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"Effect of Gamma Radiation on Growth, Oxidative Stress, Antioxidant System, and Alliin Producing Gene Transcripts in *Allium sativum*"

[1]Rashad Kebeish (Ph.D)*, [2]Hanan, E. Deef (Ph.D), [3]Nagwa El-Bialy (M.Sc)

Faculty of Science/Botany Department/Plant Biotechnology Laboratory (PBL)
Zagazig University, Zagazig, Egypt
[1]rkebeish@gmail.com, [2]Hanandeef@yahoo.com, [3]Nagwa_Ali_Belal@yahoo.com

Abstract: *The cloves of garlic (Allium sativum L.) were exposed to variable doses of gamma rays ranging from 10 to 150 Gy in order to assess their effects on plant growth, morphological variation, biochemical, and molecular traits. There was a clear correlation between gamma radiation doses and plant growth. Pigments fractions and total carbohydrate contents were also decreased with increasing γ- radiation doses. The level of Proline contents and the activity of antioxidant enzymes; CAT, POD, PPO, and SOD showed gradual increase with increasing the level of γ- radiation up to 100 Gy and thereafter decline. It is interesting to note that abundance of Alliinase gene transcripts which was gradually reduced with the increase of γ-radiation doses.*

Keywords: *Allium sativum L, Gamma radiation, Antioxidant enzymes, Alliinase transcripts.*

1. INTRODUCTION

Garlic (*Allium sativum L.*), belongs to the Liliaceae family, is a common food spice, used widely in many parts of the world. For many centuries, various species of genus Allium have been used as vegetables, spices and as folk medicines for curing of various diseases **[1].** Garlic has been a subject of considerable interest as a medicine world-wide since ancient times. Since ancient times, garlic has been used worldwide as a seasoning spice and herbal remedy **[2].** Garlic is known to possess a vast variety of biological functions. It was reported as an antimicrobial **[3],** antithrombotic **[4],** anticancer **[5]**, antioxidant **[6],** and it could improve the immune-system **[7].** Garlic has the capacity to lower serum lipid, glucose levels **[8]** and blood pressure **[9].** Garlic has demonstrated beneficial effects in a large number of pathological conditions, including hyperlipidemia **[10]**, cardiovascular disorders and arteriosclerosis **[11].** Cancer preventative properties of garlic have also been reported **[12].** Epidemiologic studies have revealed the lower risk of stomach cancer in people with high garlic intake **[13].**

Gamma rays, belong to ionizing radiation, can be energetically charged particles such as electrons, or high-energy photons. The biological effect of gamma rays is based on the interaction with atoms or molecules in the cell particularly with water to produce free radicals in cells. These radicals can damage or modify important components of plant cells and have been reported to affect differentially the morphology, anatomy, biochemistry and physiology of plants. These effects include changes in the plant cellular structure and metabolism e.g. dilation of thylakoid membranes, alteration in photosynthesis, modulation of the antioxidative system and accumulation of phenolic compounds **[14,15].** The primary effects of ionizing radiation are ionization, dissociation and excitation. The excitation cause a weak interaction, whereas the ionization and dissociation resulted in strong interaction. Absorption of ionizing radiation in biological materials acts directly on critical targets in the cell **[16].** The effects observed after exposure were deeply influenced by several factors, some related to plant characteristics (e.g., species, cultivar, stage of development, tissue architecture and genome organization) and some related to radiation features (e.g., quality, dose, duration of exposure) **[17].**

The objectives of the current study are to investigate growth, pigments fractions, proline, carbohydrate content, antioxidant enzyme activities, and Alliinase gene expression in garlic plants (*Allium sativum* L) after exposure of their bulbs to different doses of γ-radiation.

2. MATERIALS AND METHODS

2.1. Plant Materials

The garlic cloves were obtained from the Agricultural Research Center, Giza, Egypt. It's genotypes, namely 'Balady', a locally adapted garlic cultivar widely grown in Egypt. It is an early cultivar with large number of relatively small clove per bulb (60).

2.2. Plant Cultivation

γ-irradiation pretreatment by Indian Co-60 gamma cell at National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo , Egypt. Bulbs were pachaged in paper bag, coverd with aluminum foil , and exposed to low doses (10, 20, 30, 40, 50, 70, 100, 120, and 150 Gy). Cloves of garlic used in this investigation were germinated in plastic pots filled with about 1cm of tap water in a place illuminated by natural light.

2.3. Methods

2.3.1. Shoot growth measurements

The length of shoot system of the germinated bulbs of *Allium sativum* was recorded after 15 days of growth. The mean of the length of the shoot system was calculated:The mean length of the shoot system (at each γ-radiation dose) = (The total length of germinated bulbs after 15 days / Total number of germinated bulbs).

2.3.2. Determination of total pigments

The chlorophyll content was measured as described by **[18,19].** 100 mg of fresh plant leaves were harvested in a 2 ml eppendorf tube and immediately frozen in liquid nitrogen. The leaf samples were ground and 1 ml of 80% acetone were added. Samples were mixed vigorously then spun down (30000 x g/10 min). The basic extinction at 663, 645, and 440 nm corresponding to Chl A, Chl B, and Carotenoids; respectively were measured.

2.3.3. Determination of total carbohydrates content

80% hot ethanol was added to a known fresh weight of plant material then boils till the leaf become colorless .Discard the leaf rests and evaporates all ethanol. Dissolve in 0.5 ml dist. H_2O **[20].**Carbohydrate content was estimated according to the method described by **[21].** In a clean test tube 1ml of 5% phenol solution was added to 0.1 ml of the extract was mixed well, with 5 ml of conc. H_2SO_4 The tubes were equally agitated during the acid addition . After 10 minutes, the tubes were re-shaken and placed in a water bath at 25-30°C for 20 minutes. The absorbance of the developed yellow brown color was measured at 560nm and compared with calibration standard curve. Glucose was used as a standard.

