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Sensitivity to Gamma Irradiation of Post-harvest Pathogens of Pear

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ABSTRACT

Recently, radiation has been used as a fungicidal treatment in the post harvest technology of fruits. The effect of gamma irradiation at doses of 5 - 3000 Gy, on spore germination and mycelial growth of four fungi (*Alternaria tenuissima*, *Botrytis cinerea, Penicillium expansum* & *Stemphylium botryosum*) pathogenic to stored pears were studied. Inhibition of spore germination was found to be directly related to the strength of the radiation dose. *B. cinerea* and *P. expansum* were radiation sensitive, while *A. tenuissima* and *S. botryosum* were radiation resistant. Exposure of mycelial mat to different radiation doses showed that a dose level of 1000 and 3000 Gy could be considered sufficient for decontamination by the radiosensitive and radio-resistant species, respectively. Regardless of index of mycelial age, young mycelia were more resistant than mature mycelia. The lower doses of gamma radiation increased total proteins and total soluble sugars of all the tested fungal species but did not effect lipid synthesis.

Key Words: Gamma; Radiation; Pathogen; Fungi and pears

INTRODUCTION

Radiation is the emission of any rays or particles from a source (Uvanov & Isaacs, 1986). The effects of gamma radiation on the viability of microorganisms have attracted a great deal of attention. Gamma rays, electromagnetic waves with high penetrating power, pass through materials without leaving any residue, an advantage comparing to other disinfection treatments (Adamo *et al*., 2001). The exposure of fungi to gamma radiation sets of chain of reactions, which give rise to chemical and metabolic or physiological changes, so irradiation represent an additional stress on the cells, which tend to disturb their organization (Lawrence, 1971). Gamma radiation was used for killing fungi by Sedlackova (1992).

Gamma radiation applied to microbial community leads to negative effect on microbial metabolic capacity (Jones *et al*., 2004). Fungi are more susceptible to radiation damage than bacteria (Chou *et al*., 1971). El-khawas *et al*. (1999) reported that 2, 4 and 6 KGy of gamma rays reduced microbial count. Fungi have been successfully inactivated with gamma radiation doses ranging from 6 to 15 kGy (McNamara *et al*., 2003). The effect of gamma radiation on *Botrytis cinerea* was studied by Tiryaki (1993). Several fungal species (*Acremonium, Aspergillus, Cladosporium, Fusarium, Penicillium* & *Trichosporon*), were eliminated by using CO- 60 irradiation unit with doses ranging from 14.5 to 25 kGy, the minimum dose required to kill these fungi was 16 kGy (Silva *et al*., 2006).

The problem of survival of living organisms in extreme environments has always been in focus. Among living organisms, microorganisms, including the filamentous fungi are well known for their high radioresistance (Magan, 1997). It is evident that some fungi have developed very efficient mechanisms to protect themselves against various sources of radiation, while growing in highly radioactive polluted environments (Strike & Osman, 1993). Tiryaki (1990) stated that the sensitivity of storage pathogenic fungi isolated from pears to gamma rays was as follow: *Alternaria tenuissima* and *Botrytis cinerea* were the most resistant, while *Penicillium expansum* and *Rhizopus stolonfer* were the most sensitive. *Alternaria alternata* can be inactivated at relatively high doses of gamma irradiation ranging between 1.15 and 1.39 kGy and was dominates other fungi inhabiting with radioactivity locations (Saleh *et al*., 1988). In addition, two other *Alternaria* species (*A. tenuis & A. citri*), were demonstrated to tolerate doses of 4.2 - 4.57 kGy (Beraha *et al*., 1960). ONeil *et al*. (1991) showed that *Alternaria spp,* were more resistant to radiation than *Penicillium spp*. Irradiation to 0.5 kGy did not prevent fungal spoilage but irradiation with 1.5 or 2 kGy controlled blue and green moulds (*Penicillum italicum & P. digitatum*) (Watanabe *et al*., 1976). Ziombra (1994) recorded that spore germination of *Pleurotus sp* was accelerated by irradiation with 100 and 200 Gy dose of gamma rays.

