The Pharma Innovation Journal 2020; 9(11): 87-91

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2020; 9(11): 87-91

www.thepharmajournal.com
Received: 16-09-2020
Accepted: 19-10-2020

Thokchom Ronald Meitei
Department of Horticulture, Pandit Deen Dayal Upadhyay Institute of Agricultural Sciences, Utlou, Bishnupur, Manipur, India

Rocky Thokchom
Department of Horticulture, Pandit Deen Dayal Upadhyay Institute of Agricultural Sciences, Utlou, Bishnupur, Manipur, India

Thoudam Bhaigyabati
Department of Botany, Pravabati College, Mayang Imphal, Manipur, India

Akoijam Merina Chanu
Department of Horticulture, Pandit Deen Dayal Upadhyay Institute of Agricultural Sciences, Utlou, Bishnupur, Manipur, India

Corresponding Author:
Thokchom Ronald Meitei
Department of Horticulture, Pandit Deen Dayal Upadhyay Institute of Agricultural Sciences, Utlou, Bishnupur, Manipur, India

Response of radioactive cobalt (^{60}Co) teletherapy on M_1 plants of *Capsicum chinense* Jacq

Thokchom Ronald Meitei, Rocky Thokchom, Thoudam Bhaigyabati and Akoijam Merina Chanu

DOI: https://doi.org/10.22271/tpi.2020.v9.i11b.5320

Abstract
In the present investigation, the seeds of *Capsicum chinense* (Jacq.) were irradiated with different doses of gamma rays (0, 10, 25, 50, 75 and 100 Gy) using ^{60}Co source for studying the effect of different dose of gamma rays as induced mutagen on physiological, morphological, anatomical and biochemical parameters during seed germination and seedling growth. Results revealed that, the non-irradiated seeds (Control) performed better than those of the irradiated ones for few parameters such as germination percentage, vigour index and chlorophyll content. In case of total phenolic content, leaf area index and leaf relative water content, irradiated plants showed better results. Stomata count was found maximum when the seeds were treated at a dose of 25 Gy and 50 Gy respectively.

Keywords: *Capsicum chinense*, Gamma ray, ^{60}Co

1. Introduction

Capsicum chinense Jacq., locally known as U-morok in Manipuri, is a semi-perennial herb belonging to the family Solanaceae. It is one of the globally fame highly pungent chilli landraces of Manipur (Sanatombi *et al*. 2010) [1]. It is considered as India's hottest chilli and was previously acknowledged as the world's hottest chilli having Scoville Heat Units (SHU's) rating of 1,001,304 (Bosland and Baral 2007) [2]. The species of the genus *Capsicum* have vast variability in its main morphological characters such as form, size, colour and position of flowers and fruits. Narrow genetic diversity is a main problem restricting the progress of breeding in *Capsicum chinense*. One way to improve genetic diversity of plant is through mutation (Kusmiyati *et al.*, 2017) [3]. In this light, mutation breeding in crop plants is an effective tool in hands of vegetable breeders especially in crops having narrow genetic base such as *Capsicum chinense*. The irradiation of seeds may cause genetic variability that enable plant breeders to select new genotypes with improved characteristics such as precocity, salinity tolerance, yield and quality (Ashraf, 2009) [4]. It is an important supplementary approach to crop improvement (Mudibu *et al*., 2012) [5]. Mutation breeding is one of the conventional breeding methods in plant breeding. It is related with various fields like, morphology, cytogenetic, biotechnology and molecular biology etc. Induced mutations are effectively used in improving the quality of natural genetic resources and have been applied in developing improved cultivars of cereals, fruits and other crops (Lee *et al*., 2002) [6]. Induced mutation is highly instrumental in plant biology to induce genetic variability in a great number of crops. The technology is simple, relatively cheap to perform and equally usable on a small and large scale (Siddiqui and Khan, 1999) [7].

