

Effect of Gamma Irradiation on *Penicillium expansum* Isolated from “Golden Delicious” Apples

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ABSTRACT

Postharvest diseases reduce postharvest quality and limit storage period of fruit. One of the alternative methods against the postharvest diseases is to use gamma irradiation. In this study, ten *P. expansum* isolates were collected from ‘Golden Delicious’ apples in cold storages of Kayseri. According to pathogenicity test, O2 isolate of *P. expansum* was found as the most virulent isolate and used in the experiment. *In vitro*, cultures were irradiated with ⁶⁰Co gamma sources (with dose rate of 0.668 kGy h⁻¹ and specific radioactivity of 1026.2 Curie), incubated at 3-4°C and 23±1°C. At 27th day while mean the colony diameter of untreated cultures was 25.87 mm, the cultures treated with 3.0 kGy had a diameter of 9.12 mm in 3-4°C. Similarly, at 9th days 3.0 kGy treated cultures and untreated cultures had a mean colony diameter of 37.50 mm and 54.87 mm, respectively, at 23±1°C. The optimum dose was 3.0 kGy for both incubation temperatures. Differences between doses were also significant at $p < 0.05$ level. The area under the disease progress curve (AUDPC) was calculated for each individual treatment and observation day *in vitro*. Confirming statistical analysis, the lowest AUDPC value was found at 3.0 kGy treatment.

Keywords: Postharvest diseases, *P. expansum*, gamma irradiation.

INTRODUCTION

Gamma radiation is a physical treatment used against the microbial spoilage. It does not create any radioactivity in the irradiated foods (WHO, 1999; CAC, 2003; Farkas, 2006). FAO/IAEA/WHO Expert Committee for Food Irradiation (JECFI) declared that foods irradiated up to 10 kGy (1 kGy=100 krad) are wholesomeness (Anonymous, 1987; WHO, 1981). The limit of 10 kGy is accepted in Turkish Food Codex in 1999 (Anonymous, 1999).

Penicillium spp. are the most important postharvest pathogens. *Penicillium expansum* is commonly reported as the most destructive pathogen that cause of soft rot on apple (Barkai-Golan, 2001). The effect of gamma radiation on seedborn fungi (*in vitro*) on *Oryzasa tiva* was investigated by Maity et al. (2011). The responses of fungi to gamma radiation (0–4.2kGy; 0.12 kGy/h) were studied in individual cultures of major seedborne fungi including *Alternaria alternata*, *Aspergillus flavus*, *Trichoderma viride* and *Curvularia geniculata*. The inactivation of individual fungal-viability was noted in the ranges of 1.0–2.0 kGy for *A. alternata* and *A. flavus* and, and in ranges of 0.5–1.0 kGy for *T. viride* and *C. geniculata*. Complete inhibition was observed at <2.5kGy. Formations of multiple germ tubes were noted in *A. alternata* and *A. flavus* at 2.0 kGy and 2.5 kGy, respectively. *A. flavus* required a higher dose to reduce ability to 10% (D_{10}) value in comparison to other selected fungi. The dose range of 2.0–2.5 kGy were effective in killing all selected fungi (Maity et al., 2011).

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In a recent study, Mostafavi et al., (2011) worked on the effect of gamma radiation on *P. expansum* *in vitro*. The *P. expansum* cultures were exposed to gamma rays at the doses of 0.0 (control), 1.5, 2.0, 2.5, 3.0 and 3.5 kGy from a ^{60}Co gamma resource (with dose rate of 0.3 Greysecond⁻¹ and specific activity of 2300 Curie). The gamma irradiation dose above 3 kGy inhibited mycelial growth completely.

Tiryaki (1990) worked on gamma radiation doses against the postharvest decay of pear Cultivar ‘Ankara’ (*Pyrus communis* L.) *in vitro*. Radio resistance of fungal pathogens, in other words, degree of sensitivity of pathogens to gamma rays at 3-4°C *in vitro* on PDA, was found as follows: from resistant to sensitive, *Botrytis cinerea*>*Alternaria tenuissima*>*Penicillium expansum*>*Rhizopus stolonifer*.

