

# Combination of Irradiation and Sodium Carbonate to Control Postharvest *Penicillium* Decay of Apples

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## ABSTRACT

*Penicillium expansum* is an important pathogen causing considerable postharvest losses in apples. Due to some restrictions applied for chemicals, there is a need for new approaches and combined treatments for inhibition of fungi. In present study, O2 isolate of *P. expansum*, isolated from apples in cold storages of Kayseri, was used. Inoculated apples were irradiated with 3.0, 3.5 and 4.0 kGy and stored at 3-4°C for 40 days. While the lesion diameter of untreated apples at 40<sup>th</sup> day was 20.28 mm, the apples treated with 3.5 kGy had a diameter of 7.85 mm. Differences in lesion diameters were found to be significant ( $p < 0.05$ ). In second part of study, inoculated apples were treated with sodium carbonate (SC,%3)+2.5 kGy, SC+3.0 kGy and SC+3.5 kGy combinations and stored at 3-4°C for 40 days. At the 40<sup>th</sup> day, lesion diameters were measured as 49.00 mm and 19.18 mm for control samples and SC+3.0 kGy treatment, respectively. The area under the disease progress curve (AUDPC) was evaluated to calculate individual and total AUDPC. Long-term impacts of treatments on *P. expansum* were also evaluated by performing re-isolation from untreated and SC+3.0 kGy treatments. There were no significant differences between control and treated ones with regard to cultural characteristics and pathogenicity of fungus.

**Key words:** Postharvest disease; *Penicillium expansum*; Irradiation; Combined treatment

## INTRODUCTION

Postharvest losses are estimated to be 30-40% in Turkey and sometimes it may reach to 50% (Karabulut and Baykal, 2004; Anonymous, 2013). The major postharvest pathogen of apples in Turkey is *Penicillium expansum* Link. Today, customers demand residue-free food products in one hand; several fungi develop resistance against common fungicides on the other hand. Alternative control strategies are under investigation due to health concerns with regard to chemicals. However, such alternatives have not been able to provide a control as effective as chemical agents have. Combinations of several alternatives increase their effectiveness and decrease negative effect of application by exposure to lower doses when compared to individual treatments (Beraha et al., 1960; Ragsdale and Sisler 1994; Tiryaki et al., 1994;

Conway et al., 2004). Temur and Tiryaki (2013a) reported advantages and disadvantages of irradiation and combined treatments of sodium carbonate (SC), cold storage, chemicals, heat treatment, modified atmosphere packaging and bio-control agent in details.

The purpose of all food production techniques is to minimize decay and extend shelf life. Previous studies have demonstrated that irradiation, particularly gamma ray, produced from  $^{60}\text{Co}$ , is used for controlling postharvest decays and extending the shelflife of fresh fruit (Mostafavi et al., 2011; Duvenhage et al., 2012). Gamma irradiation not only decreases postharvest decays and but also decreases the chemical needs for postharvest treatments (Geweely and Nawar 2006; Cia et al., 2007). The penetrating power of gamma rays is more than fungicides. They can uniformly penetrate deep into fruit and easily treat every kind of fruits regardless of the shape and size (Jarrett, 1982). Irradiation increases major phenolic compounds of fruit peels. Such an increase is related to phenylalanine ammonia-lyase enzyme activity. Ionizing radiation can induce fruit resistance against pathogens and stimulate the biosynthesis of constitutive phenolic. Researchers also concluded that the direct effects of irradiation on fungal structures growing in the rind were more important for disease reduction than a possible indirect effect on fruit defense mechanisms. This assumption is further supported by considerable inhibition of fungi sporulation through irradiation (Palou et al., 2007). DNA fragmentation is the basic mechanism of irradiation to kill microbes. Complex organisms are more sensitive to irradiation than simple ones (Shea et al., 2000).

