Arab Journal of Nuclear Sciences and Applications

Vol 50, 3, (159-173) 2017

The Egyptian Society of Nuclear Sciences and Applications

ISSN 1110-0451 | *Web site: esnsa-eg.com* | (ESNSA)

Management of Potato Soft Rot by Gamma Irradiation

H. Abd El-Ghany[1], Z. Moussa[1], E.A. Salem[2] and A.F. Abd El-Rahman[1]

[(1)] *Bacterial Diseases Research Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt*

[(2)] *Food Irradiation Department, National Center for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt*

Received: 1/1/2017 **Accepted: 12/2/2017**

ABSTRACT

This investigation aims to apply a safe practice to minimize potato losses due to soft rot disease of tubers kept under ambient temperature. In this regard, gamma irradiation was used to extend keeping quality through its effect on soft rot bacteria. Eight bacterial isolates were recovered on Logan's medium from kitchen kept tubers with symptoms of soft rot disease. Five isolates were found pathogenic and tentatively identified as *Pectobacterium atrosepticum* and *Pectobacterium carotovorum* subsp. *brasiliense* on the basis of the usual bacteriological methods. A molecular method using 16SrDNA sequence analysis for verification of the identity of two isolates was made. The two bacterial isolates, *Pectobacterium atrosepticum* and *Pectobacterium carotovorum* subsp. *brasiliense*, were irradiated by different doses of gamma rays. Complete inhibition occurred at doses 2.5 and 2.0 KGy for high densities (Approximately 4.0 x10^9 CFU/ml) of *P. atrosepticum* and *P. carotovorum* subsp. *brasiliense*, respectively. The D_{10} value of gamma irradiation was 0.24 KGy for *P. atrosepticum* and 0.20 KGy for *P. carotovorum* subsp. *brasiliense*. Irradiation of artificially infected tubers with soft rot bacteria using the two mentioned D_{10} doses for the two bacterial species increased the shelf life of tubers kept under ambient temperature. The internal chemical quality of tubers was shown to be improved by keeping the tubers under ambient temperature after irradiation by the two D_{10} doses 0.24 and 0.20 KGy.

Keywords: ***Identification/ P. atrosepticum/ P. carotovorum subsp. Brasiliense /Irradiation doses/ D_{10} value / Potato / Shelf life/ Chemical quality of potatoes/ Starch change in potatoes/ Sugar change in potatoes/Solanum tuberosum.***

INTRODUCTION

Postharvest diseases limit storage period and marketing life of vegetables and fruits and may cause losses up to 50 % or more **(Temur and Tiryaki, 2013).** Soft rot *Pectobacterium* spp. and *Deckeya solani* cause important disease on potato and other arable and horticulture crops, and are responsible for causing blackleg disease of potato in the field; and tuber soft rots in the field, storage and in transit (**Czajkowski *et al.*, 2015).** Bacterial soft rot leads to losses of potato that may reach up to 60 % in field, transit, and during storage **(Mantsebo *et al.*, 2014).** In 2014, the total cultivation area

Corresponding author E-mail: aabdelrahman2012@gmail.com

of potatoes in Egypt reached 172,005 hectares (409,536 feddans) which produce 4,611,056 tons of tubers with an average yield 11.23 tons/feddan (http://www.fao.org/faostat/en/#data/QC). Potato crop in Egypt is economically important for local consumption and exportation. Potato growers in Egypt complained about losses due to soft rot bacteria **(Hajhamed *et al.*, 2007)**.

Identification of pectolytic *Erwinia* and differentiation species and subspecies were made simply based on pectolytic activity, colony characteristics on crystal violet pectate (CVP) medium and limited number of morphological, biochemical and physiological tests **(De Boer and Kelman, 2001)**. Molecular methods based on 16S rDNA sequence analyses supported the revival that soft rot *Erwinia* species placed in a separate genus called *Pectobacterium* **(Hauben *et al*., 1998)**. Recently, 16S rDNA sequences analysis is used to confirm identification, genetic relationships and taxonomic classification of soft rot bacteria **(Zhu *et al*., 2010; Nabhan *et al*., 2013; Gasic *et al*., 2014 and Wu *et al*., 2015)**.

Potato storage in cold temperature (2 – 4 °C) is beneficial for long-term storage and limit pathogen and microbial contamination. This storage is unsuitable for processing due to the conversion of starch to sugars and accumulation of sugars **(Karim *et al.* 2008)**. In addition, cold storage requires high energy for refrigerators. Therefore, potato storage at ambient temperature is required **(Matho and Das, 2014)**. The use of pesticides for long-term storage and limiting pathogen and microbial contamination is disapproving, due to their environmental and health hazards. Furthermore, the microbial resistance against pesticides limits the use of them (**Karabulut and Baykal, 2004**). Therefore, finding new safe tools to control postharvest pathogen is necessary.

Gamma irradiation is one of the promising tools to manage pests of vegetables and fruits during storage **(Temur and Tiryaki, 2013)**. During the irradiation process, food moves through an energy field, but never touches the energy source and does not become radioactive. Irradiated foods are safe and wholesome **(Bruhn, 2001)**. The irradiation of foods up to 10 KGy is nontoxic and safe **(WHO, 1981).** Irradiation of fresh, vegetables and fruits is permitted to an irradiation dose of 1 KGy **(US FDA, 2004).** Irradiation of fresh fruits and vegetables has been utilized as a postharvest intervention measure to inactivate microbial pathogen on products and extending the shelf life as well as reducing quality losses in food or products **(Prakash *et al.,* 2000 and Olanya *et al.,* 2015)**.Gamma radiation was successfully applied to control postharvest fungal pathogens **(Kim and Yook, 2009)**. Studies on the use of radiation to control bacterial plant pathogens and their potential modes of action are few compared with fungal plant pathogens. Gamma irradiation was used to reduce bacterial soft rot of tomato and paprika **(Spalding and Reeder, 1986 and Jeong *et al.*, 2016)**. **Mukhopadhyay *et al*., (2013)** illustrated that gamma irradiation inactivated *Escherichia coli* O157:H7 and *Salmonella enterica* on grape tomatoes. **Olanya *et al*., (2015)** used gamma irradiation to inactivate *Pseudomonas fluorescens* on romaine lettuce and baby spinach. **Abdullah *et al*., (2016)** indicated that gamma irradiation reduced microflora count on carrot leading to extending the shelf life of carrot.

Ionizing radiation can affect the materials in a direct way through the interaction of radiation with molecules causing ionization or excitation and then causing damage in the molecules. The indirect effect usually refers to the damage done to molecules by radiolytic products of irradiated water, oxygen or other materials in the medium **(Nather, 2001)**. The D_{10}-value (the decimal reduction dose) is the radiation dose required for reducing the microbial population by 90 % or defined as the radiation dose required to surviving 10 % of the initial bacterial count. The D_{10} radiation dose varies with types of species and strains (**Rajkowski, 2008 and Olanya *et al*., 2015**). The use of gamma irradiation for improving the shelf life of potato at a safe level was considered at D_{10} dose in this study.