Previous studies have demonstrated that irradiation, particularly gamma ray which was produced from ^{60}Co used for controlling postharvest decays and extending the shelflife of fresh fruit (Mostafavi et al., 2011; Prakash et al., 2000; Tauxe, 2001; Duvenhage et al., 2012). A few researchers reported that irradiation cannot eradicate pathogens but it delays only fungal development at different levels (Beraha et al., 1960; Mostafavi et al., 2011; Tiryaki and Maden, 1991).

Lethal radiation doses required for pathogens in the host are higher than in the culture media (Beraha et al., 1960; Temur and Tiryaki, 2013). Dose rate is also important for inhibition of fungal development. Beraha (1964) investigated the effect of dose rate and reported higher effects of high dose rates than low rates. *B.cinerea* infection was inhibited with 1.25-1.50 kGy of irradiation with an implementation at rate of 250 Gy min⁻¹, whereas infection was not inhibited with 2 kGy at Gy min⁻¹.

Low irradiation doses stimulated fungal development for both *in vitro* and *in vivo*. After 40 days of irradiation, lesion diameters on fruit were 36.21 mm and 34.75 mm for 1 kGy and control treatment, respectively, in Ankara pears inoculated with *Penicillium expansum* (Tiryaki and Maden, 1991).

The objective of the current study was to evaluate the effect of gamma irradiation on *P. expansum* isolated from Golden Delicious apples *in vitro*.

Isolation of pathogen

Decayed Golden Delicious apples (Fig. 1) were collected from cold storage of Yahyalı and Yeşilhisar region of Kayseri, Turkey. *Penicillium expansum* was isolated from apple fruit showing blue mold symptoms. Pieces (0.5 cm) of fruits with blue mold symptoms were disinfected by dipping on a 0.5% sodium hypochloride solution. The pieces were placed on the surface of PDA (Potato dextrose agar, PDA, Meck KGaA Darnstadt, Germany) and incubated at 23±1°C for 7 days (Fig. 2). Single spore isolation were performed for each isolate. Ten *P. expansum* isolates were collected. Code of isolates and their origins were: EL8 and EL5 (Elbuz Apple Marketing Co.), EM3 and EM10 (Eminsu Cold Storage Co.), G2 and G9 (Gülüm Agriculture Cold Storage Co.), D1 and D8 (Demir Medical Cold Storage Co.), O2 and O3 (Special Provincial Administration Cold Storage Co). These isolates in slant agar which include PDA, were stored at 3-4°C.



