EFFECT OF IONIZING RADIATIONS ON FOOD DECAY FUNGI

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Maggy Kopelman

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ABSTRACT

EFFECT OF IONIZING RADIATIONS
ON FOOD DECAY FUNGI

by Maggy Kopelman

Spores of Aspergillus flavus, Aspergillus niger, Botrytis cinerea, Penicillium sp. and Rhizopus stolonifer were exposed to gamma rays from Cobolt 60, under various conditions. The lethal effect of the irradiation was determined. Spores of A. flavus were also exposed to 1 Mev elec- from a resonant transformer accelerator.

A. flavus, A. niger and Penicillium sp. spores were found to have a D value in the range of 30 to 35 krad of gamma rays, whereas B. cinerea spores had a D value of 55 krad and R. stolonifer a D value of 100 krad.

A. flavus and Penicillium sp. spores irradiated at pH 3, 4, 5, 6 and 7, showed almost no change in their radiosensitivity. B. cinerea spores showed a distinct decrease in their radioresistance at the higher pH levels (6 and 7).

Penicillium sp. spores irradiated in sucrose and dextrose solutions (0 to 20%) showed no significant change in their radioresistance in comparison to irradiation in water. B. cinerea spores displayed higher radioresistance

when they were irradiated in 5 to 20 per cent sucrose solution than in water. A. flavus spores showed an increase in their radioresistance to destruction by gamma rays, when the dextrose concentration of the suspension media increased from 0 to 40%.

A. flavus spores were considerably more radioresistant when they were irradiated in the dry state than in water suspension.

A comparison of gamma with cathode rays indicated no significant difference in their lethal effect on A. flavus spores irradiated in water.

The age of Penicillium sp. spores was found to have a very significant effect on their radioresistance: the older the spores, the higher the sensitivity to gamma rays, after the age of 20 days.

Strawberries inoculated with a B. cinerea spore suspension at 5, 2 and 0 days before irradiation with 200 krad of gamma rays, showed that the longer the period between inoculation and irradiation, the less the protection by irradiation against spoilage. The best protection was achieved when irradiation immediately followed the inoculation.

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By

Maggy Kopelman

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To my family

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ABBREVIATIONS

D value = The radiation dose (in krad) necessary to kill ninety per cent of the microorganisms.

krad = one thousand rad.
Rad is a radiation unit that represents an energy absorption of 100 ergs per one gram of material.

Mev = one million ev.
ev is the energy gained by an electron in moving through a potential difference of one volt and is equivalent to 1.602×10^{-12} ergs.

 = This symbol appears in the figures. It shows the upper and the lower limits of the distribution and its mean average.

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INTRODUCTION

The discovery of ionizing radiation by Roentgen and Bequerel (32) in 1895 initiated research on its bactericidal properties. The ionizing forms of radiation were the ones suggested for use in "cold sterilization." The ability to cause ionization of a receptor atom by ejection of an orbital electron, is the characteristic property of ionizing radiations. When an atom is ionized, the molecule of which it is a part almost certainly undergoes chemical changes.

Some types of ionizing radiation cannot be applied to foods, since they have the further property of bringing about a nuclear transformation, which can form radioactive atoms. Only X-rays, gamma rays, beta rays and cathode rays remain for consideration, when they are used at energy levels which do not exceed 10-15 Mev.

In this study the radiation forms used were gamma rays from Cobalt-60 and 1 Mev electrons generated in a General Electric resonant transformer.

In the last few years, in addition to research on sterilization of foods by irradiation, the partial eradication of food decay microorganisms, radio-pasteurization, has been explored.

The radio-pasteurization of fresh foods, such as fruits, vegetables and fish, appears to be a very promising application of irradiation. Fungi are one of the major group of decay organisms found on fruits and vegetables. The literature concerning irradiation of food decay fungi is not very extensive.

The objective of this study has been to investigate the effect of a number of different factors likely to affect the survival of fungi exposed to ionizing radiations.

The factors studied were: presence or absence of water in the suspension medium, pH levels, and sucrose or dextrose concentrations in the suspension medium.

The fungi studied were Aspergillus flavus, Aspergillus niger, Botrytis cinerea, Penicillium sp. and Rhizopus stolonifer.

A. flavus was found to be a cause of poisoning in animals consuming moldy peanuts or cereals (41).

A. niger (black mold), is widely distributed in the air and soil. It is often found on exposed foods and causes their decay (1).

B. cinerea was found by Powelson (28) to be responsible for 90% of the decay losses of west coast strawberries.

Penicillia are as common as the Aspergilli. They are

also food decaying microorganisms (1).

R. stolonifer is one of the decay organisms found in peaches (4), strawberries, grapes, tomatoes, etc.

The spores from the aforementioned fungi are all one-celled. The R. stolonifer has sporangiospores, while all the other fungi have conidiospores. Both types of spores will be referred to later only as spores.

LITERATURE REVIEW

Attempts to sterilize with ionizing radiation date back to early researches on its bactericidal properties (29), soon after the discovery of X-rays, and the natural radioactivity by Roentgen and Becquerel respectively (32). In 1930 a French patent was issued to O. Wörtfor for using ionizing radiation to preserve food (15). A strong interest in applying ionizing radiation to the preservation of foods began only around 1940, after the electron accelerators were developed. Brasch and Huber (8) were the first to report on the possibility of food preservation by accelerated electrons. During the past twenty years considerable interest has been expressed in the lethal effect of gamma and cathode rays on microorganisms as a means of full or partial eradication of food decay organisms. Only few of the early publications deal with fungi.

Lea's classical book (20) is a summary of considerable studies done by him and others on the actions of radiations on living cells. Lea's book does not give any suggestions on the use of radiation as a practical means for destroying microorganisms for purposes of sterilization. Others however, appeared to think in terms of such a purpose.

Dunn (10) reported in 1962 his and his co-workers' studies on the lethal doses of a large variety of micro-organisms, including a few molds, when exposed to X-rays and cathode rays.

In 1955, Hannan's (15) excellent review of all phases and aspects of the radiation preservation of food appeared.

Lethal doses of cathode and gamma rays of some fruit spoilage fungi was also determined by Bridges (9), Beraha and co-workers (5), Saravacos et al. (31) and others.

Today one of the most active groups in the study of the effect of ionizing radiation on food decay fungi is located at the University of California, Davis. Some of their publications deal with pure cultures of fungi irradiated with gamma rays (33, 36, 35, 25). Other papers of the same group report on the irradiation of the fruit itself with or without artificial inoculation with fungi (23, 22, 21, 26).

METHODS AND MATERIALS

1. Organism

Of the fungi studied here, *Botrytis cinerea* and *Penicillium sp.* were isolated from moldy strawberries. *Aspergillus flavus*, *Aspergillus niger* and *Rhizopus stolonifer* (nigricans) were obtained from Dr. E. S. Beneke of the Department of Botany and Plant Pathology, Michigan State University.

2. Growth media

(a) Difco Potato Dextrose Agar (P.D.A.) slants were used for maintaining the stock cultues. The same agar was used in the plates for counting colonies subsequent to irradiation.

(b) V8 agar was used as a sporulation agar. It was prepared by centrifuging commercial V8 Vegetable Juice manufactured by Campbell Soup Company. The supernatant liquid was separated and adjusted to pH 6.5-7.0. Two percent Difco Bacto Agar was dissolved in the separated supernatant and autoclaved in Roux type flasks for 20 minutes at 250^{o}F.

3. Spore growing and harvesting

A modified method of Bridges and co-workers (9) was used for the spore growing and harvesting. The fungus tested was transfered from the stock slant to V8 agar in the Roux type bottles. It was incubated at room temperature (25°C.) for two weeks, unless otherwise cited. The spores were harvested with sterile demineralized water containing 0.01% Triton X-100 as a wetting agent. Sterile glass beads were added to facilitate the harvesting. The spore suspension was filtered through sterile filter disks, to prevent micelia filaments from passing through. In order to eliminate clumping, the filtered suspension was shaken in an Erlenmeyer flask with glass beads. The suspension was centrifuged and washed twice by centrifugation with sterile demineralized water containing Triton X-100 and resuspended in it.

4. Preparation of sample for irradiation

Depending on the radiation source used, the samples were prepared as follows:

(a) Cobalt-60 source. The washed spore suspension was stirred with a magnetic stirrer having a sterile bar. One ml. portions of the suspension were transfered to sterile test tubes containing 3 ml. of the liquid in which the irradiation was carried out.

(b) Cathode ray source. When the irradiation was carried out in the electron accelerator, the preparation procedure was the same, but the suspension was transfered to sterile petri dishes, and irradiated in them.

5. Suspension media for spores during irradiation

(a) Water. All the fungal spores cited earlier were irradiated with gamma rays in water demineralized by means of a Bantam demineralizer. To this water 0.01% Triton X-100 was added as a wetting agent.

(b) pH. Citrate buffer was used to obtain suspending liquid of a definite pH. The final buffer concentration was 0.075 M, unless otherwise cited.

(c) Dextrose. Pure dextrose crystals (Baker reagent), were dissolved in demineralized water. The final concentrations used will be cited in the different cases.

(d) Sucrose. Pure sucrose crystals (Baker reagent) were dissolved in demineralized water. The final concentrations will be cited when describing the individual experiments.

(e) Dry state. The spores were harvested in the same way as was described earlier in paragraph 3. The

irradiation was carried out using gamma rays and cathode rays. One ml. portions of the spore suspension in demineralized water were dried in test tubes or Petri dishes under 27.5" of vacuum at 25°C. The test tubes were left in the drier for 20 hours. The Petri dishes were dried for 4 hours under vacuum (27.5") and then placed in a desiccator overnight.

6. Irradiation sources

All fungi were irradiated with gamma rays. A. flavus was irradiated with both gamma and cathode rays.

(a) Cobalt-60 served as a source of gamma rays. The Phoenix Radiation Facility and the Phoenix Memorial Laboratory sources at the University of Michigan in Ann Arbor were used. The activity of the sources used was 2500 and 10000 curies respectively.

(b) A General Electric resonant transformer generator was used as a source of cathode rays. The electron beam had the energy of 1 Mev. The stated doses are at the surface. The maximum ionization occurs at .5 m"m water and the maximum penetration in 3.5 m"m of water.

7. Irradiation

(a) Cobalt-60 source. The test tubes containing the fungal spores were placed in a styrofoam holder cut to fit around the cage of the Co-60 rods. The distance of the tubes from the cage was calculated on the basis of dosimetry performed by the University of Michigan personnel, in order to expose the samples to the desired level of irradiation. The dose rate varied according to the source used and the distance of exposure.

(b) Resonant transformer generator. The Petri dishes containing the fungus spores were placed on a conveyor belt which carried the plates under the electron beam. The velocity of the conveyor was adjusted according to the dose desired and was based on available dosimetry data. Here again the dose rate was not the same when different doses were applied. During irradiation the Petri dish covers were removed and were immediately replaced following irradiation. Control plates containing sterile water indicated that the contamination using this technique was negligible. The irradiation was performed at room temperature.

8. Survival determination

The irradiated spores were held overnight at a temperature of 40°-42°F. The test tubes and the Petri dishes containing the irradiated spore suspension were well mixed before diluting the plating with the aid of a vortex mixer. For plating, suitable water dilutions were made to give a count of 20-200 colonies per plate. In order to obtain the desired range, at least triplicate samples were run on P.D.A. at each of 2 to 3 dilution levels. The plates were incubated at room temperature (25°C.) for 10 hours to 3 days, according to the organism studied. The exact time will be cited later in each case. The number of survivors was determined by their ability to form colonies on the P.D.A. sub-culture. A Quebec Colony Counter was used for counting, with the exception of Rhizopus stolonifer, where a binocular microscope was used. The Quebec Counter facilitated the counting of visible colonies as it has a light source, a low magnifying glass and a grided viewing surface.

9. An experiment on established infection

The effect of irradiation on established growth of the fungus Botrytis cinerea in fresh strawberries was studied in one experiment.

Strawberries were placed in small egg crate dividers with their tip upward. The strawberry tips were slightly wounded and inoculated with the aid of a wire brush which was dipped in a 10^6 spores/ml. suspension of the fungus spores. After inoculation, the crates were covered with a plastic bag to eliminate further contamination and to form a high humidity atmosphere. The inoculations were performed at intervals of 5, 2 and 0 days before irradiation. The strawberries were incubated at 52°F. following inoculation. For each inoculation time a control was used which was not irradiated. For each treatment 18 berries were used.

Following the irradiation, the berries were stored at 52°F. The berries were checked for visual fungal growth over a period of 18 days.

RESULTS AND DISCUSSION

Most of the results were expressed as percent survival of organism.

$$\% \text{ survival} = \frac{\text{number of spores after irradiation}}{\text{number of spores before irradiation}} \times 100$$

The percent survival was plotted on a logarithmic scale on the y-axis and the dose level or the solution concentrations were plotted arithmetically on the x-axis. The reason for this way of plotting is that generally the biological effects of irradiation are exponential functions of dose (20).

The dose rate was not kept the same in all the experiments, as it was found in the literature that there is no rate dependence of irradiation on the lethality of yeasts and bacterial spores exposed to cathode, gamma and X-rays (20, 39, 38, 17). They found that the mean lethal dose was the same, whether the irradiation was performed at a low intensity and spread over a prolonged time, or at high intensity for a short time.

1. General observations

(a) Production of small colonies

All the tested fungi produced small colonies after irradiation. The number of small colonies among

survivors increased with increasing dose of irradiation, in an irregular manner. The small colonies did not reach the size of the regular colonies, even if they were held for longer incubation times.

Laser found in 1964 (18) similar results when yeasts were exposed to X-rays.

(b) Delayed growth

A temporary inhibition of division and formation of visible colonies was observed among the spores that survived the irradiation. The duration of the delay increased with increasing dose. It is most apparent in the doses approaching the lethal dose. A similar retardation was reported by Lea (20) to be a general action of irradiation in a great variety of living cells.

Demineralized water was used in all the liquid suspensions prepared for irradiation because it was shown by Dunn (11) that the presence of certain inorganic cations can affect the sensitivity to some extent.

The first experiment was the isolation of some fungi from moldy strawberries. The fungi genera* were identified

*Dr. Beneke assisted in the identification of the species.

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using the Handbook of Barnett (3) on imperfect fungi.

The species isolated were Alterneria grisea, Alterneria tenuis, Helmintho sporium sp., Botrytis cinerea and Penicillium sp. Only the last two fungi were chosen for the irradiation work.

2. Sporulation media

In order to find good sporulation media, the following media were tested:

(a) Potato dextrose agar (P.D.A.), Difco.

(b) Corn Meal Agar plus 0.5% Yeast Extract (Difco).

(c) Mycological Agar (Difco).

(d) V8 Agar, prepared from either:

(1) the whole V8 juice, or

(2) the centrifuged juice in its natural pH (around pH 4), or

(3) the centrifuged juice adjusted to pH 6.5-7.0.

Of these the V8 media gave the best results in terms of luxuriant sporulation. Among the V8 media (d-3) was preferred, because it solidified uniformly and strongly. The results will be presented on a species basis.

3. A. falvus

This fungus was irradiated with both gamma and cathode rays under various conditions.