Lethal radiation doses required for pathogens in the host are higher than in the culture media (Beraha et al., 1960). 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. *Botrytis cinerea* infection was inhibited with 1.25-1.50 kGy of irradiation with an implementation rate of 250 Gymin<sup>-1</sup>. Whereas the infection was not inhibited with 2kGy at 25Gy min<sup>-1</sup>. Darras et al., (2010) emphasized the importance of order of application. The irradiation after inoculation with *B. cinerea* was found to be more effective than before inoculation. The time elapsed between inoculation and irradiation is also significant point (Kuhn et al., 1968). An irradiation dose of 2kGy after 24 h of inoculation yielded 10% decrease in lesions over peaches. Researchers observed 60, 80 and 90% increase in lesion incidence by postponing irradiations 36, 48 and 60 h after inoculation, respectively. Low irradiation doses stimulated fungal development for both in vitro and in vivo. After 40 days of irradiation, lesion diameters were 36.21 mm and 34.75 mm respectively for 1 kGy and control treatment in Ankara pears inoculated with *Penicillium expansum* (Tiryaki and Maden 1991). Similarly, 1kGy stimulated aflatoxin incidence, whereas 3-4 kGy inhibited fungal and mycotoxin development (Kabak and Var 2005).

SC solutions have been used to control postharvest fungi on fruit since 1928 (Conway et al., 2004). It is an inexpensive readily available method with minimal risk of injury over the fruits (Palou et al., 2002; Zamani et al., 2009). Inhibitory activity of SC mostly depends on the presence of salt residues within the infected sites and the interactions between residual salt and peel constituents. SC primarily act

as fungistatic and not provide persistent protection if fruit are infected after treatment (Smilanick et al., 1999; Usall et al., 2008).

Palou et al., (2007) investigated the effects of X-ray and SC treatments on postharvest *Penicillium* decay in mandarins. SC treatment with 0.875 kGy was found to be more effective for the inhibition of fungus. *P. digitatum* lesion diameter was significantly reduced only by the highest dose of irradiation as 0.875 kGy with storage at 20°C. In a recent study carried out with *P. digitatum* and *P. italicum* in mandarins revealed that low pressure-fruit rinsing is considered as an efficient method to eliminate adverse effects of SC treatments on quality parameters of mandarins. On the other hand, Smilanick et al., (1999) reported significant loss of SC effectiveness against green mold by rinsing. Palou et al., (2007) therefore observed higher SC effectiveness on un-rinsed Clementine mandarins.

While heat treatment is very effective in eradicating decay if infection occurs prior to heating, it has little protective effect if infection occurs after heating (Klein et al., 1997). Synergistic effects of heat and SC solutions to control *Penicillium* decay on citrus fruit were also observed in previous research (Smilanick et al., 1999; Palou et al., , 2001). Heating SC solution until 50°C was considered as an effective treatment to control *Penicillium* decay in mandarins (Palou et al., 2002). Similar findings were also reported by Conway et al., (2004), suggesting the use of heated SC solution (38°C) against the *P. expansum*.

The safety of foods irradiated up to 10 kilogray (kGy, 1kGy=100 krad) is guaranteed by joint FAO/IAEA/WHO Expert Committee for Food Irradiation (JECFI) (Anonymous, 1987; WHO, 1999). The limit of 10 kGy is accepted in Turkish Food Codex in 1999 (Anonymous, 1999). Radioactivity is impossible since any chain reactions occur to make foods radioactive. Irradiation disappears when the energy source is removed (Brennand, 1995; Farkas, 2006). Unless the specified optimum doses are not exceeded for certain foodstuff, ionizing radiation has minimal or almost zero effect on nutritional characteristics, taste and quality (Diehl and Josephson, 1994).

The aim of this study was to investigate the effects of combined treatment of SC and gamma irradiation on the growth of *Penicillium expansum* in artificially inoculated Golden Delicious apples. The most virulent *P. expansum* O2 isolate from cold storages of Kayseri Province was used to evaluate the effects of individual and combined treatments.