2.3.4. Determination of proline contents

Extraction and determination of proline was performed according to the method of **[22]**. Plant cells were homogenized in 10 ml of 3% sulphosalicylic acid .Supernatant was obtained by centrifugation at 5000 rpm for 10 minutes.2 ml of supernatant was reacted with 2 ml acid ninhydrin and 2 ml glacial acetic acid in a test tube for 1 hour at 100°C, and the reaction was then terminated by placing the tubes in an ice bath. The reaction mixture was extracted with 4 ml toluene mixed vigorously for 15-20 sec. The absorbance of the developed blue color was measured at 520 nm. Proline content was calculated as µmol/dry weight. Proline was used as a standard.

2.3.5. Antioxidant enzyme activities

Estimation of the activities of oxidative enzymes (catalase, peroxidase and polyphenol oxidase):

2.3.5.1. Enzyme extraction

Frozen leaves were ground in liquid nitrogen to a fine powder with a mortar and pestle**.** plant material was homogenized in 0.005M cold phosphate buffer (KH_2PO_4 , K_2HPO_4) (PH 6.5) and centrifuged at 10.000 rpm for 10 min. The supernatant was completed to a total known volume and used as enzyme source **[23].**

2.3.5.2. Assay of catalase activity

Catalase activity (CAT) was determined spectrophotometrically at 25°C according to the methods described by **[24].** The reaction mixture containing (3ml) of 50mM phosphate buffer, PH 7.0 ,to which 10Mm, 30%(w/v) H_2O_2 was added until reaching an absorbance at 240 nm. The reaction was started by adding the reaction solution to 10µl of crude extract and the activity followed by monitoring the decrease in absorbance at 240 nm as a consequence of H_2O_2 consumption.Catalase activity was expressed as µ mol H_2O_2 destroyed/mg protein/minute.

2.3.5.3. Assay of Peroxidase enzyme

The assay mixture of peroxidase (POX) contained 2.3 ml of 0.1M of phosphate buffer (pH 6.0) at 4°C. The reaction mixture (0.5 ml) consisted of 0.01 M pyrogallol and 0.1 ml of 0.025 M hydrogen peroxide. The addition of 0.1 ml of crude enzyme extract initiated the reaction, which was measured spectrophotometrically at 420 nm. The enzyme activity was expressed as the change in the optical density/mg protein/minute according to **[25]** and **[23].**

2.3.5.4. Assay of Polyphenoloxidase enzyme

Polyphenol oxidase (PPO) assay was performed according to the method described by **[25]** and **[23]** The assay mixture contained 1.5 ml of 0.1 M phosphate buffer (pH 6.0) at 4°C. The reaction mixture (0.5 ml) consisted of 0.01 M pyrogallol. The addition of 1.0 ml of crude enzyme extract initiated the reaction, which was measured spectrophotometrically at 420 nm at 30 s interval for 3 min**.** The enzyme activity was expressed as the change in the optical density/mg protein/minute.

2.3.5.5. Measurement of Superoxide Dismutase activity (SOD)

SOD activity was measured according to **[26]**. Three milliliters of the reaction mixture contained 50mM phosphate buffer (pH 7.8), 0.1 mM EDTA-Na_2, 13 mM methionine, 75 µM nitroblue tetrazolium chloride (NBT), 2 µM riboflavin, and 50 µl of the enzyme extract. Riboflavin was added last, The reaction started by placing the tubes two 40-W fluorescent lamps for 10 min. The reaction was finished by keeping the tubes in the dark for 10 min. The developed purple colour was then measured at 560 nm using a UV/VIS spectrophotometer (PG Instrument, UK). One unit of SOD activity was defined as the corrected amount of enzyme required to result in a 50% inhibition of the rate of NBT reduction measured at 560 nm in comparison with the positive control under the assay conditions described. The activity was expressed as units/ mg protein.

2.3.5.6. RNA extraction and Real-time RT-PCR analysis

RNA was prepared from garlic leaves following the BCP (1-bromo-3-chlorpropane) protocol **[27].** Preparation of first strand cDNA was performed as described by **[28].** Quantitative PCRs were performed on an ABI PRISM® 7300 Sequence Detection System (Applied Biosystems, USA) following the manufacturer's instructions. Amplifications were performed in the presence of SYBR Green (SYBR® GreenER™ qPCR SuperMixes; Invitrogen), and oligonucleotides were purchased from Metabion, Planegg, Germany. For the detection of Alliinase transcripts, primers were 5'-TGACCTCAACACATTCGGTTT -3' and 5'- CGTTTCAAACCCAGAGCAGT -3'. For the detection of ACTIN2 transcripts, primers were 5'-GGTAACATTGTGCTCAGTGGTGG-3' and 5'-GGTGCAACGACCTTAATCTTCAT-3'. The final primer concentration was 200 nM in the reaction mixture. Amplification conditions were 10 min of initial denaturation at 95 ˚C, followed by 40 cycles each of 15 s denaturation at 95 ˚C and 1 min combined annealing and extension at 60 ˚C.

