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Impact of Gamma Irradiation Stresses II. Control of Sugarbeet Pathogens *Rhizoctonia solani* Kühn and *Sclerotium rolfsii* Sacc.

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Abstract: To control the fungal pathogens attacked sugarbeet plant, we subjected *R. solani* and *S. rolfsii* to different gamma irradiation doses (0.0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 kGy for *R. solani* and 0.0, 0.5, 1.0, 2.0, 3.0 and 4.0 kGy for *S. rolfsii*). The growth activities of *R. solani* were completely inhibited at dose 7.0 kGy, while *S. rolfsii* at 4.0 kGy. The infection percentage was inhibited with gamma irradiation doses increased for both *R. solani* and *S. rolfsii*. This was confirmed by the SDS-PAGE for cellular proteins of both *R. solani* and *S. rolfsii*, which showed a great variation in protein bands for the same fungus at different gamma irradiation doses. Finally, we can conclude that the gamma irradiation dose to control pathogenic fungi varies with different pathogenic fungi to control the pathogenicity of *R. solani*, we irradiate it at 7.0 kGy dose while at 4.0 kGy for *S. rolfsii*.

Key words: Gamma irradiation, pathogenicity, *R. solani*, *S. rolfsii*, growth activities, protein profile

Introduction

Gamma radiation is high-energy radiation emitted from certain radioactive isotopes as ^{60}Co. These isotopes are potential sources of gamma radiation (Rosenthal, 1992). Gamma rays are of short wavelength. They are capable of great penetration into matter and they are lethal to all life including microorganisms.

When a population of microorganisms is irradiated with a low dose, only a few of the cells will be damaged or killed. With increasing radiation dose the number of surviving organisms decreases exponentially. Different species and different strains of the same species require different doses to reach the same degree of inactivation (Grant and Patterson, 1992).

The control of either plant or human pathogens by irradiation was not studied until relatively recently. Cuero *et al.* (1986) investigated the influence of gamma irradiation and sodium hypochlorite sterilization on maize seed microflora and germination. The germination of the maize seed was not adversely affected by radiation doses up to 1.2 kGy and the microflora were eliminated. Ramakrishna *et al.* (1991) compared sodium hypochlorite (12.5, 25, or 50%), mercuric

chloride (0.1 or 0.2%), methyl bromide, propylene oxide and gamma irradiation for their effectiveness in killing microorganisms on or within barely seeds. Gamma irradiation at 4 kGy eliminated most ***Alternaria***, ***Fusarium*** and ***Epicoccum*** spp. but 12 kGy was required to kill ***Bacillus*** spp. Germination was improved up to 8 kGy but gradually decreased at doses up to 15 kGy.

Gamma radiation response of some decay pathogens was stated (Aly, 1978; O'Neill ***et al.***, 1991). It was found that there was a variability in genera sensitivity of isolated fungi at higher doses (0.25-1.0 KGy) and variability in species of fungi of the same genus. In order to characterize organisms by their radiation sensitivity, the mean lethal dose (MLD) is sometimes used. It is the dose required that kill 63% of a population leaving 37% survive (D_{37}). A more commonly used measure of radiation sensitivity is the D_{10} dose which is required to kill 90% of a population (Diehl, 1995).

Kiljajic (1960) determined the lethal doses of ^{60}Co-gamma rays of some pathogenic fungi isolated from various plants. Lethal doses were very high 200-1000 Krad particularly for ***Aspergillus solani***, ***A. pisi***, ***Bacillus ceneria***, 600-1000 Krad for ***Fusarium oxysporum*** and 800-1000 Krad for ***Aspergillus niger***. Many hypotheses have been proposed and tested. Radiation effect on enzymes or on the energy metabolism was postulated. It is now universally accepted that DNA in the chromosomes represents the most critical target of ionizing radiation (Diehl, 1995). Effects on the cytoplasmic membrane possibly play an additional role in some circumstances (Greez ***et al.***, 1983).