(a) Irradiation in demineralized water

Two week old spores were suspended in demineralized water and exposed to 0, 50, 100, 150, 200, 250 and 300 krad of gamma or cathode rays.

The absolute numbers and percentage of surviving spores are given in Tables 1 and 2 of Appendix 1 for gamma and cathode rays, respectively. The percent survivals are presented in graphic form in Figures 1 and 2. An approximate D value of 35-37.5 krad can be calculated, indicating that A. flavus spores are rather sensitive to irradiation.

In this experiment the radiosensitivity of A. flavus spores was not influenced significantly by the nature of radiation (gamma or cathode rays). Available information indicates that differences in the biological effect caused by these two types of rays are not large, but if the conditions of irradiation are not exactly the same, as for example in the irradiation of spore suspension in test tubes vs. Petri dishes, in which the ratio of surface to depth differs, the effect may be different (13, 14).

Figure 1. Effect of gamma rays on the viability of Aspergillus flavus spores irradiated in demineralized water.

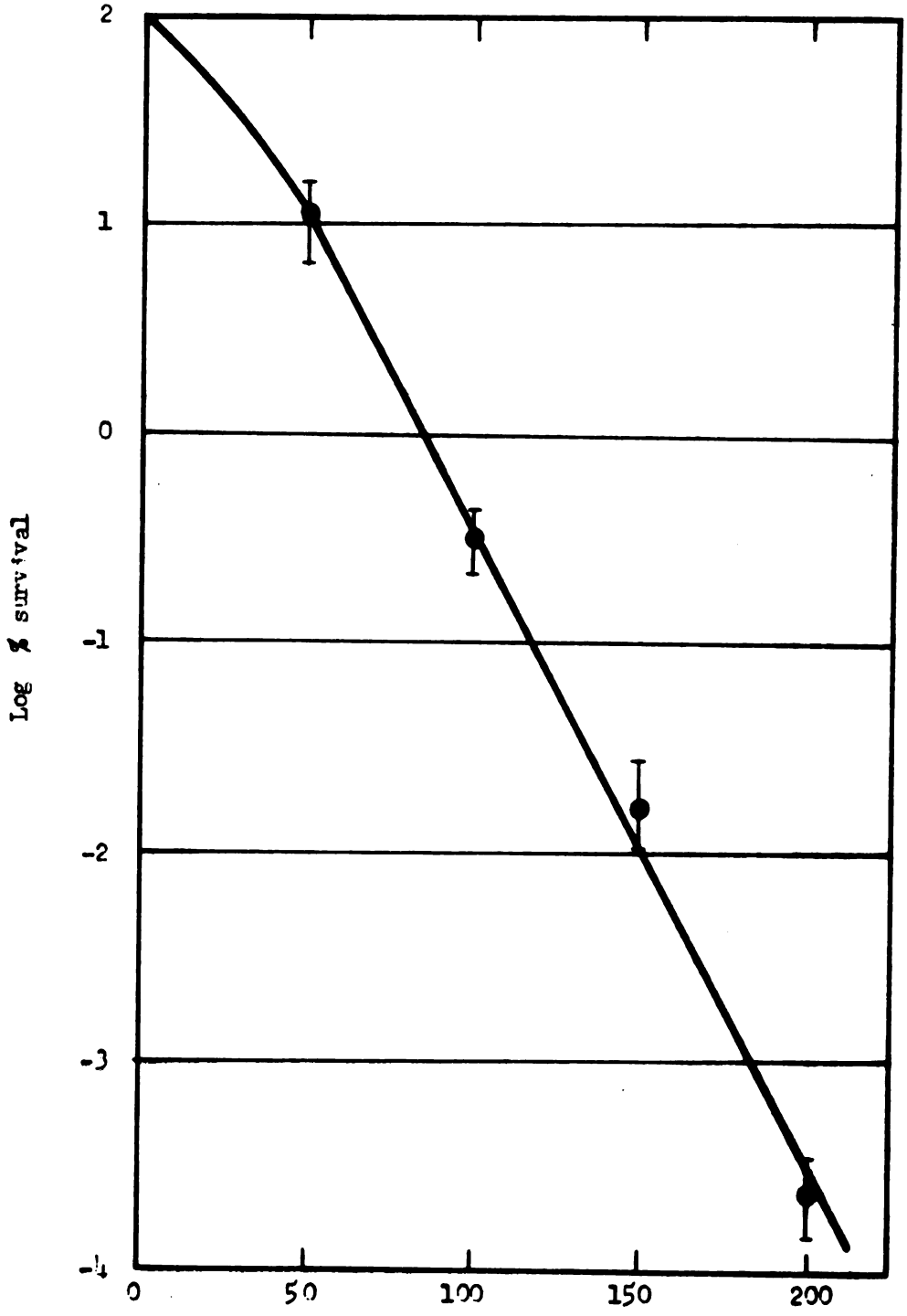
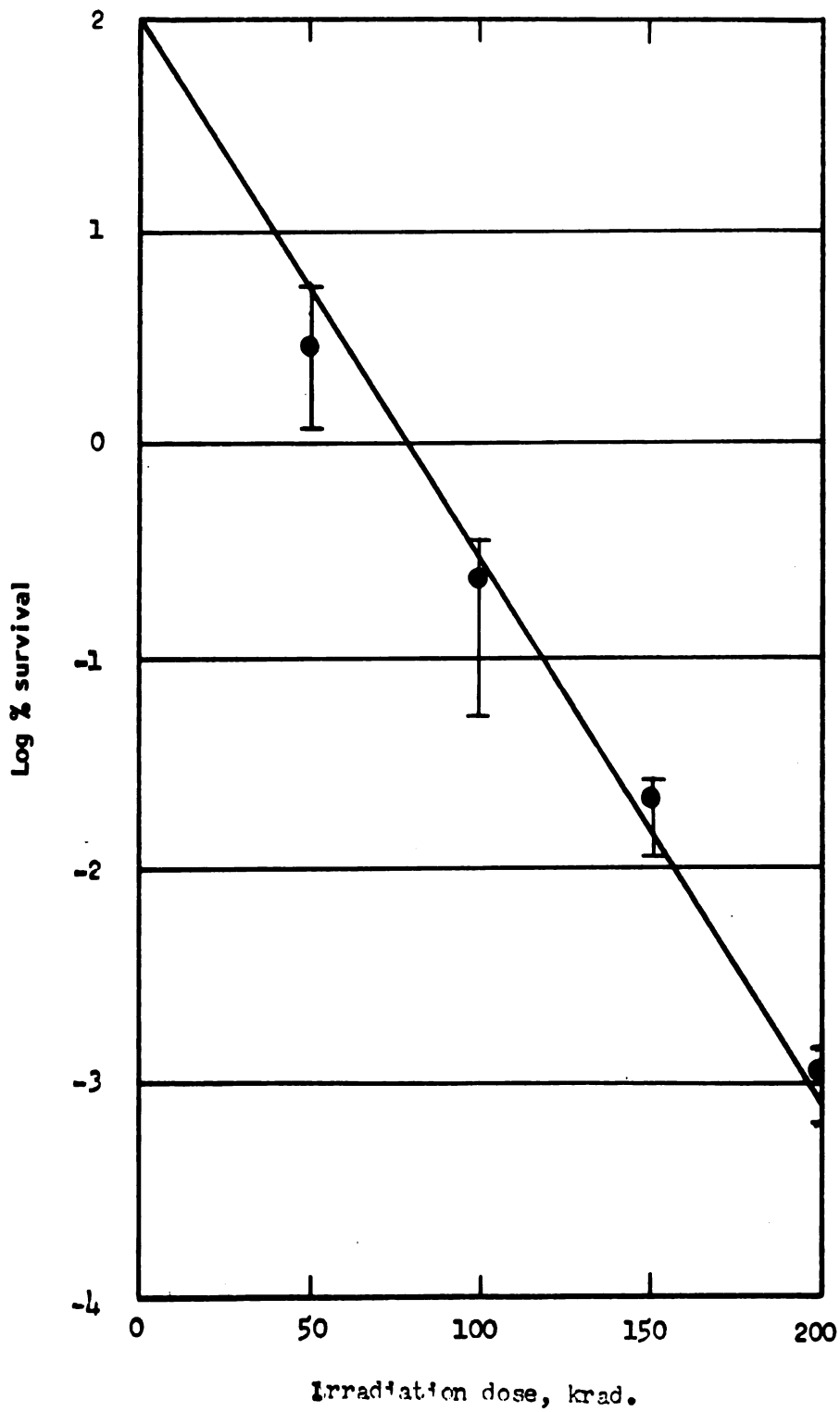


Figure 2. Effect of cathode rays on the viability of Aspergillus flavus spores irradiated in demineralized water.



(b) Irradiation at various pH levels

Two-week old spores of A. flavus were suspended in 0.075M citrate buffers at pH 3, 4, 5, 6 and 7, and exposed to 0, 50, 100, 150 and 200 krad of gamma and cathode rays. The absolute numbers and percentage of surviving spores are given in Tables 3 and 4 of Appendix 1. The percent survivals are graphically presented in Figures 3 and 4. It is apparent that pH has not an appreciable effect on the radio-sensitivity of the spores.

Similar results were obtained by Edwards and co-workers (12) who irradiated Bacillus subtilis spores with cathode rays. Alper and Gillies (2) found some dependence of the pH on the survival of irradiated E. coli. The higher the acidity of the media, the higher was the number of survivors.

(c) Irradiation in dextrose solutions

Two-week old spores of A. flavus were suspended in dextrose solutions to give a final concentration of 0, 10, 20, 30 and 40 percent (w/v) of the sugar. The suspensions were exposed to gamma and cathode rays at different levels up to 250 and 200 krad, respectively.

The results are given in Tables 5 and 6 of Appendix 1, and are illustrated in Figures 5 and 6. A. flavus spores suspended in dextrose solutions show a slight decrease in

Figure 3. Effect of gamma rays on the viability of Aspergillus flavus spores irradiated in .075 M citrate buffer of various pH.

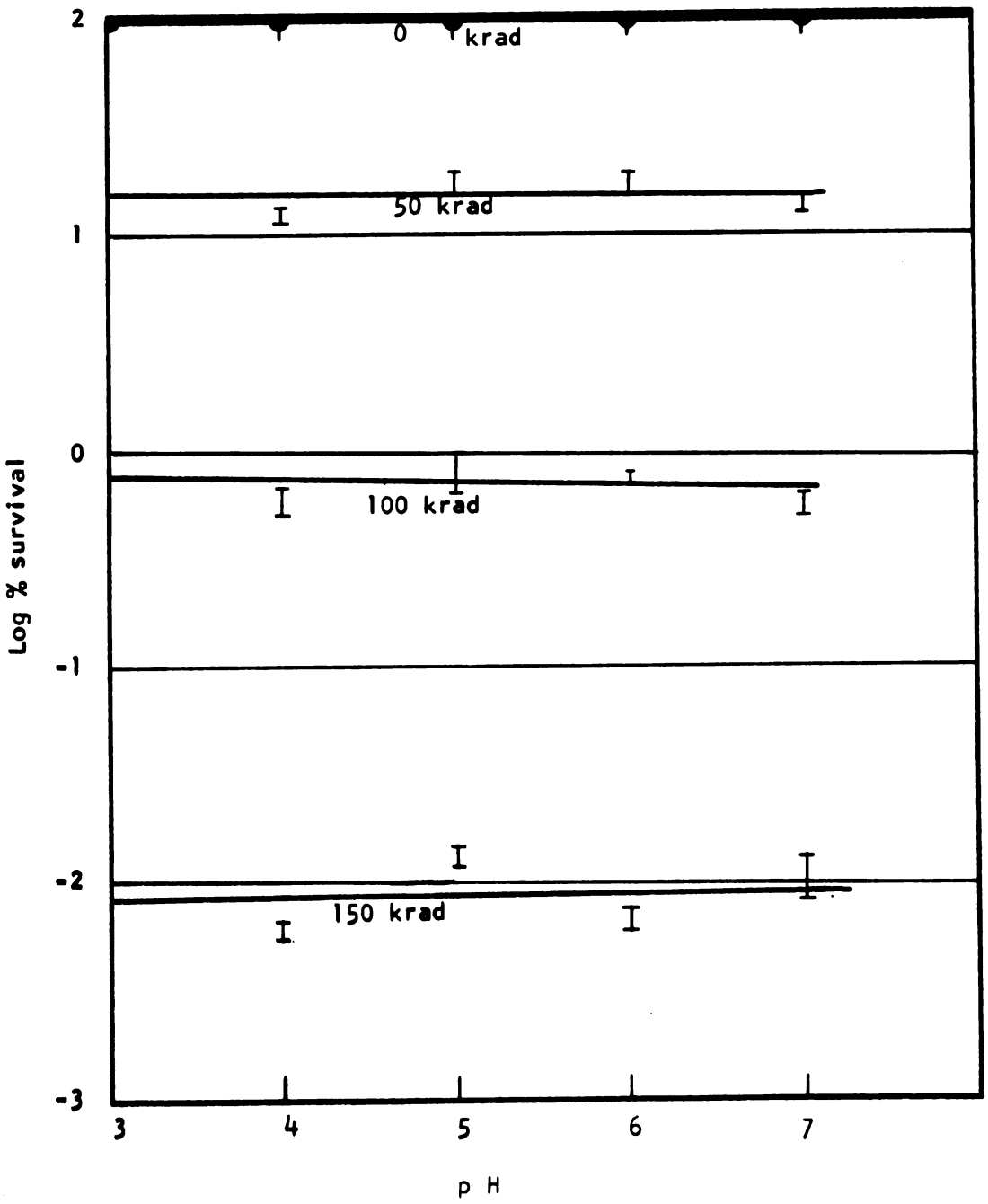


Figure 4. Effect of cathode rays on the viability of Aspergillus flavus spores irradiated in .075 M citrate buffer of various pH.