The present study investigates the effects of gamma radiation doses on fungi pathogenic to stored Pears. The direct effects of gamma irradiation on spore germination and development of fungal growth, were examined. Irradiation of mycelia at different ages, was carried out. The effect of gamma radiation on mycelial total protein, total

soluble sugars and total lipids were examined.

MATERIALS AND METHODS

Test pathogens. The microorganisms (*Alternaria tenuissima*, *Botrytis cinerea, Penicillium expansum & Stemphylium botryosum*) used in this study were isolated from infected Pear fruits. The developing fungal colonies were identified up to the species level by microscopic examination according to the keys of Gilman (1957), Raper and Fenell (1965), Barnett and Hunter (1972) and Moubasher (1993).

Source of gamma irradiation. The source of irradiation used for the tested fungal species was Cobalt- 60 gamma cell 3500. This source is located at amiddle Eastern Regional Radioisotopes Center for the Arab countries (Dokki, Cairo). The dose rate was 2.4 Gy/min.

Exposure of the spores of the tested fungal species to gamma radiation. Spore suspension (10^2 spore/mL) from pure 14 days old culture of each of the tested fungal species were irradiated by gamma radiation at dose levels of 5, 10, 50, 100, 250, 500, 750, 1000, 1500, 2000 and 3000 Gy for different exposure times. Several preliminary trials were made to estimate the time interval needed for inhibition of spore germination of each species. This estimated time interval was taken as the maximium time limit for exposure of the treated plates to radiation. Three replicates were used for each dose as well as for the control (non-irradiated). An aliquot of 0.25 mL of irradiated and non-irradiated spore suspension were plated on Czapek-Doxś agar medium in a Petri dish. The percentage of germination of irradiated and non-irradiated spores (control) were examined under the microscope at different intervals (1, 2, 3 & 4 days).

Radiosensitivity of tested fungi. A loopful of non-irradiated and irradiated fungal spore suspension were placed separately in the center of sterile plate cultures containing 15 mL Czapek-Doxś agar medium. In the meantime, 0.5 mL of irradiated and non-irradiated fungal spore suspension were inoculated separately in sterile 250 mL conical flasks each containing 50 mL Czapek-Doxś media. Three replicates were prepared for each treatment from both plates and flasks. All flasks were incubated at 27°C for 7 days after, which every flask was filtered and the produced mycelial mats were oven dried at 80°C until constant weights. The plates were incubated at 27°C for 7 days and the radial growth (mm) was measured every day during the incubation period.

Relation of sensitivity to gamma radiation to mycelia age. Regardless of index of sample size utilized, spore suspension was obtained from different mycelial ages (1, 3, 7 & 12 days old cultures). The spore suspension was irradiated by gamma radiation to compare gamma sensitivity to mycelial age.

Effect of gamma radiation on total protein, total soluble sugars and total lipids contents of the tested fungal species. 0.5 gm of dry mycelium of each tested fungal species were added to 50 mL phosphate buffer pH 8.04 (5.3 mL of 0.2 M sodium dihydrogen phosphate solution + 94.7 mL of 0.2 M sodium Phosphate dibasic anhydrous and then diluted to a volume of 200 mL), then left over night at room temperature and filtered and the filtrate was used for the subsequent work. Total proteins were quantitivaly determined using the method adopted by Bradford (1976). Total soluble sugars was determined by the phenol- sulfuric method as descibed by Dubosis *et al*. (1956). Lipid extraction was carried out twice by blending 2.5 gm dry weight of fungal species with 40 mL of chloroform/methanol mixture (2:1 v/v) in a waring blender. The total volume of the extract was measured, then mixed in a separating funnel with 0.2 its volume of water. The mixture was allowed to separate into two phases by standing (Folch *et al*., 1957). The lower phase was then measured and dried at 40°C to constant weight for quantitative estimation of total lipids (mg/100 mg dry wt).

