ENHANCEMENT OF SAFETY AND QUALITY OF *ALLIUM CEPA* BY OPTIMIZING GAMMA RADIATION DOSE ENDURING REDUCTION OF PATHOGENIC MICROFLORA

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خلاصہ

پیاز (آلیمیم سیپاایل) دنیامیں سب سے زیادہ بڑے پیانے پر بڑے سبزیوں میں سے ایک ہے اور بہت سے ممالک کے لئے قیمتی غیر ملکی کرنسی کو حاصل کرنے کا ایک ذریعہ ہے. پیاز کا مائکروبیلی خرابی ایک اہم وجہ ہے جس کا نتیجے میں ہر سال پودھاری فصل کا نقصان ہوتا ہے. موجودہ مطالعہ میں، گاماتابکاری کے علاج کا استعمال کیا گیا تھا جس میں خوراک کی اشیاء میں زہریلا استحصال کے ذخیرہ کے بارے میں کیمیکل علاج پر فائدہ اٹھاناپڑا. پیاز بلب 0.05، 0.10اور 0.15 KGy کی خوراکوں میں چراغے ہوئے تھے. اس کے بعد پیاز کے مائکروبیل آبادی کے مقابلے میں کنٹرول اور نکاسی آب نمونے کے درمیان بنایا گیا تھا. تابکاری کی خوراک کا اثر وزن میں کمی اور پیاز کے آرگنولیپٹک خصوصیات کا بھی جائزہ لیا گیا تھا. نتائج سے پتہ چلتا ہے کہ Gamma Rays کی irradiation نے bioburden، وزن میں کمی اور organoleptic خصوصیات کو نمایاں طور پر متاثر کیا. 0.15 کلو گرام کی خوراک میں تابکاری کی پیاز بلبوں کو کم سے کم وزن میں کمی، مایکیبلی لوڈ کم کرنے اور پیاز کے آرگنولوپی خصوصیات کے تحفظ کی وجہ سے زیادہ سے زیادہ وزن کی کمی دکھائی دی.

Abstract

Onion (*Allium cepa* L.) is one of the most widely grown vegetable in the world and a means to earn valuable foreign exchange for many countries. Microbial spoilage of onion is a major cause resulting in postharvest loss every year. In the present study, gamma radiation treatment was used which have benefit over chemical treatments regarding the accumulation of toxic residues in the food commodities. The onion bulbs were irradiated at the doses of 0.05, 0.10 and 0.15 kGy. Then the comparison of microbial populations of onion was made between control and irradiated samples. The impact of radiation dose on weight loss and organoleptic qualities of onion was also evaluated. The results revealed that gamma irradiation significantly affected bioburden and weight loss protecting organoleptic properties. Onion bulbs irradiated at the dose of 0.15 kGy showed minimum weight loss, high safety due to reduced microbial load and preservation of organoleptic qualities of onions.

Introduction

Onion (*Allium cepa* L.), belonging to family Alliaceae, is one of the most primitive vegetables cultivated in the world. It is universally important and has remarkable medicinal properties. Onions are extensively utilized for culinary purpose as per the unique seasoning spice flavour associated with pungency (propyl disulfides and other disulfides) and sugars (glucose, fructose, sucrose). The presence of flavonoids and pigments such as anthocyanin in onion contributes towards the antioxidant, anti-cancer, anti-microbial, anti-inflammatory, neuro protective biological activities (Nath *et al.*, 2010). In addition, it is scientifically proven that it has potential use against diabetes and cardiovascular diseases due to hypoglycemic, cardio protective and hypolipidemic activities (Sohail *et al.*, 2011).

In comparison to other fresh vegetable crops, mature and treated (cured) onion bulbs can be stored for fairly extensive periods of time due to their prolong dormancy and slow respiratory and metabolic mechanisms. Nevertheless, substantial losses still occur at some point in long standing storage, particularly under hostile environmental conditions. Postharvest deterioration of onion bulbs may be credited to physical damages (mechanical injury, cuts, bruises, dry scale loss), physiological phenomenon (sprouting, dehydration, respiration) or pathological invasions particularly by fungi (neck rot-*Botrytis alli*, Black mould rot-*Aspergillus niger*, *Fusarium* bulb rot-*Fusarium oyxsporum*, smudge-*Colletotricum circinans* (Dris and Jain, 2004). According to a report from Poland, most common bacterial pathogens of onions are *Burkolderia cepacia*, *Burkolderia gladioli* pv. *alliicola*, *Pectobacterium carotovorum*, subsp. *Carotovorum*, *Psuedomonas* sp.(Kowalska and Smolińska, 2008).