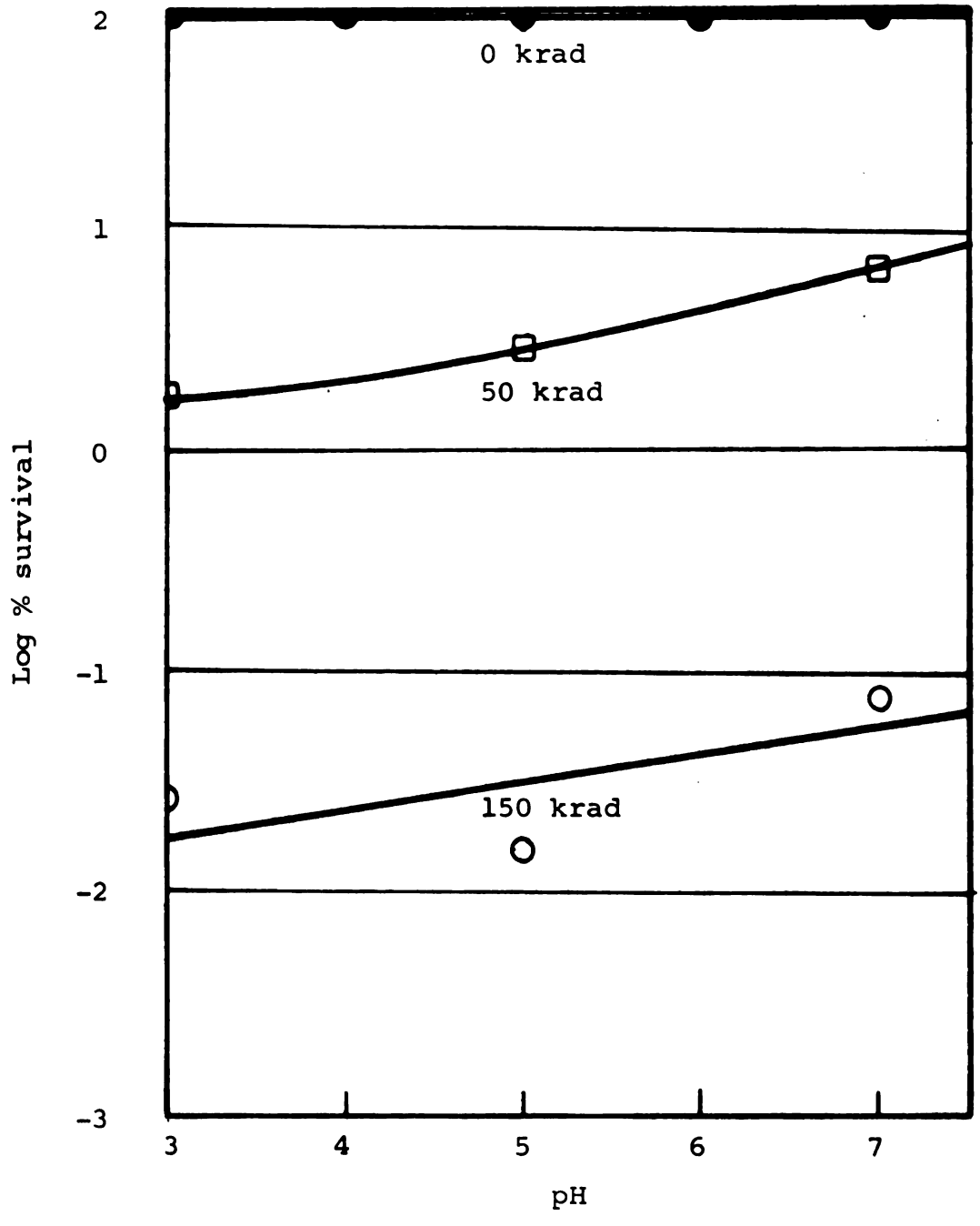


Figure 5. Effect of gamma rays on the viability of Aspergillus flavus spores suspended in dextrose solutions.

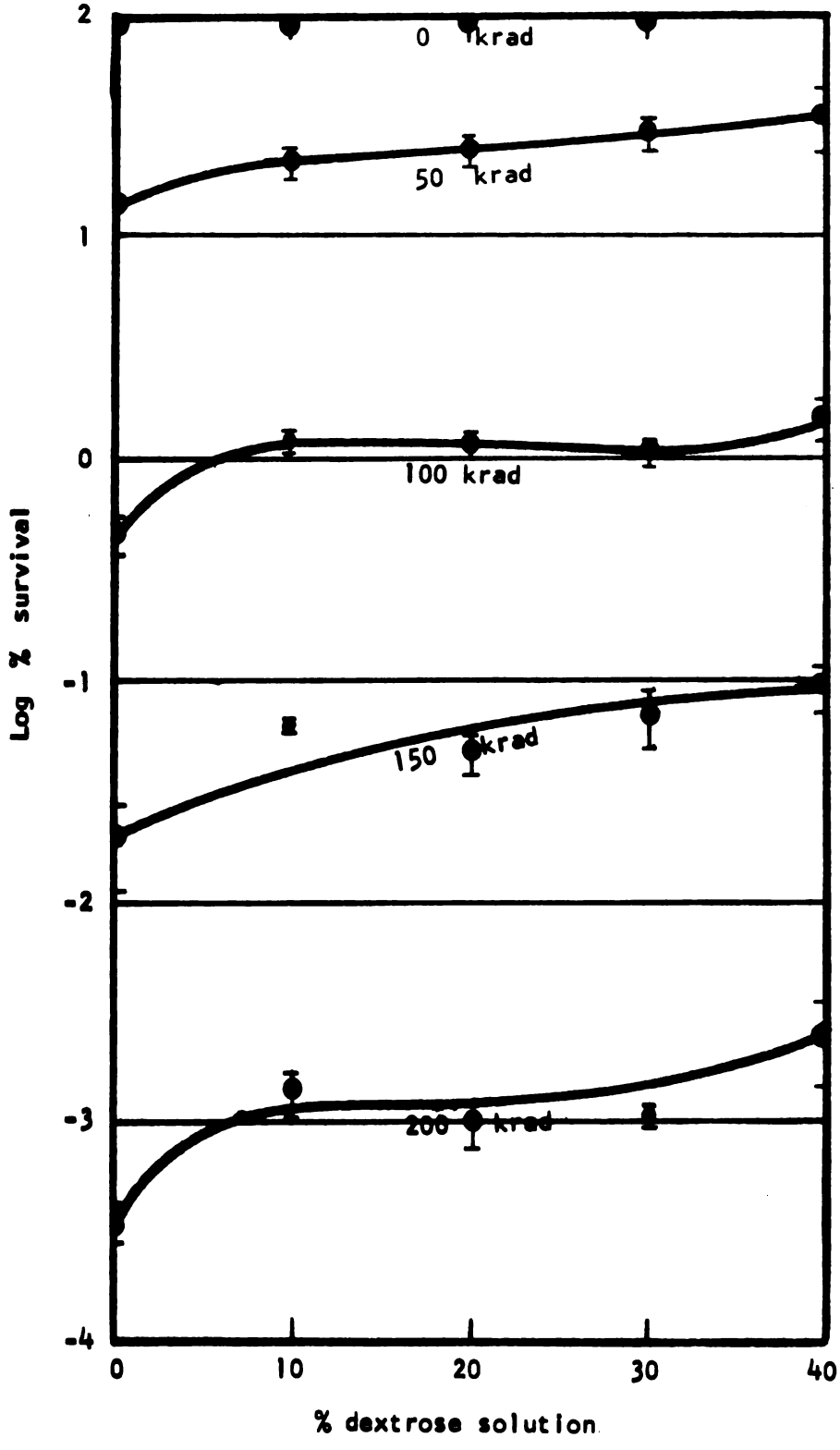
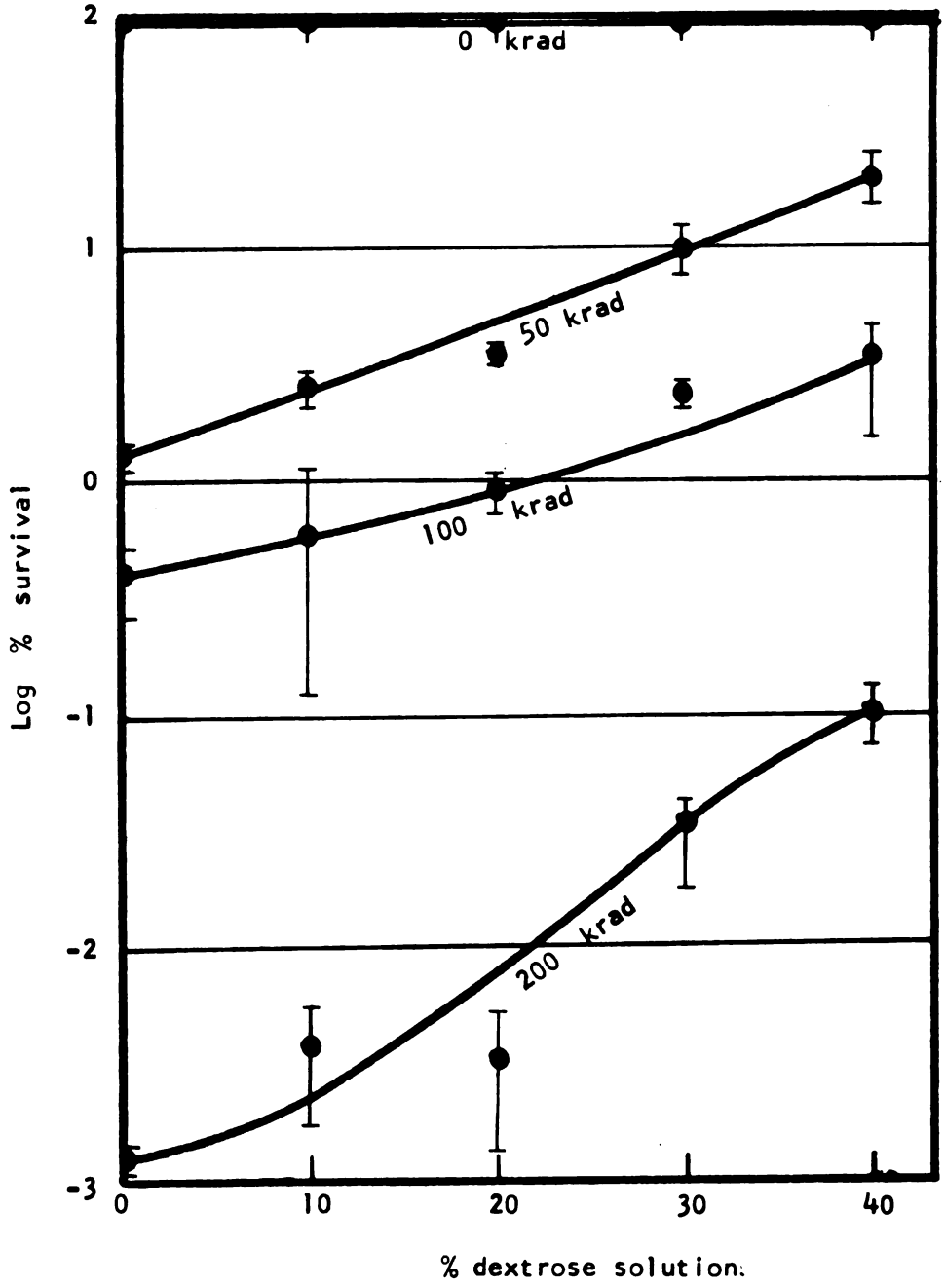


Figure 6. Effect of cathode rays on the viability of Aspergillus flavus spores suspended in dextrose solutions.



their sensitivity and this decrease is more apparent at the high doses.

Several workers (27, 16, 40, 43) studied the effect of dextrose present in the growth media or in the suspension media used for irradiation, on the resistance of micro-organisms. They found also that there is a slight increase in resistance of the microorganisms to irradiation, in the presence of dextrose.

Latarjet and Loiseleur (19), 1942, quoted by Nickson (27) and later Hollaender and Stapleton, 1953 (16) offered some explanations for its protective action.

(d) Irradiation in the dry state

Two-week old spores of A. flavus were suspended in demineralized water, containing 0.01% triton X-100. A number of these suspensions were dried as described under Materials and Methods. The wet and dried suspensions were exposed to 0, 50, 100, 150, 200, 250 and 300 krad of gamma and cathode rays.

The results of this experiment are summarized in Tables 7 and 8 of Appendix 1 and in Figures 7 and 8.

It is apparent that the dried spores of A. flavus are considerably more resistant than the spores suspended in water. Bhattacharjee (7) obtained similar results when he

Figure 7. Effect of gamma rays on the viability of dry and wet spores of Aspergillus flavus.

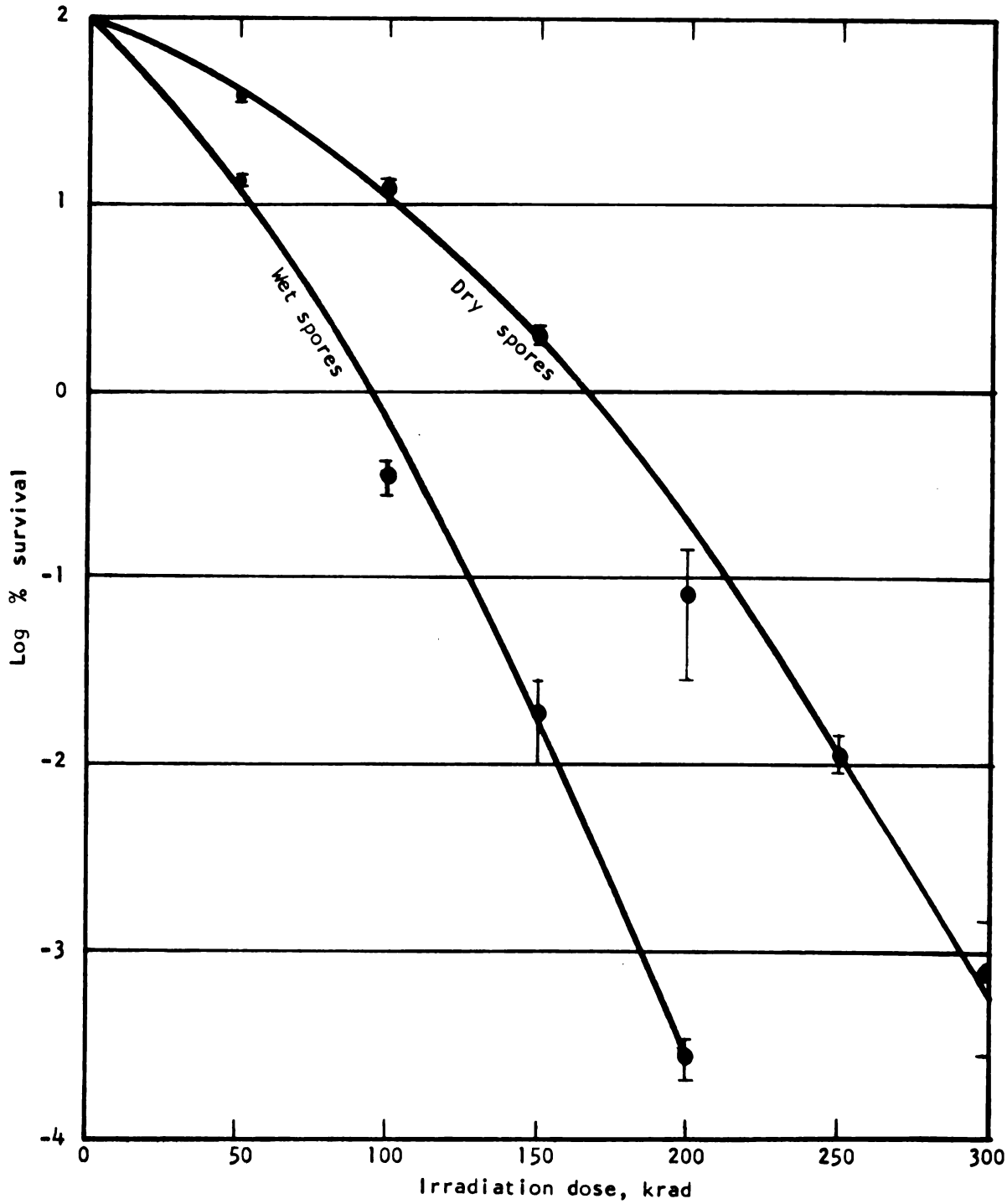
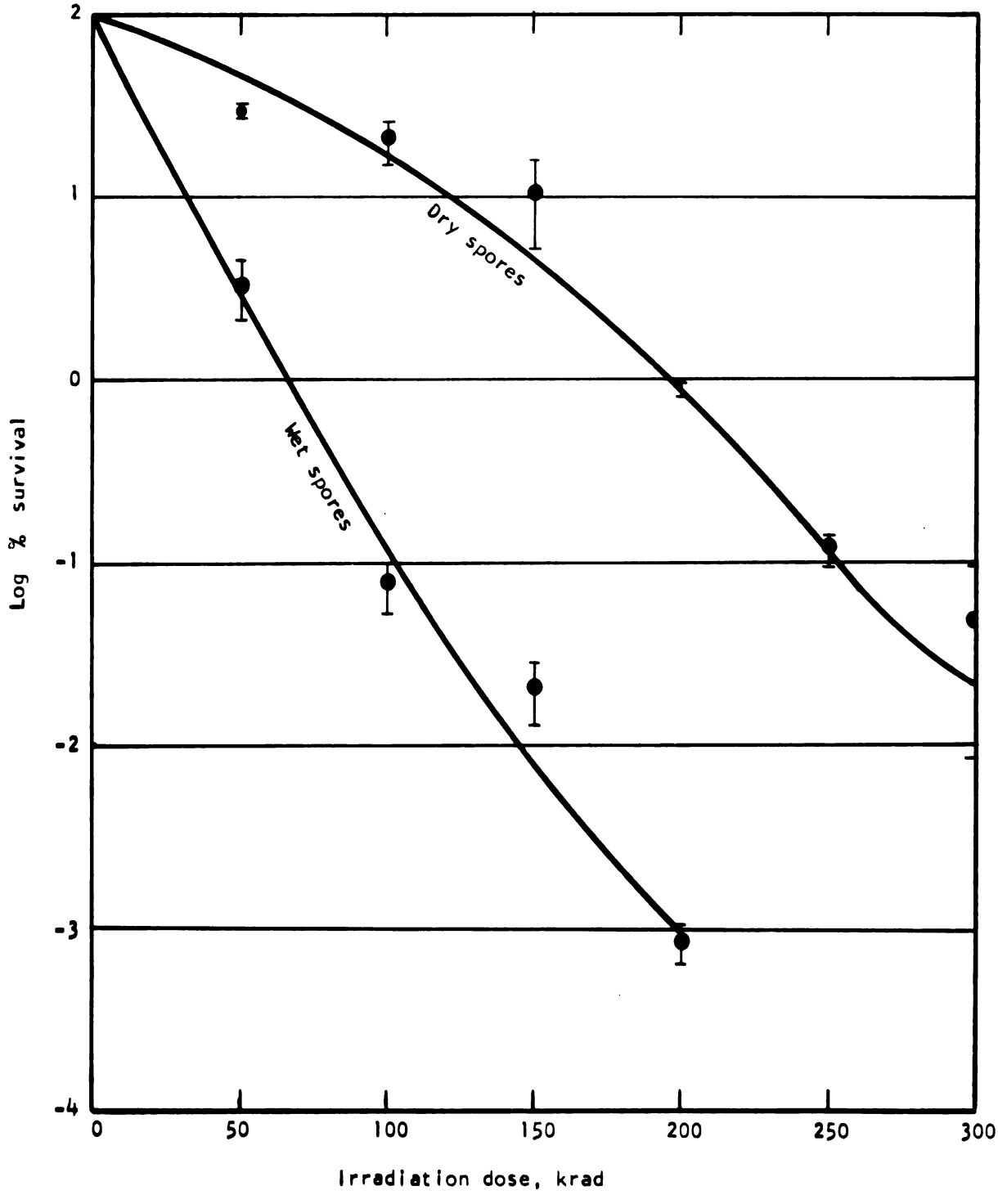