Irradiation of potato by gamma rays is an effective tool to extend the shelf life, moreover, it leads to a decrease in weight loss, the decrement of rotting range, a decrease of or preventing sprouting and decreases the weakening in physical and chemical quality **(Nouri and Toofanian, 2001; Rezaee *et al*., 2011 and Mahto and Das, 2014)**.

This study aims to isolate and identify the soft rot bacteria of potato to study the effect of gamma rays on the causing bacteria, the effect of radiation on decreasing the number of infected tubers and the effect of radiation on increasing the shelf life of potato tubers at ambient temperature. The effect of gamma rays on chemical tubers quality at ambient temperature was also considered.

MATERIAL AND METHODS

Isolation of the Pathogens

Rotten potato tubers collected from kitchen kept potato were slightly washed with water to remove excess soil without breaking down the skin. The macerated tissue margin was cut and homogenized in sterile water and left for 5 min. to allow the bacterial diffusion **(Perombelon and Van der Wolf, 2002)**. The resulting suspension was streaked onto Logan's medium **(Logan, 1963)** plates, and incubated at 28 °C for 24-48 hrs. Single colonies were streaked on King's B agar (KBA) slants and were incubated for 48 hrs. at 28ºC.

Pathogenicity tests

All tools and materials used were sterilized in an autoclave at 121 °C for 20 min. Healthy potato tubers were surface sterilized by flaming after dipping into 70 % ethyl alcohol, cut into thick slices under aseptic conditions and transferred to sterile petri dishes with filter paper moistened with sterilized water. Potato slices were inoculated in the center with 50 µl bacterial suspensions (10^8 CFU/ml) and incubated at 28°C for 72 hrs. Three replicates were used for each isolate. The diameter of rotted area was measured **(Czajkowski *et al*., 2012)**.

Identification of Pathogens

Morphological, physiological and biochemical characterization of pathogenic isolates were carried out as recommended by **De Boer and Kelman, (2001).**

Confirmation of Identity

Further to the confirmation of the identification of the bacterial isolates, two selected pathogenic isolates were sent to Sigma Scientific Services Co., Giza, Egypt and GATC Company, Germany for 16S rDNA analysis as follows:

DNA extraction was made by using of GenJet genomic DNA purification Kit (ThermoK0721) according to the manufacture protocol by gram-negative bacteria genomic DNA purification protocol. 16S rDNA DNA gene was amplified using Maxima Hot Start PCR Master Mix (Thermo K 1051) according to manufacture protocol by using forward primer 5/AGAGTTTGATCCTGGCTCAG3/and reverse primer 5/GGTTACCTTGTTACGACTT3/ (**Eden *et al*., 1991**). The PCR products were purified using GeneJet™ PCR purification Kit (Thermo K0701). Finally, the PCR products were sequenced on GATC Company using ABI 3730x1 DNA sequencer using forward and reverse primers by combining traditional Sanger technology with the new 454 technology. Obtained sequences were compared to those available in data- bases GeneBank network services at http://blast.ncbi.nlm.nih.gov/Blast.cgi for significant alignments. The phylogenetic tree was constructed by MEGA 6.0 with Neighbor-Joining method **(Tamura *et al*., 2013)**.Bootstrap analysis with 1000 replicates was performed to calculate the support of branches.

Effect of Gamma Rays on Soft Rot Bacteria

Two bacterial isolates (E2) and (E4) were shaking grown in King's B broth in a shaking incubator (150 rpm) at 28°C for one day. The bacterial growth was harvested, washed twice and suspended in physiological saline solution (0.85% NaCl) to give approximately $4x10^9$ CFU/ml. Eppendorf tubes of each species (1 ml suspension/tube) were then exposed to 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 KGy of gamma radiation in Indian Co-60 gamma cell at the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt. Five replicates for each dose were considered. The surviving numbers of bacterial cells for each dose were determined by tenfold serial dilution method

by plating on King's B agar plates. The plates were incubated at 28°C for 48 hrs. and colonies were counted and the value of log (CFU/ml) for each treatment was calculated.

Determination of D_{10} Value

The dose-response curve is made by plotting log CFU/ ml of the surviving counts on the vertical coordinate and radiation dose (KGy) on the horizontal coordinate. From the straight-line plots, the D_{10} value (decimal reduction dose) of bacteria was calculated from the regression linear equation according to the formula: D_{10} value = - 1/slope (**Rajkowski, 2008).**

Effect of Irradiation Treatments on Soft Rot and Potato Tubers Contents

Standardized fresh Spunta Potato tubers were kindly received from Brown Rot Disease Project, Giza, Egypt,and used in this investigation. Treatment of potato tubers using gamma radiation by D_{10} value was made in the Indian Co-60-gamma cell at NCRRT. The dose rate was 2.45 kGy/hr at the time of the experiment. Potato tubers were washed under running water to remove excess soil, surface disinfected with 1% sodium hypochlorite, washed with distilled water and dried in air. Two cross shape wounds (2 cm in equal direction) were made in the middle of each potato tuber and densely inoculated with the bacterial suspension ($4x10^9$ CFU/ml). Tubers were packed in plastic bags and exposed to radiation doses. Two treatments (40 potato tuber for each) for each isolate were followed: 1-potato tuber was densely inoculated with bacterial suspension(1ml/tuber) and irritated with the D_{10} value of the isolate, 2- potato tuber was densely inoculated with bacterial suspension(1ml/tuber) without irradiation (control). Five replicates per treatment were used. All tubers were kept at ambient temperature and were examined for the presence of soft rot. Storage period commenced from May 5th, 2016 and extend to July 13th, 2016, the temperatures were recorded at night and day during this period which ranged from 18 to 43 °C.

The potential of gamma radiation to prevent soft rot by the two studied bacterial species in potato tubers was expressed as a decrease in infected tubers percentage and was determined by using the formula:

Reduction in infected tubers percentage = ([No. of infected tuber in the control group] – [No. of infected tuber in the treated group]/ No. of infected tuber in the control group) X 100**.**

Effect of Gamma Irradiation on Tuber Quality

Tubers of Spunta potato variety were arranged into three groups. The first group was kept without irradiation as a control treatment. The second and the third groups were irradiated by the D_{10} dose for spp. *atrosepticum* and spp. *carotovorum*, respectively. All tubers were kept for 9 weeks (from May 5th, 2016 to July 13th, 2016) as bench incubated treatments at ambient temperature. The temperatures were recorded at night and day during this period which ranged from 18 to 43 °C. The quality evaluation was considered as:

Total Soluble Sugars and Starch Changes

Potato tubers were used to determine total soluble sugars and starch using anthrone reaction as described by **Elayaraj *et al.* (2015).**

Total phenolic compounds changes

Potato tubers (1gm) were macerated in 10 ml 80% ethanol for at least 24 hrs. at 0°C. After centrifugation, the remained residue was re-extracted with 10ml 80% ethanol 3times. The supernatant was completed to 50 ml using 80% ethanol. Phenolic compounds were determined according to the method of **Daniel and George, (1972)** as described by **Elagamey *et al*.(2013)**. The standard curve was established using gallic acid (GA). Total phenolic compounds were expressed as mg GA /100 g fresh weight.