RESULTS AND DISCUSSION

Effect of gamma radiation on spore germination of the tested species. Table I indicates that after one day of incubation, the non-irradiated spores (control) of all tested species did not show any sign of germination. For all doses of irradiation the spore germination increased with the increase of the incubation period. After four days of incubation, higher germination percentages of non-irradiated spores (19.0, 10.4, 9.4 & 12.7%) were achieved for *A. tenuissima, B. cinerea*, *P. expansum* and *S. botryosum,* respectively. The low experimental doses of gamma radiation up to 100 Gy were significantly stimulatory to spore germination of *B. cinerea* and *P. expansum*, compared to 250 Gy in case of *A. tenuissima* and *S. botryosum*. Salama *et al*. (1977) reported that low doses of gamma rays stimulated spore germination and growth of some fungi. The percentages of spore germination of the tested species decreased with further increasing the radiation dose and were completely inhibited at 1000 for *B. cinerea* and *P. expansum* (radiosensitive) and at 3000 Gy for *A. tenuissima* and *S. botryosum* (radioresistant) and these were considered as the lethal doses. These results are in agreement with that of Golan *et al*. (2002), who stated that inhibition of spore germination was found to be directly correlated to gamma radiation dose and that *Penicillium expansum* the causal organism of the rot of stored melons was the most sensitive to radiation, while *Alternaria tenuis* was radiation resistant with 300 krad. Difference in sensitivity to gamma radiation among genera may be explaned on the basis that *Botrytis cinerea* and *Penicillium expansum* with lighter pigmentation and thin cell walls are more sensitive to gamma rays than the dark walled *A. tenuissima* and *S. botryosum* spores and that the effect of radiation on spores diminish as pigmentation increased (Wilson *et al.,* 1998). Kimura and Tsuge (1993) reported that *A. alternata* is also well known for producing melanin,

Table I. Effect of different doses of gamma rays on the spore germination of the tested fungal species

Age (day)	Percentage of spore germination at different periods															
Dose (Gy)	*A. tenuissima*				*B. cinerea*				*P. expansum*				*S. botryosum*			
	1 day	2 days	3 days	4 days	1 day	2 days	3 days	4 days	1 day	2 days	3 days	4 days	1 day	2 days	3 days	4 days
Control	0.0	5.2	10.6	19.0	0.0	3.5	7.0	10.4	0.0	2.0	5.3	9.4	0.0	4.1	8.5	12.7
5	0.0	6.5	12.5	20.1	0.0	4.8	9.1	12.0	0.0	3.5	6.8	10.0	0.0	5.0	11.0	15.8
10	0.0	7.2	17.0	22.4	0.0	5.5	11.5	15.4	0.0	4.0	9.0	12.4	0.0	6.5	15.2	19.0
50	4.2	8.0	18.4	25.0	2.1	6.0	12.0	18.0	1.0	5.1	10.2	15.0	3.0	7.3	14.0	22.6
100	5.8	12.5	20.3	32	3.5	8.7	14.7	20.5	2.2	6.4	11.6	17.5	4.3	10.0	17.4	27.0
250	7.5	15.7	23.5	50	2.5	4.0	10.9	17.0	0.9	2.7	7.0	12.3	6.1	13.5	20.1	42.3
500	4.8	11.3	14.4	39.4	1.8	2.5	8.5	12.7	0.3	1.6	4.5	9.5	3.0	9.0	11.6	30.0
750	2.1	5.2	8.5	18.0	0.1	1.4	4.0	8.5	0.1	0.8	2.0	6.0	1.2	4.0	6.0	14.3
1000	1.1	3.0	4.1	7.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	2.2	3.0	5.0
1500	0.7	2.1	3.0	3.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	1.0	1.7	3.1
2000	0.4	1.3	2.0	2.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.8	1.1	1.5
3000	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
LSD at 5%	1.6	2.7	3.0	7.0	0.9	1.9	2.7	2.2	0.9	1.1	1.3	2.5	1.2	3.3	2.5	6.4

Table II. Mycelial growth of the tested fungal species influenced by irradiation by gamma rays

