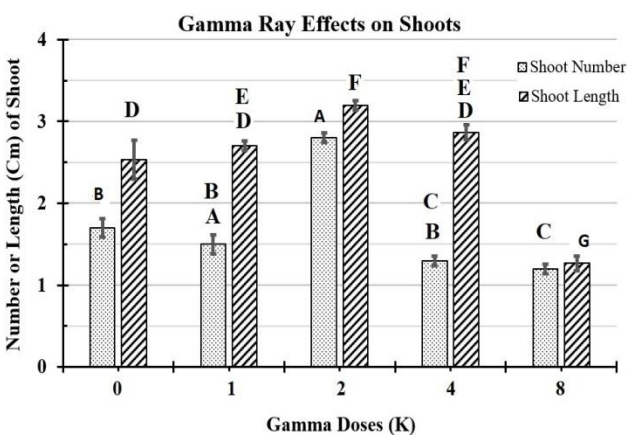


Fig. 2. Histogram showing the plant changes in shoot numbers and length due to gamma radiation exposure. All results have statistically significant ($p \leq 0.05$) differences between groups, except for the groups annotated with same letter have non-significant differences between means.

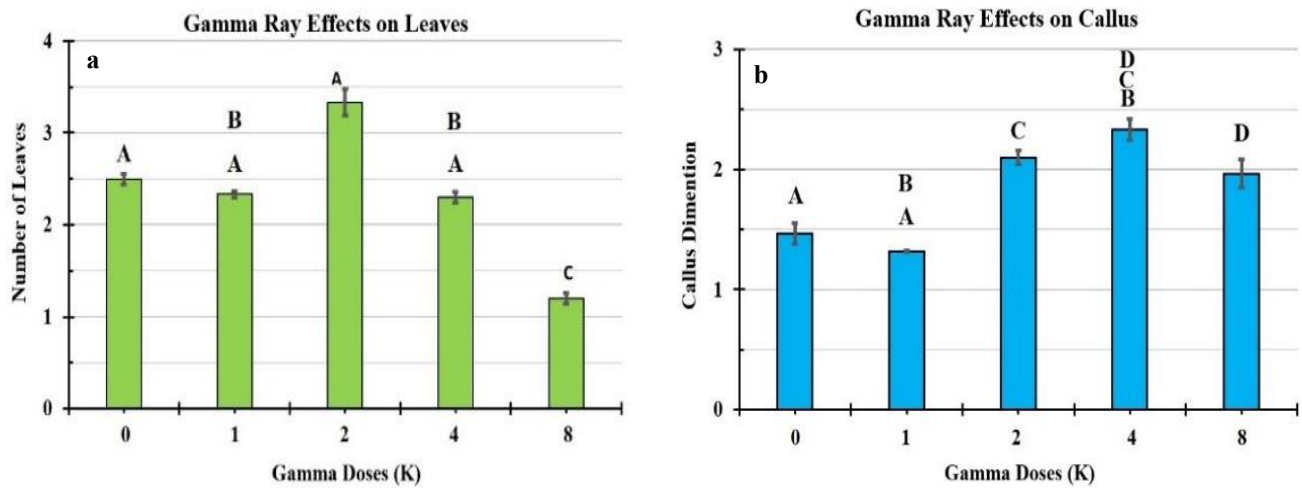


Fig. 3. Histograms showing the plant changes in (a) leaf numbers, and (b) callus induction due to gamma radiation exposure. All results have statistically significant ($p \leq 0.05$) differences between groups, except that the groups annotated with same letter have non-significant differences between means.

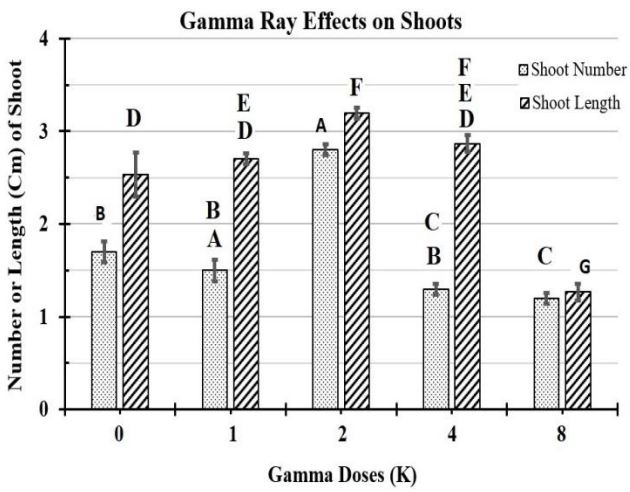


Fig. 4. Histogram showing the plant changes in shoot numbers and length due to treatment with mannitol. All groups have statistically significant ($p \leq 0.05$) differences between means.