Statistical Analysis

The completely randomized design was used in all experiments. Data were analyzed with the statistical analysis software CoStat (version 6.4). Duncan's multiple range test **(Duncan, 1955)** was used to compare the means at probability (*P*) level 0.05.

RESULTS

Isolation, Pathogenicity and Identification

Eight bacterial isolates were recovered using Logan's medium from potato tubers obtained from kitchen kept potato. Five isolates were able to produce soft rot on potato slices (Table 1) .E2 and E3 were colorless with about 0.5-1.0 mm in diameter; and E4, E6 and E8 with a pink center and with about 1.5-2.0 mm in diameter (Figure 1). The largest rotten area dimensions were recorded with E4 (42.0 mm) and E2 (39.0 mm). These five isolates were subjected to a complete identification using morphological, physiological and biochemical characteristics as traditional preliminary methods along with confirmation of identity by molecular methods for certain isolates.

Table (1): Pathogenicity test of eight isolates using potato tuber slices test

Isolate No.	pathogenicity	Diameter of rotted area (mm) after 72 hrs of incubation at 28 °C
E1	-	00.0 [d]
E2	+	39.0 [a]
E3	+	33.3 [b]
E4	+	42.0 [a]
E5	-	00.0 [d]
E6	+	32.0 [b]
E7	-	00.0 [d]
E8	+	27.7 [c]

Means shared a letter within the table are not significantly different using Duncan's Multiple Range Test ($p \leq 0.05$).

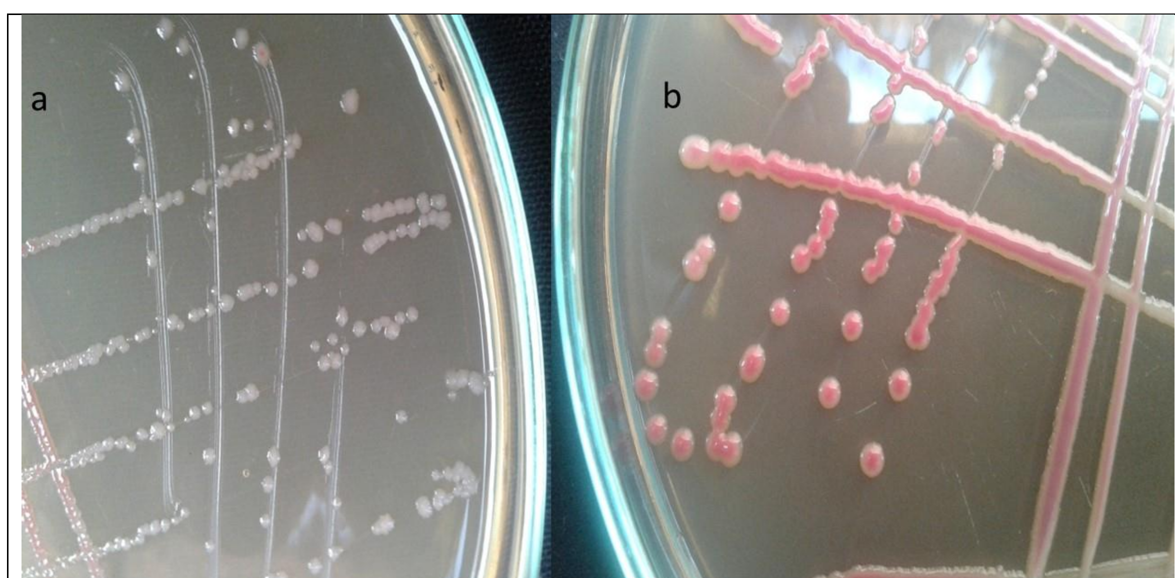

Fig. (1): Colonies shape on Logan's medium of (a): *Pectobacterium atrosepticum* (E2) 0.5-1.0 mm in diameter and colorless and (b): *Pectobacterium cartovorum* subsp. *brasiliense* (E4) 1.5-2.0 mm in diameter with a pink center

Data in Table (2) show that the five pathogenic isolates were gram-negative, rod shape, facultative anaerobic, catalase positive, oxidase negative, did not produce yellow colonies on YDC medium, no fluorescent pigment on KBA medium, not sensitive to erythromycin, have the ability to reduce sugars from sucrose and utilize keto-methyl glucoside. The isolates did not produce indol from tryptophan. The five pathogenic isolates produced acid from Melibiose and lactose. They did not produce acid from sorbitol. Isolates E2 and E3 did not grow at 37 °C, and E4, E6 and E8 can grow at 37 °C. These results suggested that E2 and E3 were identified as *Pectobacterium atrosepticum* while E4, E6, and E8 are *Pectobacterium cartovorum* subsp. *brasiliense*.

Table (2): Morphological, physiological and biochemical characteristics of soft rot bacteria

Character	Bacterial isolate				
	E2	E3	E4	E6	E8
Colony color on Logan's medium	colorless	colorless	With a pink center	With a pink center	With a pink center
Colony diameter(mm)on Logan's medium	0.5-1.0	0.5-1.0	1.5-2.0	1.5-2.0	1.5-2.0
Gram reaction	-	-	-	-	-
Cell shape	Rod	Rod	Rod	Rod	Rod
Anaerobic growth	+	+	+	+	+
Catalase	+	+	+	+	+
Oxidase	-	-	-	-	-
Yellow colonies on YDC medium	-	-	-	-	-
Fluorescent pigment on KBA medium	-	-	-	-	-
Growth at 37 °C	-	-	+	+	+
Reducing sugars from sucrose	+	+	+	+	+
Sensitivity to erythromycin	-	-	-	-	-
Indole production	-	-	-	-	-
Utilization of keto-methyl glucoside	+	+	+	+	+
Acid production from:					
Sorbitol	-	-	-	-	-
Melibiose	+	+	+	+	+
Lactose	+	+	+	+	+

+: positive reaction -: negative reaction

Confirmation of Identity

Bacterial isolates tentatively identified as *P. atrosepticum* (E2) and *P. cartovorum* subsp. *brasiliense* (E4) on the basis of the usual bacteriological methods were further confirmed by 16S rDNA identity tests. *P. atrosepticum* (E2) showed 97 % similarity to *P. atrosepticum* strain CFBP 1526 available in NCBI database. Meanwhile *P. cartovorum* subsp. *brasiliense* showed 96% similarity with the reference strain to *P. cartovorum* subsp. *brasiliense* strain 212 in NCBI database. Figures (2 and 3) show Phylogenetic trees of *P. atrosepticum* (E2) and *P. cartovorum* subsp. *brasiliense,* and some of their closest phylogenetic relatives.

