

Full Length Research Paper

Evaluation of gamma irradiation effect and *Pseudomonas fluorescens* against *Penicillium expansum*

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Antagonistic effect of *Pseudomonas fluorescens* and influence of gamma irradiation on the development of *Penicillium expansum*, the causal agent of postharvest disease on apple fruit was studied. *P. fluorescens* was originally isolated from rhizosphere of the apple trees. Suspension of *P. fluorescens* and *P. expansum* were mixed in test tubes in proportions of 1:5, 2:5, 3:5 and 4:5 (V/V). The inhibitory effect depended on the proportion of the bacterium to the fungus in the mixture. The best inhibition was observed after 48 h at the ratio of 3:5. Gamma irradiation above 3 KGy completely inhibited mycelial growth, while the highest dose around 600 Gy mostly killed *P. expansum* spores. Our experiment demonstrates that the combination of gamma irradiation and *P. fluorescens* was more effective in reducing *P. expansum* growth, than either treatment alone and that the integration of irradiation and antagonist treatments can be more effective. The results of this study show that improved control by irradiation at labeled dose in combination with antagonist could allow direct incorporation of the biocontrol agent. Thus, the combination of the *P. fluorescens* with gamma irradiation showed an impressive effect on increasing applied range of irradiation for postharvest control by decreasing of dose rate.

Key words: Antagonist treatment, gamma irradiation, *Penicillium expansum*, *Pseudomonas fluorescens*, postharvest disease.

INTRODUCTION

Penicillium spp. is among the most important postharvest pathogens. *Penicillium expansum* is commonly reported as the most destructive cause of soft rot (Barkai-Golan, 2001). Irradiation, particularly gamma ray which is produced from Cobalt-60 is used for controlling postharvest losses (Tauxe, 2001) and extending the shelf life of fresh produce by delaying the ripening processes (Prakash et al., 2000). For a time, irradiation cannot eradicate pathogens but it may have effects in cell damage and directly damage the chromosomal DNA of

living cell (Smith and Pillai, 2004). The *Pseudomonads* have been used in the biocontrol of important diseases and *Pseudomonas fluorescens* is regarded as a useful biocontrol agent (Loper et al., 2007). The aim of this study was to evaluate the effect of gamma irradiation and *P. fluorescens* as biocontrol agents against *P. expansum*.

MATERIALS AND METHODS

In this study, isolate of *Pseudomonas* spp. was originally isolated from rhizosphere of the apple trees. It was identified as *P. fluorescens* according to Schaad et al.'s (2001) key. Bacteria were cultivated at 25°C on NA complemented with 1.5% glucose, for the preparation of the suspension. After 24 h, the cells were washed down from the medium with sterile water. Concentration was

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confirmed by spectrophotometer at 420 nm and adjusted to 10^6 cfu/ml by serial dilution. *P. expansum* was separated from apple fruit showing blue mold symptoms. Pieces (0.5×0.5 cm) of fruits with blue mold symptoms were disinfected by dipping on a 10% sodium hypochlorite solution. The pieces were placed on the surface of PDA and incubated at 25°C for 48 h. A suspension of *P. expansum* conidia was obtained by washing a 7-day-old culture with sterile water. The concentration of spores in all suspensions was determined using microscope and adjusted to 10^4 per ml. The species of fungus was identified according to Barnett and Hunter's (1972) key.

Antagonism between bacterium and fungus

The interaction between bacterium and fungus was investigated *in vitro* as follows: suspension of bacteria and conidia of (*P. expansum*) were mixed in test tubes in the following proportions: 1:5, 2:5, 3:5 and 4:5 (V/V). The control consisted of conidial suspension in sterile water. After 12, 24 and 48 h of co-cultivation, 30 µl aliquots were placed on sterilized microscope slides and observed under a light microscope. The percentage of spore germination was determined in 10 different microscopic fields. A total of 500 spores (100 spores per replicate) were observed at each sampling time.

Gamma irradiation

A 0.5 cm diameter mycelial disc from 3 days old cultures of *P. expansum* was placed in the center of PDA plates. The plates 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 Cobalt-60 gamma resource (with dose rate of 0.3 grey/second and specific activity of 2300 curie) at the Agricultural, Medical and Industrial Research School, Karaj. The plates were placed at room temperature and radial growth was recorded daily for 7 days. Each treatment consisted of four replicates. Moreover, the conidia from 2 days old cultures were washed from the PDA plates with sterile water and diluted to the respective concentrations. The spore suspension of *P. expansum* was adjusted to 10^4 per ml and was cultured by streaking on the PDA medium. The plates were exposed to gamma rays at the doses of 0, 100, 300 and 600 Gy. The plates were kept at room temperature and spore germination was recorded after 12, 24 and 48 h. Each treatment consisted of four replicates.

