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BIOPHYSICS AND MEDICAL PHYSICS

The Effect of Ionizing Radiation on the Phytopathogen Growth of *R. solani*

U. A. Bliznyuk[1*], V. A. Leontiev[1], A. A. Malyuga[2], V. V. Hankin[3], A. P. Chernyaev[1,3], N. S. Chulikova[2], and D. S. Yurov[3]**

[1] *Department of Physics, Moscow State University, Moscow, 119991 Russia*
[2] *Siberian Federal Research Center of Agricultural Biotechnology, Russian Academy of Sciences, Novosibirsk oblast, Krasnoobsk, 630501 Russia*
[3] *Skobeltsyn Institute of Nuclear Physics, Moscow State University, Moscow, 119234 Russia*

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Abstract—Growth of the plant pathogenic fungus *Rhizoctonia solani* Kuhn, which is the causative agent of one of the most harmful and widespread potato diseases (black scab), has been experimentally studied. Sclerotia of *Rhizoctonia solani* are irradiated with accelerated electrons with an energy of 1 MeV at various doses in the range from 0.02 to 38 kGy. The results of the study made it possible to conclude that the exposure to electrons in the dose range both inhibited and stimulated the growth of *R. solani* plant pathogenic fungi; the dependence of the growth rate of *R. solani* fungi on the absorbed dose is not linear. Germination of *R. solani* sclerotia was completely suppressed when the absorbed dose was more than 4.5 kGy.

Keywords: electron irradiation, radiation dose, sclerotia of *Rhizoctonia solani* Kuhn, inhibition and stimulation of phytopathogen growth.

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INTRODUCTION

Ensuring of the safety of human life is based not only on compliance with sanitary and hygienic standards in relation to various harmful substances and effects, but also on maintaining the quality of the environment and the sustainable development of natural ecosystems. In various sectors of environmental management (fishing, agriculture, and industry), increasing attention is paid to the development of environmentally sensitive policy. One urgent problem is the development of physical technologies associated with irradiation of agricultural products with ionizing radiation [1]. Radiation technologies have proven to be cost effective and safe methods of processing a wide range of food products for the purpose of disinfectation, sterilization, and extension of shelf life.

Scientists are investigating the effect of ionizing radiation on various microbiological, biochemical, and organoleptic characteristics of food [2–7]. With regard to grain crops, the viability of plant feeders and the quality of grain products after their radiation treatment are being investigated [8]. The possibility of presowing irradiation of seeds in order to stimulate the growth of seedlings and reduce their fungosity is being studied [8].

The virulence and aggressiveness of pathogens can both decrease and increase under ionizing radiation. Thus, irradiation with gamma rays of urediospores of stem rust *Puccinia graminis Pers. f. sp. tritici Erikss. et Henn.* (race 11) with dose of 15–50 Gy increases their germination and virulence; there is no strict dose-dependent sporulation of the pathogen [9].

Agricultural crops, namely potatoes, are affected by a wide range of fungal, viral, and bacterial diseases. One of the most common and harmful diseases is Rhizoctonia (black scab), whose causative agent is the plant pathogenic fungus *Rhizoctonia solani* Kuhn. The annual crop losses due to black scab in the Russian Federation reach 50%, while the global crop losses due to black scab are 7–36% [10–15]. This is because there is no seed multiplication system in the Russian Federation and Russian agricultural technologies for growing potatoes are underdeveloped. Consequently, the degree of infection of potato

*E-mail: uabliznyuk@gmail.com
**E-mail: vleon-98@yandex.ru

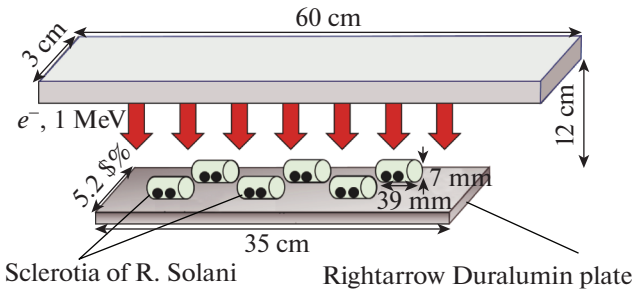

Fig. 1. Irradiation of *R. solani* sclerotia.