Dose (Gy)	Radial growth (mm/days)								Dry weight (mg/50 ml)							
	Mean (mm/days)				% of control				Mean (mg)				% of control			
	At	*Bc*	*Pe*	*Sb*	*At*	*Bc*	*Pe*	*Sb*	*At*	*Bc*	*Pe*	*Sb*	*At*	*Bc*	*Pe*	*Sb*
Control	7.1	11.0	10.3	9.5	100.0	100	100	100	9.51	10.7	8.5	10.0	100.0	100	100	100
5	7.4	11.7	10.5	9.7	104.2	106.3	101.9	102.1	9.70	12.1	9.2	10.2	101.9	113.1	108.2	102
10	8.0	11.2	10.0	10.0	112.7	101.8	97.1	105.3	9.87	11.6	8.3	10.5	103.8	108.4	97.6	105
50	8.5	10.4	9.6	10.3	119.7	94.5	93.2	108.4	10.00	10.2	7.6	10.8	105.2	95.3	89.4	108
100	8.7	10.4	9.8	10.4	122.5	94.5	95.1	109.5	10.20	9.1	7.1	11	107.3	85.0	83.5	110
250	8.9	9.8	7.4	10.7	125.4	89.1	71.8	112.6	10.33	8.4	6.3	11.2	108.6	78.5	74.1	112
500	6.1	9.2	6.6	6.5	85.9	83.6	64.1	68.4	7.11	7.6	6.0	9.0	74.8	71.0	58.3	90
750	4.2	5.0	4.5	3.1	59.2	45.5	43.7	32.6	6.70	6.0	4.2	7.2	70.5	56.1	49.4	42
1000	2.0	0.0	0.7	1.8	28.2	0.0	0.0	22.1	3.89	0.0	0.0	4.0	40.9	0.0	0.0	26
1500	1.5	0.0	0.2	0.0	21.1	0.0	0.0	7.4	2.3	0.0	0.0	1.1	24.2	0.0	0.0	11
2000	0.8	0.0	0.0	0.0	11.3	0.0	0.0	2.1	1.2	0.0	0.0	0.6	12.6	0.0	0.0	6
3000	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
LSD at 5%	0.2	0.5	0.3	0.9					0.1	0.5	1.0	0.2				

At= Alternaria tenuissima, Bc= Botrytis cinerea, Pe= Penicillium expansum, Sb= Stemphylium botryosum

a black pigment that is produced and accumulates inside the mycelium and there are some indications that the radioresistance of microorganisms may result from melanization of their cells (Pointing *et al*., 1996). Melanized fungal genera were also found to dominate other fungi in soil communities affected by long-living radionuclide (Zhdanova *et al*., 1994). Maxie *et al*. (2005) found that some physiological effects of gamma irradiation on *Alternaria* rot did not protect the lemon from the injury. Inactivation of spores of *Penicillium expansum, Botrytis cinerea* and *Alternaria tenuis* was achieved by using gamma radiation (50 Krad) (Arie & Golan, 2002). Schindler *et al*. (1980) stated that exposing spores to gamma radiation lead to fungal mutations and EL-Bazza (1991) found that high inoculum levels were reduced by irradiation.

Mycelial growth of the tested fungal species as effected by gamma irradiation of spores. The lower doses of gamma rays significantly increased radial growth rate and dry weight of *A. tenuissima* and *S. botryosum* (radio-resistant) and the highest increase (8.9 & 10.7 mm/day) and (10.33 & 11.2 mg/50 mL) were observed at 250 Gy, respectively. As radiation dose increased over 250 Gy, the growth parameter gradually decreased to reach a minimum at 2000 Gy and 3000 Gy was the lethal dose (Table II). The obtained lethal dose value was in accordance with Hassanein (1987), who reported that lower doses of gamma radiation increased the growth of *Aspergillus flavus* and complete inhibition was observed at 3000 Gy. On the other hand, the highest growth (11.7, 10.5 mm/day) and (12.1, 9.2 mg/50 mL) were achieved at five Gy for the radio-sensitive species (*Botrytis cinerea* & *Penicillium expansum*), respectively and the lethal dose was 1000 Gy. The difference in the response of the tested fungal species to gamma radiation may be due to the presence of the mycelial water content, which act as a natural radioprotector (El-Sherbeny, 1982) and also to the induction of certain enzymes, which leads to recovery of radiation damage after irradiation (Nahed, 1999) or could be due to the formation of chemical substances, which stimulate or retard the process of growth. Variation in radiation resistance among filamentous fungi are extremely variable ranging from supersensitive to highly resistant to radiation (Mironenko *et al*., 2000). 50 Krad of gamma radiation did not prevent the rotting of fruits inoculated with *B. cinerea* and *Alternaria sp*. but delayed disease development (Arie & Golan, 2002). Gamma radiation acted on fungal development resulting in

