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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2019 16(2):1528-1537. **OPEN ACCESS**

Pretreatment with low-doses of gamma irradiation enhances *Vicia faba* plant tolerance to lead stress

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The present work is intended to study the effect of different doses of gamma-irradiation (0, 10, and 25 Gy) and/ or lead (0, 0.48, 4.8 and 48mM) on broad bean (*Vicia faba*) (Misr 1) plants. This was achieved by estimation of total soluble protein and some non-enzymatic antioxidants such as proline, total phenol and ascorbic acid contents. Gamma irradiation of seeds had a stimulatory effect on all measured parameters as well as it enhanced the plant resistance towards lead stress.In general, *Vicia faba* plants grown from irradiated-seeds with10 and 25 Gy and treated with lead nitrate possessed higher values of soluble proteins content and proline (due to interactive treatments) than those treated with lead only.

Keywords: *Vicia faba,* Lead nitrate, Gamma irradiation, Non-enzymatic antioxidants

INTRODUCTION

There has been an increasing concern over heavy metal contamination, due to their toxicity to living organisms. Heavy metals are non-biodegradable and capable of accumulation in living tissues.

Lead is one of the most common and dangerous environmental contaminants (Needleman, 2004). Accumulation of lead in plant tissues results in numerous disturbances of physiological processes. The most harmful effects of Pb phytotoxicity include alterations in proteins (Rastgoo and Alemzadeh, 2011), proline (Abo-Hamad et al., 2013), total phenol (Michalak, 2006), ascorbic acid (El-Beltagi and Mohamed, 2010) and sugars (Kaur et al., 2010).

Broad bean is one of the important legumes in the Middle East countries especially in Egypt. It can be used as a dietary item alone or can serve as potential supplement to cereal diets, especially for the preparation of inexpensive protein-rich food for children (Al-Kaisey et al., 2000).

Contamination by lead resulted in the oxidative stress and the phytotoxicity was mediated by reactive oxygen species (ROS) accumulation in *Vicia faba* seedlings (Wang et al., 2008). However, the results obtained by Piechalak et al., (2002) and Kamel (2008) considered *Vicia faba* as a Pb hyperaccumulator.

It has been reported that the low dose irradiation induced the growth stimulation by increasing the antioxidant capacity of the cells to easily overcome daily stress factors (Wi et al., 2007).

The current study aimed to investigate the effects of lead ions on *Vicia faba* L. plants of 15-day old germinated from 0, 10 and 25 Gy-irradiated seeds through the measurement of some physiological parameters including total soluble proteins, total proline, total phenol and ascorbic acid.

MATERIALS AND METHODS

Plants:
Seeds of *Vicia faba* (Misr 1) were purchased from the Crop Institute, Agriculture Research Center, Giza, Egypt.

Chemicals:
$Pb(NO_3)_2$ (Sigma, USA) was used in this study.

Seed irradiation:
Uniform (healthy seed of equal size and the same color) dry *Vicia faba* seeds were irradiated for a time equivalent with 10 and 25 Gy using a Cobalt-60 gamma cell (GC 220 Excel Atomic Energy of Canada Ltd.), at a dose rate of 1.88 kGy/ h at the National Center for Irradiation Research and Technology, Cairo, Egypt. Non-irradiated seeds were served as control.

Growth Conditions:
Irradiated and control seeds were sterilized with 1% sodium hypochlorite solution for about 2 min and thoroughly rewashed three times with distilled water, soaked in distilled water for six hours, then germinated in the dark on filter papers moistened with distilled water at 24 ^{0}C. Two-day old seedlings were transferred to a 16 cm diameter polyethylene pot (5 seedlings / pot) containing 2 kg sandy soil (pH 7.6, Ec 0.33 ds/m, organic matter 0.44 %, SAR 0.85). Pots were kept under controlled conditions (light intensity of 100 µmol m^{-2} s^{-1}, 10-h light/ 14-h dark cycle at 28 ^{0}C), and irrigated with full strength Hoagland's nutrient solution (pH 5.8). Soil water content was maintained at about 65% of field water capacity. Faba bean seedlings were harvested after 15 days of sowing.

