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Effects of X-Ray irradiation on germination and phytosanitary condition of cereal and oilseed crops

Ulyana Bliznyuk[1,2*], *Polina* Borshchegovskaya[1,2], *Anna* Malyuga[3], *Natalia* Chulikova[3], *Victoria* Ipatova[2], *Yana* Zubritskaya[1], and *Maria* Chibisova[1]

[1]Lomonosov Moscow State University, Physics Department, 119991, Moscow, Russia
[2]Skobeltsyn Institute of Nuclear Physics, Lomonosov Moscow State University, 119991, Moscow, Russia
[3]Siberian Federal Scientific Centre of Agro-BioTechnologies of the Russian Academy of Sciences, 630501, Krasnoobsk, Russia

Abstract. The laboratory study explores the impact of X-ray radiation on the germination of flax, rapeseeds and wheat seeds naturally infected with fungi, as well as on their phytosanitary condition after irradiation applying different doses. During the research, it was found that pre-planting treatment of naturally infected wheat, rapeseeds, and flax seeds with X-rays with the doses ranging from 4 Gy to 150 Gy had both stimulating and inhibitory effects on the growth rate of seeds and their phytosanitary status. To ensure a superior quality of the new crop, pre-planting irradiation doses were established, on the one hand, to maximize the seed germination rate of seeds, and on the other hand, to completely inhibit the activity of phytopathogen in seeds. It was established that X-ray radiation with the doses of 30-50 Gy, 16 Gy, 16 Gy and 12 Gy is the most efficient method for pre-planting treatment of Novosibirskaya 29 wheat seeds harvested in 2021 and in 2022, Builder rapeseeds and Severny flax seeds, respectively.

1 Introduction

In the current environmental and political situation coupled with a dramatic increase in the global population, food safety, security, and sustainability have become a major concern for the years ahead [1]. While cultivation of plants resilient to specific climate conditions contributes to food security, treatment of soil and crops at different stages of plant formation to ensure biodiversity and maintain the balance of ecosystems has become a pivotal driver for a sustainable development of agriculture [2].

The quality of crops depends on phytosanitary condition of seed material. Phytopathogens found in seed material can spread across the whole plant population through the soil, water and air [3]. To prevent this, inorganic pesticides, insecticides, fungicides, and fertilizers are commonly used in agriculture [4,5]. Currently, the future of the food industry is largely secured by the extensive use of agrochemical substances. However, it is important to find a

* Corresponding author: uabliznyuk@gmail.com

sensible approach to pre-planting treatment to preserve and improve the quality of crops while ensuring microbial and fungal biodiversity of the local environment.

Being an environmentally friendly and high-performance solution to compare with methods which use chemical compounds, radiation technologies have a great potential in agrobiotechnology for increasing crop yields and quality, inhibiting pathogenic microflora present in seed material as well as inhibiting root crop sprouting [6-11].

Irradiation treatment in doses ranging from 5 to 20 Gy is used for pre-planting treatment of agricultural crops in order to increase the rate of plant germination, thereby reducing the vegetative period and decreasing the risk of fungal and bacterial diseases spread from the soil [12]. Higher doses ranging from 50 Gy to 150 Gy inhibit sprouting and partly inhibit scabs and rot in harvested root crops [13]. A further increase in the irradiation dose up to 500 Gy disinfects root crops for long term storage or export [9].

Three types of irradiation are applied in the food industry as per ISO 14470:2011 [14]: gamma radiation emitted by isotopes ^{60}Co and ^{137}Cs, electron beams with the energy up to 10 MeV, and bremsstrahlung radiation with the energy up to 5 MeV generated by electron accelerators.

There is evidence proving that low-energy X-ray radiation is effective for surface treatment of vegetables, fruit and seeds [15-17], since the dose generated by low-energy photons is primarily absorbed by the surface layers of the treated object, and it does not affect the internal biological structures and tissues [18].