Several works were carried out to know the influence of gamma radiation on the germination of fungal spores. The percentage of conidial germination of ***Penicillium expansum*** was reduced and the maximum reduction was recorded (83%) at the dose of 300 rad (Chou ***et al.***, 1970a). Mohyuddin and Skoropad (1972) studied the effect of gamma radiation doses ranging from 0.25-2.0 kGy on non-germinated conidiophores and mycelia. They found that 0.12% of spores survived at 1.25 kGy for 6 h while the mycelium was very sensitive to radiation damage as compared with non-germinated spores. It was found that gamma radiation caused marked inhibition to the spore germination of certain soil fungi, ***Aspergillus niger***, ***A. sulphunereus*** and ***Trichoderma lignorum*** especially at the level of 500 krad (Osman, 1973).

Furthermore, Osman ***et al.*** (1991) found that gamma-irradiation inhibited significantly the spore germination of non-pigmented and pigmented cultures of ***Verticillium agaricinum***.

Our investigation designed to evaluate the effect of different gamma irradiation doses on the pathogenicity, growth activities and fungal cell protein profile for the two serious pathogens ***R. solani*** and ***S. rolfsii*** of sugarbeet in saline areas in Egypt (El-Abyad ***et al.***, 1988).

Materials and Methods

Pathogenic fungi, culture conditions, plant material and soil

Rhizoctonia solani (AG 2-2) Kühn and ***Sclerotium rolfsii*** Sacc. were isolated from diseased sugarbeet roots (El-Abyad ***et al.***, 1988) and maintained on the medium described by (Johnson and Curl, 1972).

Seeds of sugarbeet (***Beta vulgaris*** L.) cultivars Raspoly, Kawemira and Farida were obtained from the Agricultural Research Center, Giza, Egypt.

Pathogenicity experiment

The pathogenic fungi were irradiated by using Russia ^{60}Co gamma chamber, in National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt. The dose rate was 1 kGy/h. The irradiation doses for ***R. solani*** were 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 kGy, while *S. **rolfsii*** subjected to doses 0.5, 1.0, 2.0, 3.0 and 4.0 kGy.

Effect of γ-irradiation doses on growth activities of *R. solani* and *S. rolfsii*
***In vitor* sclerotial germination and mycelial growth**

These experiments involved determination of the percentage germination of sclerotia and average length of mycelial growth of sclerotia for non-irradiated and gamma irradiated ***R. solani*** and ***S. rolfsii*** on sterial tap water agar (1.5%, w/v). Five surface sterilized sclerotia of either pathogen of regular shape and size were placed on the agar surface of each petri dish and the plates were incubated at 27°C. The percentage of germinated sclerotia and average length of mycelia were estimated. Three plates were prepared for each treatment and the means were compared.

***In vivo* mycelial growth**

The soil tube method described by El-Abyad and Saleh (1971) was studied in these experiments. The tubes were filled with air-dried sieved soil and autoclaved for 20 min at 120°C. On cooling, sterile water was added and the tube were inoculated at one end, each with a 6 mm disc bearing mycelium of non-irradiated and gamma irradiated for either ***R. solani*** or ***S. rolfsii*** and incubated at 27°C. Growth was followed daily and loss of water was stored. Three tubes were set for each treatment and means compared.

Dry mass

Czapek-Dox medium was distributed in 50 ml aliquots in 250 ml Erlenmeyer flasks. Each flask was inoculated with a 6 mm disc of agar bearing mycelium of non-irradiated and gamma irradiated of either ***R. solani*** or ***S. rolfsii*** cut from the margin of 7 days actively growing colonies. The flasks were incubated for 15 days at 27°C, the mycelium was then harvested dried at 80°C to constant weight and the dry mass yield was recorded. Three flasks were prepared for each treatment and the means were compared.

Production of sclerotia

Potato-dextrose-agar was poured into petri dishes that were inoculated each with 1 ml of blended mycelial suspension of either non-irradiated or gamma irradiated ***R. solani***. The plates

were incubated at 27°C for 7 days. The number of sclerotia produced per plate in each treatment was visually counted. Three plates were prepared for each treatment and the means were compared.