Figure 1. *P. expansum* decay in the cold storage

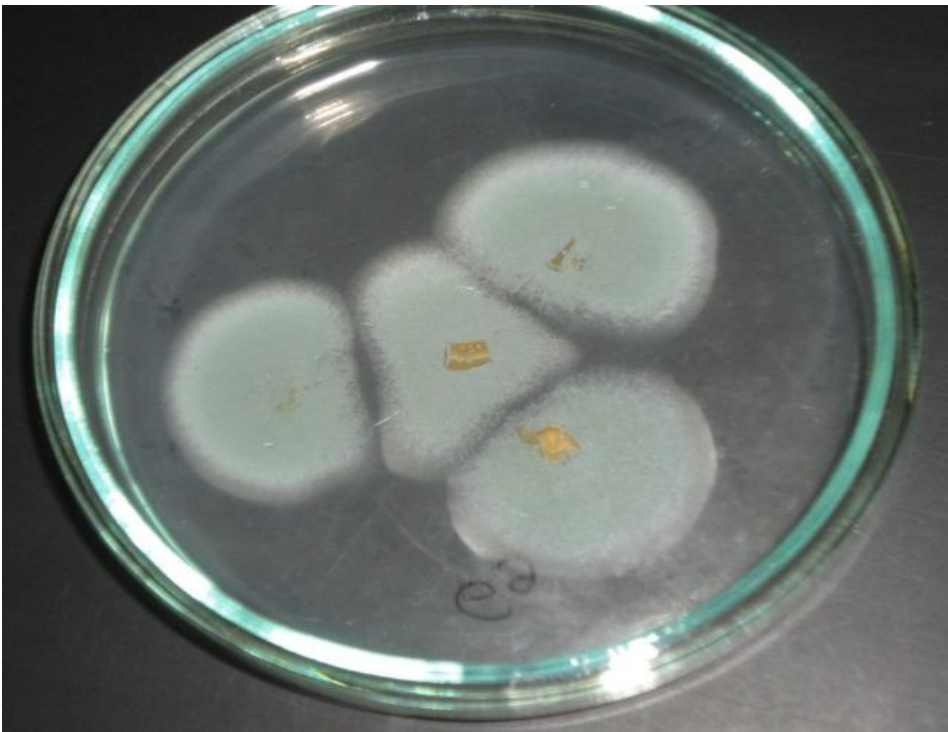


Figure 2. *P. expansum* developed on apple tissues on PDA

Pathogenicity Test

To determine the high virulent isolate of *P. expansum* pathogenicity, tests were performed by using 10 *P. expansum* isolates. A spore suspension of *P. expansum* isolates were obtained by washing a 7-day-old culture with sterile water. A high-density conidial suspension was prepared in Tween 80 (0.05%, w/v) in sterile water (Palou et al., 2007), passed through two layers of cheesecloth, adjusted with a hemacytometer. The conidial suspensions were adjusted to 10^6 spore ml^{-1} (Fig. 3).

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Figure 3. Spore suspensions prepared from *P. expansum* isolates for the pathogenicity test.

The “Golden Delicious” apples were disinfected with 0.5% NaOCl solution. After drying, apples were wounded at two sites (the opposite sides of fruit tip with 50 mm distance) with a dissecting needle (1.5 mm diameter X 2.0 mm deep). On each fruit, both wounds were inoculated with *P. expansum* by immersing needle into a suspension of 10^6 conidia ml^{-1} (Karabulut and Baykal, 2004). That inoculum density was recommended for evaluation of postharvest treatments to control green and blue molds (Palou et al., 2007). For each isolate 2 apples were inoculated with *P. expansum*, totally 4 measurement were taken. Experiments were conducted with randomized parcel design.

The apples were incubated under dark condition for 12 days at $23 \pm 1^\circ\text{C}$. The diameter of lesions on apples was measured at 6th, 9th and 12th days of incubation. Lesions diameters each isolates were statistically evaluated and SPSS software (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. Duncan’s multiple range test was used performed compare virulence of the isolates. The most virulent pathogen was selected. The identification of selected *P. expansum* isolate was performed with the images of trinocular microscope after Anniline Blue treatment to isolate. Morphological structures of fungus such as conidiophore branching, conidia shape and size, colour of culture were examined (Frisvad and Samson, 2004).

***In vitro* studies**

High virulence *P. expansum* isolate obtained from decayed apple were isolated, identified, and cultured on PDA. Cultures were irradiated with doses of 0.0 (control), 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 kGy. Irradiation carried out by using ^{60}Co gamma source (with dose rate of 0.668 kGy h^{-1} and specific radioactivity of 1026.2 Curie) located at the Saraykoy Nuclear Research and Training Center, Ankara, Turkey. After irradiation a 5 mm diameter mycelial disc from irradiated cultures of *P. expansum* was placed in the center of fresh PDA plates with 4 replications. The petri dishes were held 4 hours at $23 \pm 1^\circ\text{C}$ and then were held for 4 weeks at $3-4^\circ\text{C}$, paralleling with *in vivo* conditions (Tiryaki, 1990). Other 4 replicates were held for 2 weeks at $23 \pm 1^\circ\text{C}$, paralleling with fungal development conditions (Seçer and İç, 2003). The diameter of colonies was measured in 3 day intervals. Initial diameter of colony was 5 mm.