Considering that pre-planting treatment aims at increasing crop germination rate to reduce the risk of infection from the soil and decreasing the rate of infection in seed material to reduce the rate of infection in new crop yield, it is important to determine the irradiation method and the dose range to achieve both objectives.

The study explores the impact of low-energy X-ray radiation on the germination of flax, rape and wheat seeds naturally infected with fungi, as well as on their phytosanitary condition after irradiation applying different doses.

2 Materials and methods

2.1 Research Stages

The research was conducted on Novosibirskaya 29 wheat seeds harvested in 2021 and 2022, Builder rape seeds and Severny flax seeds harvested in 2022 to investigate the influence of X-ray pre-planting irradiation on germination and overall condition of the crops. All the seeds were naturally infected with fungi.

Seed samples of each species were placed into 52 polyethylene 6 cm x 4 cm airtight zipper bags (Figure 1a). The number of wheat seeds, rapeseeds and flax seeds was 30, 200 and 130 in each bag, respectively, to ensure that the weight of each bag filled with seeds was 1 gram.

After irradiation with thirteen doses ranging from 0 Gy to 150 Gy, 30 seeds from each bag were planted and monitored to study the impact of pre-planting irradiation on germination rate coefficient and health of crops (Figure 1b). Fricke solution was used to estimate the dose absorbed by the samples (Figure 1c).

The germination rate and phytosanitary condition of irradiated seeds were compared with those of control samples to find the optimal X-ray irradiation method for pre-planting treatment of cereal and oilseed crops (Figure 1d,e).

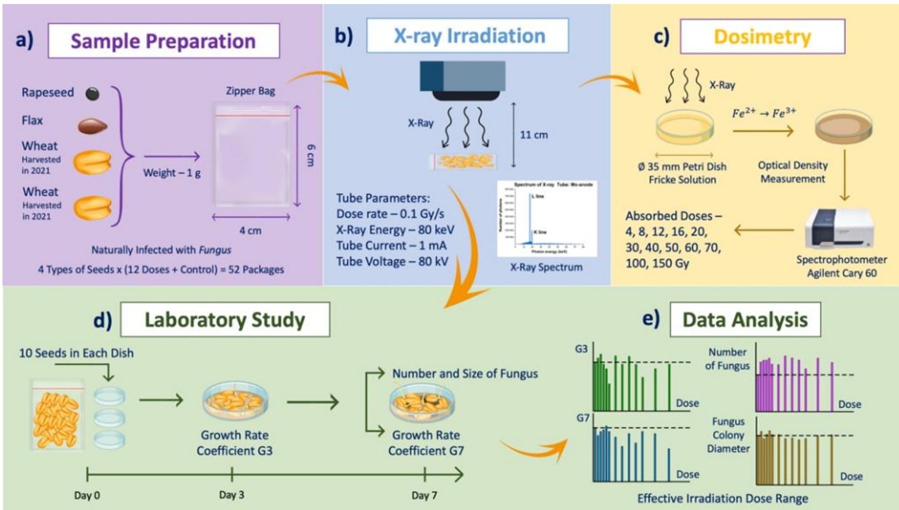

Fig. 1. Stages of the research: a) sample preparation; b) X-ray irradiation method; c) estimation of the dose absorbed by the samples using Fricke solution; d) laboratory studies to determine the growth rate coefficient and phytosanitary conditions of seeds; e) data analysis to determine the effective dose range.

2.2 Object of study

Wheat variety: Novosibirskaya 29; pedigree: PPG-38/1«B» (Mexico) x Novosibirskaya 22 (Russia). Novosibirskaya 29 is a mid-early variety with the vegetative period of up to 70-78 days and resistant to loose smut, powdery mildew and brown rust. The variety is zoned for Western Siberian and Eastern Siberian regions.

Oilseed rape variety: hybrid Builder; pedigree: Brassica napus var. napus. Type-00 hybrid (BAYER CROPSCIENCE AG, Germany). Hybrid Builder is a mid-early variety with the vegetative period of up to 93 days. The variety is zoned for Central and Western Siberian regions.