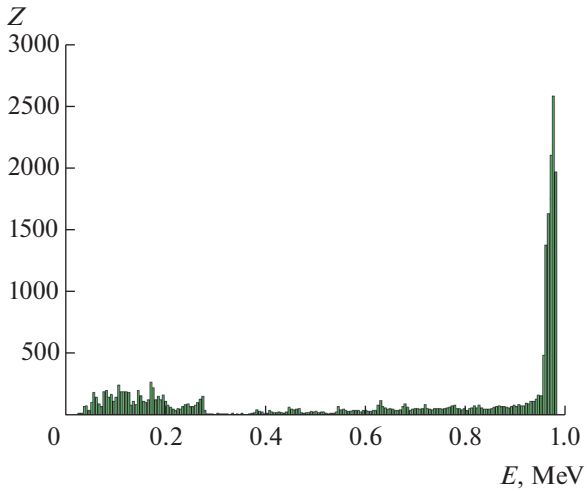

Fig. 2. The spectrum of the electron beam at the yield of the UELR-1-25-T-001 accelerator.

tubers with Rhizoctonia is at least 20%, and 25–50% of the surface of potato tubers is covered with sclerotia of *R. solani* [10].

The purpose of this work was to study the growth of the plant pathogenic fungus *Rhizoctonia solani* Kuhn grown from sclerotia irradiated with different doses of electron radiation.

EXPERIMENTAL

Irradiation of Sclerotia

The subject of this research was sclerotia of *Rhizoctonia solani* Kuhn. The sclerotia were exposed to electron radiation, whose source was a UELR-1-25-T-001 continuous electron accelerator with an energy of 1 MeV and maximum beam power of 25 kW [16]. Spherical sclerotia with a mean diameter of (3 ± 1) mm in two pieces were placed in sterile Eppendorf tubes with a volume of 2 mL. The samples were placed on a duralumin plate with size of 35 cm × 5.2 cm in six pieces (Fig. 1). The distance from the electron beam yield to the irradiated samples was 12 cm. The samples were irradiated with 12 different doses. During the irradiation, the temperature in the room was 18°C; the control samples were in the same conditions as the samples exposed to electron radiation.

The radiation doses absorbed by the sclerotia of *R. solani* were determined using computer simulations. The GEANT 4 program code based on the Monte Carlo method was used. The simulation was carried out taking the geometric parameters of the samples and the technical characteristics of the accelerator such as the spectrum of electrons obtained from it into account (Fig. 2).

The values of the charge absorbed by the plate were measured in each session of irradiation; the inaccuracy of charge measurements did not exceed 3%. The irradiation time was also recorded. The mean values of charge, beam current, irradiation times, and calculated values of doses absorbed by the samples are presented in Table 1.

The test tubes were modeled with polypropylene cylinders 39 mm long with an inner diameter of 7 mm; sclerotia of *R. solani* were modeled with water phantoms of appropriate sizes. The number of electrons in model calculations required to achieve statistical significance is $Q_{\text{model}} = 10^8$ units. The dose absorbed by the phantoms was determined by the formula $D_{\text{model}} = \frac{\Delta E_{\text{model}}}{M_{\text{model}}}$, where ΔE_{model} is the energy absorbed by the water phantom and M_{model} is the mass of the phantom. The absorbed dose in the samples is proportional to the value of the charge Q_{exp} absorbed by the plate during the irradiation. Thus, the value of the absorbed dose in the sample was calculated by the formula $D_{\text{phantom}} = \frac{Q_{\text{exp}}}{Q_{\text{model}}} \times D_{\text{model}}$, where D_{model} is the value of the absorbed dose in the water phantom corresponding to the calibration value of the charge Q_{model} absorbed by the plate and Q_{exp} is the value of the charge recorded during the irradiation.