Lead treatment and sample collection:
The irradiated and non-irradiated collected seedlings were transferred to containers with full strength Hoagland's nutrient solution (Stephan and Prochazka, 1989) to which KH_2PO_4 was replaced with an equivalent molar concentration of KCl to avoid Pb^{+2} precipitation by PO_4^{+3} ions (Antosiewicz, 2005), the nutrient solution supplemented with 0, 0.48, 4.8, and 48 mM of $Pb(NO_3)_2$ for 24, 48, 72 and 96h. Each treatment was conducted in triplicate.

Biochemical analysis:
The total soluble protein was estimated quantitatively in the borate buffer extract using the method described by Bradford (1976). Free proline was estimated according to the method of Singh et al. (1973). Extraction of total phenol was carried out according to Bahorun et al. (2004). Total phenol were determined by the method of Singleton and Rossi (1965) using the Folin-Ciocalteu reagent. L-Ascorbic acid (Vitamin C) was estimated according to the method of Bajaj and Kaur (1980) using ammonium molybdate solution (5 % W/V), oxalic acid solution 0.05M freshly prepared containing 0.2 mM EDTA and 5% V/V sulphuric acid.

Statistical analysis
The data were subjected to one way ANOVA to compare each treatment with the control using SPSS software version 10 (SPSS, Richmond VA, USA) as described by Dytham (1999). The effect of different time intervals was performed using Tukey's test (T-test) at $p > 0.05$.

RESULTS AND DISCUSSION

It must be mentioned here that toxicity symptoms (blacking of leaves and stems) appeared first due to 48mM Pb^{+2} after 48h and blacking of leaves increased with the time till complete wilting after 96h (Photo 1 and 2). These symptoms appeared later after 48h were elapsed due to 4.8 mM but the plant remained survived. These toxicity symptoms may be due to destroying of the defense systems of cells. When leaves or stems of *Vicia faba* are injured, dark substance is formed. This substance is a melanin-like compound produced by oxidation of 3, 4-dihydroxyphenylalanine (dopa) that is present in *Vicia faba* (Takahama and Oniki, 1991).

Results implicated significant increases in the total soluble proteins in plants grown from gamma irradiated seeds compared with control plants (Table 1). *Vicia faba* plants grown from 25Gy gamma-irradiated seeds attained the highest value of soluble proteins content (23.39, 22.98, 22.99 and 22.45 mg/g D. wt), while the control possessed the lowest value (16.13, 16.19, 16.87 and 18.31 mg/g D. wt). Thus, it seems likely that treatment with gamma irradiation at 10 and 25 Gy has stimulatory effect on total soluble proteins. In support an increase in protein content under radiation exposure has been reported in soy bean (Afify and Shousha, 1988; Stajner et al., 2007; Alikamanoglu et al., 2011; Mahdy, 2016), *Oryza sativa* (Khanna and Meherchandani, 1985), wheat (Singh and Datta, 2010) and sesame and sunflower (Mahdy, 2016).

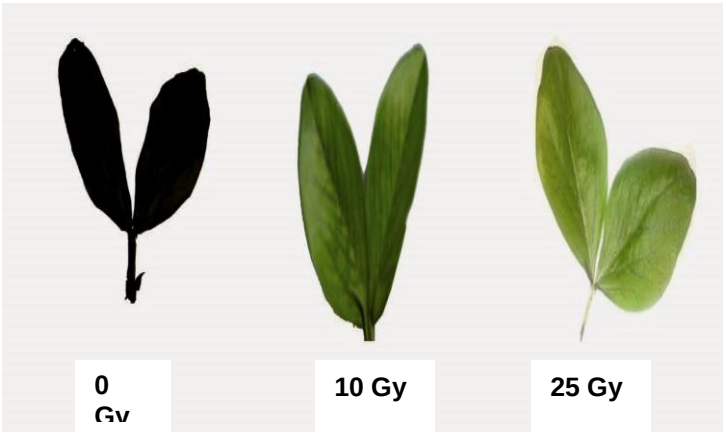

Figure 1: Leaves of *Vicia faba* plants grown from 0, 10 and 25 Gy gamma irradiated seeds and treated with 48 mM Pb^{+2} in Hoagland's nutrient solution for 96h