Oilseed flax variety: Severny; pedigree: K-1994 (Morocco) x breeding line 157 (Russia). Severny is an early maturing variety with vegetative period of up to 80-104 days and highly resistant to Fusarium. The variety is zoned for Western Siberia, Lower Volga, Ural, Eastern Siberia, Volga-Vyatka regions.

2.3 X-Ray irradiation

The samples were irradiated at Burnazyan Federal Biophysical Center using RAD-100 with 1BPV23-100 molybdenum anode X-ray tube. Before being irradiated, the seeds were evenly distributed in one monolayer in each bag to ensure the absorbed dose uniformity and placed at the distance of 11 cm from the beryllium window of X-ray tube (Figure 1b). Each irradiation session involved recording the time of exposure, tube current, and tube voltage permitting a margin of error below 0.1%. The average tube current was 1.0 mA, the average voltage – 80.0 kV. The irradiation was carried out in the room with the ambient temperature of 20 ºC.

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2.4 Dose absorbed by samples

The dose rate absorbed by the samples was determined using the ferrous sulfate Fricke solution (Figure. 1c). To make the samples equal to the dosimeter solution by mass, 1 ml of the solution was poured into ∅35 mm Petri dish to estimate the dose absorbed by the seeds. The irradiation method using Fricke dosimeter fully corresponded to that used for the irradiation of seeds. Further, the optical density of the irradiated solution was measured using a Cary 60 spectrophotometer (Agilent, USA) at wavelength 304 nm to estimate the dose absorbed by the solution [19]. The dose rate absorbed by the samples amounted to (0.10 ± 0.03) Gy/s, allowing for the margin of error of the Fricke solution within 3%.

The seeds were exposed to X-ray radiation at the doses 0, 4, 8, 12, 16, 20, 30, 40, 50, 60, 70, 100, and 150 Gy.

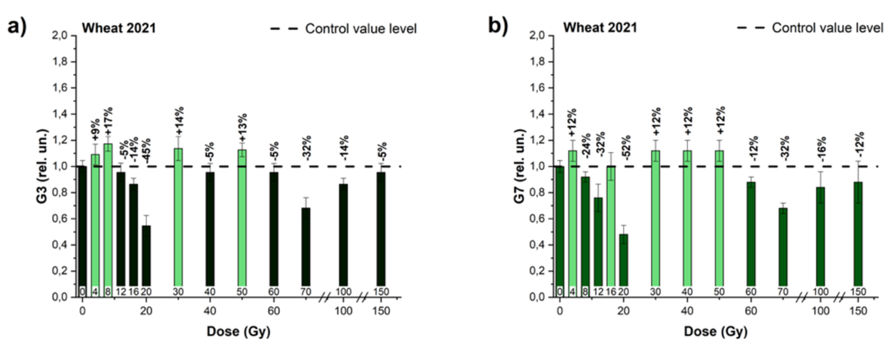
2.5 Laboratory study

After irradiation 30 seeds were placed in groups of 10 in ∅90 mm Petri dishes containing modified Czapek-Dox agar (0.5 g/l potassium chloride, 30.0 g/l sucrose, 0.5 g/l magnesium glycerophosphate, 0.01 g/l iron sulfate, 0.35 g/l potassium sulfate, 2.0 g/l sodium nitrate and 12 g/l bacterial agar). All the seeds germinated at a constant ambient temperature of 20 °C (Figure 1d). During the experiment, the ratio of germinated plants in the total number of plants under study was recorded on day 3 and day 7 after planting [20]. The number and diameter of fungi colonies in the irradiated and non-irradiated seeds were determined on day 7 of the research (Figure 1d).

3 Results and discussion

3.1 Seed germination rate

Pre-planting irradiation of wheat, flax, and rape seeds showed the non-linear dependencies of plant germination rate on the irradiation dose. Figures 2 show plant germination rate of seeds irradiated with different doses relative to that of non-irradiated samples, measured at day 3 (G3) (Figures 2 a,c,e,g) and day 7 (G7) (Figures 2 b,d,f,h) after planting.