According to recent researches, pre-harvest chemical sprays have proved to prolong the storage life of onions. Maleic hyrazide is the most efficient of these, but concentration of the chemical and timing of applying spray was shown to be of great concern as they have the potential of causing damage to plant or bulbs (Nouri and Toofanian, 2001). Gamma irradiation of food is effective in reducing spoilage, disinfecting, decontaminating and extending the storage life of foods in environment friendly manner. Currently, more than 120 commercial irradiation facilities have been established all around the world to radiate food commodities. Irradiated food is safe for the human consumption, unlike the chemically treated food which tends to have certain hazardous residues. The food irradiation has been declared acceptable and wholesome by the Codex Alimentarius Commission Regulation, International Atomic Energy Agency, World Health Organization and Food and Agriculture Organization (Sharif, 1990). The major advantages of gamma irradiation over conventional pasteurization procedures are deep penetration and considerable uniformity of dose in the food commodity with low risk to the environment.

Globally, onion is being cultivated over an area of 3.06 million hectares with an annual production of 55 million tons. China and India are being the world's top most onion producers, are accounted for nearly half of world onion production.

Materials and Methods

Sample collection, packaging, irradiation and storage

Desi Red variety of onion was collected from Mandi of Lahore. The onions obtained were about 2-4 weeks postharvest age. The samples were apparently of good quality and without any physical injury. The average size of onions used was approximately 2 inches in diameter. Onions were packed in perforated paper bags and labelled with desired dose. Afterwards these packets were subjected to gamma radiations (0.05-1.5kGy) using Co^{60} as a radiation source (Kader, 1986). Both control and irradiated onions were stored at room temperature (30-37°C). At ambient conditions, scope for natural ventilation around the shelves and bags were made (Khan, 1993). The period of storage was three weeks.

Periodical investigations and evaluations

Control and irradiated samples were examined periodically for odour, colour, internal sprout growth, textural compactness, weight losses and microbial rot (Dahlhelm and Matejko, 1990). The weight (g) of onions belonging to each group was measured to determine the weight loss percentage difference.

The marketable bulbs were cut open longitudinally and observed for the incidence of internal sprouting and appearance of radiation induced darkness after every week. Organoleptic qualities of raw samples were evaluated by using 9-point hedonic scale where 9= like extremely and 1= dislike extremely. Acceptance test was also performed to find out the liking of each sample with respect to a set of attributes such as overall liking, flavour, texture and appearance (Stone *et al.*, 2012).

Weekly microbiological analysis

Irradiated onions were analysed at 7 days interval to estimate the bioburden. Four types of culture media were employed for the enumeration and identification of bacteria and fungi associated with onions. Nutrient agar (for bacterial isolation), MacConkey agar for (Gram-negative enteric bacilli isolation), PDA (for fungus isolation) and Salmonella-Shigella Agar (for *Salmonella* spp. and *Shigella* spp. isolation), were used. Isolation of microflora was done using serial dilution method. The onions were washed with 250 ml of 0.9% sterile saline water. Dilutions were made out of this stock solution. 100 µl of each dilution was transferred to the media plates containing nutrient agar, MacConkey agar, salmonella shigella and potato dextrose agar media. They were then kept at 37°C and 30°C for 24 and 72h for bacterial and fungal growth respectively. The average colony count (arithmetic mean) of all replicates was calculated after the incubation. Viable bacterial and fungal count was determined by a standard formula of Colony Forming unit per ml (cfu/g) (Gent and Schwartz, 2005).

The colonies of enterobacteriaceae were identified using API strips. The identification of fungi was carried based upon microscopic and macroscopic features.

Statistical analysis

The results were statistically validated by using Costat 6.4 program. In this regard, One- way ANOVA with completely randomized block design was applied to the data. The standard deviation of each treatment from the mean value was also calculated. The significance of mean values of data was analysed by Duncan's multiple range test.