Exposure of plants to radiation has both direct and indirect effects on seed germination, plant growth and reproduction through the change in both cellular and tissue structure or genetic aberration which leads to different phenotypic development (De Micco *et al*., 2011) [8]. Several researches on the stimulative effects when using gamma rays as induced mutagens were reported. Recently, low doses of gamma-rays of seed treatment resulted significant effects on germination, shoot and root systems of Chilli (Omar *et al*., 2008) [9], Wheat (Melki and Marouani, 2010) [10], Physic nut (Sangsiri *et al*., 2011) [11], Rice (Harding *et al*., 2012) [12], Maize (Marcu *et al*., 2013) [13], Pigeon pea (Neelum *et al*., 2014) [14], Chick pea (Umavathi and Mullainathan, 2015) [15], Lathyrus (Beyaz *et al*., 2016) [16], Castor bean (Lopes *et al*., 2017) [17], Soybean (Kusmiyati *et al*., 2017) [3]. However, the impact of gamma rays on *Capsicum chinense* Jacq. is still mystery. The major objective of this research is to investigate effect of different dose of gamma rays as induced mutagen on physiological, morphological, anatomical and biochemical markers during seed germination and seedling growth of *Capsicum chinense* Jacq.

2. Materials and methods

Healthy seeds were collected from KVK, Bishnupur. The seeds were irradiated with gamma rays using ^{60}Co source at the Department of Radiology and Cancer Centre, Regional Institute of Medical Sciences (RIMS), Imphal. Different doses were applied with non-radiated seeds taken as control. Each dose comprise of 100 seeds. After irradiation, the seeds were kept at room temperature for 24 hours. A greenhouse experiment was conducted at Horticulture Farm, PDDUIAS, Utlou by sowing the seeds of different doses in polybags which are arranged in a Completely Randomized Block Design (CRD) with three replicates. The experimental field lies in 24.71°N latitudes and 93.84°E longitudes. It is situated at an altitude of 820 m above mean sea level. All the pots will be placed equally spaced and filled with equal soil contents comprising of sand, clay, compost and cocopeat in the ratio of 1:1:1:1. The following procedures were followed for recording the observations:

2.1 Leaf area index: Canopy area were measured by using digital camera image processing software as suggested by Easlon *et al.* (2014) [18].

2.2 Stomata count: Stomata count was carried out by collecting the fresh leaf sample of treated and untreated plants and washed them gently under running tap water to remove the dust and the debris. The number of stomata present in the sample was counted by using the methodology of Paul *et al.* (2017) [19].

2.3 Leaf relative water content (Barr & Weatherley, 1962) [20]: Each leaves between 4 to 6 samples (replications) was taken from a single treatment. Leaf discs were cut from the leaves, to obtain a total of about 5-10 cm^2/sample. Avoid large veins and leaf discs should be large enough (around 1.5 cm in diameter) so as to reduce the area of cut leaf surface/sample. Each sample was placed in a pre-weighed airtight (possibly also oven proof) vial. Vials should be immediately placed in a picnic cooler (around 10 ^{0}C-15 ^{0}C) but not frozen on ice. The samples should reach the lab as soon as possible. In the Lab vials were weighed to obtain leaf sample weight (W), after which the sample were immediately hydrated to full turgidity for 3-4hr under normal room light and temperature. Leaf discs and small leaflets were usually hydrated by floating on de-ionized water in a closed petri dish. After hydration the samples were taken out of water and were well dried of any surface moisture quickly and lightly with filter/tissue paper and immediately weighed to obtain fully turgid weight (TW). Samples were then oven dried at 80^{0}C for 24hr and weighed (after being cooled down in a desiccator) to determine dry weight (DW).

Calculation

RWC (%) = [(FW-DW) / (TW-DW)] x 100,
Where,
FW – Sample fresh weight
TW – Sample turgid weight
DW – Sample dry weight.

2.4 Extraction of Chrorophyll (Porra 1989[21], Lichtenthaler 1987[22] and Lichtenthaler and Wellburn 1983[23]): The fresh plant leaf sample of 0.5g were measured precisely and taken. It was homogenized in mortar and pestle with 10 ml of 80% acetone as extractant solvent. The homogenized sample mixtures of different treatments were centrifuged at 10,000 rpm for 15 minutes at 4. After that, 0.5 ml of the supernatant were taken and mixed with 4.5 ml of the solvent. The solution mixtures were analyzed for chlorophyll a (Chl a) and chlorophyll b (Chl b) by observing the absorbance at two different wavelengths using spectrophotometer (Thermo Fisher UV 2700) as per the equation given below (Porra 1989, Lichtenthaler 1987 and Lichtenthaler and Wellburn 1983) [21-23].