RESULTS

Result of pathogenicity test of isolates

The determine highest virulence of *P. expansum* isolate, pathogenicity test were performed with EL8, EL5, EM3, EM10, G2, G9, D1, D8, O2 and O3 isolates by the method described above. Pathogenicity test data of isolates, i.e., the diameters of lesions on apple statistically analyzed by using SPSS software at 5% level (Table 1). Table 1 showed that there were no statistical differences between ten *P. expansum* isolates. However, at 9th and 12th days measurement, lesion diameters of O2 isolates was bigger than other isolates. Lesion developments of *P. expansum* isolates at 12th days was shown in Fig. 4. Therefore, O2 isolate was the most aggressive isolate among them.

Table 1. Lesion diameters (mm) on apple obtained from pathogenicity test at 23±1°C

Code of isolate	Day after inoculation		
	6	9	12
G9	25.12 C*	39.12 B*	53.75 A*
EL8	25.37 C	40.00 A	56.00 A
O3	25.62 BC	40.00 A	55.25 A
D8	26.75 ABC	40.50 A	56.75 A
EM10	28.25 ABC	43.87 A	58.62 A
EL5	28.37 ABC	43.50 A	57.87 A
EM3	29.50A	43.50 A	57.87 A
D1	29.50 A	43.50 A	56.50 A
O2	29.62A	44.25A	59.25A
G2	29.75A	43.62A	57.50A

* Values followed by same letter within the same column were not significantly different at $p < 0.05$

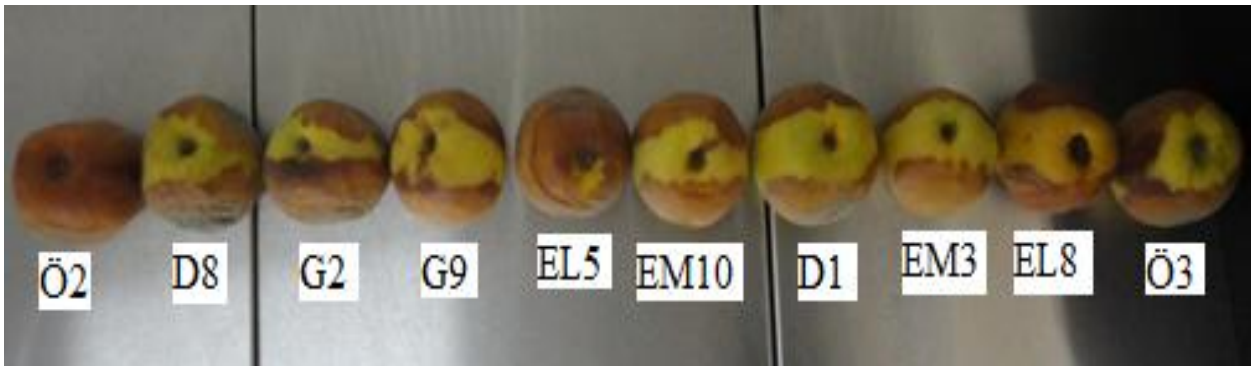


Figure 4. Lesion developments of *P. expansum* isolates at 12th days

Identification of O2 *P. expansum* isolate

The identification of most virulent O2 isolate of *P. expansum* was performed by mentioned method above. The fungal characteristics were shown in Fig. 5.

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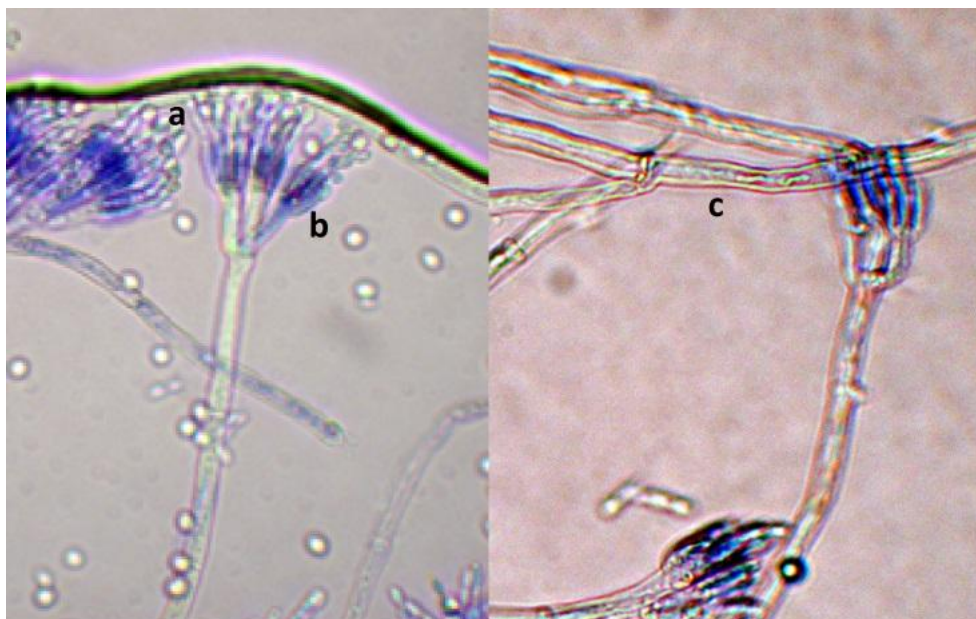