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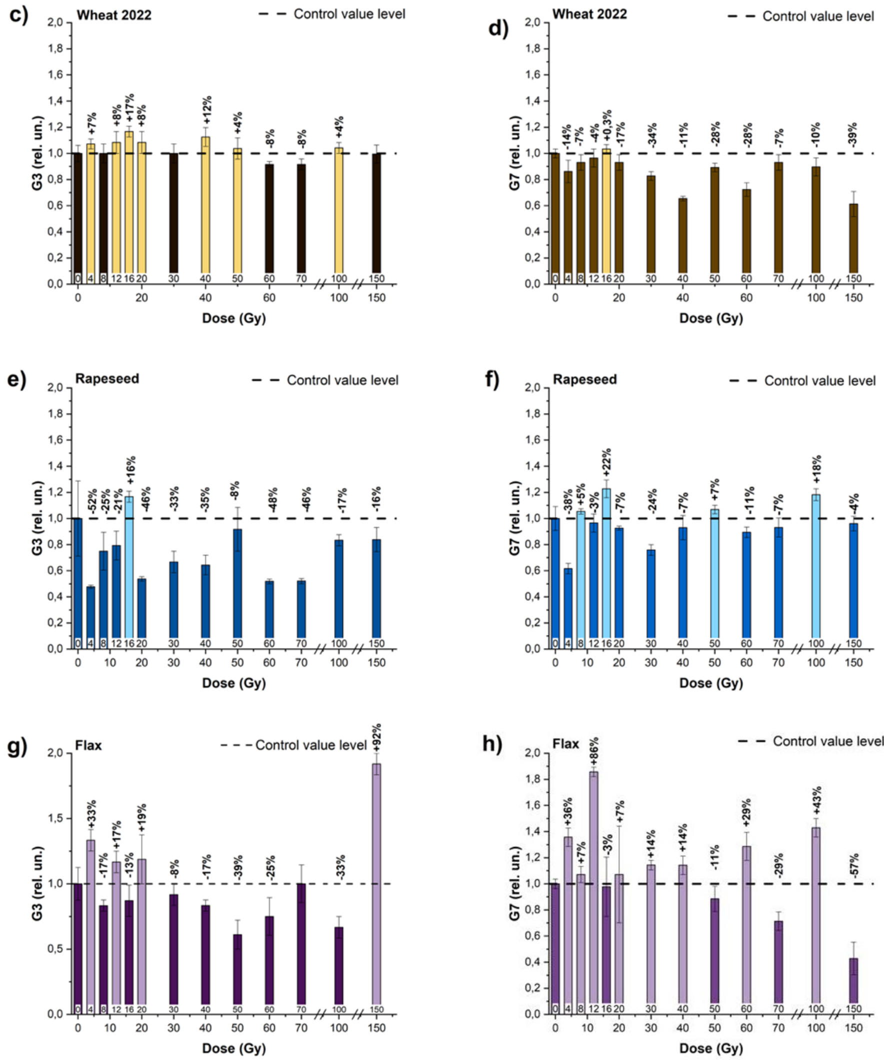

Fig. 2. Growth rate coefficients of seeds irradiated with different doses relative to that of non-irradiated samples, measured at day 3 (G3): (a) wheat seeds harvested in 2021; (c) wheat seeds harvested in 2022; (e) rapeseeds; (g) flax seeds; and at day 7 (G7): (b) wheat seeds harvested in 2021; (d) wheat seeds harvested in 2022; (f) rapeseeds; (h) flax seeds.

It was found that irradiation of wheat seeds harvested in 2021 with the doses of 4 Gy, 8 Gy, 30 Gy and 50 Gy increased the germination rate of seeds by 9%, 17%, 14% and 13% respectively, relative to non-irradiated samples 3 days after planting (Figure 2a). This tendency persisted over the following 4 days for the seeds irradiated with the doses of 4 Gy, 30 Gy, 40 Gy and 50 Gy, and the seed germination rate was 12% higher compared with non-irradiated seeds (Figure 2b). On the 7th day of monitoring, the growth rate of wheat seeds treated with 16 Gy corresponded to that of control samples while all other doses inhibited the seed germination.