Solvent	Equations
80% Acetone	Chl a = 12.7(A_{663}) - 2.69 (A_{645})
	Chl b = 22.9 (A_{645}) – 4.68 (A_{663})

2.5 Phenolic content: The total phenolic content was determined using Folin - Ciocalteu reagent (Spanos GA and Wrolstad RE 1990) [24] in aqueous leaves extract of untreated and treated gamma irradiated plants of *C. chinense* . 2.5ml of 10% Folin - Ciocalteu reagent and 2ml of Na_2CO_3 (2% w/v) were added to 0.5 ml of (3 replicates) extract solution (1mg/ml). The resulting mixture was incubated at 45˚C for 15 mins. The absorbance was measured at 760 nm UV Visible Spectrophotometer (UV-2700). Gallic acid (10-50 µg/ml) was used as a standard compound.

Total phenolic contents of the plant extracts in Gallic Acid Equivalent (GAE) were calculated by using the following formula (Chakraborthy GS and Ghorpade PM, 2010) [25]:

T = (C x V) / M

Where,
T = total content of phenolic compound, mg/g plant extract in GAE;
C = concentration of gallic acid established from the calibration curve, µg/ml;
V = volume of extract, ml;
M = weight of the plant extracts, g.

3. Results and Discussion

Table 1: Effect of gamma irradiation on *Capsicum chinense*

Doses	Leaf area index (cm^2)	Stomata count (mm^{-2})	LRWC (%)	Total phenolic content (mg/g)
0 Gy	19.45 ± 0.81	23.00 ± 3.61	65.06 ± 6.40	7.87 ± 0.08
10 Gy	10.80 ± 1.27	20.33 ± 2.08	69.27 ± 3.92	10.83 ± 3.35
25 Gy	15.04 ± 2.16	23.33 ± 10.07	71.60 ± 4.28	14.01 ± 2.25
50 Gy	10.70 ± 1.00	23.33 ± 4.93	70.97 ± 3.49	27.24 ± 0.84
75 Gy	19.68 ± 0.42	19.00 ± 3.61	68.90 ± 1.13	14.09 ± 0.69
100 Gy	15.49 ± 5.93	23.00 ± 2.65	67.20 ± 7.61	10.70 ± 0.61
CD (0.05)	4.78	9.27	8.77	3.06
CV%	0.176	0.023	0.071	0.122

Values are taken in triplicates and expressed as mean ± SD

3.1 Leaf area index
The mean leaf area index of treated and untreated plants is shown in Table 1. Results show that different doses of gamma irradiation had a significant effect on leaf area index of *Capsicum chinense*. The maximum leaf area index was observed in 75 Gy (19.68 cm^2) which is statistically at par with the non-irradiated plants (19.45 cm^2), 100 Gy (15.49 cm^2) and 25 Gy (15.04 cm^2) irradiated plants. Least leaf area index (10.70 cm^2) was observed at 50 Gy treated plants.
The leaf areas were found to reduce in other irradiated plants when compared to control. Çelik *et al.* (2014) [26] observed reduction of leaf area in the irradiated soybean plants by an average of 98.86% and 98.00%, compared to control, at 14 and 21 days after irradiation.

3.2 Leaf relative water content (RWC)
The relative water content of the leaf had a non-significant effect and was found higher in irradiated plants when compared to control (Table 1). With increase in the dose from 10Gy to 25Gy the RWC was found to increase and then gradually decrease as the dose increase from 25Gy to 100Gy. Similar results were also reported by Yamasaki and Dillenburg (1999) [27] where the water-stressed plants showed a decreased in RWC by 12% after 48 hours imbibition period while the control plant have 6% decreased in RWC. The difference in decreased RWC for control and stressed plant might be related to both physiological and structural changes that typically occurred in leaves of stressed plant (Vance and Zaer, 1991) [28]. The decreased RWC in treated plants with increase in dose in our study may be due to gamma radiation which might have caused physiological and structural changes in the treated plant's leaves.

3.3 Stomata count
The stomata count (mm^{-2}) of irradiated and non-irradiated plants had a non-significant effect (Table1). However, maximum number of stomata (23.33) was observed in 25Gy and 50Gy treated plants; while the least (19.00) stomata count was observed at 75 Gy treated plants. The stomata count were clearly inconsistent (decrease or increase) with increase in gamma radiation doses. In contrary, Celik *et al.* (2014) [26] observed that soyabean plant leaves developed from gamma irradiated seeds showed reductions in stomatal count compared to the control plant. The function of stomata is correlated with process of transpiration and photosynthesis that occurred in the leaves (Peksen *et al.* 2006) [29]. The higher density of stomata in 25 Gy and 50 Gy allows the higher gas exchange; consequently, the rate of photosynthesis is higher than control. The higher photosynthesis supports the plant growth. Similar result was observed by Widiastuti *et al.* (2010) [30] in *Garcinia mangostana*.