Results

Physiological weight loss of onions after irradiation

The radiation treatment had significantly affected weight loss (Table 1). Maximum % weight loss (6.5%) was found in controls while minimum weight loss (3.25%) was recorded in onions irradiated at the dose of 0.15 kGy. However, weight loss at radiation doses of 0.05 kGy and 0.10 kGy was 5.18% and 5.69%, respectively which is also less than that of controls. The weight loss after 7, 14 or 21 days of storage were 4.02%, 5.7%, 5.6% respectively.

Sensory evaluation using 9-point hedonic scale

Significance test for consumer acceptance was carried out to evaluate the significance of attributes for both radiated and un-irradiated samples. The consumer acceptance for radiated sample was found to be more as compared to un-irradiated onions (Table 2). The characterization of trends of sensory qualities was also performed by using a sample size of 25 people by filling the questionnaire (Table 3). However the statistical analysis (ANOVA) indicated that there was no significant difference between the irradiated and control samples at $p \leq 0.05$. This indicated that irradiation did not cause any undesirable changes in the sensory attributes of the onions.

Effect of radiation on total viable count of bacteria on Nutrient Agar

Microbiological analysis of irradiated and control groups of onions displayed substantial difference in total viable bacterial count on nutrient agar (Figure 1). The samples irradiated at 0.15 kGy showed greater reduction in bacterial count (5.7×10^4 cfu/g) as compared with control samples (11.9×10^4 cfu/g) during the first week of storage. This bacterial colony count was 8.7×10^4 cfu/g and 7.6×10^4 cfu/g at 0.05 kGy and 0.10 kGy respectively which were again less than that of controls. The same trend was observed for the next intervals of analysis. The highest dose of 0.15 kGy was inhibiting the total viable bacterial count (10.4×10^4 cfu/g) as compared to the lower doses 0.05 kGy (10.4×10^4 cfu/g) and 0.10 kGy (10.4×10^4 cfu/g) and controls (21.0×10^4 cfu/g) after the third week as well.

Effect of radiation on total viable count of bacteria on onions using MacConkey agar

The ability of gamma radiation to retard the bacterial growth on the surface of the onion was observed on MacConkey agar as well. The control group exhibited bacterial count of 2.81×10^4 cfu/g which kept on augmenting with storage time until the third week of analysis (3.19×10^5 cfu/g). The irradiated group of onions once again harboured reduced total viable bacterial count than the control one and the effect was more pronounced with the escalation in the applied doses of gamma radiation (Figure 2). The total viable bacterial count was least at 0.15 kGy during the whole storage period (0.2×10^4, 0.3×10^4 and 0.4×10^4 cfu/g after 7, 14 and 21 days of storage respectively). The bacterial colonies were circular and convex having entire margins.

Effect of radiation on total viable count of bacteria on Salmonella Shigella Agar

The analysis of bacterial count obtained from irradiated and control samples was measured on Salmonella-Shigella agar. The results revealed that controls carried 0.7×10^4 cfu/g of bacteria, whereas experimental samples had microbial load of 0.3×10^4 and 0.1×10^4 cfu/g at the doses of 0.05 kGy, 0.10 kGy respectively at the end of first week. The highest dose of 0.15 kGy was effective in completely inhibiting the bacterial growth, hence no colonies were observed in culture medium after the first week of storage (Figure 3). The bacterial colony count amplified after each successive week, but significant difference was witnessed between control and irradiated samples. The colonies grown on Salmonella Shigella Agar were round, flat having entire margins.

Identification of bacteria using API 20E strips

The most common and dominating colonies identified by using API 20 E strips were *Escherichia coli*, *Shigella sonnei* and *S. flexneri*. The colonies of *E.coli*, *Shigella sonnei* and *S. flexneri* were found on Salmonella Shigella agar at all doses except 0.15 k Gy. However, a dose of 0.10 kGy also inhibited *Shigella sonnei*. Similarly, the colonies of *E.coli*, non-fermentor specie and *Shigella sonnei* were identified on MacConkey agar at all doses but at 0.15 kGy *E.coli*, *Shigella sonnei and S. flexneri* were inhibited (Table 4).