For wheat harvested in 2022, a 7-12% increase in plant growth rate 3 days after sowing was observed for seeds irradiated with 4 Gy, 12 Gy, 16 Gy, 20 Gy and 40 Gy 3 days after sowing, while a slight increase in germination rate was detected in seeds irradiated with 50

Gy and 100 Gy (Figure 2c). G3 parameter for seeds irradiated with the doses 8 Gy and 30 Gy corresponded to that of control samples. Further analysis of the wheat seed germination rate conducted on the 7th day of monitoring showed that irradiation with 16 Gy had an insignificant effect on the increase of the germination rate in comparison with the control values while all other doses inhibited the seed germination (Figure 2d).

Considering the germination rate of wheat seeds harvested in 2021 and 2022 it can be concluded that X-ray pre-planting treatment more efficiently stimulated the germination of wheat seeds harvested in 2021, which can be explained by more favourable climate conditions for the development of plants and ripening of grain in 2021 compared with 2022.

For rapeseeds, an increase in the germination rate by 16% compared to the control values was observed in samples irradiated with 16 Gy (Figure 2e). On day 7 after sowing, the germination rate was higher in seeds irradiated with 8 Gy, 16 Gy, 50 Gy and 100 Gy by 5.4%, 22%, 7% and 18%, respectively, compared to the control samples (Figure 2f). The other doses showed a decrease in growth rate on day 7 after planting compared with the control values.

For flax, irradiation with 4 Gy, 12 Gy, 20 Gy and 150 Gy increased the value of G3 coefficient 3 days after sowing by 33%, 17%, 19% and 92%, respectively (Figure 2g). On day 7 practically all doses, except for 16 Gy, 50 Gy, 70 Gy and 150 Gy, stimulated the plant germination by 7-43% compared with control samples (Figure 2h). On day 7 a more significant decrease in germination rate of 57% was observed after irradiation with 150 Gy.

3.2 Phytosanitary condition of seeds

Analysis of the phytosanitary condition of control wheat, rape and flax seeds revealed a wide range of both phytopathogenic fungi such as Alternaria, Fusarium and Birolaris, and mold-causing fungi Aspergillus, Mucor and Penicillium. Table 1 represents the phytosanitary parameters of non-irradiates wheat seed harvested in 2021 and 2022, rapeseeds and flax seeds. As evident from Table 1, the extent of contamination with phytopathogens was higher for wheat seeds compared to rapeseeds and flax seeds, which is related to the presence of a longitudinal furrow and hairs at a blunt end of the grain in wheat seeds where fungal spores can cling. Rapeseeds and flax seeds have a smooth shape which makes it harder for spores to latch on to these seed varieties.

Table 1. Phytosanitary parameters of non-irradiated wheat, rapeseeds, and flax seeds.

	Alternaria	*Fusarium*	*Birolaris*	*Aspergillus*	*Mucor*	*Penicillinium*	Total Number	Colony diameter, mm
Wheat harvested in 2021	90.3%	9.3%	0.3%	ND*	ND	ND	5.7±0.3	43.1±0.6
Wheat harvested in 2022	91.1%	7.0%	0.3%	0.3%	ND	1.3%	9.7±0.3	32.8±1.3
Rapeseed	33.3%	26.7%	ND	ND	20.0%	20.0%	1.3±0.3	21.3±7.0
Flax	75.0%	25.0%	ND	ND	ND	ND	0.3±0.1	20.0±5.8

*Not Detected

A non-linear dependency of the number and the diameter of colonies on the irradiation dose was observed for all seeds under study (Figure 3).