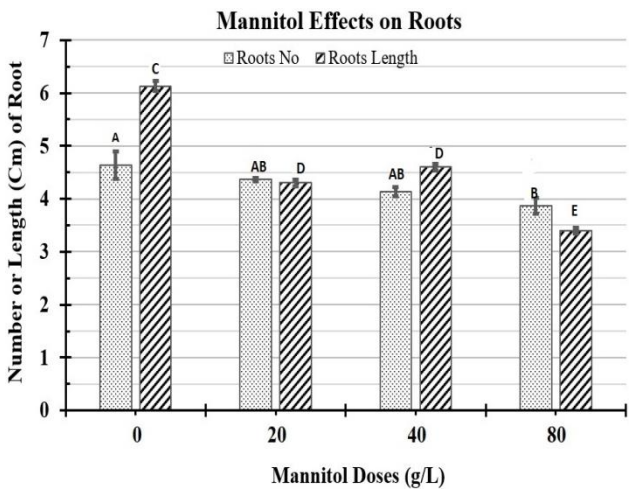


Fig. 5. Histogram showing the plant changes in shoot numbers and length due to gamma radiation exposure. All groups have statistically non-significant difference between means, except for the groups annotated with the same letter have significant differences ($p \leq 0.05$) between mean.

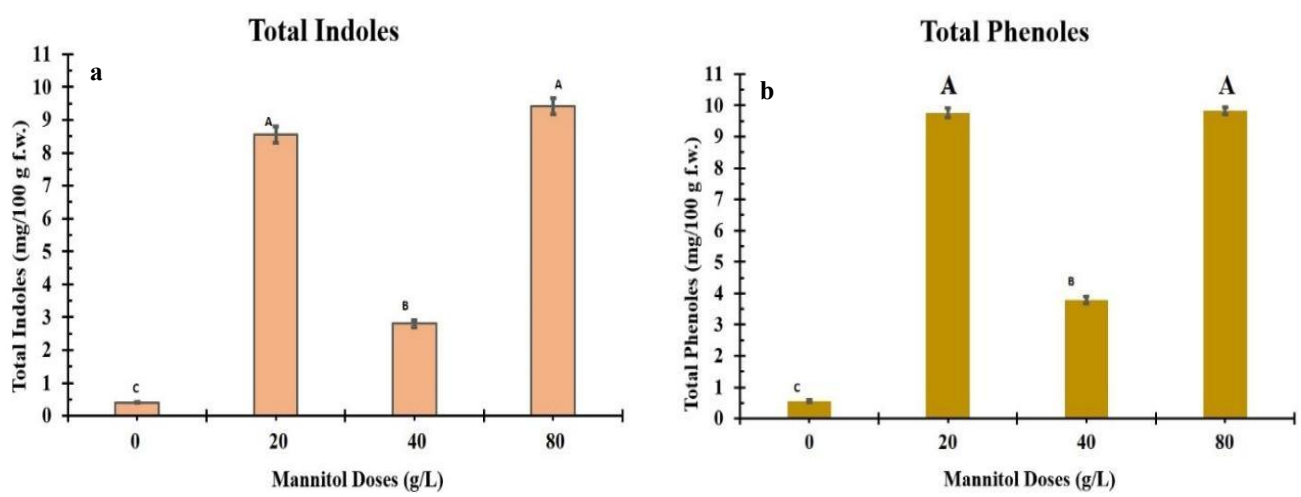


Fig. 6. Histograms showing the plant changes in (a) total indoles, and (b) total phenols due to treatments with mannitol. All results have statistically significant ($p \leq 0.05$) differences between groups, except that the groups annotated with the same letter have non-significant differences between means.