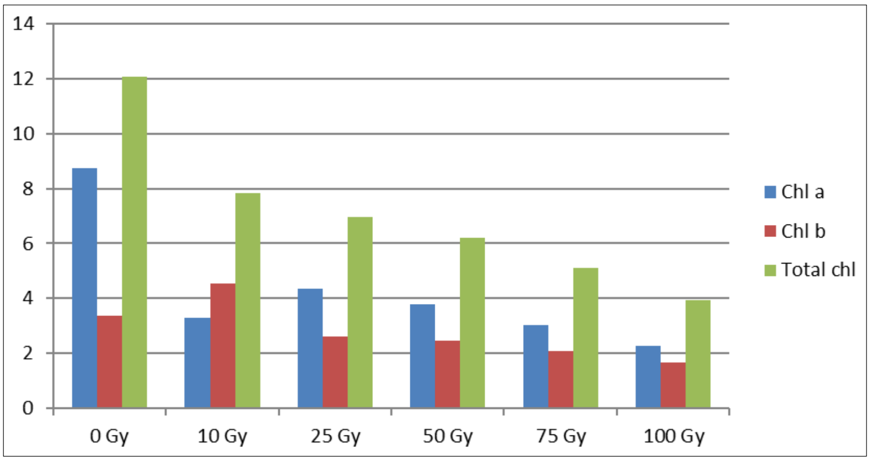

Fig 1: Effect of gamma irradiation on chlorophyll content in the leaves of *C. chinense*

3.4 Chlorophyll content
The chlorophyll content of the irradiated and non-irradiated plants is given in Figure 1. Content of Chlorophyll a and total chlorophyll content in the leaves had significant effect, whilst chlorophyll-b content had non-significant effect. Maximum Chlorophyll a (8.73 µg/g) and total chlorophyll (12.09 µg/g) content was observed in non-irradiated plants. Meanwhile, maximum (4.55 µg/g) chlorophyll-b content was observed in the plants irradiated at 10 Gy. Least chlorophyll content in the leaves was obtained from those plants treated at highest irradiation dose (100 Gy). Chloroplasts being extremely sensitive to gamma radiation compared to other cell organelles may be the reason for decreasing in the overall chlorophyll content. Similar findings were reported by Wi *et al.* (2007) [31]. Kiong *et al.* (2008) [32] also reported that the reduction in chlorophyll-b is due to a more selective destruction of chlorophyll-b biosynthesis or degradation of chlorophyll-b precursors. Furthermore, Kim *et al.* (2004) [33] have evaluated the chlorophyll content on irradiated red pepper plants and showed that plants exposed at 16 Gy may have some significant increase in their chlorophyll content that can be correlated with stimulated growth.

3.5 Total phenolic content
Total phenolic content had a significant effect and the data has been presented in Table 12 and Figure 11. Highest concentration (27.24 mg/g) of total phenol content was observed in 50 Gy which is significantly higher than other irradiated and non-irradiated plants. When compared to control, the total phenolic content of the irradiated group was more or less higher. Similar results were reported by Behgar *et al.* (2011) [34]; Harrison and Were (2007) [35]; Kim *et al.* (2008) [36], where irradiated plants showed a higher total phenolic content than the control. De Toledo *et al.* (2007) [37] found that the effects of ionizing irradiation on phenolic compounds are dose dependent. Universally, gamma radiation studies have shown different effects that were attributed to the different phenolic compounds present in the various plant materials. Some of the materials have appreciable amounts of hydrolysable compounds, which may be more susceptible to

gamma-irradiation compared to the condensed compounds present in other product. Kim *et al.* (2009) [38] also found a non-significant increase in the phenolic content in irradiated cumin. Variyar *et al.* (2004) [39] found increased amounts of phenolic acids in irradiated cloves and nutmeg.

4. Conclusion

From the result of irradiation of seeds using radioactive Cobalt (^{60}Co) on *Capsicum chinense*, it can be concluded that gamma rays are capable in inducing damage to plants at molecular level and is capable of inducing mutation. Higher dose of irradiation resulted in more damage and hence, it may result in increasing higher chances of variability. Irradiation at 50 to 75 Gy proved better by improving certain plant characteristics such as higher root length, total phenolic content, higher leaf area index, lower content of stomata in leaves etc. responsible for better adaptability in adverse conditions. Higher dose of gamma rays proved detrimental to plant growth and development. The present research clearly proved that induced mutation can be successfully employed to create genetic variability when it is preferred to improve definite traits in plants. So, physical mutagenic treatments may be employed in inducing superior genotypes with significant alterations in growth and metabolism of the plant body. However, further trial is needed to check the results in next generations of the present investigation.