Figure 6. Effect of cathode rays on the viability of dry and wet spores of Aspergillus flavus.



irradiated E. coli with X-rays.

Stapleton and Hollaender (37) showed that the higher the water content of A. terreus spores, the lower the lethal dose. Several other investigators also demonstrated this striking increase of microorganism radioresistance when irradiated in dry state rather than in wet state. The explanation offered to this phenomenon is that the removal of free water decreases the indirect effect,* thus the radiosensitivity decreases too (7, 24, 44, 20).

4. A. niger

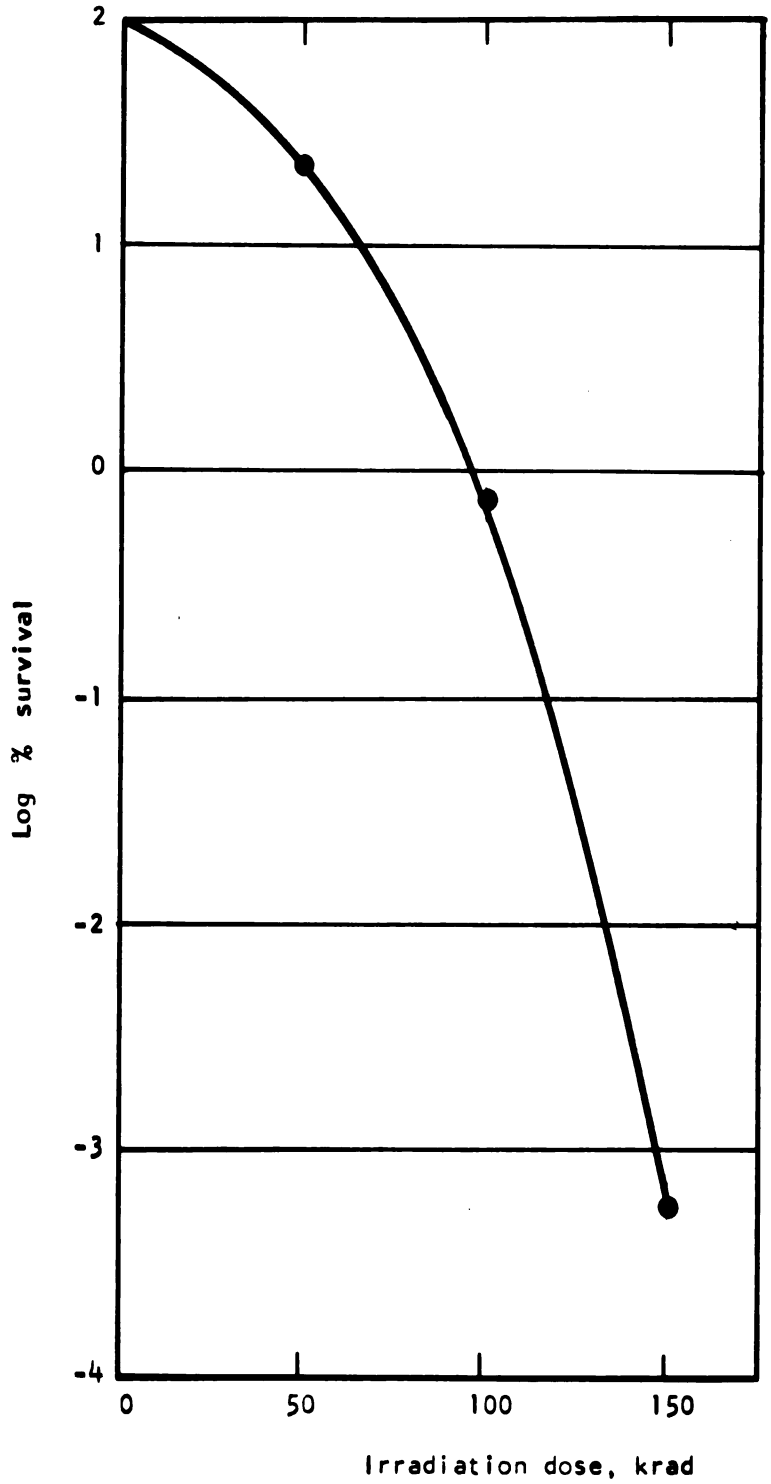
One-week old spores of A. niger were harvested, suspended at the concentration of 10^6 spores per ml. of demineralized water containing 0.01% Triton X-100, and exposed to 0, 50, 100, 150, 200, and 250 krad of gamma rays.

The results are presented in Figure 9 and summarized in Table 9 of Appendix 1.

It is apparent that this fungus is quite sensitive to gamma rays. At 200 krad no growth could be observed.

*Damage is caused to a microorganism when the radiation hits its sensitive area directly (Lea's Target Theory). When the cause of the damage is due to the ionization in the outside environment of the target, the effect is called an indirect effect.

Figure 9. Effect of gamma rays on the viability of Aspergillus niger spores suspended in demineralized water.



Saravacos and his co-workers (31) found that 250 krad is the lethal dose of A. niger. The difference in the results may be due to the suspension media during the irradiation and the size of the original population. In their experiment the fungus was irradiated on malt extract agar slants with an undetermined population of cells.

5. B. cinerea

Nineteen-day old cultures of B. cinerea were harvested and the spores were irradiated in several suspension media.

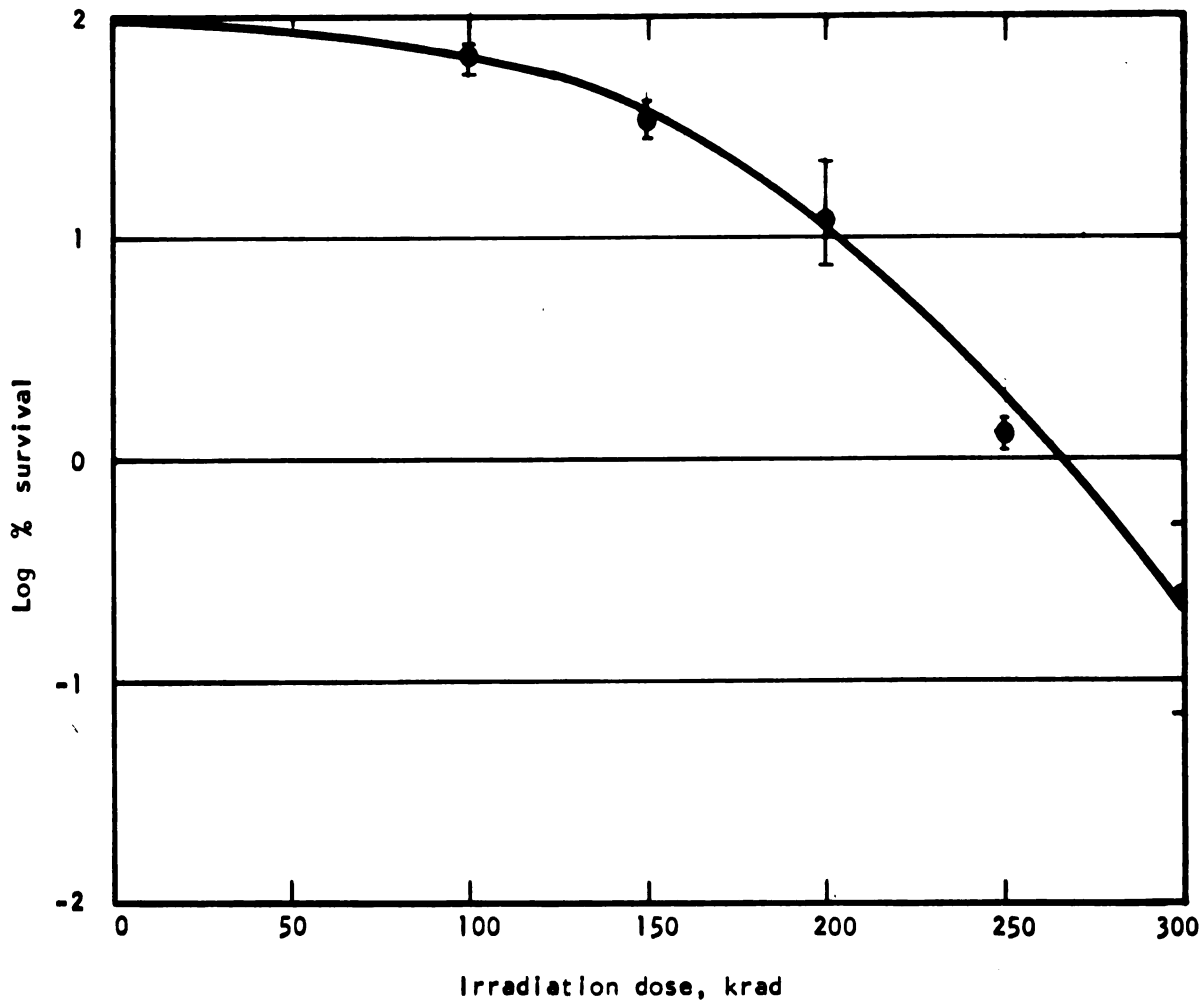
(a) Irradiation in demineralized water

Aqueous spore suspensions containing 10^5-10^6 spores per ml. were exposed to gamma rays at the dose range of 0 to 300 krad. The results are presented in Table 10 of Appendix 1 and in Figure 10. The figure is based on five separate experiments. The survival curve obtained was not a straight line. The results indicated a D value of about 55 krad at the straight part of the line. The irradiation destroys only a small number of spores at the low doses, resulting in a lag which extends to 100-150 krad.

(b) Irradiation at various pH levels

Spores of B. cinerea were suspended in 0.075M citrate buffers pH 3, 4, 5, 6 and 7 and exposed to gamma rays in

Figure 10. Effect of gamma rays on the viability of Botrytis cinerea spores suspended in demineralized water.



the dose range of 0 to 300 krad. The results are presented in Table 11 of Appendix 1 and Figure 11. The results indicated that the lower the pH, in the range studied, the higher the percent survivors at all doses. As the dose increased, the number of survivors decreased more sharply at higher rather than at lower pH levels.

(c) Irradiation in sucrose solutions

Spores of B. cinerea were suspended in 0, 5, 10, 15 and 20 percent (w/v) sucrose solutions and exposed to gamma rays at the range of 0 to 300 krad. The results are summarized in Table 12, Appendix 1, and Figure 12. From these data it appears that a greater number of spores are likely to survive radiation destruction in sucrose solution than in water. However, no clear differences among the various sucrose concentrations tested can be ascertained in regard to radiation protection of the spores.

6. Penicillium sp.

In order to study the possibility of a change in the radioresistance of fungi spores, due to difference in their age, Penicillium sp. spores at the age of 9, 15, 19, 26, 40 and 60 days were irradiated with 50 krad of gamma rays. The results are summarized in Table 13, Appendix 1 and in Figure 13. The curve shows a very small reduction in the radio-

Figure 11. Effect of gamma rays on the viability of Botrytis cinerea spores irradiated in .075 M citrate buffer of various pH.