For non-irradiated and gamma irradiated *S. rolfsii*, a 6 mm disc agar bearing mycelium was inoculated on Czapek-Dox agar plates, incubated at 27°C for 7 days and the number of sclerotia/plate was visually counted and the means were compared.

Protein isolation

Mycelia were harvested and washed three times by cold 0.8% NaCl (4°C) in glass tubes. Then 10 ml of buffer (0.8% NaCl, 2 mM EDTA, 20 mM Tris, 0.4 mM phenylmethylsulfonyl fluoride) was added and the tubes were agitated for 10 min. After centrifugation (6000 g, 10 min, 0°C) supernatant were lyophilized. The total protein content concentration was estimated as described by Lowry *et al.* (1951).

SDS-PAGE

Samples were dissolved in 50 ml of sample buffer (5 mg/ml) containing 1% SDS, 10% glycerol, 5% 2-mercaptoethanol, 0.125 m TRIS-HCl, pH 6.8 and 0.002% bromphenol blue. Before loading samples were kept at 95°C for 5 min (Laemmli, 1970). SDS-PAGE was done on 7.5-15% gradient acrylamide gel at 250 V for 3 h. Proteins were visualised by silver staining (Hochstrasser *et al.*, 1988). Protein used as molecular weight standards were Myosin, 220 Kda; phosphorylase b, 94 KDa; bovine serum albumin, 67 KDa; ovalbumin, 43 KDa; carbonic anhydrase, 30 KDa; trypsin inhibitor, 20.1 KDa; lysozyme, 14.4 KDa.

Results and Discussion

Pathogenicity

In absence of gamma irradiation doses, the emergence rate was significantly decreased as compared with control without pathogen in sugarbeet cultivars with *R. solani* and *S. rolfsii*; except in Kawemira with *S. rolfsii* where no effect (Table 1).

With *R. solani*, the emergence rate of sugarbeet cultivar Raspoly was completely inhibited in all gamma irradiation doses up to 5.0 kGy and up to 4.0 kGy for cultivars Kawemira and Farida; but significantly increased with increased gamma irradiation doses in all sugarbeet cultivars. The infection percentage decreased at all gamma irradiation doses and completely inhibited at 7 kGy (Table 1).

With *S. rolfsii*, the emergence rate was completely inhibited in cultivar Raspoly and increased gradually at higher doses (3.0 and 4.0 kGy); while in cultivars Kawemira and Farida the emergence rate significantly decreased at lower doses (0.5, 1.0 kGy) and highly significantly increased at 3.0 kGy, then decreased again at 4.0 kGy. The infection percentage was significantly decreased with increased gamma doses till no infection at higher doses (3.0 and 4.0 kGy) in cultivar Farida,

Table 1: Effects of different γ ray doses (KG) of ***Rhizoctonia solani*** and ***Sclerotium rolfsii*** on the emergence (E%) of three sugarbeet cultivars and infection (I%) in emerged seedlings 15 days after sowing

	Gamma irradiation doses (kGy)								
	0	0.5	1.0	2.0	3.0	4.0	5.0	6.0	7.0
Raspoly									
(E%)	37.5	0.0**	0.0**	0.0**	0.0**	0.0**	0.0**	8.0**	33.0**
R. solani									
(I%)	67.1	0.0**	0.0**	0.0**	0.0**	0.0**	0.0**	11.6**	0.0**
(E%)	54.2**	0.0**	0.0**	0.0**	25.0**	100.0	-	-	-
S. rolfsii									
(I%)	43.2	0.0**	0.0**	0.0**	0.0**	8.4**	-	-	-
Kawemira									
(E%)	33.3**	0.0**	0.0**	0.0**	0.0**	0.0**	33.0**	37.0**	54.0**
R. solani									
(I%)	70.1	0.0**	0.0**	0.0**	0.0**	0.0**	30.4**	12.6**	0.0**
(E%)	75	50.0**	71.0*	75.0	83.0**	62.5**	-	-	-
S. rolfsii									
(I%)	35.5	53.2**	41.7**	24.6**	16.8**	0.0**	-	-	-
Farida									
(E%)	41.6**	0.0**	0.0**	0.0**	0.0**	0.0**	25.0**	3.5**	45.0*
R. solani									
(I%)	40.2	0.0**	0.0**	0.0**	0.0**	0.0**	32.3**	29.0**	0.0**
(E%)	66.6**	29.0**	62.5*	67.0	100.0**	67.0	-	-	-
S. rolfsii									
(I%)	66.5	63.4*	34.5**	22.7**	0.0**	0.0**	-	-	-