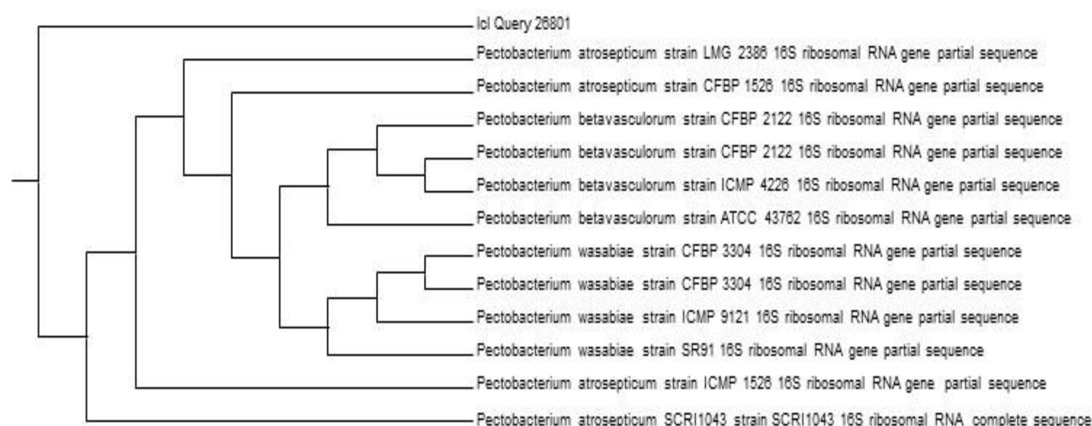

Fig. (2): Phylogenetic tree based on a comparison of the 16S rRNA sequences of *P. atrosepticum* (E2) isolate and some of their closest phylogenetic relatives

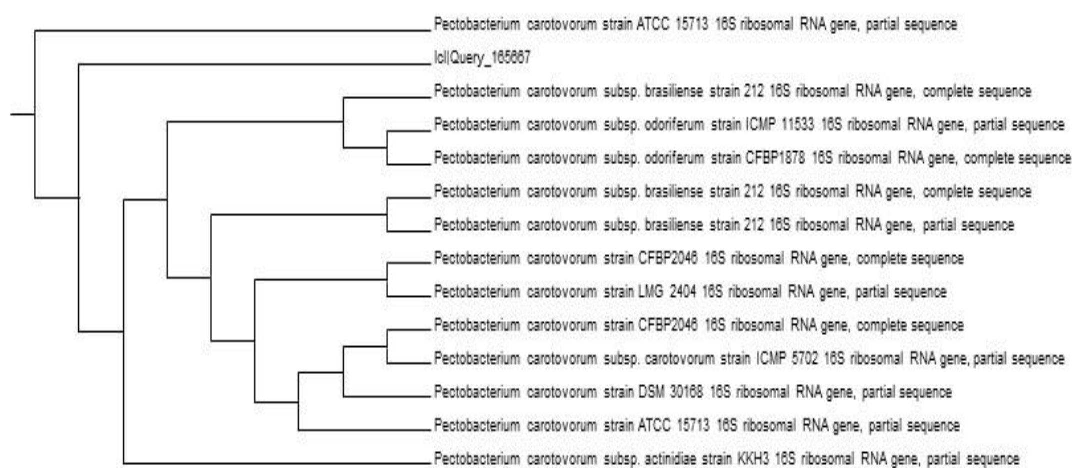

Fig. (3): Phylogenetic tree based on a comparison of the 16S rRNA sequences of *P. cartovorum* subsp. *brasiliense* isolate and some of their closest phylogenetic relatives

Effect of Gamma Rays on Soft Rot Bacteria

P. cartovorum subsp. *brasiliense* showed a greater sensitivity to gamma rays than *P. atrosepticum*. Exposure of bacterial suspension to gamma radiation doses 0.5, 1.0, 1.5, 2.0 and 2.5 KGy led to a drop in logs of CFU/ml of *P. cartovorum* subsp. *brasiliense* compared to logs of *P. atrosepticum* for the same dose (Figures 4 and 5). Bacterial suspension exposed to 0.5 KGy of gamma rays showed a decrease in the log CFU/ml of *P. cartovorum* subsp. *brasiliense* from 9.68 to 8.18 while the same gamma rays dose led to a decrease in log CFU/ ml of *P. atrosepticum* from 9.61 to 8.83. A similar trend could be recognized for greater doses used as 1.0 and 1.5 KGy. The dose of 2.0 KGy caused a complete inactivation of *P. cartovorum* subsp. *brasiliense* (log CFU/ ml of and was zero) while log CFU/ ml of *P. atrosepticum* was 1.7. The complete inactivation of *P. atrosepticum* occurred with radiation dose 2.5 KGy.