Effect of radiation on fungal count of onions using potato dextrose agar

The enumeration of total fungal count was carried out on potato dextrose agar. The fungal growth was gradually retarded as the strength of gamma radiation increased. The control samples had highest total viable fungal count of 8.2×10^4 cfu/g on the 7th day of storage, which progressed steadily afterwards and was found to be 14.9×10^4 cfu/g after 21 days. The most notable results were obtained at the highest dose of 0.15 kGy (Figure 4). The earlier total viable fungal counts of 5.6×10^4, 4.7×10^4 and 3.9×10^4cfu/g were obtained for the doses of 0.05 kGy, 0.10 kGy and 0.15 kGy respectively. The count for the above doses increased to 11.8×10^4, 10.9×10^4 and 9.6×10^4cfu/g till the end of the third week.

The macroscopic and microscopic analysis of fungal growth showed the species identified to be *Aspergillus niger, Aspergillus flavus, Fusarium oxysporum, Colletotrichum circinans* and also some *yeast* species (*Saccharomyces cerevisiae*, *Rhodotorula* and *candida* species).

Discussion

The cultivation, harvesting, transportation, storage and marketing of onions play important role in international market since it is a major source of foreign exchange for the producers. The application of improved post-harvest systems resulted in greater trade opportunities owing to better quality and longer storage life of food commodities (Thompson, 2008). The main obstacle pertaining to limited marketability of onions is postharvest losses, mainly due to microbial spoilage (Snowdon, 2010). Developing postharvest handling techniques emphasize on maximum extension of storage life under given conditions with minimum depressing effects on fresh produce. Therefore, the main focus of this study was the administration of gamma radiations to extend the safety and storage life of onions by controlling the growth of spoilage microorganisms associated with the produce. Depending upon the dose, some or all microorganisms are destroyed, slow down or rendered incapable of reproduction as gamma radiation induces direct cell damage by having deleterious effects on the chromosomal DNA along with protein coagulation of living cells (Roberts, 2014). When gamma radiation targets bacteria or other microbes, the high energy breaks the chemical bonds present in the biomolecular structures vital for the growth and integrity of the cell. Consequently, the microbes are killed and hence cannot replicate to cause food spoilage and food borne illnesses. Gamma rays have been approved by the FDA for over fifty years (Thomas, 2001). They have more penetration power and therefore more effective than ionizing radiations or x-rays. The maximum dose for onions as approved by the Codex Alimentarius Commission is 0.15 kGy (Codex, 2011). In this study, the doses of 0.05 kGy, 0.10 kGy and 0.15 kGy were administered to reduce the spoilage microflora and hence enhance the storage life of onion bulbs. The effect of these doses on weight loss, microbial load and organoleptic qualities of onion was demonstrated.

Irradiated onion bulbs showed less incidence of weight loss as compared to that of controls kept under identical conditions. The increase in weight loss in non-radiated onion bulbs may be due to high metabolic rate and subsequent respiration and dehydration of these onions while radiation inhibited these changes to some extent (Brewster, 2008). The storage time also affected the weight of the bulbs. This difference in weight after weekly analysis was due to transpiration losses. The weight loss in onion samples irradiated at 0.15 kGy was least as compared to that at 0.05 kGy and 0.10 kGy. So the highest dose was more effective in preserving the water constituents and maintaining the optimum metabolic rate of onions. These findings are in line with those of Jabeen *et al.* (2003) who reported that radiation treatment considerably reduced weight loss of onions.

Onions were irradiated 2-3 weeks postharvest. The reason to recommend irradiation at the early stage of storage is to reduce the incidence of radiation induced darkening which may occur in inner scales (Arvanitoyannis *et al.*, 2009). Enumeration of microbial load illustrated that the control samples or unirradiated had the highest microbial count throughout the storage period as compared to radiated samples. However, the most effective dose that kept onions from deterioration appeared to be 0.15 kGy. At this dose, the sanitizing efficiency was enhanced and it significantly reduced bacterial as well as fungal load on onions.

The epiphytic fungal and bacterial load of onion was recorded for three progressive weeks. Radiation treatment significantly affected total viable count of bacteria and fungi. The quantitative analysis of bacterial population grown on Nutrient, MacConkey and Salmonella Shigella Agar also showed that the total viable count was found maximum on control group and it decreases linearly with increasing radiation doses and minimum bacterial population was observed at 0.15 kGy. The radiated samples showed significant variation of bacterial load from the control group. The administered doses also had a vivid impact on total fungal count of both control and experimental groups stored at ambient conditions. These results are in line with those of Farkas *et al*. (1997) who examined the effect of gamma radiation on the microbial load of onions concluding that the treatment of gamma irradiation at all does significantly reduce the load of yeast, mold and bacteria.