5. References

1. Sanatombi K, Sen-Mandi S, Sharma GJ. DNA profiling of Capsicum landraces of Manipur. Scientia Hort 2010;124:405-408.
2. Bosland WP, Baral BJ. *Bhut Jolokia*: The World's Hottest Known Chile Pepper is a Putative Naturally Occurring Interspecific Hybrid. Hortscience 2007;42:222-224.
3. Kusmiyati F, Sutarno, Sas MGA, Herwibawa B. Mutagenic effects of gamma rays on soybean (*Glycine max* L.) germination and seedlings. IOP Conf. Ser.: Earth Environ Sci., 2017;102:012059. doi :10.1088/1755-1315/102/1/012059
4. Ashraf M. Biotechnological approach of improving plant salt tolerance using antioxidants as markers. Biotechnol. Adv 2009;27:84-93.
5. Mudibu J, Nkongolo KKC, Kalonji-Mbuyi A, Kizungu RV. Effect of Gamma Irradiation on Morpho-Agronomic Characteristics of Soybeans (*Glycine max* L.). American J. Pl. Sc 2012;3:331-337.
6. Lee YI, Lee IS, Lim YP. Variation in sweet potato regenerates from gamma-rays irradiated embryogenic callus. J. Plant Biotech. 2002;4:163-170.
7. Siddiqui BA, Khan S. Breeding in Crop Plants: Mutations and *in vitro* Mutation Breeding. 1st ed. Kalyani Publishers, Ludhiana 1999.
8. De Micco V, Carmen A, Diana P, Durante M. Effect of sparsely and densely ionizing radiation on plants. Radiation and Environ. Biophysics 2011;50:1-19.
9. Omar SR, Ahmed OH, Saamin S, Majid NMA. Gamma Radiosensitivity Study on Capsicum annuum. Amer. J. Appl. Sc 2008;5(2):67-70.
10. Melki M, Marouani A. Effects of gamma rays on seed germination and growth of hard wheat. Environ. Chem. Lett 2010;8(4):307-310.
11. Sangsiri C, Sorajjapinun W, Srinives P. Gamma Radiation Induced Mutations in Mungbean. Science Asia 2005;31:251-255.
12. Harding SS, Johnson SD, Taylor DR, Dixon CA, Turay MY. Effect of gamma rays on seed germination, seedling height, survival percentage and tiller production in some rice varieties cultivated in Sierra Leone. Amer. J. Exp. Agric 2012;2(2):247-255.
13. Marcu D, Damian G, Cosma C, Cristea V. Gamma rays effects on seed germination, growth and pigment content, and ESR study of induced free radicals in maize (*Zea mays*). J. Biol. Phy 2013;39(4):625-634.
14. Neelam D, Tabasum T, Husain SA, Mahmooduzaffar, Subhan S. Rays sensitivity of *Cajanus cajan* to gamma rays. J. Food Proc. & Tech 2014;5:394.
15. Umavathi S, Mullainathan L. Physical and chemical induced mutagenesis study for identifying lethality dose in Chick pea (*Cicer arietinum* L.) var. Co-4. Inter. Lett. Nat. Sci 2015;35:1-5.
16. Beyaz R, Kahramanogullari CT, Yildiz C, Darcin ES, Yildiz M. The effect of gamma rays on seed germination and seedling growth of *Lathyrus chrysanthus* Boiss. under *in vitro* conditions. J Environ. Radioact 2016; 162-163:129-133.
17. Lopes AM, Bobrowski VL, Silva SDDAE, Deuner S. Morphophysiological and biochemical alterations in *Ricinus communis* L. seeds submitted to $cobalt_{60}$ gamma rays. Anais daAcademia Brasileira de Ciencias 2017;89(3):1925-1933.
18. Easlon HM, Bloom AJ. Easy Leaf Area: Automated Digital Image Analysis for Rapid and Accurate Measurement of Leaf Area. Applications in Plant Sciences 2014;2(7):1400033. doi:10.3732/apps.1400033
19. Paul V, Sharma L, Pandey R, Meena RC. Measurement of Stomatal Density and Stomatal Index on Leaf/Plant Surfaces 2017.
20. Barrs HD, Weatherley PE. A Re-Examination of the Relative Turgidity Techniques for Estimating Water Deficits in Leaves. Australian Journal of Biological Sciences 1962;15:413-428.
21. Porra RK, Thompson WA, Kriedemann PE. Determination of Accurate Extinction Coefficients and Stimultaneous Equations for Assaying Chlorophyll a and b extracted with four different solvents: Verification of the concentration of chlorophyll standards by Atomic Absorption Spectrometry. Biochem. Biophys. Acta. J. 1989;975:384-394.
22. Lichtenthaler HK. Chlorophylls and Carotenoids: Pigments of Photosynthetic Membranes. Method Enzymol 1987;148:350-382.
23. Lichtenthaler HK, Wellburn AR. Determination of Carotenoids and Chlorophylls a and b of leaf extracts in different solvents. Biochem. Soc. Trans 1983;11:591-592.
24. Spanos GA, Wrolstad RE. Influence of processing and storage on the phenolic composition of Thompson Seedless grape juice. J. Agric. Food Chem 1990;38(7): 1565-1571.
25. Chakraborthy GS, Ghorpade PM. Free Radical Scavenging Activity of *Abutilon indicum* (Linn) Sweet Stem Extracts. International Journal of ChemTech Rsearch. 2010;2(1):526-531.
26. Celik Ö, Atak Ç, Suludere Z. Response of Soybean plants to gamma radiation: Biochemical analyses and expression patterns of trichome development. Plant Omics J 2014;7(5):382-391.