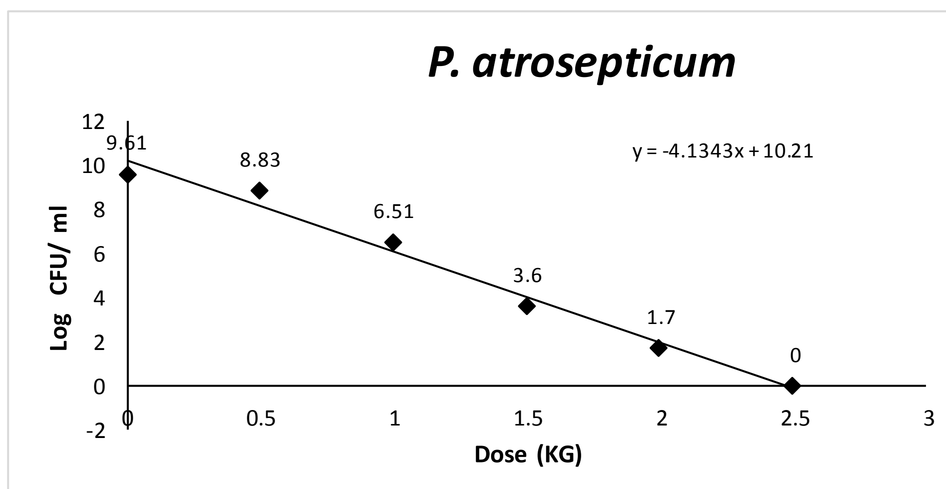

Fig. (4): Detrimental effect of gamma radiation on *P. atrosepticum*

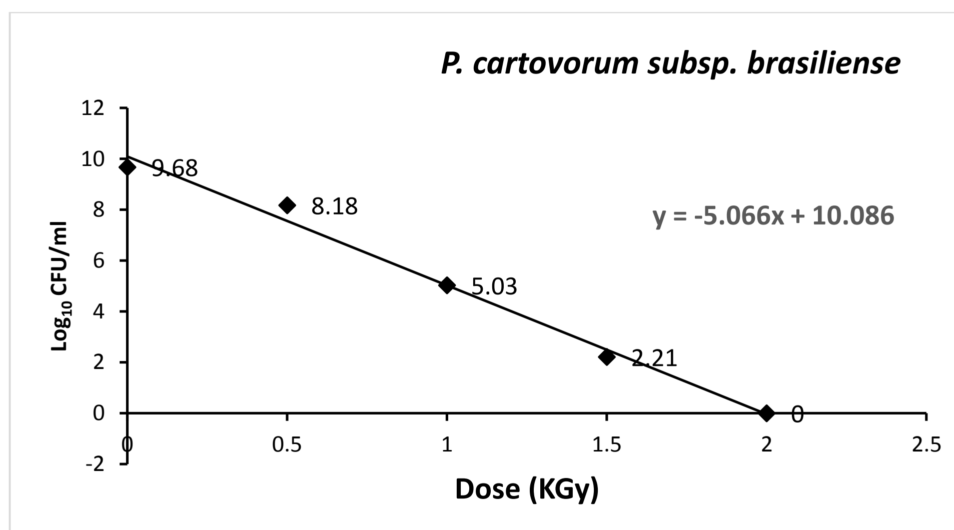

Fig. (5): Detrimental effect of gamma radiation on *P. cartovorum* subsp. *brasiliense*

D_{10} Value of Bacterial Isolates

D_{10} value = - 1/slope.

Slope (m) =change in y / change in x ($y - y_1 / x - x_1$), then: $y - y_1 = m(x - x_1)$, where the line crosses the y-axis so point (x_1, y_1) is actually at (0, b), then: $y - b = m(x - 0)$ which is: $y - b = mx$, then: $y = mx + b$. https://www.mathsisfun.com/algebra/line-equation-point-slope.html . From figures (4) and (5): for *P. atrosepticum* (E2), Slope= - 4.13429 and D_{10} = 0.24KGy; and for *P. cartovorum* subsp. *brasiliense* (E4), Slope: -5.066 and D_{10}= 0.20KGy.

Effect of Gamma Irradiation on Length of Shelf Life of Tubers

Data in Tables (3) show the increase of shelf life of artificially infected potato tubers with soft rot bacteria by irradiation. Irradiating tubers by gamma rays using D_{10} value determined for each corresponding pathogenic bacterium (0.24 KGy for *atrosepticum* and 0.20 KGy for *brasiliense*) exerted a promising effect. However, the effect was different in increasing the shelf life of potato when studied with the two subspecies of bacteria. Tubers infected with *atrosepticum* (control treatment) showed a complete rot after 50 days, compared to those infected and irradiated by gamma

rays (0.24 KGy) that extended to 70 days. Moreover, the non-irradiated tubers infected with *brasiliense* showed a complete rot after 40 days, compared to those infected and irradiated by gamma rays (0.2 KGy) that rotten totally after 60 days.

Furthermore, the gradual incidence of rotten tubers significantly decreased in irradiated tubers compared to control treatments of both species at the same period. In the case of *atrosepticum* treatments, there were no rotten tubers after 5 and 10 days in the irradiated treatment. In control treatment, however, the mean number of rotten tubers was 4.6 and 12.4 after 5 and 10 days, respectively. The mean number of rotten tubers in control was 18.8 and in the irradiated treatment was 7.8, with a decrease in the percentage of rot reaching 58.5% after 15days. Twenty percent decrease in the number of rotten tubers was recorded after 50 days, a complete collapse of tubers (40 tubers) in control treatment compared to 32.0 in irradiated treatment.

In the case of *P. cartovorum* subsp. *brasiliense*, using 0.2 KGy gamma rays led to a decrease in a number of rotten tubers. There were no rotten tubers after 5 and 10 days in the irradiated treatment, compared to those in the unirradiated control treatment that showed 7.8 and 13.4, respectively. After 15 days, the mean number of rotten tubers in control was 19.2 and in the irradiated treatment 6.4. The overall decrease in the percentage of rotten tubers was 66.7 % becoming more pronounced after the first 10 days. With prolonged keeping period, the mean number of rotten tubers increased in both two treatments. The percentage of rot decrease recorded after 40 days was 28.0 % when all tubers (40 tubers) in control treatment were collapsed and the mean number of rotten tubers in the irradiated treatment was 28.8 .

Table (3): Rot decrease in irradiated and artificially infected tubers with soft rot and storage at ambient temperature

Storage time (Days)	***P. atrosepticum* E2 (0.24 KGy)**			***P. cartovorum* subsp. *brasiliense* E4 (0.2 KGy)**		
	Infected tubers (control)	**Infected tubers (irritated)**	**Decrease in infected tubers%**	**Infected tubers (control)**	**Infected tubers (irritated)**	**Decrease in infected tubers%**
5	4.6^{m}	0.0^{n}	100.0	7.8^{j}	0.0^{l}	100.0
10	12.4^{k}	0.0^{n}	100.0	13.4^{h}	0.0^{l}	100.0
15	18.8^{h}	7.8^{l}	58.5	19.2^{f}	6.4^{k}	66.7
20	26.8^{f}	8.4^{l}	68.7	28.8^{e}	10.6^{i}	63.2
25	29.6^{e}	11.8^{k}	60.1	33.0^{d}	13.8^{h}	58.2
30	32.4^{d}	13.6^{j}	58.0	36.6^{b}	16.0^{g}	56.3
35	35.4^{c}	16.4^{i}	53.7	39.6^{a}	19.2^{f}	51.5
40	36.0^{c}	24.4^{g}	32.2	40.0^{a}	28.8^{e}	28.0
45	39.0^{ab}	30.0^{e}	23.0	40.0^{a}	32.2^{d}	19.5
50	40.0^{a}	32.0^{d}	20.0	40.0^{a}	35.0^{c}	12.5
55	40.0^{a}	35.2^{c}	12.0	40.0^{a}	39.0^{a}	2.5
60	40.0^{a}	36.0^{c}	10.0	40.0^{a}	40.0^{a}	00.0
65	40.0^{a}	38.2^{b}	4.5	-	-	-
70	40.0^{a}	40.0^{a}	00.0	-	-	-

Means shared a letter within second and third or fifth and sixth columns are not significantly different using Duncan's Multiple Range Test ($p \leq 0.05$).