2.3.5.7. HPLC analysis of Alliin production in Allium sativum

Alliin production in irradiated cloves of *Allium sativum* after 15 days of growth. Alliin was quantified by HPLC analysis of methanol extracts; 100 mg fresh leaf materials were directly frozen in liquid nitrogen and homogenized to a fine powder using mortar and pestles and extracted in 500 µl of methanol: water (9:1, v/v). Extracts and cell debris were separated by centrifugation (13000 rpm) for 20 min at 4 ºC. Cell extracts were concentrated in vacuum, and residues were taken up in methanol: water (9:1, v/v). HPLC analysis was performed using a Thermo Scientific Surveyor PlusTM HPLC System (Thermo Scientific Co, USA). The system was completed with PDA Plus detector set at 350 nm. Metabolites and parent compound were separated on Hypersil gold C18 (10µm, 100X, 4.6 mm) columns (Surveyor, Thermo scientific co, USA) using acetonitrile: water (1:1, v/v) as mobile phase

with flow rate of 1 ml/min at 25 °C and injection volume of 20 µl. Metabolites were identified by comparison with reference compound, Alliin (Sigma Aldrich, Germany) peak at a retention time of 130 seconds. The amount of Alliin produced in the leaf samples was quantified based on a standard curve of serial dilutions of Alliin.

2.4. Statistical Analysis

The data were represented as mean ± stander error (SE) of at least three independent experiments. Students t-*test* was use to determine significant differences among the data. Differences were considered significant when $P \leq 0.01$. All statistical analyses were carried out using the Microsoft Excel software.

3. RESULTS AND DISCUSSION

Bulbs of *Allium sativum* that exposed to different doses of γ- radiation were planted and irrigated for 15 days. The length of shoot system germinated bulbs of *Allium sativum* were recorded and measured after 15 days of growth. Exposure of garlic bulbs to γ- radiation caused obvious changes in plant growth. Variable biochemical, physiological, and molecular parameters have been extensively studied.

3.1. Effect of γ-Irradiation on Germination and Growth of *Allium sativum L* Plants

The cloves of *Allium sativum* exposed to different doses of gamma radiation recorded highly significant changes in seed germination. Length of germinated plants decreased by increasing the dose of γ- radiation Fig1. It is obvious that the length of shoot system decreased with increasing the γ-radiation dose. The results of the plant length analyses revealed that exposure to gamma radiation have negative effects on the germination rates and plant growth in garlic. The tallest plants were observed for plants resulted from bulbs that were exposed to the low doses of γ- rays (10, 20, 30 and 40 Gy). Extra doses of gamma radiation led to sharp decrease in the plant height, by increasing radiation dose to 50, 70 and 100 Gy. The maximum deceased in plant height was observed when cloves were exposed to 120 and 150 Gy. Above 150 Gy, a complete inhibition in plant growth was obvious. Exposure to low doses of γ- radiation of (10, 20, 30 and 40 Gy) caused 11.36%, 14.34%, 15.88%, and 23.01% reduction; respectively in total plant height. Gamma radiation doses of 50, 70 and 100 Gy show a reduction of 33.72%, 39.57%, and 45.94; respectively in the total plant height. Higher doses of γ- radiation (i.e. 120 and 150 Gy) led to significant decrease in the total shoot length by 56.29% and 57.14%; repetitively when compared to untreated control plants (Fig1). γ-rays are a part of electromagnetic spectrum belonging to ionizing radiation with energetically charged particles, such as electrons, or high-energy photons [14]. The biological effect of gamma rays is based on the interaction with atoms or molecules in the cell, particularly with water to produce free radicals. These radicals can damage or modify important components of plant cells and have been reported to affect differentially the morphology, anatomy, biochemistry and physiology of plants depending on the irradiation level. These effects include changes in the plant cellular structure and metabolism, e.g., dilation of thylakoid membranes, alteration in photosynthesis, modulation of the antioxidative system and accumulation of phenolic compounds **[15].** The effects observed after exposure were deeply influenced by several factors, some related to plant characteristics (e.g., species, cultivar, stage of development, tissue architecture and genome organization) and some related to radiation features (e.g., quality, dose, duration of exposure) **[17]**.The irradiation of seeds with high doses of gamma rays disturbs the synthesis of protein, hormone balance, leaf gas-exchange, water exchange and enzyme activity **[29].** Some authors refer to the concept of hormesis, the stimulation of different biological processes (e.g., faster germination, increased growth of roots and leaves, that accurse when seeds are subjected to pre-irradiation with low doses of a radiation source **[30].** The stimulatory effects of γ-rays on germination may be attributed to the activation of RNA synthesis as observed in castor bean (*Ricinus communis L*.) **[31].** The inhibition of seed germination at high doses could be due to the damage in seed tissue, chromosomes and subsequent mitotic retardation and the severity of the damage depend on the doses used [30]. In contrast, the growth inhibition induced by high-dose irradiation has been attributed to the cell cycle arrest at the G2/M phase during somatic cell division and (or) varying damage to the entire genome **[32].** The relationship between growth of irradiated plants and the dose of γ-irradiation has been manifested by investigating the morphological changes and seedling growth of irradiated plants. Growth inhibition by γ-irradiation in the current study may be related to auxin and DNA biogenesis.