The differential method Gram staining was employed to distinguish bacteria on the basis of their gram characteristics by using crystal violet as a primary dye. The gram positive bacteria retained the crystal violet complexed with the Gram's Iodine (mordant) and appeared purple. However, gram negative bacteria were unable to withhold the CV—I complex after decolorizing with alcohol and appeared pink when counterstained with safaranin (secondary dye). Most of the bacteria isolated on nutrient agar from the onion surface were gram negative and rod shaped. Further bacterial identification test was carried out using analytical profile index system and performing oxidase and catalase test. The bacteria identified were *Escherichia coli,* which is a Gram-negative, facultative anaerobe and rod-shaped bacterium and gave oxidase negative, catalase positive results.

The main casual agents of onion deterioration are fungal species. There was no incidence of fungal rottage during first and second week of storage in both control and irradiated samples. However, the deposits of black spores were found on the outer scales of onions of the control group after the third week whereas the irradiated samples were free of fungal rots. The dominant fungal species identified were *Aspergillus niger, Aspergillus flavus, Colletotrichum circinans, Fusarium oxysporum* and various yeast species. Nevertheless, the major spoilage fungus was found to be *Aspergillus niger* as its spores could also be seen on onion tissues.

The sensory evaluations showed that the irradiated bulbs were judged to be indifferent in quality with respect to internal and external appearance ($p \leq 0.05$) and flavor ($p \leq 0.05$), after 21 days post irradiation. This establishes that radiation treatment has an overall useful impact on enhancing the shelf life as well as the quality of onions stored at ambient conditions.

Table1. Effect of different Gamma radiation doses on the % weight loss of onions

Time	Gamma radiation doses				
	Control	**0.05kGy**	**0.10kGy**	**0.15kGy**	**Mean***
Week$_1$	6.20	3.38	4.32	2.19	4.02^{a}
Week$_2$	6.70	6.49	6.51	3.46	5.79^{b}
Week$_3$	6.74	5.54	6.25	4.11	5.66^{c}
Mean*	6.54^{a}	5.13^{b}	5.69^{bc}	3.25^{c}	

Each value represents the mean and superscripts indicate that the mean difference is significant at $p \leq 0.05$ by Duncan's New Multiple Range Test.

Table 2. Sensory evaluation of onions using 9-point Hedonic scale

Treatment (kGy)	**Storage period**			
	1st week	**2nd week**	**3rd week**	**Mean**
		Appearance (0-9)		
Control	7.2 ± 1.90	6.75 ± 0.89	5.59 ± 1.34	6.51
0.05 kGy	7.62 ± 1.24	6.81 ± 1.33	6.1 ± 1.25	6.84
0.10 kGy	7.76 ± 1.04	6.85 ± 1.45	6.7 ± 1.65	7.10
0.15 kGy	7.91 ± 2.1	7.1 ± 1.87	6.95 ± 1.11	7.32
		Flavour (0-9)		
Control	7.11 ± 1.26	6.89 ± 1.34	5.89 ± 1.43	6.63
0.05 kGy	7.21 ± 1.56	7.01 ± 0.97	6.64 ± 1.65	6.95
0.10 kGy	7.5 ± 1.43	6.75 ± 1.29	6.76 ± 1. 76	7.00
0.15 kGy	7.7 ± 1.37	7.42 ± 1.04	7.3 ± 1.80	7.47
		Texture (0-9)		
Control	7.23 ± 0.67	6.77 ± 1.78	6.01 ± 1.13	6.67
0.05 kGy	7.29 ± 0.54	7.19 ± 1.99	7.01 ± 1.76	7.16
0.10 kGy	7.33 ± 1.76	7.39 ± 1.28	7.21 ± 1.25	7.31
0.15 kGy	7.45 ± 1.33	7.5 ± 1.29	7.46 ± 1.71	7.47
		Overall Acceptibility (0-9)		
Control	7.78 ± 1.34	6.29 ± 1.90	5.99 ± 1.55	6.69
0.05 kGy	7.92 ± 1.98	7.88 ± 1.16	7.23 ± 1.21	7.68
0.10 kGy	7.97 ± 2.01	7.85 ± 1.73	7.39 ± 1.08	7.74
0.15 kGy	8.21 ± 1.44	7.9 ± 1.09	7.67 ± 1.63	7.93

Sample means ± standard deviation and significance for consumer acceptance (n=25) *****9-point Hedonic scale: **9**=like extremely, **8**=like very much, **7**=like moderately, **6**=like slightly, **5**=neither like nor dislike, **4**=dislike slightly, **3**=dislike moderately, **2**=dislike very much, **1**=dislike extremely.