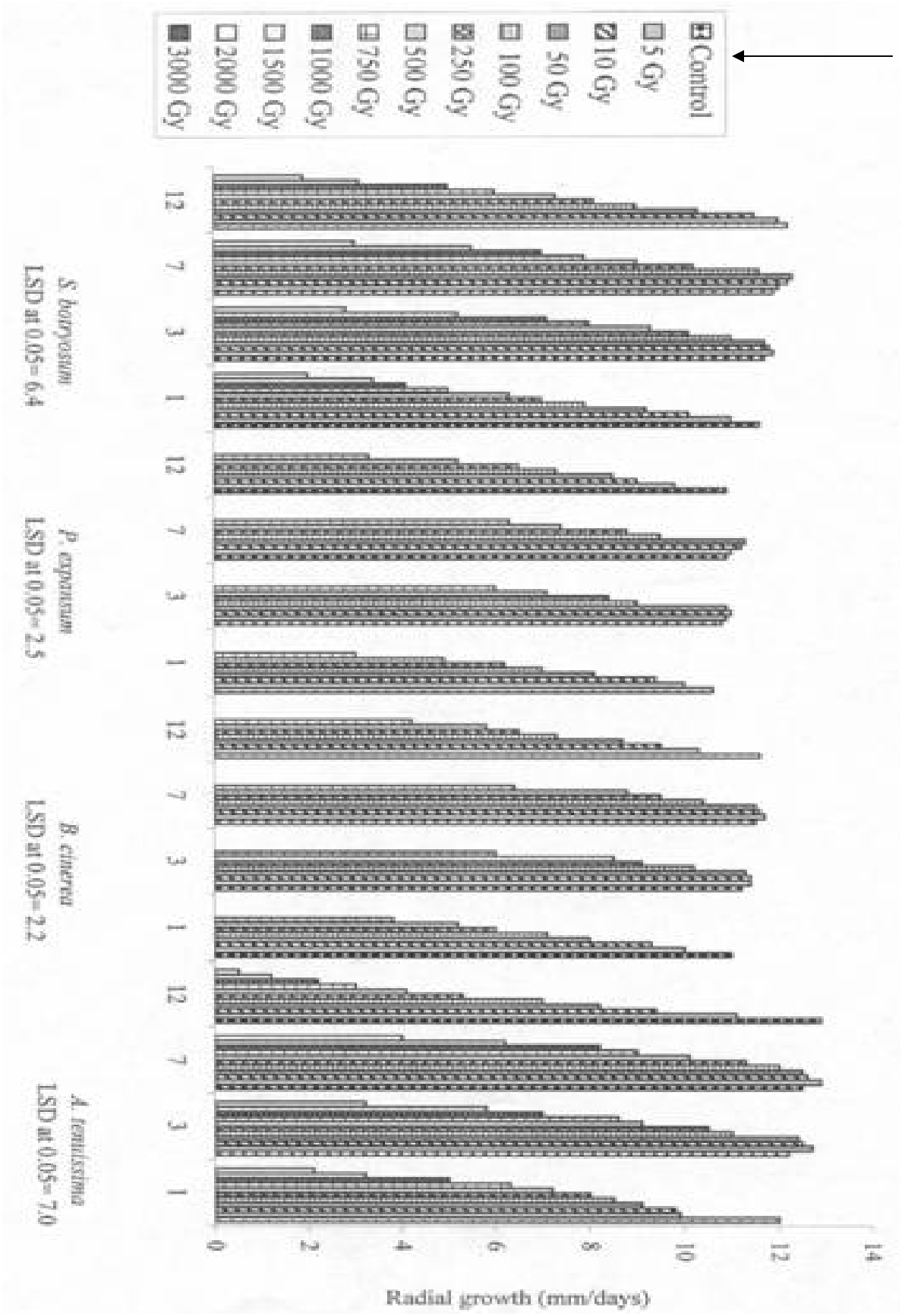


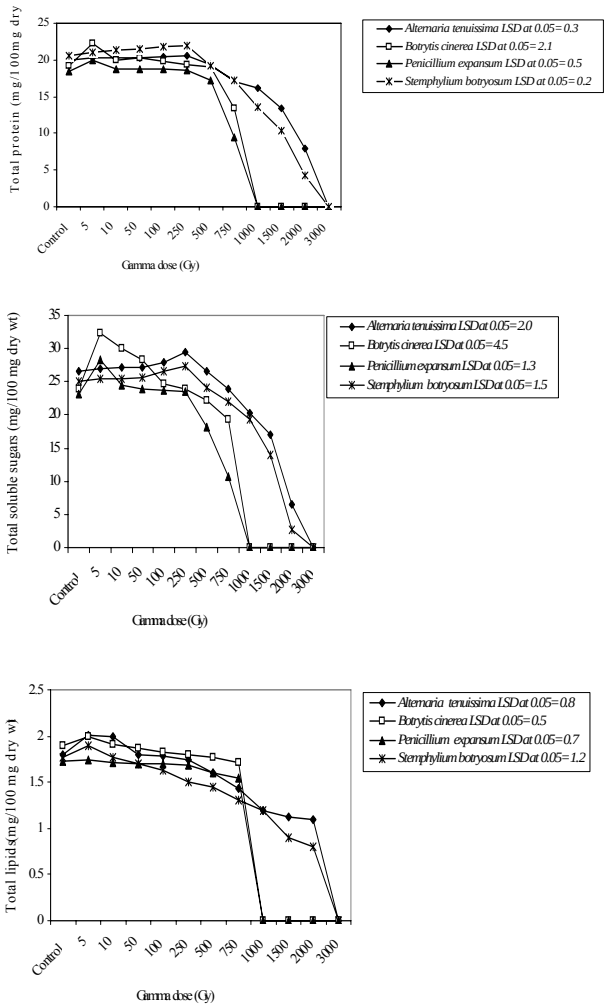
Fig. 1. Comparative sensitivity of mycelia age to gamma radiation

1.7% and 10.0% light-red tomatoes infection by *Botrytis cinerea* and *Rhizopus stolonifer*, respectively compared with 67% and 100% infection in the non-treated controls. Irradiation at 0.5 KGy totally eliminated decay by *Alternaria alternata* (Golan *et al*., 2003). Control of grey mold (*Botrytis cinerea*) of strawberry fruits by gamma irradiation was recorded by Sommer *et al*. (2005). Abo-Zeid (1987) reported that the highest dry weight was recorded at 1000 Gy of gamma rays in the case of *Aspergillus koningi* and at 1500 Gy for *Aspergillus flavus*. Gamma irradiation doses up to 1.5 kGy were ineffective to control rots due to *Alternaria citri* (Ladaniya *et al*., 2003). Radio-resistant isolates, which survived doses levels ranging from 3.0 to 5.0 kGy were; *Rhizopus oryzae, Rhizopus stolonifer, Penicillium chrysogenum* and *Penicillium corylophillum.* 6.0 kGy was the lethal dose for fungal decontamination (Mahmoud *et al*., 1992a, b). Ibrahim (1986) found that dry mycelial weight of *Paecilomyces violacea* was significantly increased at the two lower irradiation doses (100 & 250 Gy) over the control value by 11.38 and 19.89%, respectively whereas the higher irradiation doses of 2500 and 3000 Gy reduced the dry weight by 63.44 and 70.4%, respectively. Han and Yang (1991) found that the optimum irradiation dose that gave maximum production for *Auricularia auricula* was 2000 Gy but for *A. fuscosucinea* it was 100 Gy. Gamma irradiation causes inhibition of spore germination and consequent decrease in mycelial dry weight, EL-Bazza *et al*. (2001).