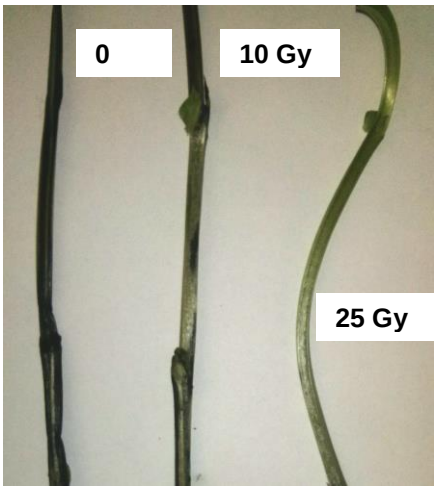

Figure 2: Stems of *Vicia faba* plants grown from 0, 10 and 25 Gy gamma irradiated seeds and treated with 48 mM Pb^{+2} in Hoagland's nutrient solution for 96h

These proteins might play a role in signal transduction, anti-oxidative defense, anti-freezing, heat shock, metal binding, anti-pathogenesis or osmolyte synthesis which were essential to a plant's functions and growth (Gygi et al., 1999).

The present results also showed that 4.8 mM-lead treatment resulted in reduction of protein content from the second to the fourth day (Table 1).

Regarding the effect of time, different significant elevations in the content of soluble proteins were recorded due to the lowest (0.48 mM) and the highest (48 mM) lead concentrations; but different significant decreases were recorded under treatment with 4.8 mM-lead.

Pankovic et al. (2000) reported that the increase in soluble protein content might be the possible mechanism for alleviating Cd toxicity as another heavy metal. This causes sequestration of the mobile form of the heavy metal to the immobile form by binding to some protein molecules. It has been reported that the protein content in *Spirulina* and *Anabaena*, increased with increasing metal ion concentration (Jetley et al., 2004; Kumar et al., 2004).

Significant increases of the soluble protein were recorded due to the interactive effect of $10\gamma+48Pb^{+2}$ (from the second to the fourth day) and also due to $25\gamma+48Pb^{+2}$ (from the third to the fourth day). However, the content was significantly lower than that of the corresponding single treatment (Table 1).

Increases in the soluble proteins due to the single effect of the highest concentration of lead ions may be due to protein hydrolysis (Habib,

2011) especially when accompanied with plant wilting. Thus, the irradiation by 10 and 25 Gy may help the plant to withstand 48mM Pb^{+2}-treatment by postpone of protein hydrolysis especially by taking into consideration the total free proline content due to these treatments; where proline may be used by the plant for more soluble proteins production due to these interactive treatments.

Biochemical differentiation based on proline content (mg/g D. wt) revealed that, the control leaves exhibited the lowest free proline content. Treatment with 25 Gy resulted in significant increases (39.85%, 46.67%, 40.26% and 45.37% of control) at 24, 48, 72 and 96h, respectively. Also, application of a 10 Gy accomplished significant increments (15.41%, 12.04%, 17.65% and 23.82%) at the end of the first, second, third and fourth day, respectively (Table 2).

Proline was altered with several environmental stresses including gamma irradiation (Al-Rumaih and Al- Rumaih, 2008). Also, the proline content was increased as reported by Moussa (2011) to cope with the problem of oxidative stresses.

Lead treatments at 0.48 mM and 4.8 mM resulted in an increase in proline. However, 48mM-treatment resulted in sharp decline in the free proline content started from the second day till the end of the experiment. It was accompanied with plant wilting with a sharp elevation in the total soluble proteins. This may be referred to uncontrolled plant processes and disturbance of nitrogen metabolism (Habib, 2011) in the presence of this high concentration of lead (Table 2).

Increased levels of heavy metals are known to affect permeability of membranes, according to Basak et al. (2001) this may lead to a water stress like condition inducing the production of proline. Tantrey and Agnihotri (2010) stated that *Cicer arietinum* plants grown with Cd and Hg showed rapid accumulation of proline. At 25 µmol/L concentration, the authors showed that proline content was enhanced up to 36% and 38% under Cd and Hg treatments, respectively.

Generally, the interactive treatments of 10 and 25Gy doses with the 0.48 mM of lead increased the total free proline content with respect to control; where the increase due to 10γ+0.48Pb^{+2} was persistence throughout the experimental period. Also, the increase due to 25γ+0.48Pb^{+2} was persistence except at the last day (Table 2).