Effect of Gamma Radiation on Tubers Quality

Results in Tables (4 and 5) reveal an inverse relation between sugar content and starch. The total sugars content increased while the starch content decreased immediately after irradiation process compared to the control. The sugar content increased from 5.21 mg/g fresh weight in control treatment

to 5.81and 5.85 mg/g fresh weight and the starch content decreased considerably from 13.82 g/100g fresh weight in control treatment to 13.40 and 13.35 g/100g fresh weight as after treatment with 0.2 and 0.24 KGy of gamma doses, respectively. The increase in the total sugars in the irradiated tubers was followed by a subsequent decrease, and the decrease in the starch content was followed by an increase in all treatments (control and irradiated treatments) regardless of irradiation during keeping periods for 3, 6, 9 weeks at ambient temperature are being clear. The rate of decreasing sugars in irradiated treatments was higher than that in control treatment. The mean values of the total sugars in the two irradiated treatments, after 9 weeks (3.58 and 3.42 mg/ g fresh weight) were low compared to the control treatment (3.83 mg/ g fresh weight). The rate of increasing starch in the irradiated treatments was higher than the rate of increasing starch in control treatment. The mean values of starch in the two irradiated treatments (15.90 and 15.40 g/100g fresh weight) after 9 weeks were higher than in the control treatment (14.63 g/100g fresh weight).

Table (4): Effect of irradiation on total sugars of potato tuber (mg/ g fresh weight)

Radiation dose (KGy)	Storage period (week)			
	0	3	6	9
Control	5.21^{b}	4.71^{c}	4.24^{e}	3.83^{f}
E4 dose (0.20)	5.81^{a}	5.09^{b}	4.38^{d}	3.58^{g}
E2 dose (0.24)	5.85^{a}	5.13^{b}	4.19^{e}	3.42^{h}

Means shared a letter within the table are not significantly different using Duncan's Multiple Range Test ($p \leq 0.05$).

Table (5): Effect of irradiation on starch of potato tuber (g/100g fresh weight)

Radiation dose (KGy)	Storage period (week)			
	0	3	6	9
Control	13.82^{ef}	13.90^{ef}	14.06^{de}	14.63^{c}
E4 dose (0.20)	13.40^{f}	13.86^{ef}	14.61^{c}	15.90^{a}
E2 dose (0.24)	13.35^{f}	13.67^{ef}	14.44^{cd}	15.40^{b}

Means shared a letter within the table are not significantly different using Duncan's Multiple Range Test ($p \leq 0.05$).

The phenolic content of potato tubers showed an increase in irradiated tubers compared to control. The mean value of phenolic content increased from 17.47 mg GA / 100 g in the control to 18.13 and 18.71 mg GA / 100 g as a result of treatment with 0.20 and 0.24 KGy of gamma doses, respectively. Phenolic content increased by increasing the storage period in the irradiated and control treatments (Table 6). Values of phenolic content after 9 weeks were kept high in the two irradiated treatments (18.99 and 19.45 mg GA / 100 g fresh weight) compared to the control treatment (17.91 mg GA / 100 g fresh weight).

Table (6): Effect of irradiation on phenolic of potato tuber (mg GA/ 100g fresh weight)

Radiation dose (KGy)	Storage period (week)			
	0	3	6	9
Control	17.47^{h}	17.60^{gh}	17.75^{fg}	17.91^{f}
E4 dose (0.20)	18.13^{e}	18.33^{d}	18.57^{c}	18.99^{b}
E2 dose (0.24)	18.71^{c}	19.00^{b}	19.16^{b}	19.45^{a}

Means shared a letter within the table are not significantly different using Duncan's Multiple Range Test ($p \leq 0.05$).

DISCUSSION

Pectobacterium atrosepticum and *Pectobacterium cartovorum* are among the most important species in the genus *Pectobacterium* that causes severe losses of many crops in the field, during storage and during marketing and transit **(Baht *et al*., 2010 and Manstebo *et. al*., 2104)**. Identification of pectolytic *Erwinia* is traditionally based on morphological, physiological and biochemical characteristics **(De Boer and Kelman, 2001)**. The five pathogenic isolates, isolated from rotten potato tubers collected from kitchen kept potato, were identified as *Pectobacterium atrosepticum* and *Pectobacterium cartovorum* subsp. *brasiliense.* A traditional identification was confirmed by molecular techniques such as 16S rDNA sequence **(Hauben *et al*., 1998; Zhu *et al*., 2010; Nabhan *et al*., 2013; Gasic *et al*., 2014 and Wu *et al*., 2015)**. The identification of the two selected pathogenic bacterial isolates E2 and E4 by 16S rDNA confirmed identity with the preliminary identification made based on morphological, physiological and biochemical characteristics. E2 was matched with *Pectobacterium atrosepticum* and showed 97 % similarity to *Pectobacterium atrosepticum* strain CFBP 1526 which is available in NCBI database. The identification of E4 isolate was confirmed to be *Pectobacterium cartovorum* subsp. *brasiliense* and showed 96% similarity to *Pectobacterium cartovorum* subsp. *brasiliense* strain 212, as available in NCBI database.

The irradiation of bacterial suspension(s) indicated that gamma rays inhibited the growth of both *P. atrosepticum* and *P. cartovorum* subsp. *brasiliense*. Increasing the doses of gamma irradiation have led to a decrease in the log CFU/ ml of the two tested bacteria, and with a complete inhibition with 2.5 KGy in the case of *P. atrosepticum*, and 2.0 KGy in the case of *P. cartovorum* subsp. *brasiliense*. These results were in agreement with those of **Jeong *et al*. (2016)** who found that log CFU/ ml of *Erwinia (Pectobacterium) cartovorum* subsp. *cartovorum* decreases with the increase of gamma irradiation dose and completely inhibited by approximately 0.6 KGy irradiation dose. The recognized difference on a complete inhibition dose in the results of this investigation and that of the last-mentioned study may be attributed to the difference in concentration of bacterial suspension irradiated, being in this study more than 10^9 CFU/ ml compared to 10^5 CFU/ ml in that study. In addition, differences between species, subspecies and different isolates evidently play an important role in the sensitivity of the bacteria to gamma irradiation.

The direct effect of radiation is due to the interaction of rays with molecules causing ionization or excitation and then causing damage in the molecules **(Nather, 2001)**. The effects of gamma radiation on bacterial cell suspension is a dramatic reduction of the viable counts as well as an increase in the amounts of DNA and protein released from the cells **(Jeong *et al.*, 2015)**. Gamma irradiation may change the shape of bacterial cells. Irradiated bacterial cells illustrated surface cracks and had rough cell surface. Gamma irradiation caused a damage in bacterial cell membrane leading to bacterial cell death. Furthermore, gamma irradiation may cause DNA fragmentation. These effects on bacterial cells varied according to the dose of gamma irradiation **(Jeong *et al*. 2016).**

The D_{10} value of *P. atrosepticum* was found to be 0.24 KGy and the D_{10} value of *P. cartovorum* subsp. *brasiliense* was 0.20 KGy. These results showed that the D_{10} value varies with different types of species. This finding was confirmed by previous studies that revealed that the D_{10} values vary depending on types of species and strains. **Olanya *et al*. (2015)** reported that the D_{10} values of *Pseudomonas fluorescence* strains 2-79, Q8R1 and Q287 were 0.10, 0.09 and 0.12 KGy, respectively. **Hwang *et al*. (2013**) stated that D_{10} values of different strains of lactic acid bacteria were different and ranged from 0.26 to 0.90 KG. **Rajkowski (2008)** showed that D_{10} values *Listeria monocytogenes* different strains ranged from 0.48 to 0.85 KGy.