Figure 5. Conidium (a), conidiophora (b) and hyphae (c) of *P. expansum* O2 isolate.

Results of irradiated cultures at 3-4°C storage

The mean colony diameters of irradiated *P. expansum* culture were presented in Table 2. Until 21 days of incubation, there was no any fungal development at 2.0, 2.5, 3.0, 3.5, 4.0 kGy applications. At 27th day while the mean colony diameter of untreated cultures was 25.87 mm, the cultures treated with 3.0 kGy had a diameter of 9.12 mm. These effects were also found to be significant at $p < 0.05$ (Fig. 6). Therefore, the effective gamma irradiation dose was assumed as 3.0 kGy *in vitro*. The colony size of *P. expansum* cultures (irradiated with different gamma rays and incubated at 3-4°C) were also illustrated in Fig. 7. The treatment of gamma irradiation on mycelial growth showed that doses of more than 3 kGy totally inhibited *P. expansum* growth.

Table 2. Colony diameters (mm) of *P. expansum* isolate O2 irradiated at different doses and cold stored (3-4°C)

Day	Dose (kGy)							
	Control	1.0	1.5	2.0	2.5	3.0	3.5	4.0
12	10.50 A*	11.00 A	0.00B	0.00 B	0.00B	0.00B	0.00	0.00B
15	13.50 A	13.00 A	0.00B	0.00B	0.00B	0.00B	0.00B	0.00B
18	16.50 A	15.00 B	12.25 C	0.00D	0.00D	0.00D	0.00D	0.00D
21	21.00 A	18.50 B	15.25 C	11.62 D	13.25 D	0.00E	0.00E	0.00E
24	22.87 A	20.75 B	18.25 C	13.62 E	16.25 D	0.00G	10.62 F	0.00G
27	25.87 A	22.87 B	21.25 BC	15.87 D	19.25 C	9.12 E	13.62 D	14.62 D
30	27.50 A	23.75 B	23.50 B	18.50 C	21.87 B	10.87 D	16.50 C	16.62 C
33	29.62 A	25.25 B	25.75 B	21.37 C	24.00 B	13.50 E	18.75 D	19.25 CD
36	31.00 A	26.50 BC	27.25 B	24.00 CD	25.50 BC	15.50 F	21.25 E	21.87 DE
39	32.75 A	27.00 BC	28.62 B	25.62 BCD	27.25 BC	18.50 E	23.50 D	24.87 CD

* Values followed by same letters within the same row (line) were not significantly different at $p < 0.05$.