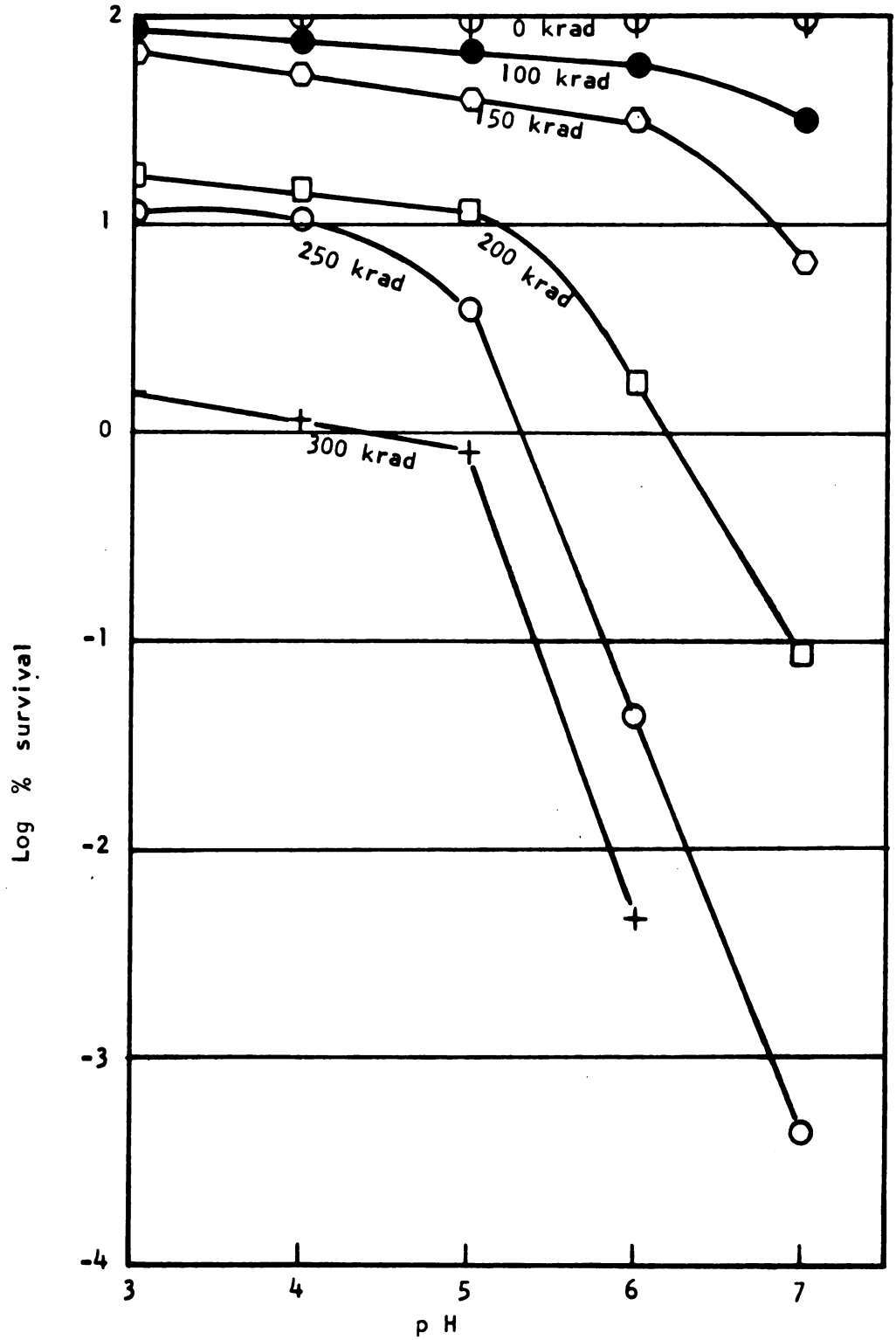
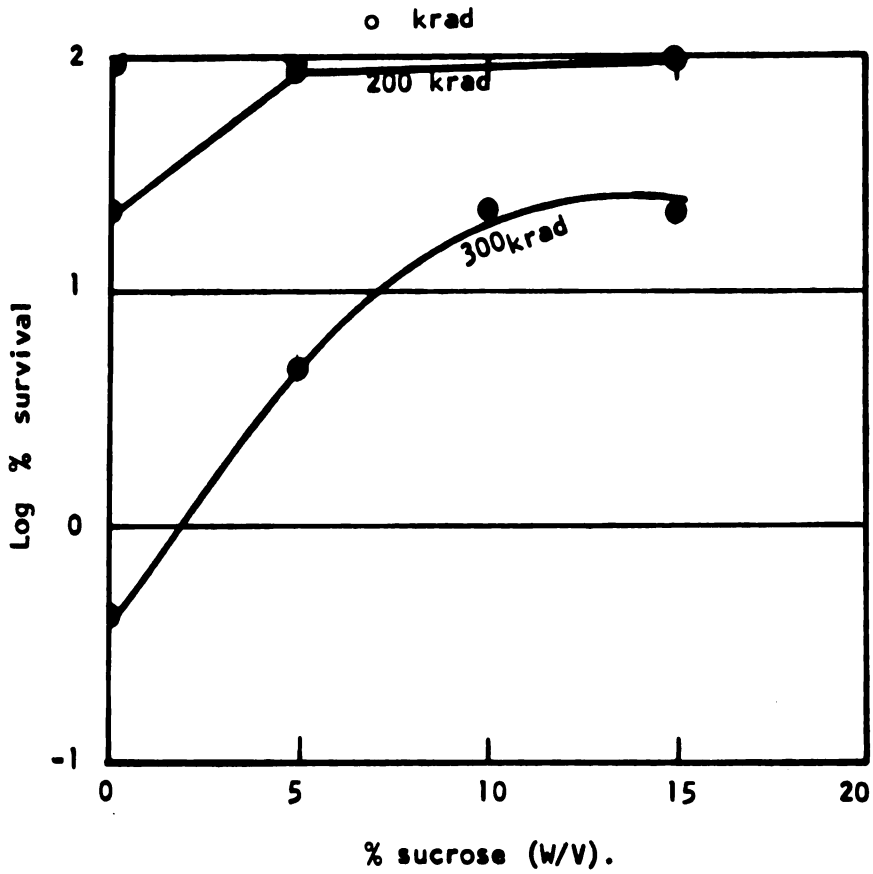


Figure 12. Effect of gamma rays on the viability of Botrytis cinerea spores suspended in sucrose solutions.



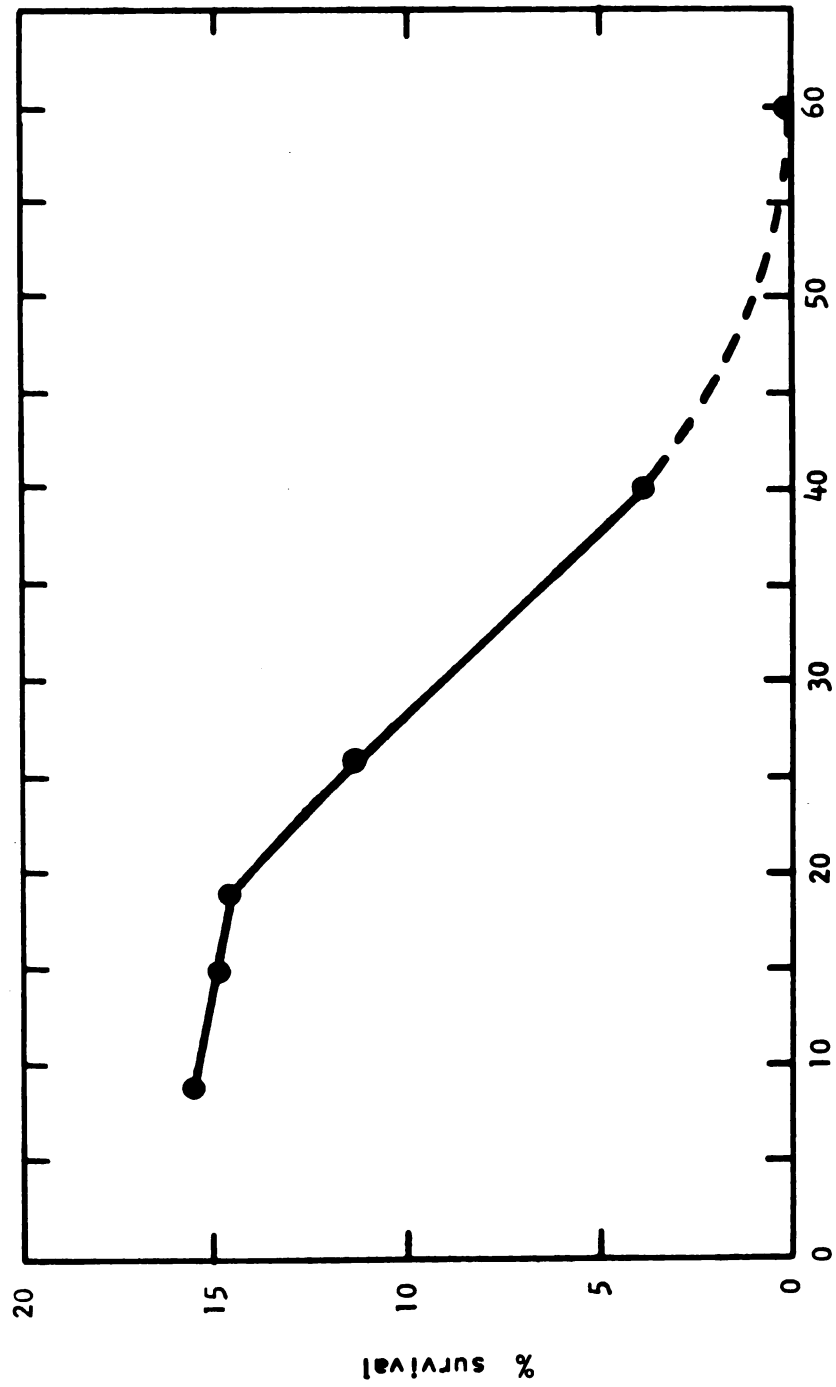


Figure 13. Effect of age of Penicillium sp. spores on their radioresistance (dose of irradiation 50 krad of gamma rays).

resistance of the spores up to the age of 20 days. At a still older age, the resistance of the spores to irradiation falls almost linearily.

The age of the fungi spores chosen to work with was 14-19 days.

Proctor and co-workers (30), working with bacteria, found that the culture age played a role in the radiosensitivity of the organism, the old cells are the most radiosensitive.

(a) Irradiation in demineralized water

Aqueous spore suspensions containing 10^6-10^7 spores per ml., were exposed to gamma rays at the dose levels of 0, 50, 100, 150, 200, 250 and 300 krad. The results are presented in Table 14 of Appendix 1 and in Figure 14. The survival curve is based on four separate experiments. Colony counting, two days after plating, indicated a D value of about 35 krad.

(b) Irradiation at various pH levels

Penicillium sp. spore suspensions at pH levels of 3 to 6 produced using citrate buffers (0.075M), were exposed to gamma rays at the doses 0, 50, 100, 150 and 200 krad. The results are summarized in Table 15 of Appendix 1 and in Figure 15. Four separate experiments were conducted.

Figure 14. Effect of gamma rays on the viability of *Penicillium sp.* spores suspended in demineralized water.

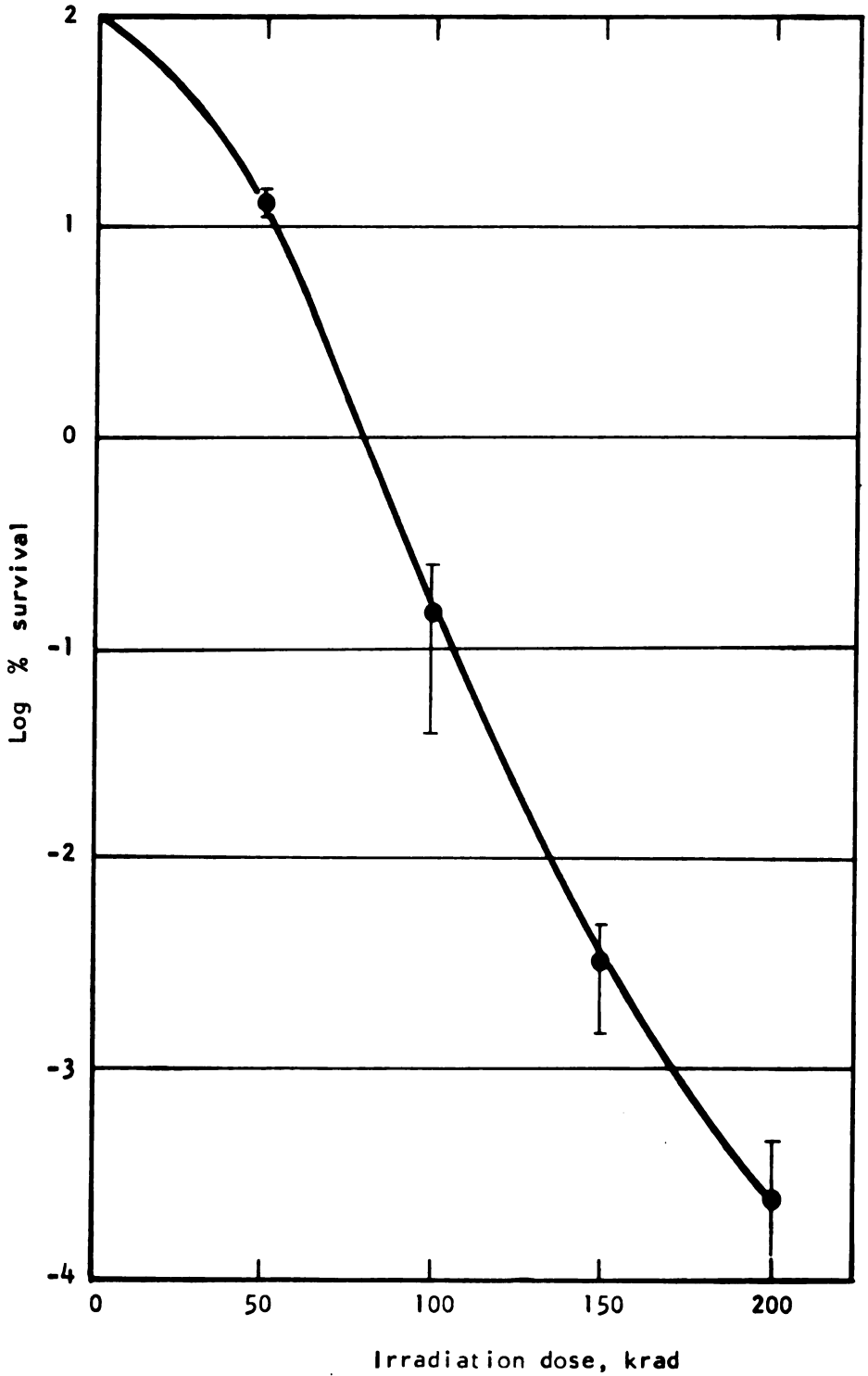
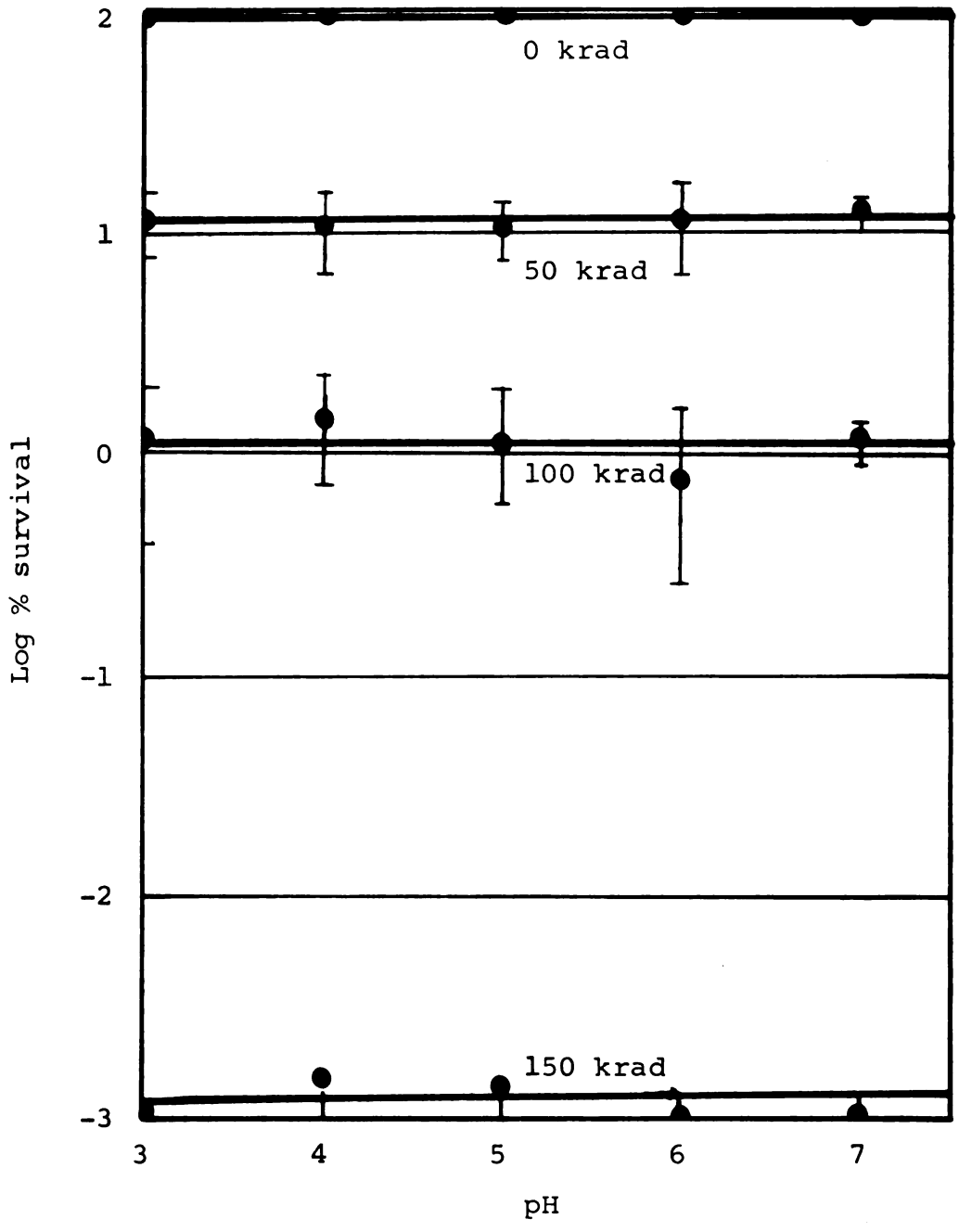