To estimate the uniformity of irradiation of the sclerotia by electrons with energy of 1 MeV, a water sphere 3 mm in diameter was divided into layers 0.1 mm thick in the direction perpendicular to the direction of the electron beam, as shown in Fig. 3. Then, the dose distribution along the radius of the sphere in the plane perpendicular to the direction of the electron beam was calculated for each layer. Figure 4 shows the dose distributions calculated in arbitrary units over the phantom radius for layers located at different depths. The ratio of the maximum value of the dose $D_{\max}$ to the minimum value of $D_{\min}$ in different layers varied within 1.2–1.6.

Table 1. The irradiation parameters of the sclerotia

Sample no.	Irradiation time, s	Electron beam current, μA	Charge of plate, nC	Absorbed dose, kGy
1	7 ± 1	0.1 ± 0.002	978 ± 29	0.02 ± 0.006
2	26 ± 1	0.1 ± 0.002	2120 ± 64	0.04 ± 0.01
3	58 ± 1	0.1 ± 0.002	4246 ± 127	0.075 ± 0.02
4	128 ± 1	0.1 ± 0.002	$10\,627 \pm 319$	0.15 ± 0.05
5	223 ± 1	0.1 ± 0.002	$21\,256 \pm 638$	0.4 ± 0.01
6	318 ± 1	0.1 ± 0.002	$31\,825 \pm 955$	0.6 ± 0.02
7	426 ± 1	0.1 ± 0.002	$42\,530 \pm 1280$	0.9 ± 0.03
8	56 ± 1	1.7 ± 0.003	$84\,870 \pm 2540$	1.8 ± 0.05
9	134 ± 1	1.7 ± 0.003	$218\,280 \pm 6550$	4.5 ± 0.14
10	231 ± 1	1.9 ± 0.003	$425\,800 \pm 12\,800$	7.5 ± 0.23
11	197 ± 1	4.8 ± 0.007	$852\,000 \pm 25\,000$	15.0 ± 0.45
12	463 ± 1	4.8 ± 0.007	$2\,125\,000 \pm 63\,000$	38.0 ± 1.14

Growth Monitoring of the Plant Pathogenic Fungus R. solani

Irradiated sclerotia were placed on a PDA (potato–dextrose agar) culture medium in Petri dishes. The Petri dishes with inoculations were cultivated in a thermostat at a temperature of 24°C. The growth of the fungal samples was monitored after 24, 48, 72, and 96 hours from the moment of inoculation. The diameter of the colonies was measured using a metal ruler [17, 18].

The degree of inhibition or stimulation of fungal growth was calculated using the Abbott formula for the growth inhibition coefficient T [19]. When stimulating the fungal growth T was negative; when inhibiting the fungal growth T was positive.

The data were statistically processed using the SNEDEKOR software package based on the standard methods of mathematical processing [20].

RESULTS AND DISCUSSION

Table 2 shows the diameters of the colonies of the *R. solani* fungus grown from sclerotia irradiated with different doses that measured at 24, 48, 72, and 96 hours after the irradiation. The growth of the *R. solani* fungus for 4 days after the irradiation treatment was monitored in order to reveal the dependence

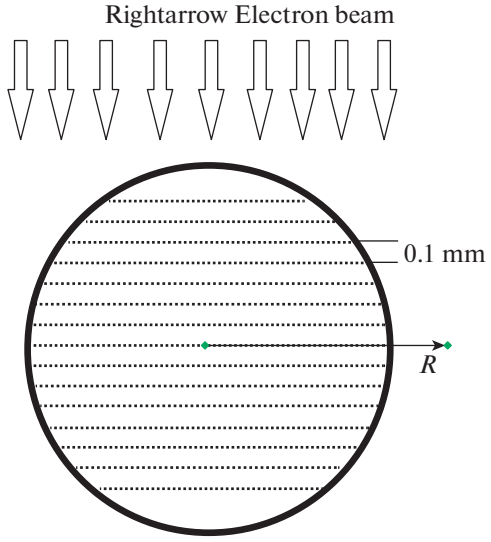

Fig. 3. Division of the water sphere into layers in depth along the direction of the electron beam.