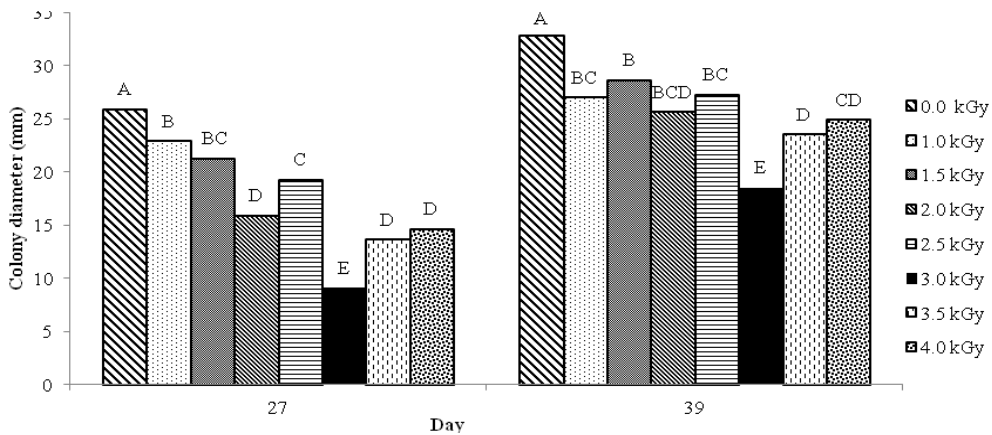


Figure 6. Colony size of *P. expansum* culture irradiated with different gamma rays and incubated at 3-4°C

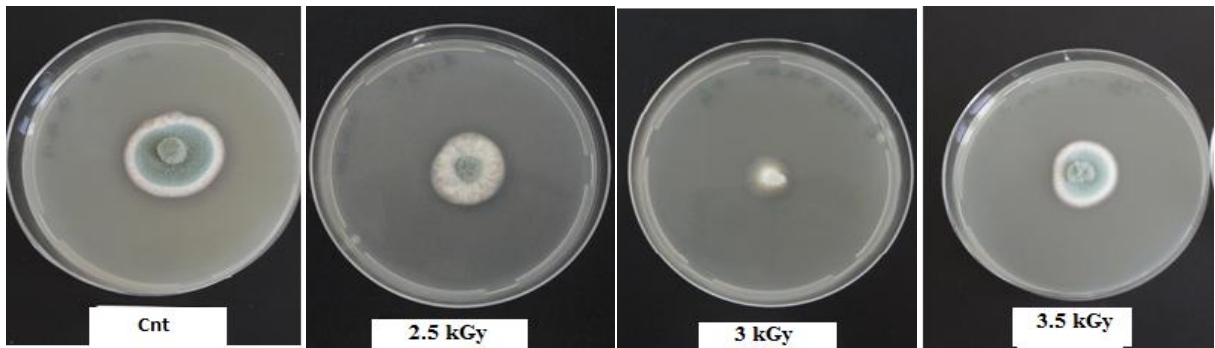


Figure 7. Colony developments of *P. expansum* cultures irradiated with different gamma rays, at 27th day of 25 incubation at 3-4°C.

Results of irradiated cultures at 23±1°C storage

As it can be seen in Table 3, fungal development started at 3rd day of incubation. At 9th days 3.0 kGy treated cultures had a mean colony diameter of 37.50 mm while untreated culture had 14.25 mm. These effects were significant at $p < 0.05$ (Fig. 8). However, at 12th and 15th days of observation, there was no significant difference between 3.0 kGy and other treatments, but the smallest colony diameter was still in 3.0 kGy treatment. Colony development at 23±1°C was also illustrated in Fig. 9. Similarly cold storage results, the effective gamma irradiation dose was found as 3.0 kGy in this part of experiment.

Table 3. Colony diameters (mm) of *P. expansum* irradiated at different doses and incubated at 23±1°C

Day	Dose (kGy)							
	0.0	1.0	1.5	2.0	2.5	3.0	3.5	4.0
3	14.25 A*	14.12 A	14.12 A	10.75 B	12.75 AB	10.75 B	11.00 B	12.25 Ab
6	37.00 A	35.50 AB	34.37 ABC	30.62 CD	31.62 BC	21.50 F	27.12 DE	24.00 EF
9	54.87 A	54.12 A	52.75 AB	50.00 AB	48.50 BC	37.50 D	43.62 C	43.75 C
12	69.37 A	68.37 A	68.12 A	63.62 AB	67.62 AB	54.37 C	59.87 BC	62.62 AB
15	74.50 A	73.12 A	75.37 A	70.00 A	73.75 A	61.37 B	69.00 AB	72.50 A

* Values followed by same letters within the same row (line) were not significantly different at $p < 0.05$