Figure 15. Effect of gamma rays on the viability of *Penicillium sp.* spores irradiated in .075 M citrate buffer of various pH.



These data indicate that the effect of pH on the radioresistance of the spores is not pronounced. There might be a slight increase in the sensitivity at pH 6 over that at lower levels. The survivors at 200 krad were too few for any significant counting.

(c) Irradiation in sucrose solutions

Nineteen-day old Penicillium sp. spores were suspended in 0, 5, 10 and 15 percent (w/v) solutions of sucrose, and were exposed to 0, 50, 100 and 200 krad of gamma rays. The results are presented in Table 16, Appendix 1.

From these data it appears that sucrose, at the concentrations used, had little or no effect on the radioresistance of the fungus spores.

Another irradiation of 40 day old Penicillium sp. spores in sucrose solutions was performed. Spores suspended in 0, 10 and 20 percent (w/v) of the sugar were exposed to 0, 50, 100, 200 and 300 krad of gamma rays. The results are presented in Table 16 (2) of Appendix 1. No data were reported for 200 and 300 krad doses, because no plate growth was observed under the conditions of plating used.

The radioresistance of these spores in plain water and in the sugar suspensions is slightly lower than that observed in the previous experiment. The main reason may be the age of the spores used.

(d) Irradiation in dextrose solutions

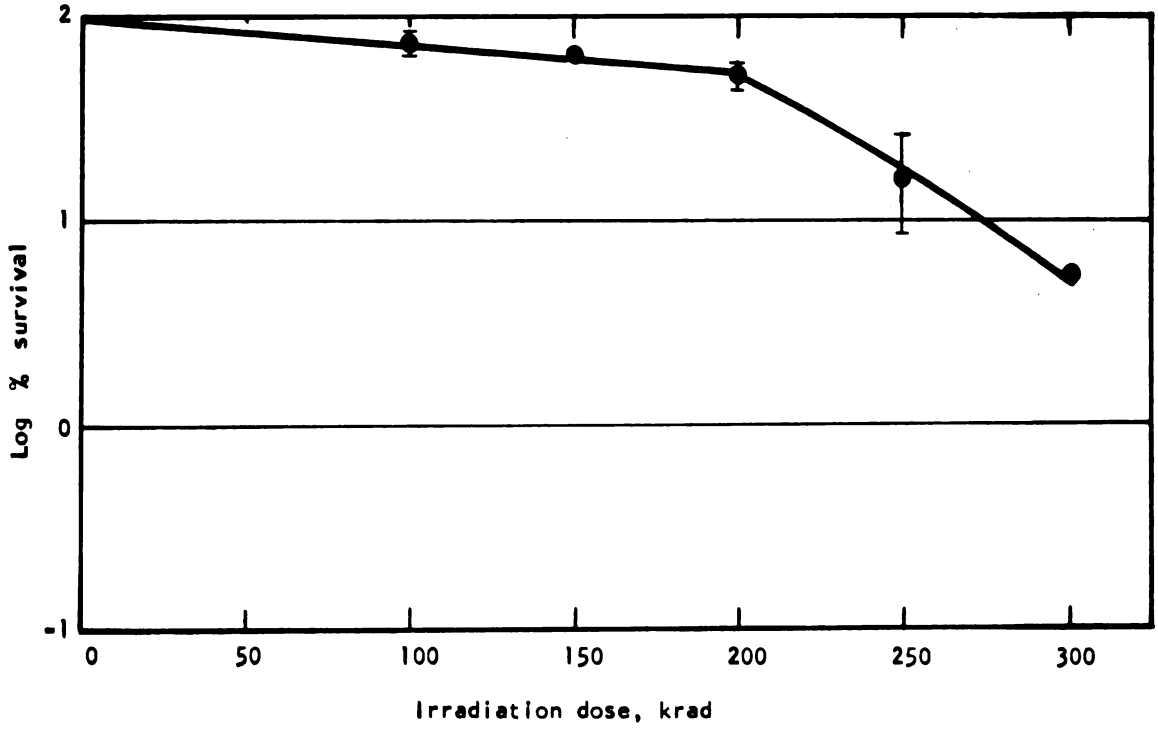
Forty-day old spores of Penicillium sp. were suspended in 0, 10 and 20 percent w/v of dextrose and were exposed to 0, 50, 100, 200 and 300 krad of gamma rays. The results are presented in Table 17 of Appendix 1. No data were reported for 200 and 300 krad, because no plate growth was observed under the conditions of plating used. The radioresistance of this spore in plain water is slightly lower than that observed in previous experiments.

7. Rhizopus stolonifer

Fourteen-day old spores of the fungus were suspended in demineralized water containing 0.01% Triton X-100, and were exposed to 0, 100, 150, 200, 250 and 300 krad of gamma rays. The survivals were counted after 20 to 24 hours of plating with the aid of a binocular. The reason for this way of counting is that colonies of this fungus older than 24 hours spread over adjacent colonies hindering the enumeration. The results are presented in Table 18 of Appendix 1, and graphed in Figure 16.

Rhizopus stolonifer is the most resistant fungus checked. The results obtained are quite similar to those reported in the literature (6, 31, 34), although a different procedure

Figure 16. Effect of gamma rays on the viability of Rhizopus stolonifer spores suspended in demineralized water.



of harvesting, irradiating and determining the survival was used.

8. Irradiated inoculated strawberries

Strawberries, inoculated with B. cinerea spore suspension 5, 2 and 0 days before irradiation, were exposed to 0 and 200 krad of gamma rays, and were checked for visual spoilage for 18 days. The results are summarized in Table 19 of Appendix 1 and presented in Figures 17 and 18. It is apparent that the earlier the berries were inoculated before irradiation, the less protection against spoilage was achieved. The best protection against spoilage was at the zero (0) time of inoculation, as shown in Figure 18 in which the 50% spoilage was plotted vs. days elapsed between inoculation and irradiation.

9. General discussion on food irradiation

Irradiation is a novel method of food preservation. It involves the use of gamma rays, cathode rays and X-rays. It differs from heat preservation procedures in that the temperature of the food does not increase appreciably. Radiation preservation of food might have some application in sterilization (pork), pasteurization (fruits, vegetables), disinfection (water), disinfestation (cereal) and sprout inhibition (potatoes).

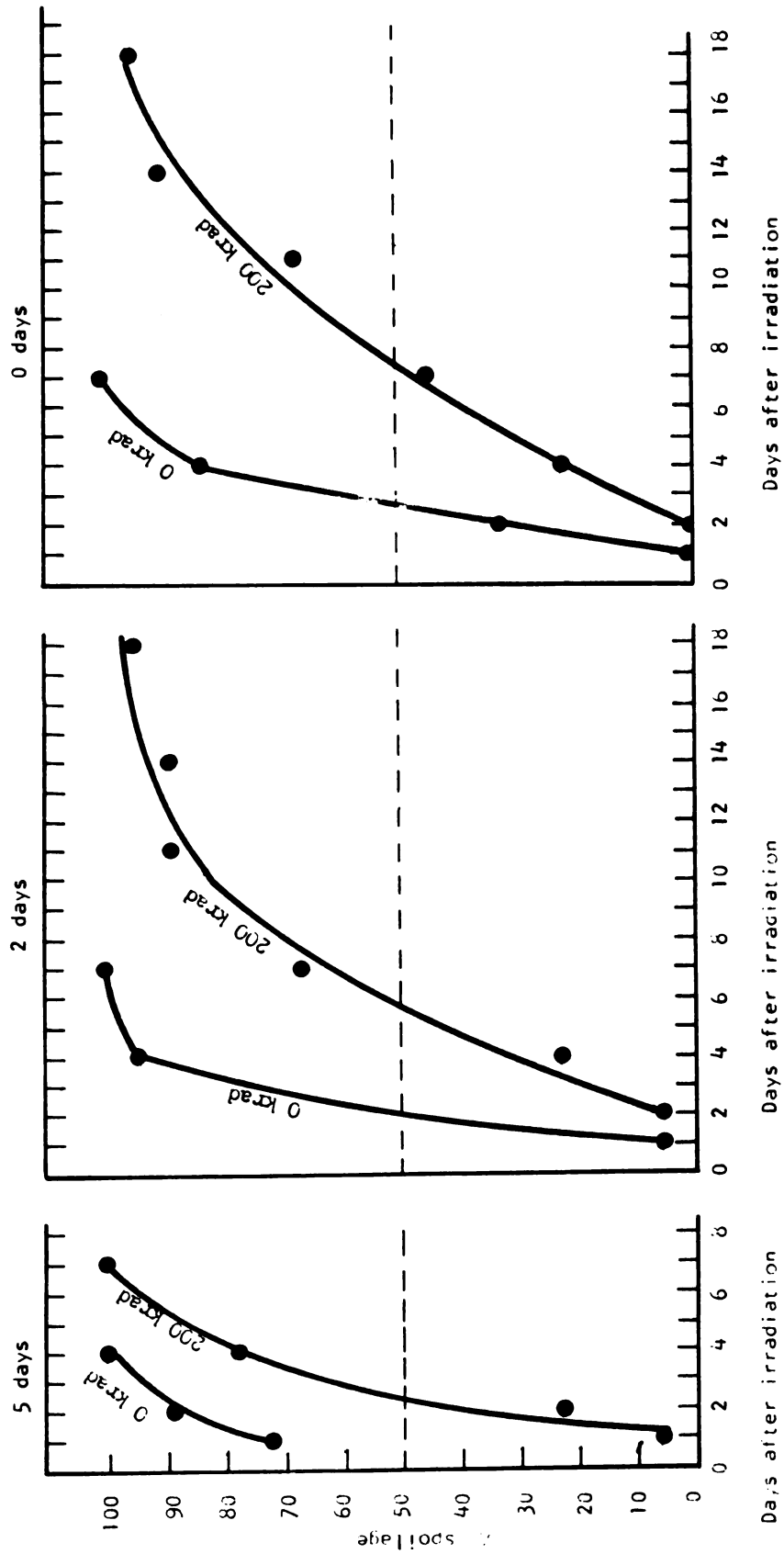
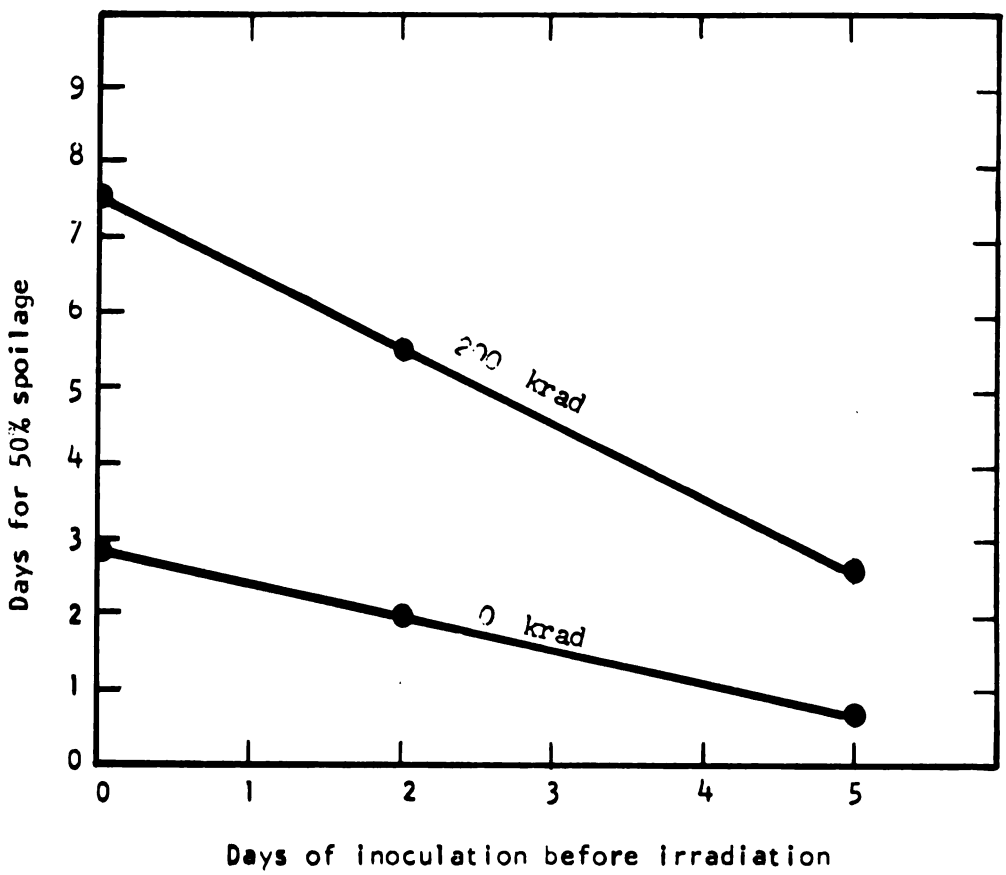


Figure 17. Effect of gamma rays on the % spoilage of strawberries inoculated with Botrytis cinerea 5, 2, and 0 days before irradiation.