According to Danilin et al. (2004) and Abo-Hamad et al. (2013) the bio-positive effects of low-dose gamma irradiation on plant growth under Pb stress may be attributed to the increased synthesis of phytochelatin or secondary metabolites associated with stress tolerance during seedling growth after seed irradiation. Also, Qi et al. (2015) reported that low-dose gamma irradiation modulated the physiological responses and expression levels of genes related to Pb-resistance in *Arabidopsis* seedlings.

A proportional increase of phenol content was observed by increasing gamma dose throughout the experimental period relative to control. Application of 10 and 25 Gy accomplished significant increments (7.42%, 14.34%, 10.29%, 12% and 19%, 25.66%, 17.60%, 27.76%) at 24, 48, 72 and 96h, respectively. This suggests the development of a protective mechanism due to the stimulative effect of low doses of irradiation. Ionizing radiation increased formation of phenolic compounds in a number of plant tissues as reported by Tomás-Barberán and EspÍn (2001). Such increase in total phenols may be due to the release of phenolic compounds from glycosidic components and the degradation of larger phenolic compounds into smaller ones by gamma irradiation as suggested in almond by Harrison and Were (2007).

In the present study, there was a sharp significant increase in the total phenol content as observed after treatment with 0.48 and 4.8 mM. The increase was 170.68% and 251.56%, respectively after 24h. The high level of lead (48 mM) caused the least increase in the phenol content (24.33% relative to control) compared to the effect of low (0.48 mM) and medium (4.8 mM) treatments (Table 3).

The total phenolic content of all lead-treated *Vicia faba* plants in the present study was decreased significantly after 96h when compared with their counterparts after 24h. Also, total phenol content decreased significantly and gradually by increasing lead concentration in the nutrient medium. Regarding the control, the total phenolics decreased by about 33%, 40% and 77% after treatment with 0.48, 4.8 and 48 mM Pb^{+2} treatments, respectively.

The concept of antioxidant activity of phenolic compounds is not novel. There have been many reports of induced accumulation of phenolic compounds in plants treated with high concentrations of metals (Michalak, 2006). Antioxidant activity of phenolic compounds is due to their high tendency to chelate metals. Phenolics possess hydroxyl and carboxyl groups, able to bind heavy metals (Jung et al., 2003). The roots of

many plants exposed to heavy metals exude high levels of phenolics (Winkel, 2002).

Table 1. Single or interactive effect of gamma irradiation of seeds and lead on the total soluble protein (mg /g D. wt) of *Vicia faba* plants

Treatment		Total Soluble Protein (mg /g D. wt)			
		Days			
		1	2	3	4
Control	(0Pb^{+2}, 0γ)	16.13 ±0.41h	16.19 ± 0.48g	16.87 ± 0.92 f	18.31 ± 0.12 f*
γ(Gy)	10γ	20.28 ±0.02f	20.04 ± 0.64de	19.48 ± 0.59 e	20.29 ± 0.05 e
	25γ	23.39 ±0.15d	22.98 ± 0.92c	22.99 ± 0.00 c	22.45 ± 0.20 d*
Pb^{+2}(mM)	0.48Pb^{+2}	19.48 ±0.02g	31.05 ± 0.25a*	37.57 ± 0.73 a*	28.09 ± 0.55 b*
	4.8Pb^{+2}	24.05 ±0.05c	17.37 ±0.81fg*	9.82 ± 0.39 h *	9.78 ± 0.13 i *
	48Pb^{+2}	22.93 ±0.03d	28.13 ± 0.82b*	32.43 ± 0.69 b*	29.75 ± 0.64 a*
γ(Gy)+	10γ+0.48Pb^{+2}	21.23 ±0.06e	20.55 ± 0.02d*	14.4 ± 0.58 g *	17.42 ± 0.51fg*
Pb^{+2}(mM)	10γ+4.8Pb^{+2}	35.97 ±0.09b	26.61 ± 0.28b*	16.97 ± 0.18 f*	24.05 ± 0.19 c *
	10γ+48Pb^{+2}	20.23 ±0.07f	21.25 ± 0.21d*	24.46 ± 0.12 c*	30.31 ± 0.41 a *
	25γ+0.48Pb^{+2}	22.84 ±0.56d	21.29 ± 0.37d	18.39 ±0.05 ef*	13.31 ± 0.05 h *
	25γ+4.8Pb^{+2}	39.72 ±0.05a	18.52 ±0.04ef*	21.08 ± 0.47 d*	16.34 ± 0.81 g *
	25γ+48Pb^{+2}	13.45 ±0.22i	14.60 ± 0.55h	19.35 ± 0.66 e*	27.31 ± 0.75 b *