## **MATERIALS and METHODS**

### **Pathogens**

*Penicillium. expansum* was isolated from decayed Golden Delicious apples in cold storages of Kayseri. Ten *P. expansum* isolates were collected, namely, EL8 ve EL5 (Elbuz Apple Marketing Co.),

EM3 ve EM10 (Eminsu Cold Storage Co.), G2 ve G9 (Gülüm Agriculture Cold Storage Co.), D1 ve D8 (Demir Medical Cold Storage Co.), O2 ve O3 (Special Provincial Administration Cold Storage Co). Pathogenicity test was performed with the 10 *P.expansum* isolates in our previous work (Temur and Tiryaki, 2013b). High virulent isolate of *P. expansum* was O2 isolate. This isolate was identified and cultured on potato dextrose agar (PDA Merck, KGaA Damstadt, Germany) in Petri dishes at  $23\pm 1$  °C for 7–10 days. A high-density conidial suspension ( $10^6$  conidiamL<sup>-1</sup>) was prepared in Tween 80 (0.05%, w v<sup>-1</sup>) in sterile water, passed through two layers of cheesecloth, measured with a hemacytometer, and diluted with sterile water to achieve the desired inoculum density (Palou et al., 2007).

### **Inoculation of apples with *P.expansum***

The “Golden Delicious” apples were surface disinfected with 0.5% NaOCl solution. Dried 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  $10^6$  conidiamL<sup>-1</sup> (Karabulut and Baykal 2004).

### **Irradiation treatments**

Under in vitro conditions (culture media), effective gamma irradiation dose for *P. expansum* was found to be 3.0 kGy in a previous study (Temur and Tiryaki 2013b). Hence, 0.0 kGy (control) 3.0 kGy, 3.5 kGy, and 4.0 kGy were used in in vivo studies. Each treatment was applied to 8 replicates with 1 apples each.

The apples were inoculated with *P. expansum* by using the procedure described above, and placed in polyethylene bags and stapled. They kept until the irradiation in a sample storage box at 3-4°C. Irradiation was carried out by using <sup>60</sup>Co gamma source located at the Saraykoy Nuclear Research and Training Center, Ankara, Turkey, with the 1026.2 Ci radioactivity and 0.668 kGyh<sup>-1</sup> dose rate. Irradiation was performed by placing apples in 1 L irradiation chamber of <sup>60</sup>Co gamma source. Irradiation application was illustrated in Fig. 1. After the irradiation, treated and untreated apples were incubated at 20°C for 24 h, then stored at 3-4°C for 46 days. Lesion diameters were measured again in 3-day intervals.



**Figure 1.** Irradiation of apples in  $^{60}\text{Co}$  gamma source.

### **Combined treatment of irradiation and sodium carbonate**

About 2 hours after inoculation, apples were placed in stainless steel grid baskets and submerged into 22.5 L stainless steel buckets with aqueous 3.0 % (w v<sup>-1</sup>) SC solutions for 150 s (Merck KGaA 64271 Darmstadt/Germany) at 40 °C (Palou et al., 2007). Temperature of the solutions were not allowed to change  $\pm 0.5$  °C during the treatments. Inoculated and SC-treated apples were placed in polyethylene bags and stapled. Each treatment was applied to 9 apples. SC-treated and control apples were incubated at  $23\pm 1$  °C for 24 h and stored at 3-4 °C for 39 days. Lesion diameters were measured in 3-day intervals.

Irradiation dose of 2.5, 3.0 and 3.5 kGy were combined with SC (3%) to control *P. expansum* decay. Inoculated and SC-treated apples irradiated in accordance with the methods described above. Treated and untreated apples were incubated at  $23\pm 1$ °C for 24 h, then stored at 3-4°C for 46 days. Lesion diameter of fruits were measured in 3-day intervals. In this part of experiment, the total number of treatment, including control samples (with neither SC nor irradiation treatment) are four. Similarly, each treatment was applied to 8 replicates with 1 apples each.

### **Long-term effects of combined treatment on pathogenicity and change on cultural characteristics of *P. expansum***

*P. expansum* re-isolated from combine treated (3.0 kGy+3.0% SC) fruits and the fungus were cultured on PDA. For the pathogenicity test, apples were inoculated with re-isolated *P. expansum* suspension. Then, incubated at  $23\pm 1$ °C for 24 h. Control samples were also passed through same methods. Each treatment was applied to 5 replicates with 1 apples each. Inoculation was performed as described above. After inoculation, one lot of fruit (5 replicates) was placed in storage at 3-4°C and another similarly treated lot was stored at  $23\pm 1$ °C. Later on, the diameter of lesions were measured in 3-day intervals (Tiryaki et al., 1994).