Each value represents the mean and ± indicates the standard deviation.

Table 3: Effect of gamma irradiation on color, firmness and pungency

Sensory Parameters	Storage period	Gamma Radiation Treatment (kGy)			
		0.0	0.05	0.10	0.15
Color	1st week	No change	No change	No change	No change
	2nd week	Yellowness	No change	No change	No change
	3rd week	Yellowness	No change	No change	No change
Firmness	1st week	No change	No change	No change	No change
	2nd week	Decrease	No change	No change	No change
	3rd week	Decrease	No change	No change	No change
Pungency	1st week	No change	Less than control	Less than control	Less than control
	2nd week	Increase	Increase	Decrease	Decrease
	3rd week	Increase	Increase	Decrease	Decrease

Table 4: Types of Bacterial colonies observed on MacConkey agar, Salmonella- Shigella agar and the identified microorganisms.

Growth media	Irradiation doses(kGy)	Total no of colonies	Colony types	Identified microorganisms
MacConkey agar	0	281	3	*E.coli, S.sonnei, S. flexneri*
	0.05	18	3	*E.coli,S. sonnei ,S. flexneri*
	0.10	10	2	*S. flexneri, S. sonnei*
	0.15	2	1	*non fermentor*sp
Salmonella-shigella agar	0	7	2	*E. coli, S. sonnie, S. flexneri*
	0.05	3	2	*E. coli, S. sonnie, S. flexneri*
	0.10	1	1	*E. coli*
	0.15	0	0	---

The total number of colonies obtained is the sum of the colony results for two consecutive microbiological analysis performed with an interval of 7 days.

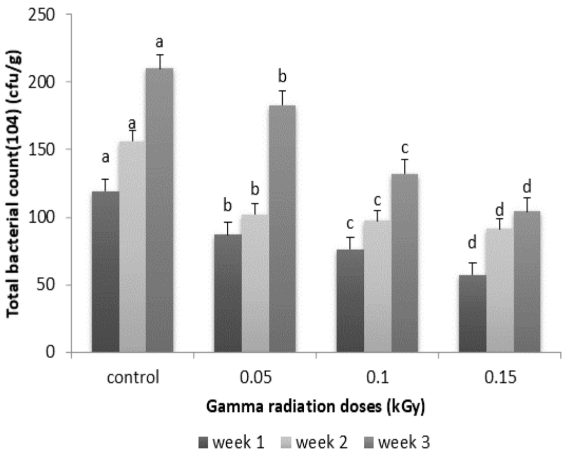

Fig. 1. Impact of different gamma radiation doses on total viable bacterial count of onion using Nutrient agar as testing medium. Each value is the mean of five parallel replicates. The error bars indicate the standard deviation from the mean value. The values vary significantly at $p \leq 0.05$.

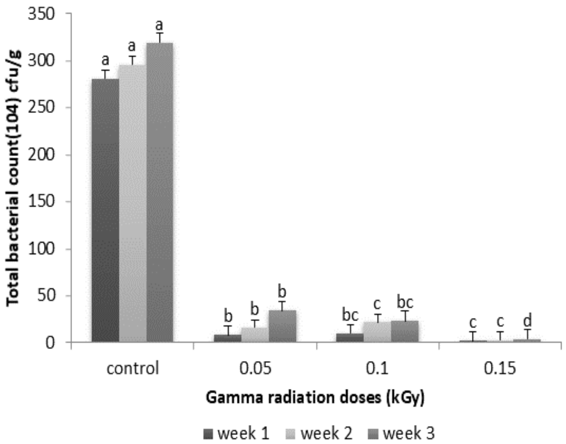

Fig. 2. Impact of different gamma radiation doses on viable bacterial count of onion using MacConkey agar as testing medium. Each value is the mean of five parallel replicates. The error bars indicate the standard deviation from the mean value. The values vary significantly at $p \leq 0.05$.