Figure 18. Number of days needed for 50% spoilage vs. the number of days of inoculation before irradiation.



Irradiation extends the shelf life of fresh fruits over regular refrigeration methods. The fruit can reach its destination in a better condition while shipped long distances.

It is predicted that irradiated fresh strawberries are going to be the first fruit to be distributed for public use.

SUMMARY

Survival curves for five species of food decay fungi were determined by exposing fungi spore suspensions in demineralized water to gamma rays.

Aspergillus flavus, Aspergillus niger and Penicillium sp. have almost the same sensitivity toward gamma rays, with D value in the range of 30 to 35 krad, whereas Botrytis cinerea has a D value of approximately 55 krad and Rhizopus stolonifer, the most resistant fungus studied, has D value of approximately 100 krad. Aspergillus flavus and Penicilium sp. spores irradiated in citrate buffer at pH 3, 4, 5, 6 and 7 showed almost no change in their radiosensitivity with pH. On the other hand, Botrytis cinerea spores showed a distinct decrease in their radioresistance at higher pH levels (6 and 7).

Penicillium sp. spores irradiated in sucrose and dextrose solutions (0 to 20%) showed no significant change in their radioresistance. Botrytis cinerea spores displayed a higher radioresistance when they were irradiated in 5 to 20 percent sucrose solution than in water. Aspergillus flavus spores showed an increase in their radioresistance to destruction by gamma rays when the dextrose concentration of the

suspension media increased from 0 to 40%.

Dry spores of Aspergillus flavus showed a considerable increase in their radioresistance when compared with spores irradiated in water.

The effect of gamma and cathode rays on Aspergillus flavus spores was compared. It was found that when the irradiation is conducted in demineralized water, there is almost no difference between the two kinds of radiation.

The age of Penicillium sp. spores was found to have a very significant effect on their radioresistance. After the age of 20 days, the older the spores, the higher the sensitivity to gamma rays.

Strawberries inoculated with a Botrytis cinerea spore suspension at 5, 2 and 0 days before irradiation with 200 krad of gamma rays, showed that the longer the period between inoculation and irradiation, the less the protection by irradiation against spoilage. The best protection was achieved when irradiation immediately followed the inoculation.

APPENDIX 1

Table 1: Effect of gamma rays on the viability of Aspergillus

Dose	0 krad		50 krad		100
Exp. No.	Cells per ml.	% survival	Cells per ml.	% survival	Cells per ml.
1	4.70×10^6	1.00×10^2	7.80×10^5	1.66×10^1	1.33×10^4
2	8.00×10^6	1.00×10^2	1.17×10^6	1.46×10^1	3.36×10^4
3	8.22×10^6	1.00×10^2	1.09×10^6	1.33×10^1	2.44×10^4
4	1.05×10^7	1.00×10^2	6.90×10^5	6.57×10^0	2.42×10^4

Table 2: Effect of cathode rays on the viability of Aspergillus

Dose	0 krad		50 krad		100
Exp. No.	Cells per ml.	% survival	Cells per ml.	% survival	Cells per ml.
1	1.98×10^6	1.00×10^2	4.40×10^4	2.22×10^0	1.03×10^3
2	4.86×10^6	1.00×10^2	5.70×10^4	1.18×10^0	1.38×10^4
3	6.38×10^6	1.00×10^2	1.62×10^5	2.54×10^0	1.97×10^4
4	6.98×10^6	1.00×10^2	3.12×10^5	4.46×10^0	6.94×10^3
5	1.12×10^7	1.00×10^2	5.95×10^5	5.32×10^0	2.03×10^4
6	1.23×10^7	1.00×10^2	1.75×10^5	1.42×10^0	6.16×10^4

flavus spores irradiated in demineralized water

krad	150 krad		200 krad	
% survival	Cells per ml.	% survival	Cells per ml.	% survival
2.83×10^{-1}	6.60×10^{2}	1.40×10^{-2}	7.50×10^{0}	1.60×10^{-4}
4.20×10^{3}	2.30×10^{3}	2.88×10^{-2}	2.85×10^{1}	3.56×10^{-4}
2.97×10^{-1}	8.60×10^{2}	1.05×10^{-2}	1.80×10^{1}	2.19×10^{-4}
2.30×10^{-1}	1.73×10^{3}	1.75×10^{-2}	-	-

flavus spores irradiated in demineralized water

krad	150 krad		200 krad	
% survival	Cells per ml.	% survival	Cells per ml.	% survival
5.20×10^{-2}	2.93×10^{2}	1.48×10^{-2}	2.10×10^{1}	1.06×10^{-3}
2.83×10^{-1}	-	-	5.13×10^{1}	1.06×10^{-3}
3.09×10^{-1}	-	-	1.14×10^{2}	1.79×10^{-3}
9.94×10^{-2}	1.94×10^{3}	2.78×10^{-2}	4.45×10^{1}	6.38×10^{-4}
1.81×10^{-1}	2.86×10^{3}	2.55×10^{-2}	1.20×10^{2}	1.07×10^{-3}
5.00×10^{-1}	-	-	1.85×10^{2}	1.50×10^{-3}

Table 3: Effect of gamma rays on the viability of Aspergillus

Dose	0 krad		50 krad	
pH	Cells per ml.	% survival	Cells per ml.	% survival
3	5.13×10^6	1.00×10^2	7.00×10^5	1.37×10^1
4	5.10×10^6	1.00×10^2	5.55×10^5	1.10×10^1
5	4.80×10^6	1.00×10^2	8.60×10^5	1.79×10^1
6	4.90×10^6	1.00×10^2	9.05×10^5	1.85×10^1
7	4.70×10^6	1.00×10^2	6.30×10^5	1.34×10^1

Table 4: Effect of cathode rays on the viability of Aspergillus

Dose	0 krad		50 krad	
pH	Cells per ml.	% survival	Cells per ml.	% survival
3	1.23×10^7	1.00×10^2	2.25×10^5	1.83×10^0
5*	8.19×10^6	1.00×10^2	2.34×10^5	2.86×10^0
7	9.55×10^6	1.00×10^2	6.28×10^5	6.55×10^0

*The data given are the mean of two separated experiments.

flavus spores irradiated in 0.075M citrate buffer of various pH

100 krad		150 krad	
Cells per ml.	% survival	Cells per ml.	% survival
4.00×10^{4}	7.80×10^{-1}	4.20×10^{2}	8.20×10^{-3}
2.70×10^{4}	5.30×10^{-1}	3.00×10^{2}	5.90×10^{-3}
3.90×10^{4}	8.10×10^{-1}	6.50×10^{2}	1.35×10^{-2}
3.60×10^{4}	7.35×10^{-1}	3.30×10^{2}	6.70×10^{-3}
2.90×10^{4}	5.30×10^{-1}	5.20×10^{2}	1.10×10^{-2}

flavus spores irradiated in 0.075M citrate buffer of various pH

100 krad		150 krad	
Cells per ml.	% survival	Cells per ml.	% survival
9.30×10^{3}	7.55×10^{-2}	3.11×10^{3}	2.52×10^{-2}
3.96×10^{4}	4.84×10^{-1}	1.41×10^{3}	1.58×10^{-2}
9.50×10^{3}	9.95×10^{-2}	8.00×10^{3}	8.36×10^{-2}

Table 5: Effect of gamma rays on the viability of Aspergillus

Dose		0 krad		50 krad		100 krad	
Exp. no.	Dextrose conc. %	Cells per ml.	% survival	Cells per ml.	% survival	Cells per ml.	% survival
1	0	8.00×10^{6}	1.00×10^{2}	1.17×10^{6}	1.46×10^{1}	3.36×10^{4}	4.20×10^{-1}
	10	7.80×10^{6}	1.00×10^{2}	1.49×10^{6}	1.91×10^{1}	8.66×10^{4}	1.11×10^{0}
	20	6.98×10^{6}	1.00×10^{2}	1.50×10^{6}	2.14×10^{1}	7.40×10^{4}	1.06×10^{0}
	30	7.18×10^{6}	1.00×10^{2}	1.76×10^{6}	2.47×10^{1}	7.06×10^{4}	9.86×10^{-1}
	40	8.14×10^{6}	1.00×10^{2}	1.87×10^{6}	2.30×10^{1}	9.32×10^{4}	1.15×10^{0}
2	0	8.22×10^{6}	1.00×10^{2}	1.09×10^{6}	1.33×10^{1}	2.44×10^{4}	2.97×10^{-1}
	10	6.92×10^{6}	1.00×10^{2}	1.62×10^{6}	2.34×10^{1}	8.92×10^{4}	1.29×10^{0}
	20	6.62×10^{6}	1.00×10^{2}	1.79×10^{6}	2.71×10^{1}	8.38×10^{4}	1.27×10^{0}
	30	5.26×10^{6}	1.00×10^{2}	1.73×10^{6}	3.29×10^{1}	5.98×10^{4}	1.14×10^{0}
	40	5.52×10^{6}	1.00×10^{2}	2.79×10^{6}	5.05×10^{1}	1.04×10^{5}	1.88×10^{0}

flavus suspended in dextrose solutions

150 krad		200 krad		250 krad	
Cells per ml.	% survival	Cells per ml.	% survival	Cells per ml.	% survival
2.30×10^{3}	2.88×10^{-2}	2.85×10^{1}	3.56×10^{-4}	5.00×10^{-1}	6.25×10^{-6}
5.00×10^{3}	6.41×10^{-2}	1.24×10^{2}	1.58×10^{-3}	-	-
3.76×10^{3}	5.37×10^{-2}	5.55×10^{1}	7.94×10^{-4}	3.50×10^{0}	5.00×10^{-5}
4.17×10^{3}	5.80×10^{-2}	6.90×10^{1}	9.59×10^{-4}	-	-
5.70×10^{3}	7.01×10^{-2}	2.96×10^{2}	3.64×10^{-3}	1.60×10^{1}	1.97×10^{-4}
8.60×10^{2}	1.05×10^{-2}	1.80×10^{1}	2.19×10^{-4}	5.00×10^{-1}	6.08×10^{-6}
4.20×10^{3}	6.06×10^{-2}	1.22×10^{2}	1.77×10^{-3}	-	-
2.50×10^{3}	3.78×10^{-2}	8.04×10^{1}	1.21×10^{-3}		
4.54×10^{3}	8.64×10^{-2}	6.28×10^{1}	1.19×10^{-3}	-	-
6.12×10^{3}	1.11×10^{-1}	8.15×10^{1}	1.47×10^{-3}	1.3×10^{1}	2.35×10^{-4}

Table 6: Effect of cathode rans on the viability of

Dose		0 krad		50 krad	
Exp. no.	Dextrose conc.	Cells per ml.	% survival	Cells per ml.	% survival
1	0	4.83×10^{6}	1.00×10^{2}	5.70×10^{4}	1.18×10^{0}
	10	7.65×10^{6}	1.00×10^{2}	1.56×10^{5}	2.03×10^{0}
	20	7.17×10^{6}	1.00×10^{2}	2.23×10^{5}	3.11×10^{0}
	30	7.82×10^{6}	1.00×10^{2}	9.53×10^{5}	1.22×10^{1}
	40	1.02×10^{7}	1.00×10^{2}	2.39×10^{6}	2.34×10^{1}
2	0	1.23×10^{7}	1.00×10^{2}	1.75×10^{5}	1.42×10^{0}
	10	8.38×10^{6}	1.00×10^{2}	2.31×10^{5}	2.76×10^{0}
	20	1.15×10^{7}	1.00×10^{2}	4.01×10^{5}	3.49×10^{0}
	30	1.08×10^{7}	1.00×10^{2}	7.72×10^{5}	7.15×10^{0}
	40	1.18×10^{7}	1.00×10^{2}	1.80×10^{6}	1.52×10^{1}

Aspergillus flavus suspended in dextrose solutions

100 krad		200 krad	
Cells per ml.	% survival	Cells per ml.	% survival
1.38×10^{4}	2.86×10^{-1}	5.13×10^{1}	1.06×10^{-3}
8.98×10^{3}	1.17×10^{-1}	4.20×10^{2}	5.50×10^{-3}
5.35×10^{4}	7.46×10^{-1}	3.85×10^{2}	5.36×10^{-3}
2.24×10^{5}	2.87×10^{0}	3.58×10^{3}	4.57×10^{-2}
1.54×10^{5}	1.51×10^{0}	7.46×10^{3}	7.33×10^{-2}
6.16×10^{4}	5.00×10^{-1}	1.85×10^{2}	1.50×10^{-3}
8.78×10^{4}	1.04×10^{0}	1.64×10^{2}	1.96×10^{-3}
1.19×10^{5}	1.03×10^{0}	1.36×10^{2}	1.18×10^{-3}
2.38×10^{5}	2.20×10^{0}	2.12×10^{3}	1.96×10^{-2}
6.34×10^{5}	5.37×10^{0}	1.82×10^{4}	1.54×10^{-1}

Table 7: Effect of gamma rays on the viability of dry and

Dose krad	Wet spores		Dry spores	
	Per ml.	% survival	Per ml.	% survival
0	8.00×10^{6}	1.00×10^{2}	6.24×10^{6}	1.00×10^{2}
50	1.17×10^{6}	1.46×10^{1}	2.43×10^{6}	3.89×10^{1}
100	3.36×10^{4}	4.20×10^{-1}	8.62×10^{5}	1.38×10^{1}
150	2.30×10^{3}	2.88×10^{-2}	1.40×10^{5}	2.24×10^{0}
200	2.85×10^{1}	3.56×10^{-4}	8.74×10^{3}	1.40×10^{-1}
250	---	---	1.04×10^{3}	1.66×10^{-2}
300	---	---	9.00×10^{1}	1.44×10^{-3}