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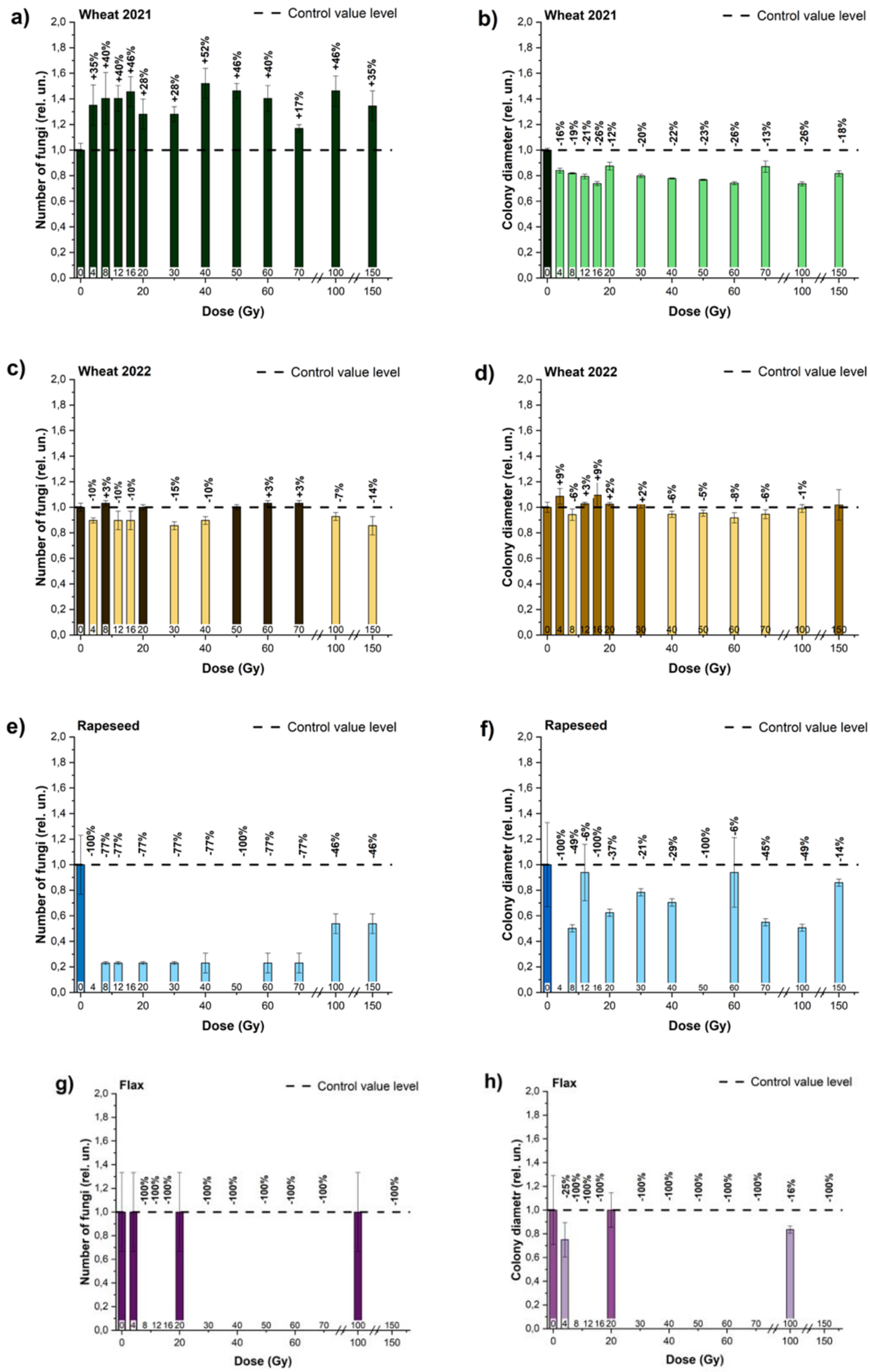

Fig. 3. The dependencies of the number of fungi colonies in wheat seeds harvested in 2021 (a), wheat seeds harvested in 2022 (c), rapeseeds (e) and flax seeds (g), and the average colony diameter found in wheat seeds harvested in 2021 (b), wheat seeds harvested in 2022 (d), rapeseeds (f) and flax seeds (h).