Data are mean of three replicates, ± standard error. Means in the same column having the same letter are not significantly differed at 5%. *Significant differences at $p < 0.05$ by Tukey's test (t- test) due to different time intervals with respect to the first day content.

Table 2. Single or interactive effect of gamma irradiation of seeds and lead on the total free proline (mg /g D. wt) of *Vicia faba* plants.

Treatment		Total Free Proline (mg /g D. wt)			
		Days			
		1	2	3	4
Control	(0Pb^{+2}, 0γ)	5.32 ± 0.07 h	5.40 ± 0.16 g	5.44 ± 0.19 g	5.29 ± 0.02 g
γ(Gy)	10γ	6.14 ± 0.04 f	6.05 ± 0.05 f	6.40 ± 0.13 f	6.55 ± 0.02 f*
	25γ	7.44 ± 0.06 d	7.92 ± 0.08 d*	7.63 ± 0.04 e	7.69 ± 0.01 d *
Pb^{+2}(mM)	0.48Pb^{+2}	4.84 ± 0.05 i	8.13 ± 0.06 c *	8.80 ± 0.04 c *	3.10 ± 0.04 i *
	4.8Pb^{+2}	9.82 ± 0.05 b	9.32 ± 0.04 a *	10.37 ± 0.31 a	12.18 ± 0.03 a*
	48Pb^{+2}	6.90 ± 0.05 e	0.53 ± 0.01 i *	0.38 ± 0.00 i *	0.29 ± 0.01 k *
γ(Gy)+	10γ+0.48Pb^{+2}	5.47 ± 0.02 h	9.37 ± 0.05 a*	8.14 ± 0.02 d *	9.93 ± 0.04 c*
Pb^{+2}(mM)	10γ+4.8Pb^{+2}	9.03 ± 0.06 c	7.53 ± 0.05 e*	6.65 ± 0.04 f *	11.44 ± 0.07 b*
	10γ+48Pb^{+2}	3.67 ± 0.05 j	2.03 ± 0.01 h *	1.76 ± 0.02 h *	1.15 ± 0.00 j *
	25γ+0.48Pb^{+2}	5.96 ± 0.04 g	7.46 ± 0.03 e*	9.72 ±0.06 b *	4.65 ± 0.02 h*
	25γ+4.8Pb^{+2}	10.61 ±0.09a	8.89 ± 0.06 b *	8.03 ± 0.05 d *	7.15 ± 0.03 e *
	25γ+48Pb^{+2}	9.79 ± 0.06 b	9.45 ± 0.01 a*	1.85 ± 0.02 h *	1.20 ± 0.02 j *

Data are mean of three replicates, ± standard error. Means in the same column having the same letter are not significantly differed at 5%. *Significant differences at $p < 0.05$ by Tukey's test (t- test) due to different time intervals with respect to the first day content.

Table 3. Single or interactive effect of gamma irradiation of seeds and lead on the total phenol (mg /g D. wt) of *Vicia faba* plants.