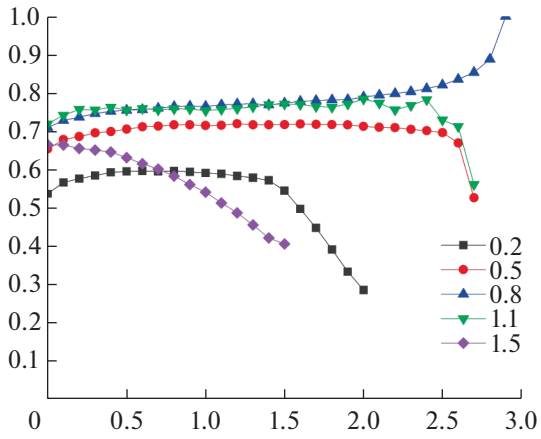

Fig. 4. The dose distribution over the radius of a water sphere with a diameter of 3 mm in different layers of the phantom located at a depth of 0.2 mm, 0.5 mm, 0.8 mm, 1.1 mm, and 1.5 mm from the upper point of the phantom.

Table 2. The growth monitoring data for *R. solani* fungus grown from sclerotia irradiated with different doses

No.	D_{exp}, kGy	Colony diameter, mm ± confidence interval ($\alpha \leqslant 0.05$)				Inhibition coefficient T, % (standard error of mean) ($\alpha \leqslant 0.05$)			
		After 24 h	After 48 h	After 72 h	After 96 h	After 24 h	After 48 h	After 72 h	After 96 h
1	0	16.9 ± 4.2	37.7 ± 6.8	60.8 ± 5.2	81.2 ± 5.3				
2	0.02	16.0 ± 3.8	37.5 ± 5.5	61.3 ± 4.0	87.5 ± 4.4	+5.3 (1.4)	+0.50 (3.8)	−0.80 (1.4)	−7.8 (1.1)
3	0.04	**19.2 ± 3.8**	**45.2 ± 4.6**	61.5 ± 3.8	83.5 ± 3.4	**−13.6** (1.1)	**−19.9** (2.2)	−1.20 (0.4)	−2.8 (0.6)
4	0.075	16.5 ± 1.6	35.8 ± 6.8	57.5 ± 4.2	80.0 ± 1.7	+2.4 (3.7)	+5.0 (2.9)	+5.4 (2.7)	+1.50 (0.1)
5	0.15	**14.0±1.8**	30.8 ± 4.2	52.0 ± 4.0	77.8 ± 3.4	**+17.2** (1.4)	+18.3 (3.6)	+14.5 (1.3)	+4.2 (1.2)
6	0.4	17.5 ± 3.0	38.7 ± 6.1	61.2 ± 3.5	83.7 ± 5.5	−3.5 (1.4)	−2.7 (3.9)	−0.70 (0.2)	−3.1 (1.1)
7	0.6	18.0 ± 1.3	**41.5 ± 2.7**	64.2 ± 2.4	89.2 ± 1.2	−6.5 (2.8)	**−10.1** (2.8)	−5.6 (1.4)	−9.9 (0.6)
8	0.9	16.3 ± 1.0	36.0 ± 3.5	59.0 ± 4.0	81.7 ± 3.8	+3.5 (2.3)	+4.5 (3.6)	+3.0 (1.4)	−0.60 (0)
9	1.8	**6.7 ± 2.2**	**26.7 ± 3.8**	**45.7 ± 4.0**	**74.0 ± 4.6**	**+60.4** (4.4)	**+29.2** (2.0)	**+24.8** (2.6)	**+8.9** (1.1)
10	4.5	0	0	0	0	100	100	100	100
11	7.5	0	0	0	0	100	100	100	100
12	15	0	0	0	0	100	100	100	100
13	38	0	0	0	0	100	100	100	100

of the radiobiological effect on time after the action of ionizing radiation on the phytopathogen.