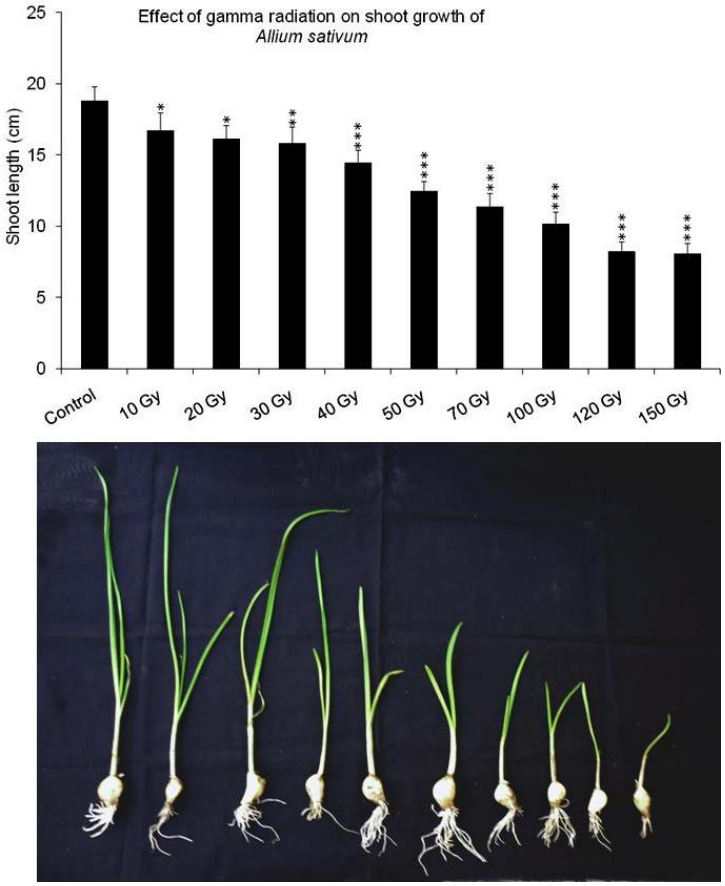

Fig1. *Effect of γ- radiation on shoot growth of Allium sativum upon exposure to different doses of gamma radiation. Vertical bars represent standard error. Data are means of three independent measurements ± SE. *, P < 0.05; **, P < 0.01 and ***, P < 0.001 according to Student's t-test.*

3.2. Effect of Gamma Radiation on Chlorophyll Content

The bioassay results, as illustrated in Fig2, show clear differences in chlorophyll A, B and carotenoids in the leaves of garlic seedlings resulted from bulbs that were exposed to different doses of γ-radiation. There is a gradual decrease in the measured chlorophyll contents. Higher doses of γ-radiation cause a great reduction in chlorophyll contents in *Allium sativum*. It is obvious that total chlorophyll pigments and carotenoid pigments were decreased with increasing the doses of γ-radiation. All the irradiated plantlets exhibited the amount of chlorophyll content as compared to the non-irradiated plantlets. Exposure to low doses of γ-radiation at 20 Gy caused 19.31% and 11.55% reduction in both chlorophyll A and B; respectively when compared to untreated control plants. However, higher doses of γ- radiation at 120 Gy led to sharp decrease in chlorophyll A and B by ~ 64.04% and 58.48; respectively. Carotenoids content of garlic plant also show subsequent decrease as the doses of γ-radiation increased. The lowest level of total carotenoids was obtained in garlic seedlings irradiated with 120 Gy, hence the carotenoids contents decreased by 73.70%. Exposure to low doses of γ-radiation (i.e. 20 Gy) caused only a reduction of 18.96% in the total carotenoids contents. These results indicate that pigments fractions were decreased regularly by increasing the dose of gamma. The chlorophyll content showed irregular distribution among the irradiated plantlets. It can be observed that the concentration of chl. A was relatively higher than chl. B in both irradiated and non-irradiated plantlets. It has been reported that γ-irradiation resulted in greater reduction in the amount of chlorophyll B as opposed to chlorophyll A **[33].** These effects include changes in the plant cellular structure and metabolism [38]. Plastids were affected by irradiation in two ways: (i) inhibition of senescence and (ii) dedifferentiation into a granal stage **[14].** The developmental regression of chloroplasts can be assumed primarily from destruction of grana **[34].** The irradiation of seeds with high doses of γ- rays disturbs the synthesis of protein, hormone balance, leaf gas exchange, water exchange, and enzyme activity as previously reported **[29].** Higher γ-irradiation inhibits chlorophyll

synthesis in wheat **[16].** Therefore, it can be concluded that the decrease in pigment fractions in irradiated plants is due to the destructive effects of γ-radiation on DNA and protein synthesis in irradiated *Allium sativum* plantlets.

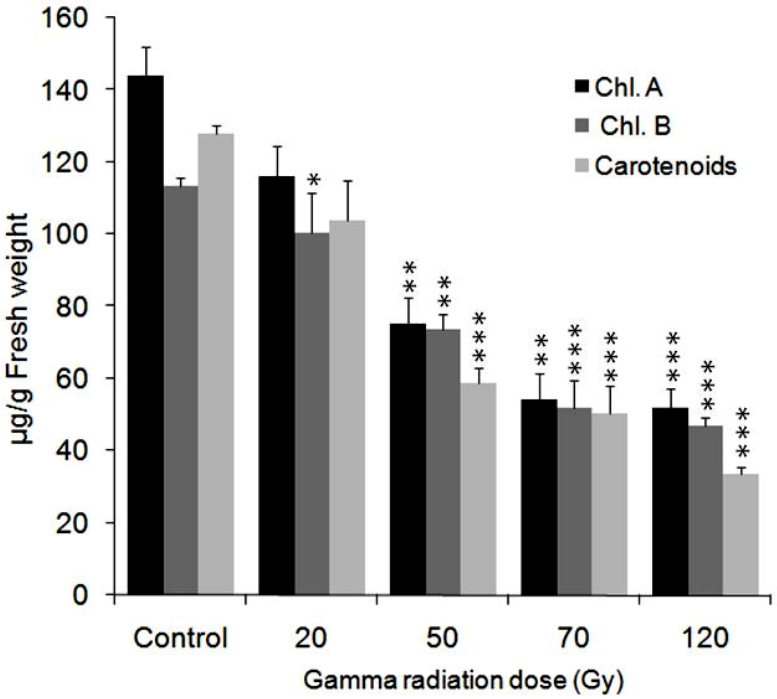

Fig2. *Effect of γ-radiation on Chl. A, Chl. B, and Carotenoids content (µg/g fresh weight) in Allium sativum. Data are means of three independent measurements. Vertical bars represent standard error. (*), (**) and (***) represent statistically significant differences when compared with untreated control at p < 0.05, at p < 0.01, and at p < 0.001 levels; respectively.*