Gamma Irradiations in this work have led to extending the shelf life of artificially infected potato tuber by soft rot bacteria and caused a decrease in a number of rotten tubers after the different durations, with considerable variations between pathogens under the study. The results reported herein are in accordance with those of **Spalding and Reeder (1986)** who found that gamma irradiation reduced the incidence of bacterial soft rot of tomato. Moreover, these results are in harmony with

those of **Jeong *et al.* (2016)** who used gamma irradiation to reduce disease incidence and severity of soft rot disease of paprika. This effect was attributed to the detrimental effect of gamma irradiation on soft rot bacterial pathogen. The shelf life of kept potato tubers varied with the difference of soft rot bacterial pathogen, the concentration of the bacterial pathogen and the dose of gamma irradiation.

This investigation showed that gamma irradiation increased the total sugars content and decreased starch content immediately after the process. The increase in the total sugars in the irradiated potato tubers was followed by a decrease during keeping periods. The decrease in the starch content was followed by an increase in the starch content during keeping periods (3, 6, 9 weeks) was recognized at ambient temperature. Immediately after irradiation, potato tubers showed a transient increase in sugar contents. The sugar contents increased in the untreated tubers when compared with the irradiated tubers after storage for a long time at ambient temperature (**Nouri and Toofanian 2001**). When potato tubers were stored at high temperatures sugars are converted into starch **(Illeperuma *et al*., 1998)**. The decrease in the sugar content and the increase in the starch content are advantageous in cooking and making crisps.

The phenolic contents in kept potato tubers significantly increased by irradiation and were significantly increased with the increase of keeping period. The increase of the phenolic content that resulted from irradiation can enhance the resistance of potato tubers against bacterial soft rot. This finding was in harmony of **Ogawa *et al.* (1968) and Ogawa *et al.* (1969)** who found that the phenolic contents of the irradiated potato tubers were higher than the non-irradiated tubers and the irradiated sample showed a larger defense action against infection by the black rot fungus, *Ceratocystis fimbriata* than the non-irradiated sample.

CONCLUSION

Gamma irradiation inhibited the growth of *Pectobacterium atrosepticum* and *Pectobacterium carotovorum* subsp. *brasiliense*. Moreover, Gamma irradiation decreased the number of rotten potato tubers during potato keeping. It extended the shelf life of potato and enhanced the quality of kept potato tubers.

ACKNOWLEDGEMENT

The authors would like to express their appreciations and thanks to Prof. Dr. A. A. Gomaa (The Former Chairman of Bacterial Diseases Research Department, Plant Pathology Research Institute, A R C, Giza, Egypt) for his encouragement and advice during this investigation.

REFERENCES

Abdullah, R.; Iftikhar, S.;Iqtedar, M.; Kaleem, A.; Iqbal, I. and Naz, S. (2016). Gamma irradiation as a quarantine treatment to prevent postharvest spoilage of *Daucus carota* L. Fuuast Journal of Biology, 6 (1): 109 – 115.

Baht, K.A.; Masood, S.D.; Baht, N.A.; Baht, M.A.; Razvi, S.M.; Mir, M.R.; Aktar, S.; Wani, N. and Habib, M. (2010). Current status of postharvest soft rot in vegetables: A Review. Asian Journal of Plant Sciences, 9(4): 200 – 208.

Bruhn, C. (2001). Frequently asked questions about food irradiation. University of California, Agriculture and Natural Resources, publication 7255.

Czajkowski, R.; de Boer, W. J.; van Veen, J. A. and van der Wolf J. M.(2012). Studies on the interaction between the biocontrol agent, *Serratia plymuthica* A30, and blackleg-causing *Dickeya* sp. (biovar 3) in potato (*Solanum tuberosum*). Plant Pathology ,61: 677–688.

Czajkowski, R.; Pérombelon, M.C.M.; Jafra, S.; Lojkowska, E.; Potrykus, M.; van der Wolf, J.M. and Sledz, W. (2015). Detection, identification and differentiation of *Pectobacterium* and *Dickeya* species causing potato blackleg and tuber soft rot: a review. The Annals of Applied Biology, 166(1): 18-38.

Daniel, H.D. and George, C.M. (1972). Peach seed dormancy in relation to endogenous inhibitors and applied growth substances. Journal of the American Society for Horticultural Science, 97: 651-654.

De Boer, S.H. and Kelman, A. (2001). *Erwinia* soft rot. In: Schaad, N.W.; Jones, J.B., and Chum, W. (Eds.). Laboratory Guide for Identification of Plant Pathogenic Bacteria, 3rd Ed. APS Press, pp. 56 – 73.

Duncan, D.B. (1955). Multiple rang and multiple F test. *Biometrics*, **11:** 1-42.

Eden, P.A.; Schmidt, T.M.; Blakemore, R.P. and Pace, N.R. (1991). Phylogenetic analysis of *Aquaspirillum magnetotacticum* using polymerase chain reaction-amplified 16S rRNA-Specific DNA. International Journal of Systematic Bacteriology, 41(2): 324-325.

Elagamey, A.A.; Abdel-Wahab, M.A.; Shimaa, M.M.E. and Abdel-Mogib, M. (2013). Comparative study of morphological characteristics and chemical constituents for seeds of some grape table varieties. Journal of American Science, 9(1):447-445.

Elayaraj, B.; Selvaraju, M. and Dhanam, S. (2015). Physiological and biochemical responses of *Ceriops roxburghiana* Arn. seedling under salt stress conditions. Journal of Aridland Agriculture, 1: 20-30.

Gasic, K.; Gavrilovic, V.; Dolvac, N.; Trkulja, N.; Zivkovic, S.; Ristic, D. and Obrodovic, A. (2014). *Pectobacterium carotovorum* subsp. *carotovorum* – the causal agent of broccoli soft rot in Serbia. Pestic . Phytomed (Belgrade), 29(4): 249 – 255.

Hajhamed, A.A.; Abd El -Sayed, W. M.; Abou El-Yazied, A. and Abd El-Ghaffar, N.Y (2007). Suppression of bacterial soft rot disease of potato. Egypt. J. Phytopathol., 35 (2): 69-80.