Control without pathogen; Raspoly, 100; Kawemira, 75; Farida, 75

P <0.05, * significant; P <0.01, **highly significant related to control without pathogen

while in cultivar Kawemira the infection percentage was significantly decreased at lower doses (0.5 and 1.0 kGy) and significantly decreased at higher doses (2.0 and 3.0 kGy) and was nil at 4.0 kGy in cultivar Raspoly the infection percentage was nil at all gamma doses (Table 1).

Gamma radiation response of some decay pathogens was stated (Aly, 1978; O'Neill ***et al.***, 1991). It was found that there was a variability in genera sensitivity of isolated fungi at high doses (0.25-1.0 kGy) and variability in species of fungi of the same genus. Kiljajic (1960) determined the lethal doses of ^{60}Co-gamma rays of some pathogenic fungi isolated from various plants. Lethal doses were very high 200-1000 krad particulary for ***Aspergillus solani, A. pisi, Bacillus ceneria***, 600-1000 krad for ***Fusarium oxysporum*** and 800-1000 krad for ***Treseum*** and ***A. niger***.

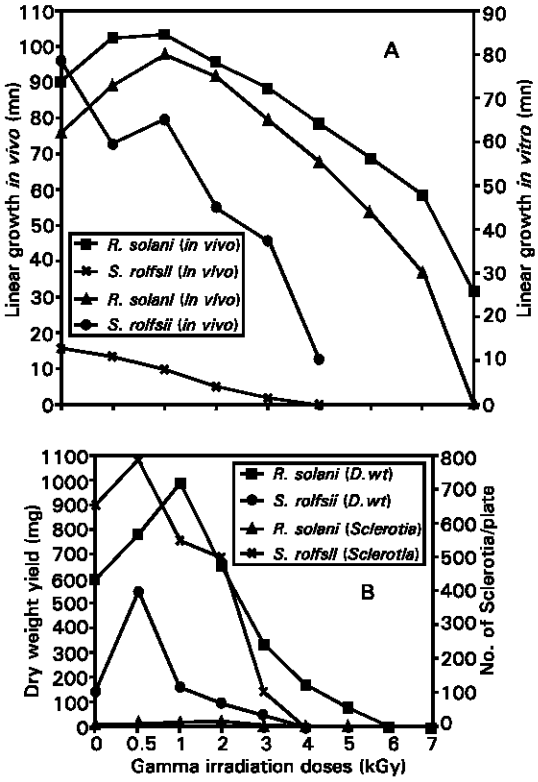

Fig. 1: Effect of different doses of gamma irradiation (kGy) on:

A: The ***in vitro*** and the ***in vivo*** linear growth

B: The dry weight yields and the number of sclerotia/plate for both ***Rhizoctonia solani*** and ***Sclerotium rolfsii***

Effect of γ-irradiation doses on growth activities of *R. solani* and *S. rolfsii*

Linear growth ***in vivo*** was significantly increased at lower gamma irradiation doses (0.5, 1.0 and 2.0 kGy) and significantly decreased at higher doses (3.0, 4.0, 5.0, 6.0 and 7.0 kGy) as compared with control for ***R. solani*** (Fig. 1A) whereas, it was significantly decreased with increased gamma irradiation doses till completely inhibited at 4.0 kGy for ***S. rolfsii*** as compared with control (Fig. 1A).