3.3. Effect of γ- Radiation on Total Carbohydrate Content

The bioassay results represented in Fig3B show clear differences in total carbohydrate contents measured from garlic plantlets after exposure to different doses of gamma radiation. The results of carbohydrate analyses revealed that γ-radiation doses have negative effects on the biosynthetic rates of carbohydrate in garlic plant. Exposure to low doses of γ-radiation of 20 Gy caused 7.08% reduction in total soluble carbohydrate contents. **γ-**radiation of 50 Gy and 70 Gy show a reduction of 24.68% and 33.76%; respectively. Higher dose of gamma radiation (i.e. 120 Gy) led to a significant decrease in the total carbohydrates contents by 56.26% compared to untreated control plants. The maximum amount of total carbohydrate contents was recorded at 20 Gy of **γ-**radiation and the minimum amounts were recorded at 120 Gy of gamma radiation. These findings support the hypothesis that gamma radiation stress cause a decrease in plant growth and in turns the total carbohydrates biosynthesis. Total carbohydrate contents decreased with increasing the dosage of γ-irradiation because of its effect on enhancing the metabolic activities of hydrolyzing enzymes in germinating bulbs. Based on previous research reports, the total protein and carbohydrate contents decreased with increasingly higher dosage of γ-irradiation caused by higher metabolic activities and hydrolyzing enzyme activity in germinating seed **[35].** Radiation resulted in the increased absorption of glucose, pyruvate, and the decreased absorption of acetate and succinate in carrot (*Dacus carrota L.*). γ-irradiation breaks the seed protein and produces more amino acids **[36].** It was reported that total proteins and carbohydrates decreased with increasing γ-ray dosage in wheat and rice plants **[37].** Al-Jassir found that the contents of arginine, methionine, lysine, phenylalanine and leucine of garlic bulbs (*Allium sativum L.*) increased slightly. However, reduction in other amino acids in irradiated samples also occurred, especially at higher doses **[38].** Consequently, it can be concluded that the decrease in total carbohydrate contents in garlic plantlets is due to the induction of hydrolyzing enzymes as a result of γ-radiation.

3.4. Effect of γ-Radiation on Proline Content

Different doses of gamma radiation support the biosynthesis of proline in *Allium sativum L* as indicated in Fig3A. The results of the proline analyses revealed that γ-radiation has positive effects on the biosynthetic rates of proline in garlic plant. **γ-** radiation increased significantly the accumulation

of proline in *Allium sativum* at almost all γ- radiation doses. Exposure to low doses of γ- radiation of 20 Gy shows induction in total proline content, higher dose of gamma radiation (i.e. 70 Gy) led to a significant increase in the total proline contents by compared to untreated control plants. The maximum amount of total proline contents was recorded at 70 Gy of gamma after that the total proline contents decreased. **γ-**radiation dose of 120 Gy show an increase in the total proline contents but still higher than untreated control plants. The overall effects indicate that gamma radiation induces stress in garlic cells with the elevation of proline contents. Gamma radiation was reported to induce oxidative stress with overproduction of reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radicals and hydrogen peroxide, which react rapidly with almost all structural and functional organic molecules, including proteins, lipids and nucleic acids causing disturbance of cellular metabolism **[39].** To avoid oxidative damage, plants have evolved various protective mechanisms to counteract the effects of reactive oxygen species in cellular compartments **[36].** Proline is a compatible osmolyte and it may interact with enzymes to preserve enzyme structure and activities. Indeed, proline has been shown to reduce *In vitro* enzyme denaturations caused due to heat, NaCl stress, and gamma radiation stress **[40].**