Treatment		Total phenol (mg /g D. wt)			
		Days			
		1	2	3	4
Control	($0Pb^{+2}$, 0γ)	8.63 ± 0.12 j	8.30± 0.07 g	8.75± 0.17fg	8.50± 0.11 c
γ(Gy)	10γ	9.27 ± 0.04 i	9.49± 0.14 f	9.65± 0.09 e*	9.52± 0.10 b
	25γ	10.2±0.07gh	10.43±0.15 e	10.92± 0.07d*	10.86±0.23 a
Pb^{+2}(mM)	0.48Pb^{+2}	23.36± 0.43 c	14.76±0.02 b*	13.57± 0.30b*	5.69± 0.13 d*
	4.8Pb^{+2}	30.34± 0.24 a	14.09± 0.18 c*	8.50± 0.39 g*	5.11± 0.08 e*
	48Pb^{+2}	10.73±0.05 g	8.18± 0.14 g*	7.39± 0.28 h*	1.97± 0.02 g*
γ(Gy)+	10γ+0.48Pb^{+2}	10.01±0.11 h	12.45± 0.08 d*	15.06± 0.39a*	6.76± 0.18 c*
Pb^{+2}(mM)	10γ+4.8Pb^{+2}	19.52±0.17 d	8.12± 0.13 g*	10.33± 0.15d*	9.74± 0.10 b*
	10γ+48Pb^{+2}	16.77± 0.10 e	8.06 ± 0.20 g*	11.77± 0.12c*	2.35± 0.08g*
	25γ+0.48Pb^{+2}	28.80±0.17 b	15.62± 0.09 a*	10.37± 0.09d*	11.01±0.50a*
	25γ+4.8Pb^{+2}	29.91±0.33 a	15.89± 0.13 a*	9.34± 0.20ef*	5.35±0.08de*
	25γ+48Pb^{+2}	12.81± 0.02 f	12.47± 0.30 d	7.31± 0.18h*	3.00± 0.02 f*

Data are mean of three replicates, ± standard error. Means in the same column having the same letter are not significantly differed at 5%. *Significant differences at $p < 0.05$ by Tukey's test (t- test) due to different time intervals with respect to the first day content.

Table 4. Single or interactive effect of gamma irradiation of seeds and lead on the ascorbic acid (mg /g D. wt) of *Vicia faba* plants

Treatment		Ascorbic Acid (mg /g D. wt)			
		Days			
		1	2	3	4
Control	($0Pb^{+2}$, 0γ)	3.06± 0.04 l	3.18± 0.08 i	3.08± 0.06 h	3.24± 0.22 g
γ(Gy)	10γ	4.40± 0.01 j	4.40± 0.02 g	4.45± 0.02 de	4.34± 0.01 e*
	25γ	5.23± 0.04 h	5.42± 0.03 ef*	5.72± 0.01 b*g	5.65±0.04ab*
Pb^{+2}(mM)	0.48Pb^{+2}	9.20± 0.06 d	6.73± 0.04 b*	5.43± 0.06 c*	5.87± 0.04 a*
	4.8Pb^{+2}	11.96± 0.04b	7.37± 0.06 a*	4.26± 0.03 ef*	2.48± 0.10 h*
	48Pb^{+2}	5.64± 0.02 g	3.98± 0.02 h*	2.76± 0.06 i*	0.38± 0.04 k*
γ(Gy)+	10γ+0.48Pb^{+2}	4.21± 0.03 k	5.23± 0.03 f*	7.18± 0.04 a*	4.78± 0.08 d*
Pb^{+2}(mM)	10γ+4.8Pb^{+2}	4.97± 0.05 i	6.38± 0.06 c*	7.37± 0.03 a*	5.44± 0.08 b*
	10γ+48Pb^{+2}	6.54± 0.05 e	3.22± 0.03 i*	4.55± 0.05 d*	2.00± 0.04 i*
	25γ+0.48Pb^{+2}	11.14± 0.05c	5.91± 0.12 d*	5.66± 0.28 bc*	5.06± 0.04 c*
	25γ+4.8Pb^{+2}	12.42± 0.05a	6.73± 0.05 b*	4.13± 0.08 f*	3.92± 0.07 f*
	25γ+48Pb^{+2}	6.22± 0.08 f	5.61± 0.15 e*	3.43± 0.06 g*	1.60± 0.09 j*

Data are mean of three replicates, ± standard error. Means in the same column having the same letter are not significantly differed at 5%. *Significant differences at $p < 0.05$ by Tukey's test (t- test) due to different time intervals with respect to the first day content.

The trend of increasing and decreasing of phenols in tested plants was approximately in most cases similar to those of ascorbic acid. It can be concluded that phenols act with ascorbic acid (ASC) and peroxidase (POD) in the H_2O_2-scavenging, phenolic/ ASC/ POD system and this is supported by the results of Michalak (2006) and Habib (2011).