Hauben, L.; Moore, E.R.; Vauterin, L.; Steenakers, M.; Mergaert, J.; Verdonck, L., and Swings, J. (1998). Phylogenetic position of phytopathogens within the Enterobacteriaceae. Systemic and Applied Microbiology, 21: 384 – 397.

Hwang, E. N.; Kang, S. M.; Kim, J. K.; Lee, J. W. and Park, J. H. (2013). Screening of radio-resistant lactic acid bacteria. Korean Journal for Food Science of Animal Resources, *33*(3), 335-340.

Jeong, R. D.; Chu, E. H.; Park, D. H. and Park, H. J. (2016). Control of postharvest bacterial soft rot by gamma irradiation and its potential modes of action. The Plant Pathology Journal, 32(2): 157–161.

Jeong, R.D.; Shin, E.J.; Chu, E.H. and Park H.J. (2015). Effects of ionizing radiation on postharvest fungal pathogens. Plant Pathology Journal, 31(2): 176-180.

Karabulut, O. A. and Baykal, N. (2004). Integrated control of postharvest diseases of peaches with a yeast antagonist, hot water and modified atmosphere packaging. Crop Protection, 23(5), 431-435.

Karim, M. R.; Khan, M. M. H.; Uddin, M. S.; Sana, N. K.; Nikkon, F. and Rahman, M. H. (2008). Studies on the sugar accumulation and carbohydrate splitting enzyme levels in post harvested and cold stored potatoes. Journal of Bio-Science, 16, 95-99.

Kim, K. H. and Yook, H. S. (2009). Effect of gamma irradiation on quality of kiwifruit (*Actinidia deliciosa* var. *deliciosa* cv. Hayward). Radiation Physics and Chemistry, 78(6): 414-421.

Illeperuma, C.; Schlimme, D. and Solomos, T. (1998). Changes in sugars and activities of sucrose phosphate synthase, sucrose synthase, and invertase during potato tuber (Russet Burbank) reconditioning at 10ºC in air and 2,53 kPa oxygen after storage for 28 days at 1ºC. Journal American Society Horticultural Science, 123(2):311-316.

Logan, C. (1963). A selective medium for the isolation of sot-rot coliforms from soil. Nature (London), 199:623.

Mahto, R. and Das, M. (2014). Effect of gamma irradiation on the physico-mechanical and chemical properties of potato (*Solanum tuberosum* L.), cv. 'Kufri Sindhuri', in non-refrigerated storage conditions. Postharvest Biology and Technology, 92, 37.

Mantsebo, C. C.; Mazarura, U.; Goss, M. and Ngadze, E. (2014). The epidemiology of *Pectobacterium* and *Dickeya* species and the role of calcium in postharvest soft rot infection of potato (*Solanum tuberosum*) caused by the pathogens: A review. African Journal of Agricultural Research, 9(19), 1509-1515.

Mukhopadhyay, S.; Ukuku, D.; Fan, X. and Juneja, V. K. (2013). Efficacy of integrated treatment of UV light and low-dose gamma irradiation on inactivation of *Escherichia coli* O157: H7 and *Salmonella enterica* on Grape Tomatoes. Journal of Food Science, *78*(7), M1049 - M1056.

Nabhan, S.; De Boer, S.H.; Maiss, E. and Wydra, K. (2013). *Pectobacterium aroidearm* sp. nov., a soft rot pathogen with preference for monocotyledonous plants. International Journal of Systemic and Evolutionary Microbiology, 63: 2520 –2525.

Nather, A. (2001). Advances of tissue banking Vol. (5).The scientific basis of tissue transplantation. World scientific publishing Co. Pte. Ltd.

Nouri, J. and Toofanian, F. (2001). Extension of storage of onions and potatoes by gamma irradiation. Pakistan Journal of biological Science, 4(10):1275-1278.

Ogawa, M.; Hyodo, H. and Uritani, I. (1969). Biochemical effects of gamma radiation on potato and sweet potato tissues. Agricultural and Biological Chemistry, 33(8): 1220-1222.

Ogawa, M.; Majima, R.; Ukitani, I. and Namiki, M. (1968). Effects of gamma-ray irradiation on metabolic changes in potato tubers in response to cutting. Plant and Cell Physiology, 9(3): 511-518.

Olanya, O. M.; Niemira, B.A. and Phillips, J.G. (2015). Effects of gamma irradiation on the survival of *Pseudomonas fluorescens* inoculated on romaine lettuce and baby spinach. LWT - Food Science and Technology, 62:55-61.

Perombelon, M.C. M. and Van der Wolf, J. M. (2002). Methods for the detection and quantification of *Erwinia carotovora* subsp. *atroseptica* (*Pectobacterium carotovorum* subsp *atrosepticum*) on potatoes: a laboratory manual.

Prakash, A.; Inthajak, P.; Hulbregtse, H.; Caporaso, F. and Foley, D. M. (2000). Effect of low dose gamma irradiation and conventional treatments on shelf life and quality characteristics of diced celery. Journal of Food Science, 65(6): 1070-1075.

Rajkowski, K. T. (2008). Radiation D_{10}-values on thawed and frozen catfish and tilapia for finfish isolates of *Listeria monocytogenes*. Journal of Food Protection, 71(11), 2278-2282.

Rezaee, M.; Almasi, M.; Majdabadi Farahani, A.; Minaei, S. and Khodadadi, M. (2011). Potato sprout inhibition and tuber quality after post-harvest treatment with gamma irradiation on different dates. Journal of Agricultural Science and Technology, 13: 829-842.

Spalding, D. H. and Reeder, W. F. (1986). Influence of hot water and gamma irradiation treatments on bacterial soft rot of tomatoes. In Proceedings of the annual meeting of the Florida State Horticultural Society (Vol. 99, pp. 145-148).

Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A. and Kumar, S. (2013). Molecular Evolutionary Genetic Analysis, Version 6.0. Molecular Biology and Evolution, 30: 2725 – 2729.

Temur, C. and Tiryaki, O. (2013). Irradiation alone or combined with other alternative treatments to control postharvest diseases. African Journal of Agricultural Research, 8(5): 421-434.

US FDA (US Food and Drug Administration) (2004). Irradiation in the production, processing and handling of food final rule. Fed. Reg. ,69:76844 – 76847.

WHO (World health organization) (1981). Wholesomeness of irradiation food, WHO Technical Reports Series, 659 (pp.34) Geneva: World Health Organization.

Wu, J.P.; Yang, C.; Jio, Z. Hu, Z.; Ding, Z., and Qiu, Z. (2015). Genetic relationships of soft rot bacteria isolated from Konjac in China by Amplified Fragment Length polymorphism (AFLP) and 16S rRNA gene sequences. Agricultural Sciences, 6: 717 – 723.

Zhu, L.; Xie, H.; Chen, S. and Ma, R. (2010). Rapid isolation, identification and phylogenetic analysis of *Pectobacterium carotovorum spp.* Journal of plant pathology, 92(2): 479 – 483.