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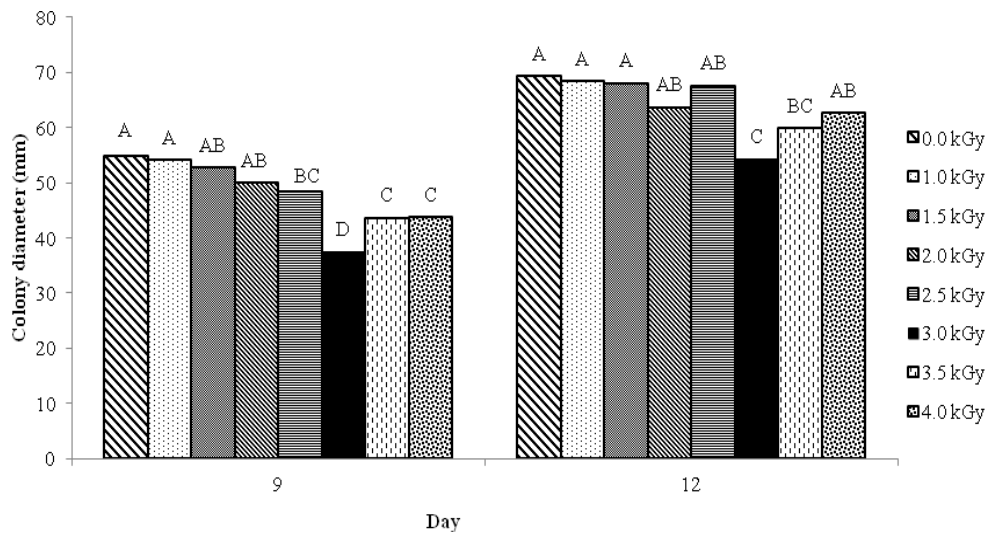


Figure 8. Colony size of *P. expansum* cultures irradiated with different gamma rays and incubated at 23±1°C

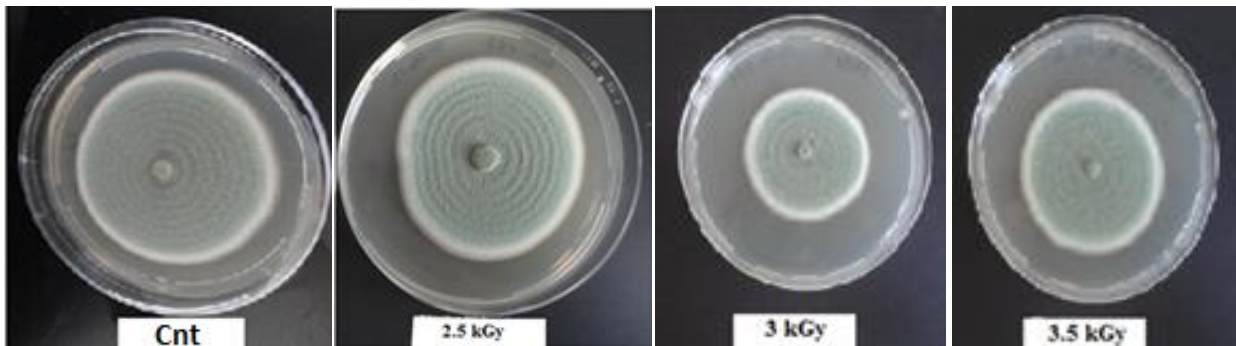


Figure 9. Colony developments of *P. expansum* cultures irradiated with different gamma rays, at 9th day at 23±1°C

In vitro AUDPC

The area under the disease progress curve (AUDPC) was calculated for each individual treatment and observation day (de Capdeville et al., 2002) *in vitro*. For this aim, the effect of irradiation on colony development of *P. expansum* evaluated with AUDPC (Fig 10). As it can be seen in the figure the lowest AUDPC value was found at 3.0 kGy treatment in all 3 observation days.