For investigation of the sporulation of fungus, 5 mm agar disk was removed from re-isolated cultures and transferred to fresh PDA and incubated at  $23\pm 1^{\circ}\text{C}$  for 24 h. Each treatment was applied to 10 replicates and each was placed into separate petri dishes. The experiment was divided into two parts; 5 replicates were stored at  $23\pm 1^{\circ}\text{C}$  and another 5 replicates were stored at  $3-4^{\circ}\text{C}$ . The diameters of colonies were measured in 3-day intervals.

### **Evaluations**

SPSS software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Duncan's multiple range test was used to compare the means of treatments. The lesion diameters were used to calculate the area under the disease progress curve (AUDPC) by using Equation 1 (de Capteville et al., 2002).

$$\text{AUDPC} = \sum[(y_i + y_{i+1})/2 \times (t_{i+1} - t_i)] \quad (1)$$

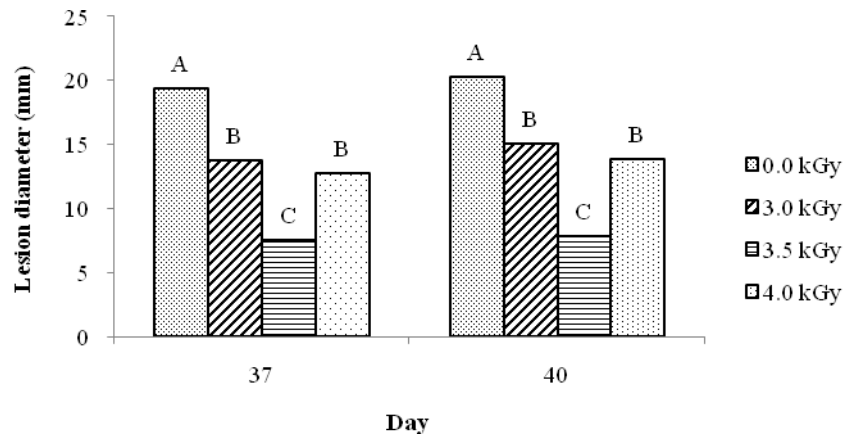
where,  $y_i$  is the diameter of a lesion at time  $t_i$ , in days,  $y_{i+1}$  is the diameter of the lesion at time  $t_{i+1}$  and  $(t_{i+1} - t_i)$  is the number of days between two evaluations.

The total AUDPC assessment was performed by the procedure specified by Palau et al., (2007).

## **RESULTS**

### **Result of irradiation treatments**

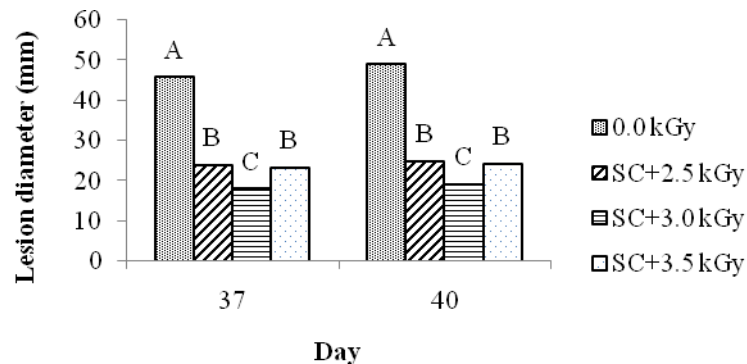
The lesion sizes on irradiated apples with gamma rays at 3.0, 3.5 and 4.0 kGy are presented in Fig.2. Irradiation dose of 3.5 kGy significantly reduced lesion diameters at 37 and 40 days of cold storage. These effects were also found to be significant at  $p < 0.05$  level. At 40<sup>th</sup> day, while the lesion diameter of untreated apples was 20.28 mm, the apples treated with 3.5 kGy had a diameter of 7.85 mm. Therefore, the effective dose was assumed as 3.5 kGy in this part of experiment. In other words, gamma-ray dose of 3.5 kGy considerably reduced the size of decay lesion on apples.



**Figure 2.** Lesion sizes on apple artificially inoculated with the and irradiated with gamma rays and incubated at 3-4°C for 37 and 40 days. Bars with same letter within same day were not significantly different at  $p < 0.05$ .