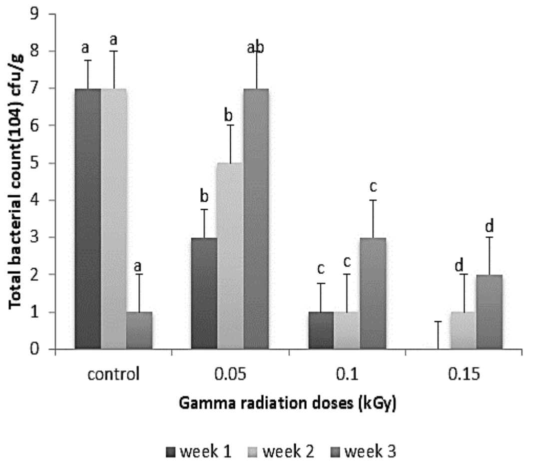

Fig. 3 . Impact of different gamma radiation doses on total viable bacterial count of onion using Salmonella Shigella agar as testing medium. Each value is the mean of five parallel replicates. The error bars indicate the standard deviation from the mean value. The values vary significantly at $p \leq 0.05$.

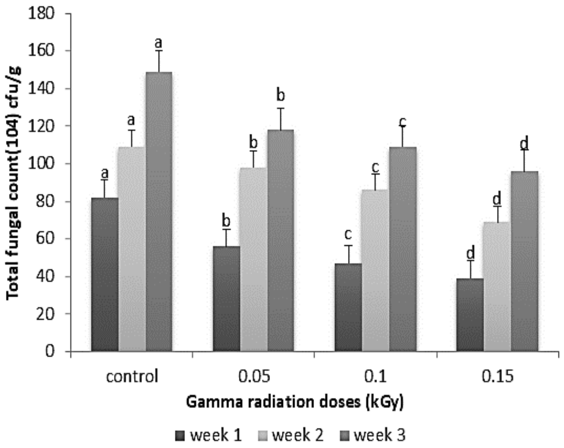

Fig. 4. Impact of different gamma radiation doses on fungal count of onion using Potato Dextrose agar as testing medium. Each value is the mean of five parallel replicates. The error bars indicate the standard deviation from the mean value. The values vary significantly at $p \leq 0.05$.

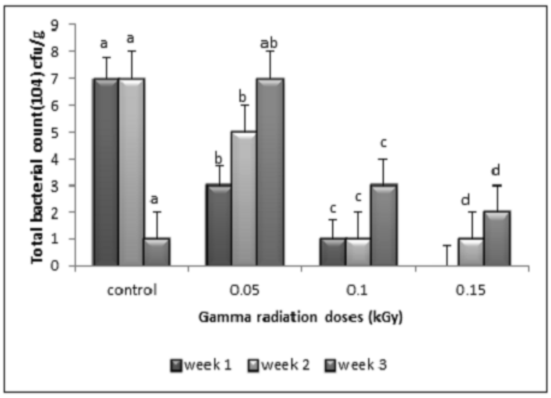

Fig. 5 . Impact of different gamma radiation doses on total viable bacterial count of onion using Salmonella Shigella agar as testing medium. Each value is the mean of five parallel replicates. The error bars indicate the standard deviation from the mean value. The values vary significantly at $p \leq 0.05$.

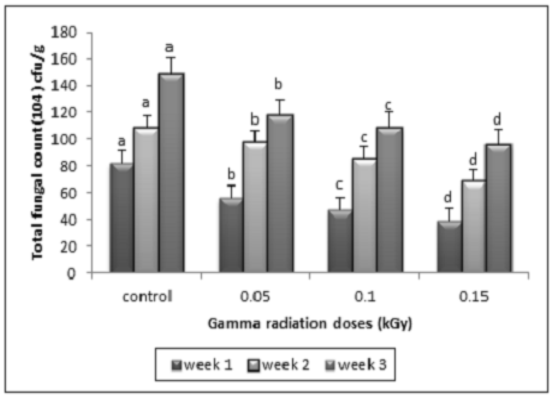

Fig. 6. Impact of different gamma radiation doses on fungal count of onion using Potato Dextrose agar as testing medium. Each value is the mean of five parallel replicates. The error bars indicate the standard deviation from the mean value. The values vary significantly at $p \leq 0.05$.

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