27. Yamasaki S, Dillenburg LR. Measurements of leaf relative water content in *Araucaria angustifolia*. Revista Brasileira de Fisiologia Vegetal 1999;11(2):69-75.
28. Vance NC, Zaerr JB. Influence of drought stress and low irradiance on plant water relations and structural constituents in needles of Pinus ponderosa seedlings. Tree Physiology 1991;8:175-184.
29. Peksen E, Peksen A, Artik C. Comparison of Leaf and Stomatal Characteristics in Faba Bean (*Vicia faba* L.). Journal of Biological Sciences 2006;6(2):360-364.
30. Widiastuti A, Sobir, Suhartanto MR. Diversity analysis of mangosteen (*Garcinia mangostana*) irradiated by gamma-ray based on morphplogical and anatomical characteristics. Nusantara Bioscience 2010;2(1):23-33. doi: 10.13057/nusbiosci/n020104
31. Wi, S.G., Chung, B.Y., Kim, J.S., Kim, J. H., Baek, M. H., Lee, J. W. and Kim, Y. S. (2007). Effects of gamma irradiation on morphological changes and biological responses in plants. *Micron*, 38: 553-564.
32. Kiong ALP, Lai AG, Hussein S, Harun AR. Physiological responses of *Orthosiphon stamineus* plantlets to gamma irradiation. Am-Eurasian J. Sustain. Agric 2008;2(2):135-149.
33. Kim JH, Chung BY, Kim JS, Wi SG. Effects of in planta gamma-irradiation on growth, photosynthesis, and antioxidative capacity of red pepper (Capsicum annuum L.) plants. J Plant Biol 2004;48:47-56.
34. Behgar M, Ghasemi S, Naserian A, Borzoie A, Fatollahi H. Gamma radiation effects on phenolics, antioxidants activity and *in vitro* digestion of pistachio (Pistachia vera) hull. Radiat Phys Chem 2011;80:963-967.
35. Harrison K, Were L. Effect of gamma irradiation on total phenolic content yield and antioxidant capacity of almond skin extracts. Food Chem 2007;102:932-937.
36. Kim JS, Kim KA, OH TR, Park CM, Kang H. Functional characterization of DEAD-box RNA helicases in Arabidopsis thaliana under abiotic stress conditions. Plant Cell Physiol 2008;49:1563-1571.
37. De Toledo T, Canniatti-Brazaca S, Arthur V, Piedade S. Effects of gamma radiation on total phenolics, trypsin and tannin inhibitors in soybean grains. Radiat Phys Chem 2007;76:1653-1656.
38. Kim JH, Shin MH, Hwang YJ, Srinivasan P, Kim JK, Park HJ *et al*. Role of gamma irradiation on the natural antioxidants in cumin seeds. *Radiat. Phys. Chem*., 2009;78:153-157.
39. Variyar PS, Limaye A, Sharma A. Radiation induced enhancement of antioxidant contents of soybean (Glycine max Merrill). J Agric Food Chem 2004;52:3385-3388.