Relationship between gamma irradiation and biological control agent

The spore suspension was prepared and adjusted to 10^4 per ml as described earlier. The spore suspension were separately exposed to gamma rays at the doses of 0 (control), 100, 300 and 600 Gy. Also, concentration of *P. fluorescens* was adjusted to 10^6 cfu/ml using spectrophotometer at 420 nm and serial dilution method. The suspensions of bacteria and irradiated conidia (*P. expansum*) were then mixed in test tubes proportionately at the ratio of 4:5 (V/V). After 12, 24 and 48 h of co-cultivation, 30 µl aliquots were placed on sterilized microscope slides and observed under a light microscope. The percentage of spore germination was determined in 10 different microscopic fields. A total of 300 spores (100 spores per replicate) were observed at each sampling time.

Statistical analysis

Results were analyzed according to completely randomized design (CRD) and statistical analyses were performed using Duncan's

multiple range test ($P \leq 0.05$), by MSTATC version 1.42.

RESULTS AND DISCUSSION

Antagonism between bacterium and fungus

Growth of *P. expansum* was significantly reduced when the fungus was applied in the mixture with *P. fluorescens*. After 12 and 24 h of co-cultivation, the spores and germ tubes were surrounded by bacteria. Considerable differences in the spore germination occurred only after 24 h in mixtures of 1:5, 2:5 and 3:5, whereas *P. fluorescens* mostly inhibited spore germination only after 48 h of incubation in mixture of 3:5 (Table 1). Deformities of non-germinated spores and also lysis of germ tubes were observed after 48 h of incubation with bacteria. The inhibition of germination of *P. expansum* conidia by *P. fluorescens* was influenced by the proportion of bacteria to spores in the mixtures and period of culture. The basis of this incompatibility is not known, it could be due to the production of antibiotic compounds by *P. fluorescens* (Weller, 2007). Incubation time of 12 h had little effect on reduction of spore germination. In mixtures 3:5 and 4:5, the antagonistic action by the bacteria was increased only after 24 h of incubation (Table 1). The greatest action of bacteria was observed after 48 h of incubation in mixture of 3:5. These results are in agreement with observations by some researchers (Janisiewicz and Korsten, 2002; Bryk et al., 2004), who demonstrated that *P. syringae* controlled growth of *P. expansum*.

Gamma irradiation

The treatment of gamma irradiation on mycelial growth showed that doses of more than 3 KGy totally inhibited *P. expansum* growth; whereas, a dose range of around 600 Gy frequently killed *P. expansum* spores (Figures 1 and 2). It showed that spores are more sensitive than hyphae to gamma irradiation. These results are in conformity with the experiments by Pongphen et al. (2005) who showed that gamma irradiation could be used to control spore germination and mycelial growth of *P. expansum*. In addition, complete inhibition of fungal growth has been reported as a result of gamma ray application which causes destruction of DNA of the cells, and cells cannot function (Smith and Pillai, 2004), while incomplete inhibition may result from slight wounding of cells (Aubrey, 2002).

Relationship between gamma irradiation and biological control agent

Effect of different doses of 0, 100, 300 and 600 Gy on spore germination in a mixed suspension (V/V) bacteria and conidia was studied after 12, 24 and 48 h (Table 2).

Table 1. Effect of *Pseudomonas fluorescens* on conidia germination of *Penicillium expansum*.

| Incubation time (h) | <i>P. fluorescens</i> | | | | |
|---------------------|-----------------------|---------------------|--------------------|-------------------|--------------------|
| | Control | 1:5* | 2:5 | 3:5 | 4:5 |
| | % of germinated spore | | | | |
| 12 | 14 ^{ef} | 12.6 ^{efg} | 11 ^{fgh} | 9 ^{fgh} | 8.6 ^{fgh} |
| 24 | 28.3 ^c | 20 ^d | 17.3 ^{ef} | 8.6 ^{gh} | 7 ^h |
| 48 | 58.3 ^a | 48.6 ^b | 28.6 ^c | 21.3 ^d | 17.6 ^{de} |

Control (conidia of *Penicillium expansum* in sterile water); *: proportion of bacteria to fungal conidia in the mixture (1:5, 2:5, 3:5, 4:5). Data followed by different letters differ significantly at $P \leq 0.05$ (Duncan's multiple range test).