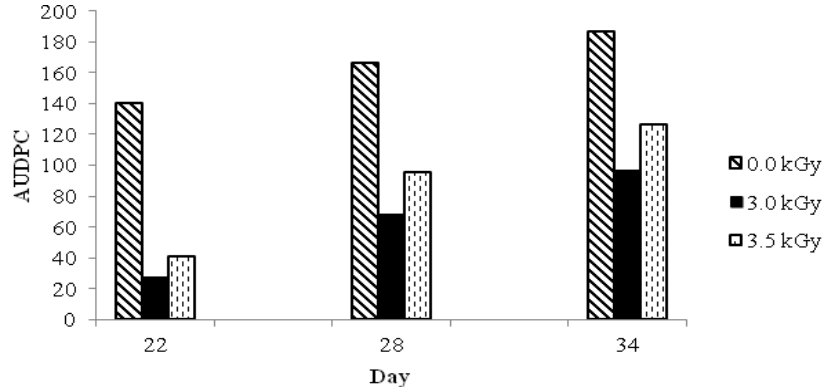


Figure 10. AUDPC evaluation of irradiation treatment on *P. expansum* development in in vitro studies at 3-4°C

DISCUSSION

This work demonstrates the applicability of gamma irradiation against the postharvest fungi. In this work for controlling *P. expansum* the effective gamma radiation dose was assumed as 3.0 kGy *in vitro*. These results are in conformity with the experiments by Pongphen et al. (2005) who showed that gamma irradiation could be used to control mycelial growth and spore germination of *P. expansum*. In our previous work, the effective gamma radiation dose against the *P. expansum* was assumed as 3.5 kGy for Golden Delicious apple (Temur, 2012). Similar findings have been reported earlier indicating that the lethal gamma radiation doses required for pathogens in the host (*in vivo*) was higher than in the culture (*in vitro*) media (Beraha et al., 1960; Temur and Tiriyaki, 2013).

Irradiation for postharvest disinfection has been for various fruits and vegetables and shows great promise in that it inhibits pathogens at doses that are low enough not to be detrimental to most fruits and vegetables (Kader, 1986; Follett, 2007). Paralleling this comment, confirmation of these study findings should be performed by *in vivo* studies for the change in qualities of irradiated apples.

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ÖZET

GAMMA RADYASYONUNUN GOLDEN DELİCIOUS ELMALARINDAN İZOLE EDİLEN *PENICILLIUM EXPANSUM* ÜZERİNE ETKİLERİ

Funguslar depolanan meyvelerde kaliteyi düşürürler ve depolama süresini azaltırlar. Hasat sonu hastalıklarına karşı alternatif yöntemlerden biri de ışınlama uygulamasıdır. Bu çalışmada, Kayseri yöresinde depolanan elmalardan 10 adet *P. expansum* izolatu toplanmıştır. Yapılan patojenisite testinde O2 *P. expansum* izolatının virulensi en fazla bulunmuş ve denemede bu izolat kullanılmıştır. *In vitro*'da kültürler ⁶⁰Co gamma kaynağında (1026.2 Ci spesifik radyoaktivite ve 0.668 kGy h⁻¹ doz oranında) ışınlanarak 3-4°C ve 23±1°C de inkübe edilmişlerdir. 3-4°C inkubasyonda 27. günde kontrol kültürlerinde ortalama 25.87 mm, 3.0 kGy ile ışınlanmış kültürlerde ise 9.12 mm koloni çapı ölçülmüştür. 23±1°C inkubasyonda 9. günde bu değerler sırasıyla, 54.87 mm ve 37.50 mm olmuştur. Her iki inkübasyon sıcaklığında *P. expansum* O2 izolatını engelleyen doz 3.0 kGy olarak bulunmuştur. Dozlar arasındaki farklılıklar da önemli bulunmuştur (p< 0.05). Hastalık gelişimini gösteren eğrinin altındaki alan (AUDPC) değerlendirmesi, her bir bireysel uygulama ve ölçüm günleri için yapılmıştır. İstatistiksel analizleri doğrular şekilde, en düşük AUDPC değeri yine 3.0 kGy uygulamasında elde edilmiştir.

Anahtar Sözcükler: Hasat sonu hastalıklar, *P. expansum*, gamma radyasyonu

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