Linear growth ***in vitro*** was significantly increased at lower gamma irradiation doses (0.5, 1.0, 2.0 and 3.0 kGy) and significantly decreased at higher doses till completely inhibited at 7.0 kGy as compared with control for ***R. solani*** (Fig. 1A); while it was significantly decreased with increased gamma irradiation doses for ***S. rolfsii*** as compared with control (Fig. 1A).

Our results are in agreement with Menasherov ***et al.*** (1992) reported that sclerotia of ***Aspergillus flavus*** and ***A. ochraceus*** isolated from groundnut, soybean, maize and wheat grains

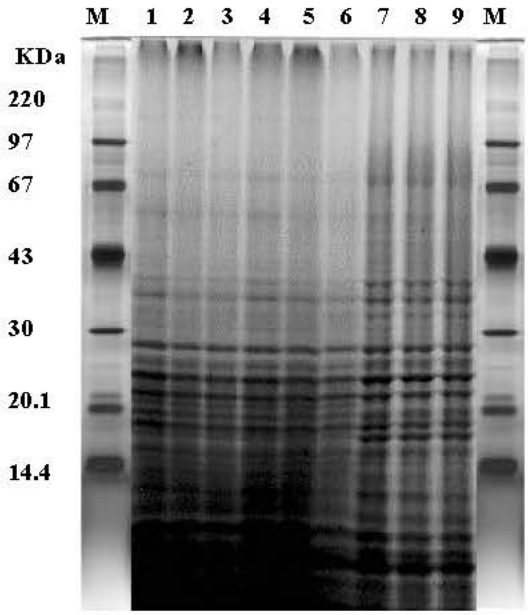

Fig. 2: SDS-PAGE of fungal protein for *R. solani* (control and irradiated); M, molecular standards; lane 1, non-irradiated *R. solani*; lane 2, dose 0.5; lane 3, dose 1.0; lane 4, dose 2.0; lane 5, dose 3.0; lane 6, dose 4.0; lane 7, dose 5.0; lane 8, dose 6.0; lane 9, dose 7.0

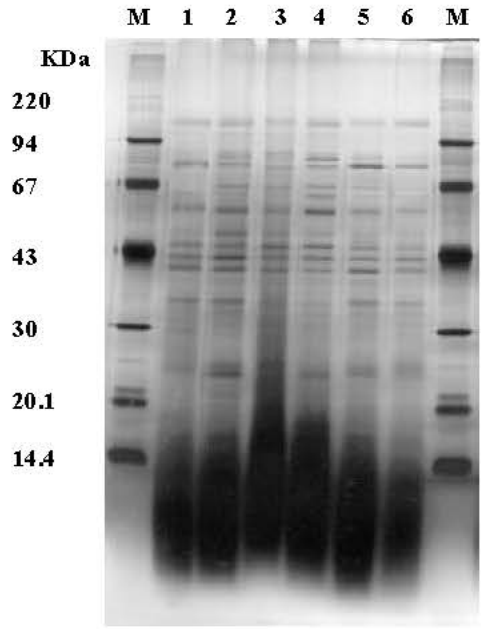

Fig. 3: SDS-PAGE of fungal protein for *S. rolfsii* (control and irradiated); M, molecular standards; lane 1, non-irradiated *R. solani*; lane 2, dose 0.5; lane 3, dose 1.0; lane 4, dose 2.0; lane 5, dose 3.0; lane 6, dose 4.0

were not germinated following irradiation with 2.5 kGy. El-Bazza (1992) found that a gamma irradiation dose of 5 kGy was sufficient to eliminate the contaminating spores of ***Aspergillus ochraceus*** on wheat samples. The mycelial growth of mushroom (***Agaricus bisporus***) was faster at lower doses of gamma irradiation (0.5-3 krad) than with higher doses (3-200 krad). The high doses significantly inhibited the mycelial growth and caused changes in mycelial colour and morphology (Hua ***et al.***, 1994). In the same connection, it was found that low doses of gamma rays stimulating growth, whereas the higher doses inhibiting the mycelial growth of ***Armillaria mellea*** (Tao ***et al.***, 1998) and that of ***Aspergillus niger*** (Gherbawy, 1998; Wang ***et al.***, 1998).