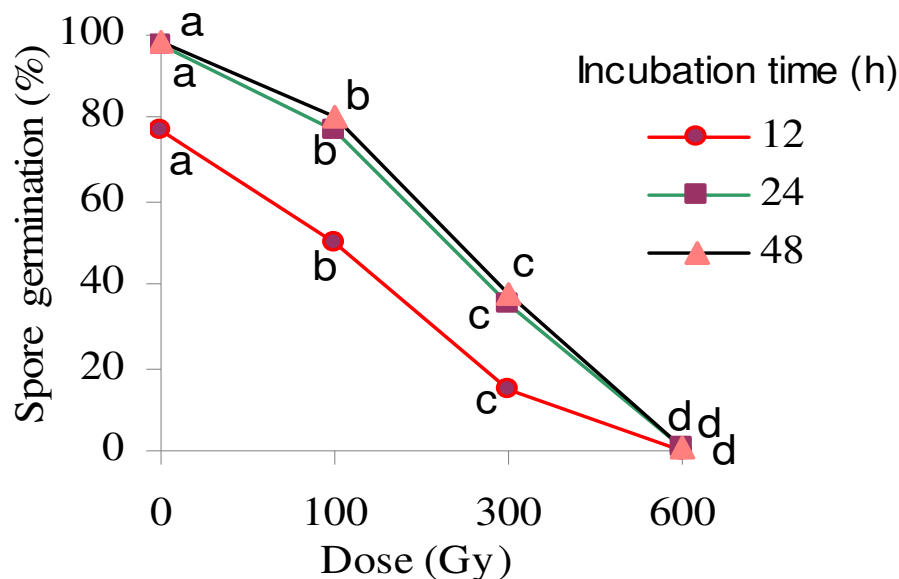


Figure 1. The effect of gamma irradiation on spore germination of *Penicillium expansum*. In each line, data followed by different letters differ significantly at $P \leq 0.05$ (Duncan's multiple range test).

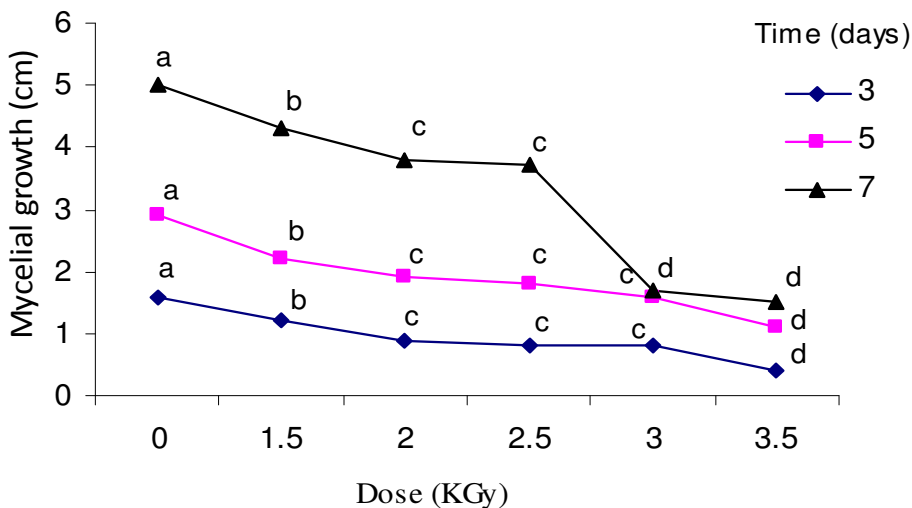


Figure 2. The effect of gamma irradiation on mycelial growth of *Penicillium expansum*. In each line, data followed by different letters differ significantly at $P \leq 0.05$ (Duncan's multiple range test).

Table 2. The combination of *Pseudomonas fluorescens* with irradiated *Penicillium expansum* on spore germination in proportion of 3:5 respectively.

| Incubation time (h) | Dose(Gy) | | | |
|---------------------|-----------------------|------------------|------------------|----------------|
| | 0 | 100 | 300 | 600 |
| | % of germinated spore | | | |
| 12 | 9.2 ^a | 6.3 ^b | 3.5 ^c | 0 ^d |
| 24 | 8.7 ^a | 6.4 ^b | 3 ^c | 0 ^d |
| 48 | 18.2 ^a | 12 ^b | 0.8 ^d | 0 ^d |

In each row, data followed by different letters differ significantly at $P \leq 0.05$ (Duncan's multiple range test).

Spore germination of *P. expansum* was least when the fungus was exposed to radiation in mixture with *P. fluorescens*. According to results of D'Hallewin et al. (2005), the combination of the yeast *Candida oleophila* strain '13L' and ultraviolet-C irradiation evidenced a synergistic effect in reducing *P. digitatum* mold. Significant differences in the spore germination were observed in all doses after 12 and 24 h, but no significant differences were observed among doses of 300 and 600 Gy after 48 h. Stevens et al. (1997) showed that when tomato, sweet potato, peach and tangerine were treated with *Debaromyces hansenii* two to three days after UV-C treatment, the reduction of storage rots was better than when UV-C was used alone and suggesting an integration of UV-C with the yeast (*D. hansenii*) treatment can be as effective as commercial postharvest treatment in reducing storage rots. Although, application of 600 Gy killed *P. expansum* spores, the combination of mixture 3:5 (V/V) bacteria and irradiated spores at 300 Gy absolutely inhibited spore germination after 48 h (Table 2).

Conclusion

The results of this study showed that integrated treatment of gamma radiation and biocontrol agent (bacteria) has potential as an alternative means of postharvest disease control. Whereas, the change in quality value caused by irradiation depends on irradiation dose to which the food has been exposed, there is a dose rate limitation for application of gamma irradiation to control of postharvest disease on fruits and vegetables. Thus, combination of irradiation and biocontrol agent increase applied range of irradiation for postharvest control by decreasing of dose rate.

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