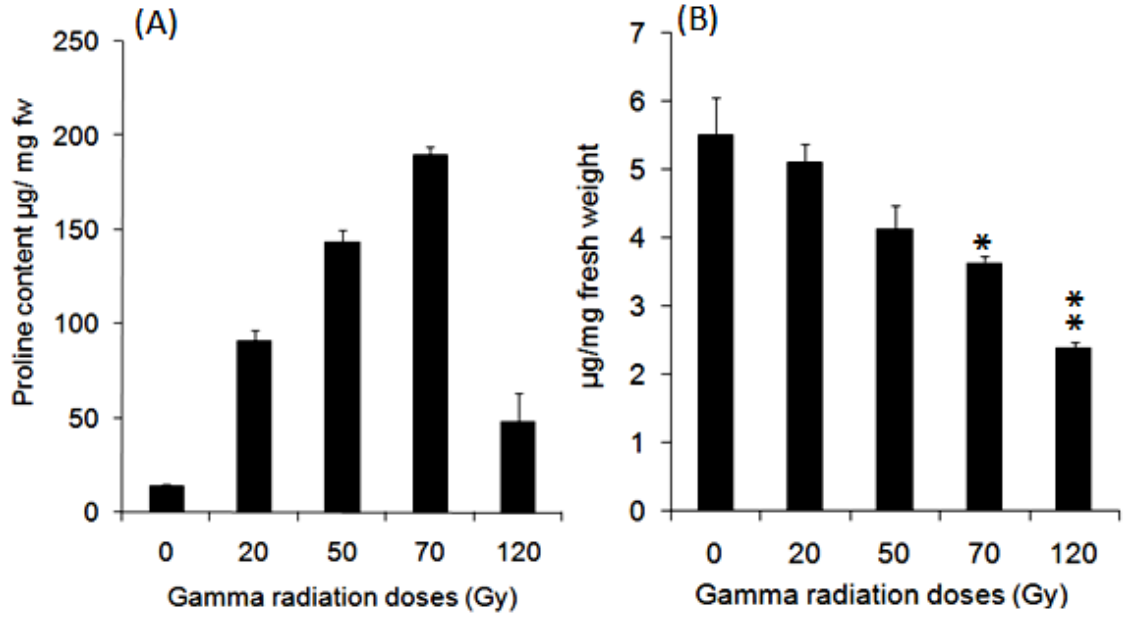

Fig3. *Effect of Gamma radiation on total proline contents (A) and total Carbohydrate contents (B) in Allium sativum L. Data are means of three independent measurements. Vertical bars represent standard error. (*) and (**) represent statistically significant differences when compared with untreated control plants at $p < 0.05$, and $p < 0.01$ levels; respectively.*

3.5. Effect of Gamma Irradiation on the Antioxidant Enzymes Activity

Different doses of gamma radiation support the biosynthesis of antioxidant enzymes in *Allium sativum* L. Gamma radiation causes a dramatic variation in the activity of the different antioxidant enzymes Catalase (CAT), Peroxidase (POD), Polyphenol oxidase (PPO), and Superoxide dismutase (SOD). Different doses of gamma radiation support the increase in the activity of catalase in *Allium sativum L* and showed highly significant changes in total catalase activity as indicated in Fig4. Irradiation dose at 10 Gy to 70 Gy enhance the catalase activity of *Allium sativum L* plants. The maximum activity was recorded in Irradiation dose of 70 Gy compared with corresponding control. After irradiation dose 70 Gy, the activity of catalase enzyme declined. The maximum amount of total catalase activity was recorded at 70 Gy of γ-radiation and the minimum amounts were recorded at 150 Gy of gamma radiation.

Similar effects were observed after measuring the peroxidase activity in irradiated garlic plantlets. The maximum peroxidase activity was recorded in at irradiation dose of 100 Gy compared with corresponding control. After irradiation dose 100 Gy, the activity of peroxidase enzyme declined.

Exposure to γ-irradiation cause changes in biochemical activity through various metabolites produced as function of γ-irradiation. The most important of these metabolites are peroxyl radicals. The radiation protective effect of peroxidase is due to removal of H_2O_2, peroxides, and especially lipid hydrogen peroxides. This accounts for the greater effectiveness of peroxidases than catalase **[41]**. The induction of POD by the irradiation would be one of the defense systems activated through the ROS-mediated cellular signaling **[42]**. It was suggested therefore that the increase in gamma doses lead to an increase in the specific activity of peroxidase enzyme **[43].**

The results of the PPO activity measurements illustrated in Fig4 revealed that doses of gamma radiation increased the activity of polyphenol oxidase in garlic plants. The maximum activity was recorded at irradiation dose of 70 Gy compared with corresponding control. After irradiation dose 70 Gy, the activity of PPO enzyme declined. Jimenez et al, found that green onion exposure to γ-radiation produces slight increases in the polyphenol concentrations **[44].** On the other hand, other studies indicated no significant differences in the residual PPO activity after the radiation dose **[45, 46].**

Different doses of gamma radiation support the activity of superoxide dismutase in *Allium sativum* L as indicated in Fig4. The maximum activity was recorded in irradiation dose at 70 Gy compared with corresponding control. After irradiation dose 70 Gy, the activity of superoxide dismutase enzyme declined. The minimum SOD activity was recorded at 150 Gy of γ-radiation.

Superoxide dismutase (SOD) isozymes are compartmentalized in higher plants and play a major role in combating oxygen radical mediated toxicity. Cells exhibiting high levels of SOD, catalase, and peroxidase activity are relatively less vulnerable to secondary effects of radiation. Since superoxide is sufficiently stable to permit diffusion within the cell. It is possible that it acts as an electron donor to transition metals in irradiated tissue and may account for sensitization of cells to the effects of H_2O_2 by radiation exposure and the protection afforded by SOD to irradiating cells **[47].** Significant differences in the changes in the two antioxidant enzymes activities were noted according to the developmental stage. Superoxide dismutase activity was conversely increased from 2.9 U/mg protein (non-irradiation) to 11.3 U/mg protein (800 Gy treatment) at the reproductive stage **[48]**. However, SOD levels of irradiated plants were lower than non-irradiated plants at vegetative stage. The activity of free radical scavenging enzymes peroxidase, catalase and superoxide dismutase showed inverse relationships with ageing period and direct proportion to reductions in the seed germination level**[49].** A major, direct target of γ-irradiation that is probably the most important one is the water molecule, which is omnipresent in organisms. The primary reactions are excitation and ionization, which produce ionized water molecules ($H_2O^{\cdot+}$) and the radicals $H^{\cdot}$ and $^{\cdot}OH$. However, in biological tissue these ionizations are induced all along the path of the radiation and lead to chain reactions, which produce secondary reactive oxygen species (ROS) as a result of $H^{\cdot}$ and e_{aq}^{-} becoming trapped. The most important ROS is H_2O_2; singlet O_2^{-} is produced to very low extent, depending on the O_2 concentration **[50]**. The $^{\cdot}OH$ radical can react rapidly with various types of macromolecules, including lipids, proteins and, in particular, DNA. However, some of the resulting injuries can be readily repaired and recovered, depending on the dose range. One result of oxidative stress is cellular damage by hydroxyl radical attack. The amount and rate of hydroxyl radicals generation from ROS is controlled partly by the cellular antioxidant status and partly by the availability of systems capable of reducing (or activating) superoxide or hydrogen peroxide. It has been shown that the effects of H_2O_2 resemble those of ionizing radiation **[51].** Therefore, the increased level of antioxidant enzymes in *Allium sativum* L plantlets is strongly dependent on the gamma irradiation doses.