Dry weight yields were significantly increased at lower doses (0.5, 1.0 and 2.0 kGy) and inhibited at higher doses till completely inhibited at dose 7.0 kGy for ***R. solani*** (Fig. 1B). Whereas, it was positively stimulated at doses 0.5 and 1.0 kGy then inhibited at higher doses till completely inhibition at 4.0 kGy for ***S. rolfsii*** (Fig. 1B). Production of sclerotia was positively stimulated at lower gamma irradiation doses (0.5, 1.0 and 2.0 kGy) and inhibited at higher doses (3.0, 4.0, 5.0 and 6.0 kGy) till complete inhibition at 7.0 kGy for ***R. solani*** when compared with control (Fig. 1B). For ***S. rolfsii*** the production of sclerotia were significantly increased at 0.5 kGy and inhibited with increased gamma irradiation doses till complete inhibition at 4.0 when compared with control (Fig. 1B).

The effect of gamma irradiation on mycelial dry weight has been studied by several workers (Chou ***et al.***, 1970b; Mohyuddin and Skoropad, 1972; Ahmed, 1987; Tamada ***et al.***, 1987; Dzhezdheva ***et al.***, 1990). These results are in agreement with Srinivas ***et al.*** (1996) who found that by increasing the level of gamma irradiation there was a decrease in mycelial dry weight of ***Aspergillus flavus***. Biomass production was found to decrease by the increase in doses, the decreased amounts were higher in the case of non-pigmented than pigmented cultures of ***Verticillum agaricinum*** (Osman ***et al.***, 1991). Szekely ***et al.*** (1991) found that irradiation at a dose of 1.0 or 2.0 kGy of ***Aspergillus alutoceus*** reduced the level mould growth greatly relative to the non-irradiated controls. El-Bazza (1992) reported that complete inhibition of the growth of ***Aspergillus ochraceus*** was observed at 4 kGy. Osman (1973) who found that gamma irradiation caused marked inhibition to the spore germination of certain soil fungi; ***Aspergillus niger***, ***A. sulphunerus*** and ***Trichoderma lignorum*** especially at level of 500 krad.

Effect of γ-irradiation on fungal cellular protein profile

Protein from mycelia of the irradiated fungi ***R. solani*** (Fig. 2) and ***S. rolfsii*** (Fig. 3) were separated according to their molecular weights. Individual samples formed the characteristic patterns with both qualitative and quantitative differences. The adverse effect of gamma irradiation doses were clearly observed and showed that radiation caused initial fragmentation of proteins up to 5 kGy and subsequent aggregation due to cross-linking of protein molecules at 6 and 7 kGy doses for ***R. solani*** and at 4 kGy dose for ***S. rolfsii***. These results suggest the gamma irradiation caused the change of secondary structure of proteins, resulting in change of physicochemical properties of proteins.

Irradiation with gamma ray results in the inhibition of protein synthesis, an inhibition which lead most probably to the increase in the amino acid content of fungal cells (Salama ***et al.***, 1977; El-Sherbeny, 1982). It was recorded that nine free amino acids in the medium of ***Paecilomyces violacea*** could be detected at different irradiation doses but were completely missed in the amino acid pool of the control sample (Awny ***et al.***, 1988). Salama ***et al.*** (1989) reported that an increase in nitate uptake and nitrogen utilization by the fungus ***Paecilomyces violacea*** when irradiated with low doses of gamma rays while higher doses inhibit them in addition to protein synthesis. Tanaka ***et al.*** (1996) found that 3 proteins 87, 60 and 46 kDa continued to be synthesized during post-irradiation incubation and the amounts of these proteins increased with higher doses in a range of 1-12 kGy. Several reports (Kitayama and Matsuyama, 1971; West and Emmerson, 1977; Tanaka ***et al.***, 1996) indicated that protein synthesis after irradiation is needed not only for cell survival but also for rejoining of DNA strand break.

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