Table 8: Effect of cathode rays on the viability of dry and

Dose krad	Wet spores		Dry spores	
	Per ml.	% survival	Per ml.	% survival
0	1.98×10^{6}	1.00×10^{2}	2.52×10^{6}	1.00×10^{2}
50	4.40×10^{4}	2.22×10^{0}	6.94×10^{5}	2.75×10^{1}
100	1.03×10^{3}	5.22×10^{-2}	6.60×10^{5}	2.62×10^{1}
150	2.93×10^{2}	1.48×10^{-2}	1.35×10^{5}	5.36×10^{0}
200	2.10×10^{1}	1.06×10^{-3}	2.35×10^{4}	9.31×10^{-1}
250	---	---	2.45×10^{3}	9.72×10^{-2}
300	---	---	2.50×10^{3}	9.90×10^{-2}

wet spores of Aspergillus flavus

Wet spores		Dry spores	
Per ml.	% survival	Per ml.	% survival
8.22×10^{2}	1.00×10^{2}	6.84×10^{6}	1.00×10^{2}
1.09×10^{6}	1.33×10^{1}	2.60×10^{6}	3.83×10^{1}
2.44×10^{4}	2.97×10^{-1}	7.84×10^{5}	1.09×10^{1}
8.60×10^{2}	1.05×10^{-2}	1.30×10^{5}	1.90×10^{0}
1.80×10^{1}	2.19×10^{-4}	2.04×10^{3}	2.98×10^{-2}
---	---	6.36×10^{2}	9.31×10^{-3}
---	---	1.87×10^{1}	2.73×10^{-4}

and wet spores of Aspergillus flavus

Wet spores		Dry spores	
Per ml.	% survival	Per ml.	% survival
6.98×10^{6}	1.00×10^{2}	4.50×10^{6}	1.00×10^{2}
3.12×10^{5}	4.46×10^{0}	1.42×10^{6}	3.06×10^{1}
6.94×10^{3}	9.94×10^{-2}	6.52×10^{5}	1.45×10^{1}
1.94×10^{3}	2.77×10^{-2}	7.46×10^{5}	1.66×10^{1}
4.45×10^{1}	6.37×10^{-4}	3.61×10^{4}	8.00×10^{-1}
---	---	6.48×10^{3}	1.44×10^{-1}
---	---	3.75×10^{2}	8.32×10^{-3}

Table 9: Effect of gamma rays on the survival of Aspergillus niger spores suspended in demineralized water containing 0.01% Triton X-100

Dose in krad	Cells per ml.	% survival
0	4.16×10^{6}	1.00×10^{2}
50	9.28×10^{5}	2.23×10^{1}
100	3.20×10^{4}	7.67×10^{-1}
150	2.30×10^{1}	5.51×10^{-4}
200	0	---

Table 10: Effect of gamma rays on the viability of Botrytis Triton X-100

Dose	0 krad		100 krad		150 krad	
Exp. no.	Cells per ml.	% survival	Cells per ml.	% survival	Cells per ml.	% survival
1	2.29×10^{5}	1.00×10^{2}	1.53×10^{5}	6.67×10^{1}	7.72×10^{4}	3.37×10^{1}
2	7.10×10^{4}	1.00×10^{2}	4.20×10^{4}	5.92×10^{1}	----	
3	1.45×10^{5}	1.00×10^{2}	7.90×10^{4}	5.45×10^{1}	----	
4	1.53×10^{6}	1.00×10^{2}	1.11×10^{6}	7.25×10^{1}	----	
5	1.20×10^{6}	1.00×10^{2}	9.10×10^{5}	7.60×10^{1}	----	

cinerea spores suspended in demineralized water containing 0.01%

200 krad		250 krad		300 krad	
Cells per ml.	% survival	Cells per ml.	% survival	Cells per ml.	% survival
2.48×10^{4}	1.08×10^{1}	3.07×10^{3}	1.34×10^{0}	1.75×10^{2}	7.64×10^{-2}
1.59×10^{4}	2.24×10^{1}	----		2.98×10^{2}	4.20×10^{-1}
1.12×10^{4}	7.74×10^{0}	----		1.00×10^{2}	6.90×10^{-2}
1.20×10^{5}	7.85×10^{0}	----		2.40×10^{3}	1.60×10^{-1}
1.20×10^{3}	1.00×10^{1}	----		6.00×10^{3}	5.00×10^{-1}

Table 11: Effect of gamma rays on the viability of Botrytis levels

Dose	0 krad		100 krad		150 krad	
pH	Cells per ml.	% survival	Cells per ml.	% survival	Cells per ml.	% survival
3	2.00×10^5	1.00×10^2	1.73×10^5	8.65×10^1	1.27×10^5	6.35×10^1
4	2.27×10^5	1.00×10^2	1.67×10^5	7.25×10^1	1.40×10^5	6.17×10^1
5	2.08×10^5	1.00×10^2	1.39×10^5	6.67×10^1	7.86×10^4	3.78×10^1
6	2.02×10^5	1.00×10^2	1.17×10^5	5.80×10^1	6.26×10^4	3.10×10^1
7	2.41×10^5	1.00×10^2	7.40×10^4	3.07×10^1	1.60×10^4	6.65×10^0

Table 12: The effect of gamma rays on the viability of Botrytis

Dose	0 krad		100 krad	
Sucrose conc.	Cells per ml.	% survival	Cells per ml.	% survival
0	7.10×10^4	1.00×10^2	4.20×10^4	5.92×10^1
5	5.00×10^4	1.00×10^2	1.57×10^4	3.14×10^1
10	7.20×10^4	1.00×10^2	3.30×10^4	4.58×10^4
15	8.50×10^4	1.00×10^2	3.80×10^4	4.47×10^1
20	3.55×10^5	1.00×10^2	2.70×10^5	7.60×10^1

cinerea spores irradiated in 0.075M citrate buffer of various pH

200 krad		250 krad		300 krad	
Cells per ml.	% survival	Cells per ml.	% survival	Cells per ml.	% survival
3.47×10^{4}	1.73×10^{1}	2.41×10^{4}	1.21×10^{1}	3.28×10^{3}	1.64×10^{0}
3.26×10^{4}	1.44×10^{1}	2.34×10^{4}	1.04×10^{1}	2.57×10^{3}	1.13×10^{0}
2.38×10^{4}	1.14×10^{1}	7.92×10^{3}	3.80×10^{0}	1.65×10^{3}	7.93×10^{-1}
3.50×10^{3}	1.73×10^{0}	8.50×10^{1}	4.20×10^{-2}	9.00×10^{0}	4.45×10^{-3}
2.00×10^{2}	8.30×10^{-2}	1.00×10^{0}	4.15×10^{-4}	---	---

cinerea spores suspended in sucrose solutions

200 krad		300 krad	
Cells per ml.	% survival	Cells per ml.	% survival
1.59×10^{4}	2.24×10^{1}	2.98×10^{2}	4.20×10^{-1}
4.50×10^{4}	9.00×10^{1}	2.25×10^{3}	4.50×10^{0}
1.80×10^{4}	2.50×10^{1}	1.59×10^{4}	2.21×10^{1}
8.50×10^{4}	1.00×10^{2}	1.77×10^{4}	2.08×10^{1}
-	-	-	-

Table 13: Effect of age of Penicillium sp. spores on their resistance to 50 krad of gamma rays

Dose	0 krad		50 krad	
Age Days	Cells per ml.	% survival	Cells per ml.	% survival
9	2.50×10^6	1.00×10^2	3.90×10^5	1.56×10^1
15	3.90×10^6	1.00×10^2	5.80×10^5	1.49×10^1
19	2.74×10^6	1.00×10^2	4.00×10^5	1.46×10^1
26	1.05×10^7	1.00×10^2	1.20×10^6	1.14×10^1
40	7.70×10^6	1.00×10^2	2.91×10^5	3.78×10^0
60	3.70×10	1.00×10	No growth	

Table 14: The effect of gamma rays on the viability of 0.01% Triton X-100

Dose	0 krad		50 krad		100
Exp. no.	Cells per ml.	% survival	Cells per ml.	% survival	Cells per ml.
1	2.50×10^6	1.00×10^2	3.50×10^5	1.40×10^1	2.92×10^3
2	1.45×10^7	1.00×10^2	1.74×10^6	1.20×10^1	3.33×10^4
3	3.90×10^6	1.00×10^2	5.80×10^5	1.49×10^1	9.10×10^3
4	1.05×10^7	1.00×10^2	1.20×10^6	1.14×10^1	4.10×10^3

Penicillium sp. spores suspended in demineralized water containing

krad	150 krad		200 krad	
% survival	Cells per ml.	% survival	Cells per ml.	% survival
1.17×10^{-1}	1.19×10^{2}	4.75×10^{-3}	5.00×10^{0}	2.00×10^{-4}
2.30×10^{-1}	-	-	6.40×10^{1}	4.40×10^{-4}
2.33×10^{-1}	6.40×10^{1}	1.64×10^{-3}	3.90×10^{0}	1.00×10^{-4}
3.90×10^{-2}	-	-	-	-

Table 15: Effect of gamma rays on the viability of Penicillium

Dose		0 krad		50 krad	
Exp. no.	pH	Cells per ml.	% survival	Cells per ml.	% survival
1	3	3.40×10^6	1.00×10^2	5.55×10^5	1.63×10^1
	4	4.20×10^6	1.00×10^2	4.00×10^5	9.50×10^0
	5	3.97×10^6	1.00×10^2	5.55×10^5	1.40×10^1
	6	3.53×10^6	1.00×10^2	5.95×10^5	1.69×10^1
	7	3.63×10^6	1.00×10^2	5.13×10^5	1.41×10^1
2	3	1.05×10^7	1.00×10^2	9.00×10^5	8.57×10^0
	4	1.16×10^7	1.00×10^2	9.50×10^5	8.20×10^0
	5	1.16×10^7	1.00×10^2	1.20×10^6	1.02×10^1
	6	1.26×10^7	1.00×10^2	9.90×10^5	7.85×10^0
3	3	1.27×10^7	1.00×10^2	2.09×10^6	1.65×10^1
	4	1.22×10^7	1.00×10^2	2.01×10^6	1.65×10^1
	5	1.56×10^7	1.00×10^2	2.00×10^6	1.28×10^1
	6	1.28×10^7	1.00×10^2	1.43×10^6	1.12×10^1
4	3	1.53×10^7	1.00×10^2	1.12×10^6	7.32×10^0
	4	1.34×10^7	1.00×10^2	8.60×10^5	6.40×10^0
	5	1.34×10^7	1.00×10^2	1.00×10^6	7.50×10^0
	6	1.44×10^7	1.00×10^2	8.80×10^5	6.10×10^0

sp. spores irradiated in 0.075M citrate buffer of various pH

100 krad		150 krad	
Cells per ml.	% survival	Cells per ml.	% survival
6.80×10^{3}	2.00×10^{-1}	3.63×10^{1}	1.07×10^{-3}
9.38×10^{3}	2.23×10^{-1}	6.69×10^{1}	1.59×10^{-3}
5.49×10^{3}	1.39×10^{-1}	5.43×10^{1}	1.37×10^{-3}
5.50×10^{3}	1.56×10^{-1}	3.66×10^{1}	1.04×10^{-3}
5.16×10^{3}	1.42×10^{-1}	3.66×10^{1}	1.01×10^{-3}
1.12×10^{4}	1.07×10^{-1}	-	-
1.30×10^{4}	1.12×10^{-1}	-	-
6.90×10^{3}	5.95×10^{-2}	-	-
3.20×10^{3}	2.54×10^{-2}	-	-
1.71×10^{4}	1.35×10^{-1}	-	-
1.60×10^{4}	1.31×10^{-1}	-	-
3.07×10^{4}	1.97×10^{-1}	-	-
1.05×10^{4}	8.22×10^{-2}	-	-
6.10×10^{3}	3.98×10^{-2}	-	-
9.50×10^{3}	7.10×10^{-2}	-	-
8.40×10^{3}	6.30×10^{-2}	-	-
4.60×10^{3}	3.20×10^{-2}	-	-

Table 16: The effect of gamma rays on the viability of

Dose		0 krad		50 krad	
Exp. no.	% sucrose	Cells per ml.	% survival	Cells per ml.	% survival
1 Spores age 19 days	0	3.01×10^6	1.00×10^2	4.00×10^5	1.33×10^1
	5	6.60×10^6	1.00×10^2	7.30×10^5	1.11×10^1
	10	5.20×10^6	1.00×10^2	7.50×10^5	1.44×10^1
	15	5.10×10^6	1.00×10^2	7.00×10^5	1.37×10^1
2 Spores age 40 days	0	7.70×10^6	1.00×10^2	2.91×10^5	3.78×10^0
	10	8.00×10^6	1.00×10^2	1.25×10^5	1.56×10^0
	20	6.36×10^6	1.00×10^2	1.14×10^5	1.79×10^2

Table 17: Effect of gamma rays on the viability of

Dose	0 krad		50 krad	
% sucrose	Cells per ml.	% survival	Cells per ml.	% survival
0	7.70×10^6	1.00×10^2	2.90×10^5	3.78×10^0
10	8.00×10^6	1.00×10^2	1.30×10^5	1.63×10^0
20	6.40×10^6	1.00×10^2	1.10×10^5	1.72×10^0

Penicillium sp. spores suspended in sucrose solutions

100 krad		200 krad	
Cells per ml.	% survival	Cells per ml.	% survival
1.49×10^{4}	4.95×10^{-1}	6.00×10^{2}	1.99×10^{-2}
1.88×10^{4}	2.85×10^{-1}	3.70×10^{2}	5.60×10^{-3}
2.16×10^{4}	4.15×10^{-1}	1.72×10^{2}	3.30×10^{-3}
1.43×10^{4}	2.80×10^{-1}	2.90×10^{2}	5.70×10^{-3}
1.10×10^{3}	1.43×10^{-2}	No growth	
2.90×10^{2}	3.62×10^{-3}	-	-
2.05×10^{2}	3.12×10^{-3}	-	-

Penicillium sp. spores suspended in dextrose solutions

100 krad		150 krad	
Cells per ml.	% survival	Cells per ml.	% survival
1.10×10^{3}	1.43×10^{-2}	No growth	
2.90×10^{2}	3.62×10^{-3}	-	-
2.10×10^{2}	3.18×10^{-3}	-	-

Table 18: Effect of gamma rays on Rhizopus stolonifer spores suspended in demineralized water containing 0.01% Triton X-100

Exp. no.	Dose in krad	Cells per ml.	% survival
1	0	6.76×10^5	1.00×10^2
	100	5.66×10^5	8.36×10^1
	150	4.48×10^5	6.64×10^1
	200	4.01×10^5	5.91×10^1
	250	1.71×10^5	2.53×10^1
	300	3.47×10^4	5.13×10^0
2	0	7.30×10^5	1.00×10^2
	100	4.93×10^5	6.73×10^1
	150	4.60×10^5	6.30×10^1
	200	3.20×10^5	4.38×10^1
	250	6.20×10^4	8.50×10^0

Table 19: Effect of gamma rays on the spoilage of strawberries before irradiation

					Days of inoculation	
	5 days				2	
Dose	0 krad		200 krad		0 krad	
Days after irra-diation	No. spoiled berries	% spoiled berries	No. spoiled berries	% spoiled berries	No. spoiled berries	% spoiled berries
1	13	72.2	1	5.6	0	0
2	16	89.9	4	22.2	9	50.0
4	18	100.0	14	77.8	17	94.4
7	all spoiled		18	100.0	18	100.0
11	-	-	all spoiled		16	88.9
14	-	-	-	-	16	88.9
18	-	-	-	-	17	94.4

inoculated with Botrytis cinerea spores at different times

before irradiation					
days		0 days			
200 krad		0 krad		200 krad	
No. spoiled berries	% spoiled berries	No. spoiled berries	% spoiled berries	No. spoiled berries	% spoiled berries
1	5.6	0	0	0	0
1	5.6	6	33.3	0	0
4	22.2	15	83.5	4	22.2
12	66.7	18	100.0	8	44.4
16	88.9	all spoiled		12	66.7
16	88.9	-	-	16	88.9
17	94.4	-	-	17	94.4

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