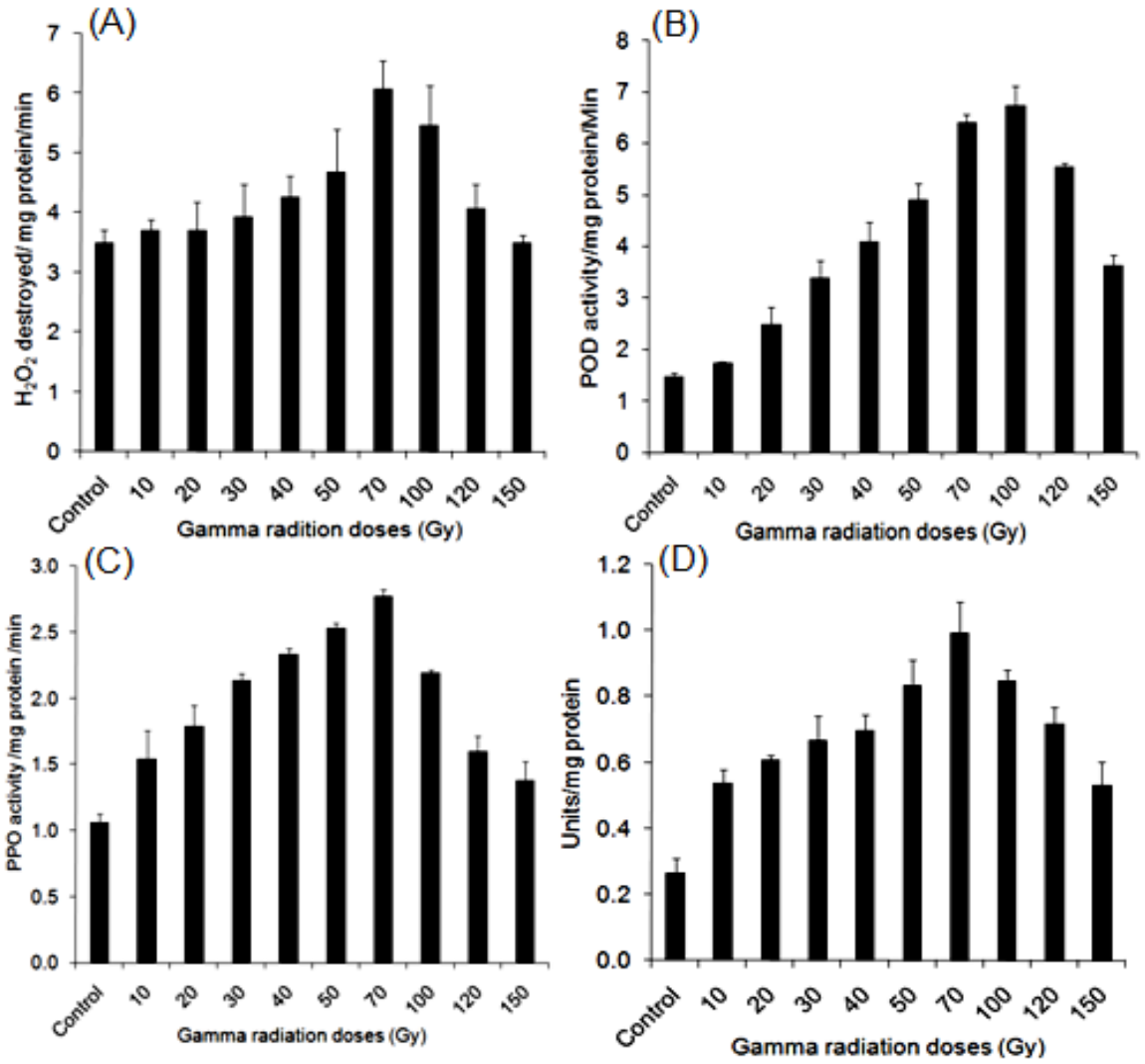

Fig4. *Effect of gamma radiation on the activity of Catalase (A), Peroxidise (B), Polyphenol oxidase (C), and Superoxide dismutase (D) in Allium sativum. Catalase (CAT), Peroxidase (POD), Polyphenol oxidase (PPO), and Superoxide dismutase (SOD) enzymatic activities measured from Allium sativum exposed to different doses of gamma radiation. Data are means of three independent measurements. Vertical bars represent standard error.*

3.6. Quantitative Real-time RT-PCR Analysis of Alliinase Transcripts

Alliinase is the basic enzyme involved in Alliin biosynthetic pathway in galic plants. Thus, the Alliinase gene expression was tested in *A. Sativum L* plants generated from garlic bulbs that was irradiated with variable doses of gamma radiation (i.e. 20, 50, 70, and 120 Gy). For this purpose, Alliinase mRNA transcript accumulation was measured by Real-Time RT-PCR as shown in Fig5. The amount of Alliinase signal in the different RNA preparations was standardized for the abundance of the transcript from the house keeping gene, *Actin2*. Variable amounts of Alliinase signals in the different RNA preparations isolated from the *A. Sativum L* plants were observed based on the level of gamma irradiation doses. It was obvious that gamma irradiation has inhibitory effects on Alliinase gene expression. Interestingly, the expression level of Alliinase gene was correlated with the strength of gamma radiation doses (Fig5). The highere levels of Alliinase expression were observed at low irradiation doses (i.e. 20 and 50 Gy) whereas the maximum inhibition of Alliinase gene expression was obsreved to be at 120 Gy. Similar approaches have been performed to study the effect of heavy metals like copper on photosynthetic related gene transcripts in some algae species **[52]**. The authors observed a great reduction in phtosynthetic related gene transcripts upon exposure to heavy metal stress. Based on these findings, it can be concluded that Alliinase gene expression is decreased by increasing the gamma irradiation dose.