Comparative sensitivity to gamma radiation of mycelia age. 1 and 12 days old cultures of all the tested fungal species of were inhibited by increase of gamma radiation dose. This could be attributed to the fact that one day old culture is in the lag phase of growth in which cells are starting to form enzymes and intermediate compounds, while the 12 days old culture is in the death phase of growth at which the activities and vitality of cells are at minimum, therefore such cultures could not tolerate the effect of radiation. On the other hand, mycelial growth at the 3rd and 7th days of incubation increased, when irradiated with low doses (5, 10 & 50 Gy) (Fig. 1). Growth at these days of incubation is in the acceleration and the exponential phases, respectively and in both phases, the cells are in active growing phases. Accordingly, they could tolerate the radiation effect (Matin *et al*., 1989; Kolter *et al*., 1993). Sensitivity of conidia and mycelia of *Botrytis sp*. to gamma radiation was studied by Sommer *et al*. (2004), who stated that regardless of index of sample size utilized, young mycelia were more resistant and mature mycelia were more sensitive than conidia. Resistance to radiation may be attributed to DNA repair mechanisms (Strike & Osman, 1993) and also the expression of heat-shock protein genes, may play an important role in the resistance to ionizing radiation among various organisms, including fungi (Boreham & Mitchel, 1994). The cells have evolved biological defense mechanisms that can protect them against a variety of harmful environmental stress including exposure to gamma rays (Boreham *et al*., 1992). Gamma radiation as sterilization treatment causes direct damage to cell DNA through ionization inducing mutation and killing the cell. It also has an indirect effect as a result of radiolysis of cellular water and formation of active oxygen species, free radicals and peroxides causing single and double strand DNA breakages (McNamara *et al*., 2003).

Effect of gamma radiation on total protein, total soluble sugars and total lipids of the tested fungal species. The lower doses of gamma radiation (5, 10, 50, 100 & 250 Gy) were non-significantly stimulatory to the production of total proteins, total soluble sugars of *A. tenuissima* and *S. botryosum* but significant inhibition was achieved above 250 Gy. The most stimulative dose of protein and sugar contents in *Botrytis cinerea* and *Penicillium expansum* was 5 Gy, whereas higher radiation doses up to 250 Gy decreased the protein content but still higher than the control

Fig. 2. Total protein, total soluble sugars and total lipida contents (mg/100 mg dry weight) for the tested fungal species influenced by different doses of gamma radiation.



(Fig. 2). Osman *et al*. (1987) reported that gamma irradiation caused increases in total protein in *Fusarium solani*, which suggested that protein might play a part in protection against the harmful effect of radiation. The appearance of some amino acids only after irradiation and their accumulation in amounts higher than the control values could be explained in the light of their probable roles as radio-protector Awny *et al*. (1982). Boreham *et al*. (1992) reported that new protein was formed to protect the cell against a varietly of harmful environmental stresses including ionizing radiation exposure. Nadia *et al*. (1988) stated that total proteins and some amino acids of irradiated *Paecilomyces violacea* were higher than the control. Low doses of gamma radiation were stimulative to the production of reducing sugars in the growth medium (Hayashi, 1986). This could be attributed to the fact that cellulose enzymes, which are responsible for cellulose degradation was proved to be gamma radiation tolerant enzymes as recorded by El-yasergy (1997). Regarding lipids, slightly non-significant increase was recorded for all tested species at radiation doses 5 and 10 Gy. Generally, total lipids production was slightly affected by gamma radiation in all tested fungal species. No clear effect of radiation on the process of lipid synthesis in *Penicillium notatum* and *Aspergillus flavus* (Osman *et al*., 1988).

CONCLUSION

In the present work, low doses of gamma radiation produced stimulatory effects but high doses caused inhibitory effects that increased with the increasing of gamma radiation dose. The lethal dose, were recorded at 1000 and 3000 Gy for radiosensitive and radio-resistant species, respectively this was attributed to the fact that these doses greatly suppressed the essential metabolic processes. The response of the fungus differed according to the fungal species, stages of growth and doses of irradiation.

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