For wheat seeds harvested in 2021, the number of fungi after irradiation increased in all irradiated samples (Figure 3a) by 17-52% compared to control samples. All the applied doses reduced the diameter of fungi colonies in seeds and the doses of 12 Gy, 16 Gy, 30-60 Gy and 100 Gy suppressed the size of phytopathogenic fungi by more than 20% compared to the control parameters (Figure 3b).

X-ray irradiation of wheat seeds harvested in 2022 to 4 Gy, 12 Gy, 16 Gy, 30 Gy, 40 Gy, 100 Gy, and 150 Gy inhibited the number of fungi by 10%, 10%, 10%, 15%, 10%, 7%, and 14%, respectively (Figure 3c). When seeds were irradiated with 8 Gy, 20 Gy, 60 Gy, and 70 Gy, the number of fungi within the margin of error corresponded to the control level. The maximum reduction of 5-8% in the diameter of fungi occurred after irradiation with 8 Gy and 40-100 Gy while all other doses did not manifest a significant decrease in the diameter of fungi after exposure to X-rays, and the average diameter was at the level of control samples (Figure 3d).

Irradiation of rapeseeds with 4 Gy, 16 Gy and 50 Gy completely inhibited fungi growth, and other doses showed a significant decrease in the number of fungi by 50-80% (Figure 3e). The diameter of fungi colonies was also reduced at all doses compared to control values (Figure 3f).

In flax seeds irradiated with 8-16 Gy, 30-70 Gy, and 150 Gy, the fungi were completely inhibited. Seeds irradiated with 4 Gy, 20 Gy, and 100 Gy had the number of fungi at the level of control values (Figure 3g). At the same time, the diameter of colonies in seeds irradiated with 20 Gy corresponded to the control values while irradiation with 4 Gy and 100 Gy decreased the fungi diameter by 25% and 16%, respectively (Figure 3h).

The absence or an insignificant suppression of phytopathogens in wheat seeds after exposure to X-rays can be explained by the fact that initially wheat seeds were more heavily infected with phytopathogens than rape and flax, so higher doses were needed to suppress fungi growth in wheat seeds.

4 Conclusion

During the research, it was found that pre-planting treatment of naturally infected wheat, rape and flax seeds with X-rays with the doses ranging from 4 Gy to 150 Gy had both stimulating and inhibitory effects on the growth rate of seeds and their phytosanitary status. While the doses 4-20 Gy were able to enhance the seed germination of seeds, the doses over 50 Gy decreased the germination rate of seeds.

To ensure a superior quality of the new crop, pre-planting irradiation doses were established, on the one hand, to maximize the seed germination rate of seeds, and on the other hand, to completely inhibit the activity of phytopathogen in seeds. It was established that low-energy X-ray radiation with the doses of 30-50 Gy, 16 Gy, 16 Gy and 12 Gy is the most efficient method for pre-planting treatment of Novosibirskaya 29 wheat seeds harvested in 2021 and in 2022, Builder rapeseeds and Severny flax seeds, respectively. Notably, the maximum effect in terms of increasing the germination rate and inhibition of phytopathogens was achieved for flax seeds, which can be explained by the difference in dose distribution across the whole volume of seeds. Therefore, it can be concluded that each type of seed requires a special approach for selecting the effective doses for pre-planting treatment considering biochemical properties of each seed type and variety, as well as the location, structure and dimensions of critical organs.

One of the factors which had an impact on the inhibition of phytopathogens in seeds was the initial contamination of seeds. The comparison of wheat seeds harvested in 2022 with a higher level of contamination with wheat seeds gathered in 2021 revealed that the former seeds required a higher irradiation dose for inhibiting fungi than the latter ones.

To establish a reliable dose range for each type of seeds which could be used as guidelines for practical implementation in Russia, it is necessary to conduct further field research at an agricultural station located in the area where the crops under study are grown considering local climate conditions and soil type.

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