At 24 h from the moment of inoculation, active growth of the fungal mycelium was observed in samples irradiated with doses of 0.02–0.9 kGy. However, there were no statistically significant differences between these samples and control samples. In this case, the largest diameter of the fungal colony compared with control values was observed after irradiation with a dose of 0.04 kGy (19.2 ± 3.8 mm). Stimulation of the phytopathogen growth is 13.6%, and the smallest diameter of the fungus colony after irradiation with a dose of 0.15 kGy is 14.0 ± 1.8 mm with an inhibition coefficient of 17.2%.

A significant decrease in the diameter of *R. solani* colonies relative to the control values was observed in samples irradiated with a dose of 1.8 kGy; the value of T was +60.4%. In this case, the diameter of the colonies was 52.4–63.5% less than the diameter of the colonies than after irradiation with doses of 0.02–0.9 kGy. In turn, the *R. solani* fungus was not developed from the irradiated sclerotia in the samples irradiated with doses more than 4.5 kGy during the entire observation period.

After 48 h of monitoring, the diameter of fungal colonies in control samples and samples irradiated with doses from 0.02 to 0.9 kGy increased by 2.0–2.3 times relative to the diameter measured at 24 h after the moment of cultivation. The value of this parameter in a sample irradiated with a dose of 1.8 kGy increased by 4.0 times over the second day. The inhibition coefficient for this dose significantly decreased in comparison with the previous value and was 29.2%. For samples irradiated with doses of 0.04 and 0.6 kGy, the inhibition coefficient T had the largest negative values in absolute value and was −19.9% and −10.1%, respectively, i.e., these doses stimulated the development of *R. solani* fungus.

After 72 h and 96 h of monitoring, an irradiation dose of 0.04 kGy no longer stimulated the growth of the *R. solani* fungus. The stimulating effect for a dose of 0.6 kGy was significant: the T values were −5.6% and −9.9%, respectively. For samples irradiated with a dose of 1.8 kGy, the inhibition coefficient was still positive, but its values decreased significantly to 24.8% and 8.9%, respectively.

CONCLUSIONS

It was found that exposure to electrons with an energy of 1 MeV in the dose range from 0.02 to 38 kGy has both an inhibitory effect and a stimulating effect on *R. solani* phytopathogenic fungi, while the dependence of the inhibition coefficient of the fungal growth on the absorbed dose was not linear within 4 days after irradiation.

It was found that electron irradiation with doses of 0.04 and 0.6 kGy stimulates the growth of *R. solani*

colonies, while the diameters of colonies grown from sclerotia irradiated with these doses were 1.1–1.2 times higher than the control values. The diameters of colonies grown from sclerotia irradiated with doses of 0.075, 0.15, and 0.9 kGy practically do not differ from the control values.

Irradiation of sclerotia with a dose of 1.8 kGy led to a significant inhibition in the growth of the fungus during the first 2 days of observation. Germination of *R. solani* sclerotia was completely suppressed when the absorbed dose was more than 4.5 kGy.

The nonmonotonic dependence of the radiobiological effect on the irradiation dose and time after exposure to ionizing radiation is associated with the complexity of the life cycle of fungi as biological systems and their nonlinear response to physical effects [21]. Further studies of the effect of accelerated electrons on *R. solani* sclerotia in the dose range of 1.5–4.5 kGy are of interest. It is also necessary to investigate the irradiation of *R. solani* sclerotia directly on the surface of seed potato tubers.

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