### Disease control ability of combined treatments

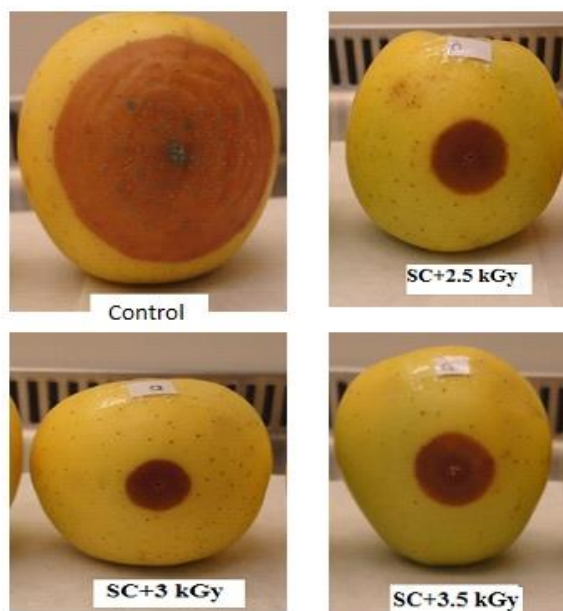
A few researchers reported that combinations of several applications increased effectiveness of single treatments (Beraha et al., 1960; Tiryaki et al., 1994; Conway et al., 2004). In present study, the combined effects of SC and irradiation treatments were also evaluated on Golden Delicious apples. Inoculated and SC-treated apples were irradiated at three gamma-ray doses of 2.5, 3.0 and 3.5 kGy. Lesion diameter of apples, subjected to SC+3kGy treatment, at 37 and 40 days of measurement are shown in Fig.3. At 40<sup>th</sup> day, the diameter of lesions caused by *P. expansum* was significantly reduced from 49.00 mm (control apples: non-irradiated and non-SC-treated) to 19.18 mm (SC+3 kGy treatment). In addition, the effect of SC +3.0 kGy combination was also photographed in Fig.4 at 40<sup>th</sup> day of observation. Therefore, the SC+3.0 kGy treatment was determined as the optimum treatment for *P. expansum* decay control.



**Figure 3.** Lesion size on apple inoculated with the *P. expansum* and combined treated with SC+2.50 kGy, SC+3.0 kGy and SC+3.5 kGy and incubated at 3-4°C for 37 and 40 days. The bars with same letter within same day were not significantly different at  $p < 0.05$ .

In present work, complying with the previous ones, while the effective gamma radiation dose for controlling *P. expansum* decay in apple was found to be 3.5 kGy; SC(3%)+3.0 kGy (by exposure lower

radiation dose) combination was considered as the optimum treatment to control *P.expansum* decay in apples. The efficiency of combined treatments was significantly superior to that of single treatments. Another issue to be highlighted here is the larger lesion size in SC + 3.5 kGy than in SC + 3.0 kGy (Fig.4) since high irradiation doses may increase sensitivity of fruits to the fungal infection.



**Figure 4.** Lesion development over apples inoculated with *P. expansum* and treated with the combination of irradiation and SC, after 40 days storage at 3-4°C.

#### **Long-term effects of combined treatment on cultural characteristics and pathogenicity of *P. expansum***

*P. expansum* was re-isolated from SC+3.0 kGy treated apples, which was found to be the optimum treatment in in-vivo studies, and development and sporulation of pathogen were evaluated. Colony diameters of *P. expansum* on PDA in Petri dishes were measured at both 3-4°C and 23±1°C incubation. In both incubation conditions, there were no significant differences between control and treated ones. Microscopic evaluations also did not show any differences on development of PDA, hypha and structure of spore.

For the pathogenicity test, apples were inoculated with re-isolated *P. expansum* from untreated and SC+3.0 kGy treated apples and incubated at 23±1°C. The lesion diameter of apples for a storage period of 16 days were measured. Any differences was not observed between control and treated ones. Lesion diameters on apples stored at 3-4°C were also measured for a storage period of 54 days. There were no differences between control and SC + irradiation treatment with regard to lesion size.

This part of study revealed that the effect of irradiation+SC treatment on pathogen was not persistent. These findings support the idea assuming food irradiation is a physical application and disappears when the energy source is removed (Urbain, 1986; Farkas, 2006).