The various doses of gamma caused an increase in the content of ascorbic acid. The increase was proportional to the applied doses; the increment percents with respect to control were 43.79%, 38.36%, 44.48% and 33.95% due to 10 Gy dose. Also, the increment percents were 70.92%, 70.44%, 85.71% and 74.38% due to the 25 Gy dose at 24, 48, 72 and 96h, respectively (Table 4). Free radicals are generated and these radicals may then stimulate a mechanism to increase antioxidant power to protect from cellular damage (Fan and Thayer, 2001). This can illustrate the effect of gamma irradiation, in the present study, regarding the time factor; where the ascorbic acid content in the irradiated plants had been kept higher than that of control throughout the experimental time. The increase in the level of non-enzymatic antioxidants such as ascorbic acid was observed under gamma rays treatment in cowpea (Mohammed et al., 2012).

Results after 24h reflected a sharp significant increase in the ascorbic acid content after treatment with 0.48 and 4.8 mM and the increment percents were 200% and 290%, respectively when compared with control. The high level of lead (48 mM) caused the least increase in the ascorbic acid content (84.31%) comparing with the effect of low (0.48 mM) and medium (4.8 mM) Pb treatments (Table 4).

The results of the present study reflected a sharp decline in the ascorbate (88%) due to the highest concentration of lead (48 mM) after 96h. This may be due to the destruction of the plant defense systems (Habib, 2011) especially when conjugates with another sharp decline in the total phenol content (77%) and significant increase in the total soluble proteins content by about 62% as an indicator for the beginning of protein hydrolysis.

This may reflect different responses of *Vicia faba* plants to different concentrations of lead. This change in the levels of ascorbic acid may play an important role against oxidative injury caused by lead ions providing a greater protection to sulfhydryl groups, a functional integrity of protein molecules (Zengin and Munzuroglu, 2005; Kováčik et al., 2017).

Lead was reported to cause an increase in ascorbic acid and α-tocopherol levels in two *Oryza sativa* cultivars (Mishra and Choudhuri, 1999). Ascorbic acid; a natural antioxidant scavenges free radicals generated by heavy metals (Halliwell and Gutteridge, 1993; Kováčik et al., 2017).

In the present study, when compared with the control, the plants showed increase in the ascorbic acid content (37.58%, 62.42% and 113.73%; 264.05%, 305.88% and 103.27%) due to the combined treatments of 10 Gy/25 Gy with 0.48, 4.8 and 48 $mMPb^{+2}$, respectively. Also, regarding the ascorbic acid content due to the single lead treatments, the 10Gy dose caused a lower content of ascorbate after treatment with $10\gamma+0.48Pb^{+2}$ and $10\gamma+4.8Pb^{+2}$ but higher content after treatment with $10\gamma+48Pb^{+2}$. However, the 25 Gy dose produced a higher content of ascorbic acid than that produced due single lead treatments (Table 4).

With respect to control, all interactive treatments produced significant differences in the ascorbic acid content. It decreased only due to $10\gamma+48Pb^{+2}$ and $25\gamma+48Pb^{+2}$ by 38.27% and 50.62%, respectively. This decline seems likely to be due to function of ascorbic acid as a co-substrate of plant peroxidases or destroying of plant defense system but the first suggestion was more realistic.

CONCLUSION

The present work indicated that *Vicia faba* plants grown from seeds irradiated by 10 and 25 Gy gamma rays were capable of tolerating 0.48, 4.8 and 48 mM lead treatments than plants grown from non-irradiated seeds. The plants grown from irradiated-seeds expressed higher values of soluble proteins content and proline (due to interactive treatments) than those treated with lead alone. Therefore, the major success of the present work was that sufficient preliminary evidence showed that low dose gamma irradiation enhanced *Vicia faba* tolerance to lead stress. However, further experimentation on lead accumulation is required to establish influence of lead accumulation on plants after seed irradiation. Moreover, a detailed study of some physiological parameters is necessary to conclude the lead toxicity observed in the plants.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

The authors gratefully acknowledge facilities introduced by Botany Department, Faculty of Science, Mansoura University and Radioisotopes Department, Nuclear Research Center, which participated for the successful completion of this work.

AUTHOR CONTRIBUTIONS

HME, HAK and HMH designed and performed the experiments and also wrote the manuscript. HME, HAK and HMH performed plants treatments, flow experiments, tissue collection, and data analysis. HME, HAK and IYM reviewed the manuscript. All authors read and approved the final version.

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