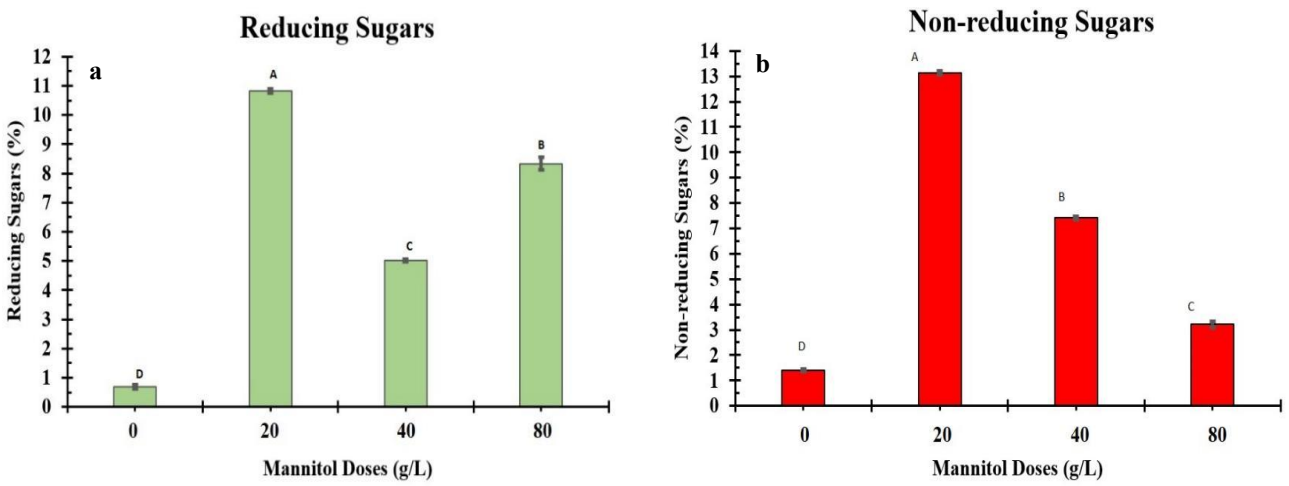


Fig. 7. Histograms showing the plant changes in (a) reducing sugars, and (b) non-reducing sugars due to treatments with mannitol. All groups have statistically significant ($p \leq 0.05$) differences between means.

## Discussion

*Lavandula angustifolia*, family (Lamiaceae), is an important aromatic plant commercially cultivated in Asia, Poland, Spain, Australia, and the USA (Brailko *et al*., 2017). The effect of mannitol levels and gamma irradiation treatments on its production and chemical contents were detected by this *In vitro* investigation.

The study data found evidence confirming that the production of lavender plant (*Lavandula angustifolia* Mill.) *In vitro* becomes increasingly degraded during *In vitro* propagation in response to increasing mannitol levels and with increasing doses of gamma irradiation to a high dose (8 krad). The same results were obtained by (Mittal *et al*., 2018), who reported that the plants respond to stressful conditions with a reduction in growth. Du & Rennenberg, (2018) and García-Caparrós *et al*., (2019) also reported a reduction of growth in *L. angustifolia* with water deficits. It was observed that one month of a water deficit or salt treatment significantly reduced growth in the two selected lavender varieties (Murashige & Skoog, 1962).

Drought stress is one of the major limiting factors affecting plant growth and yield. Plants try to maintain cell water potential through osmotic adjustment and generally respond to stressful conditions by reducing growth (Forni *et al*., 2017).

Drought generally inhibits photosynthesis, suppresses shoot growth and triggers rapid changes in leaf chemistry, and may enhance concentrations of secondary metabolites. Drought can exacerbate moisture deficits and alter both morphological and chemical traits (Zandalinas *et al*., 2018). Drought stress causes changes in the concentrations of stress-related metabolites, such as antioxidants that increase tolerance to drought (Parviz, 2016). In contrast, mild warming leads to increased plant growth and concentrations of sugars and amino acids, but not of stress-related metabolites (Zhang *et al*., 2016).

Analysis of the chemical contents of lavender plant (*Lavandula angustifolia* Mill.) showed increased total phenols and total indoles, and reduced sugar for all lavender explants exposed to any mannitol levels compared with the control. This might be due to better plant response to some mannitol and increased metabolic activity. These results are in agreement with Morosan *et al*., (2017) and indicate that both *L. angustifolia* varieties are relatively resistant to water stress, and at least are much more tolerant than most cultivars of conventional crops like *Phaseolus*. This may be due to better plant response to this level and increased metabolic activity resulting in increasing in important chemical contents of the lavender plants.

## Conclusions

In conclusion, the present study suggested adding mannitol levels to MS culturing media and exposing lavender explants to low doses of gamma rays. A 2 krad dose especially increases growth characters and some chemical contents of *Lavandula angustifolia* Mill. Increasing gamma irradiation doses and mannitol concentrations to high levels caused a decrease in growth parameters *In vitro* and chemical contents to the lowest values. That suggests the benefit of low gamma irradiation in improving and stimulating the quality of chemical contents of lavender plants (*Lavandula angustifolia* Mill) cultured *In vitro* under some levels of mannitol stress.

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