### AUDPC evaluations

The area under the disease progress curve was calculated for each individual treatment and observation day (de Capdeville et al., 2002). The effect of irradiation on *P. expansum* development was evaluated with AUDPC calculated by using Equation 1 (Fig. 5). The figure indicates the lowest AUDPC value for 3.5 kGy treatment in all 3 observations.

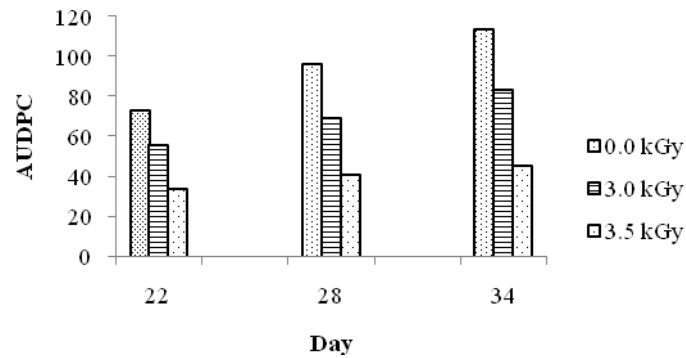


Figure 5. AUDPC evaluation of irradiation treatment on *P. expansum* development.

Similarly, the effect of combined treatment (SC+irradiation) on *P. expansum* development was evaluated with AUDPC (Fig. 6). As it can be seen in the figure, the lowest AUDPC value was found at SC+3.0 kGy treatment in all 3 observations.

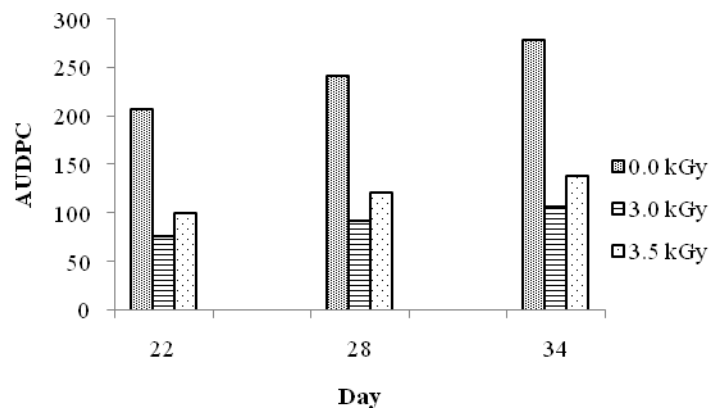
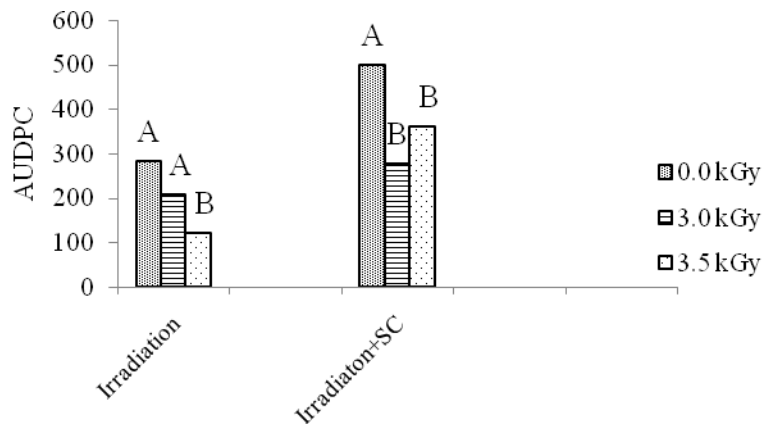


Figure 6. AUDPC evaluation of SC+ irradiation treatment on *P. expansum* development.

For the evaluation of AUDPC, Palou et al., (2007) used the total AUDPC values. Total AUDPC was calculated with the data obtained from irradiation treatment and irradiation+SC treatment parts of experiments (Fig.7). The data were subjected to Duncan's multiple range test of SPSS software. Fig.7 shows reduced rate of AUDPC in 3.5 kGy irradiation treatment and SC+3.0 kGy treatment. But the differences between the 3.0 and 3.5 kGy dose was not significant in irradiation+SC treatment.