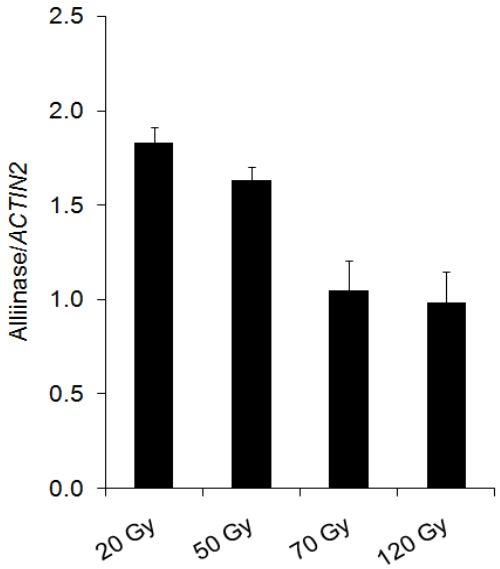

Fig5. *Reverse transcriptase RT-PCR analysis of Alliinase gene expression in A. sativum L plants.*

3.7. HPLC Analysis of Alliin Production in Allium Sativum L Plants

The amount of Alliin produced by *A. sativum* plants generated from irradiated bulbs was estimated by HPLC quantification in excised plant leaves after 15 days of growth. According to HPLC analysis, Alliin was eluted at 130 seconds after sample injection as shown in (Fig6A). The amounts of Alliin in plant leaves were quantified based on a standard dilution series of Alliin reference sample (Fig6B). The HPLC analysis show that the amount of Alliin produced in plants is strongly decreased by increasing the gamma irradiation dose when compared to the untreated control plants (Fig6C). These results together with the R-PCR results indicate the clear correlation between Alliinase expression and Alliin production in irradiated plants. These results provide clear evidence that gamma irradiation has negative impact on both biochemical and molecular levels of *Allium sativum L* plants. Moreover, increasing gamma irradiation doses will definitely affect crop yield worldwide. The decreased Alliin levels in correlation to the decreased level of Alliinase gene transcripts reflects the negative impact of gamma irradiation on *Allium sativum L* bulbs prior to cultivation. Under the currently growing climatic changes and the global warming events, further studies have to be performed in order to develop new technologies for protecting plants from the destructive actions of solar radiations.

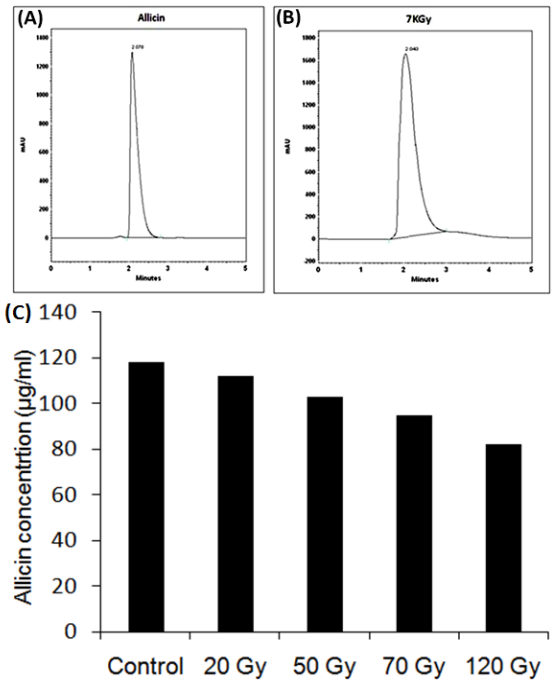

Fig6. *HPLC analysis of Alliin production in A. sativum L plants.*

A) representative chromatogram of Alliin reference sample, B) representative chromatogram of HPLC analysis of Alliin from Allium sativum L plant leaves extracts isolated from irradiated bulbs with 70 Gy gamma, and C) Alliin amounts produced in Allium sativum L plants at all gamma irradiation doses.

4. CONCLUSION

The results of the current study indicated the negative effects of gamma radiation on biochemical and molecular characteristics. Gamma radiation increased the level of antioxidant enzyme system, proline contents. However, it causes a great reduction in plant growth, chlorophyll contents, and Alliin levels in irradiated *Allium sativum L* plantlets.

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AUTHORS' BIOGRAPHY

Rashad Kebeish (Ph.D)

- Ph. D in Plant Molecular Biology from RWTH-Aachen Germany 2006.
- Assistant Professor degree in Plant Molecular Biology and plant Physiology from Zagazig University, Egypt 2012.

Permanent Affiliation:

Botany Department, Faculty of Science, Zagazig University, Egypt

Current Affiliation: Faculty of Science, Yanbu, Taibah University, KSA

Hanan, E. Deef (Ph.D)

- Ph.D in Plant Physiology from Zagazig University, Egypt.
- Assistant Professor degree in Plant Physiology from Zagazig University, Egypt.

Affiliation: Botany Department, Faculty of Science, Zagazig University, Egypt

Nagwa El-Bialy (M.Sc)

- MSc in Plant Ecology from Zagazig University, Egypt

Affiliation: Botany Department, Faculty of Science, Zagazig University, Egypt