**Figure 7.** Total AUDPC values of irradiation and SC+irradiation treatment on *P. expansum* development. The bars with same letter within same treatment were not significantly different at  $p < 0.05$ .

## DISCUSSION

Several advantages of combined treatments, including irradiation and SC, to control post harvest fungi are reported in literature. Therefore, an attempt was made in present study to evaluate the effects of combined gamma radiation and SC on growth of *P. expansum* in apples.

Although not included in objectives of present study, it was observed that ripening levels of apples was also a dominant factor in controlling postharvest decays. The more ripened the fruits are, the more they sensitive to pathogen. Lesion diameter at control apples on 37<sup>th</sup> day and 40<sup>th</sup> day reached up to 19.40 mm and 20.28 mm, respectively in initial experiment (Fig. 2). But the decay over control apples at later experiment were 45.81 mm and 49.00 mm on 37<sup>th</sup> day and 40<sup>th</sup> day of observations, respectively (Fig. 3). Such differences were mainly due to different ripening levels of apples used in both experiments. There was one month between the two set of experiments. Therefore, irradiation and irradiation+SC combination were evaluated as separate experimental designs. That is why, not only gamma doses of 3.5 kGy and 3.0 kGy, but also 2.5 kGy was used considering varying effects of irradiation based on fruit ripening levels.

In present study, it can be finally concluded that SC treatment with 3.0 kGy irradiation was the optimum implementation to control postharvest decay of *P. expansum* in apples. However, any treatment to decrease postharvest losses should be focused not only on inhibition of pathogen, but also on quality of fruit. For instance, if any treatment inhibits pathogen, this treatment should not have adverse effect of organoleptic properties of treated fruit, and vice versa. Therefore these findings should also be supported by further studies on the evaluation for organoleptic properties of treated apples (Prakash et al., 2000).

## ÖZET

### ELMALARDA HASAT SONU *PENICILLIUM* ÇÜRÜKLÜĞÜNÜN ENGELLENMESİ İÇİN IŞINLAMA VE SODYUM KARBONAT KOMBİNE UYGULAMASI

*P. expansum* elmalarda hasat sonu kayıplara neden olan en önemli fungal patojendir. Fungusun engellenmesi için, kimyasalların kısıtlamasından dolayı yeni yaklaşımlara ve kombine uygulamalara ihtiyaç duyulmaktadır. Bu çalışmada Kayseri'deki soğuk hava depolarından elmalardan izole edilen O2 kodlu *P. expansum* izolatı kullanılmıştır. İnokule edilmiş elmalar 3.0, 3.5 ve 4.0 kGy dozlarında ışınlanmış ve 3-4°C'de 40 gün muhafaza edilmiştir. 40. günde kontrol örneklerinde 20.28 mm, 3.5 kGy dozda ise 7.85 mm çürüme çapı gözlemlenmiştir. Lezyon çapları arasındaki farklılık önemli bulunmuştur ( $p<0.05$ ). Çalışmanın ikinci bölümünde inokule edilmiş elmalara sodyum karbonat (SC)+2.5 kGy, SC+3.0 kGy ve SC+3.5 kGy uygulamaları yapılmış ve 3-4°C'de 40 gün muhafaza edilmiştir. 40. günde hiçbir işlem görmemiş kontrol örneklerinde 49.00 mm lezyon çapı görülürken, SC+3.0 kGy uygulamasında bu değer 19.18 mm olmuştur. Hastalık gelişimini gösteren eğrinin altındaki alan (AUDPC) değerlendirilerek, uygulamaların bireysel ve toplam AUDPC değerleri hesaplanmıştır. *P. expansum* üzerine uygulamaların uzun süreli etkileri, kontrol ve SC+3.0 kGy uygulamalarından re-izolasyon yapılarak değerlendirilmiştir. Fungusun kültürel özellikleri ve patojenisitesinde, uygulama yapılmış ve kontrol örnekleri arasında önemli bir farklılık bulunmamıştır.

**Anahtar Kelimeler:**Hasat sonu hastalıkları; *Penicillium expansum*; Işınlama; Kombine uygulamalar

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