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ORIGINAL PAPER

Effectiveness of electron beam irradiation in the control of some soilborne pathogens

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Abstract. Electron beam (EB) irradiation was tested against *Botrytis cinerea*, *Pythium ultimum* and *Phytophthora citricola* the most dangerous pathogens causing stem and root rot of seedlings, cuttings and older plants. In the laboratory trials cultures of 3 species were irradiated with doses 0 (control), 1.5, 3.0, 4.5 and 6.0 kGy whereas peat was treated with 10, 15 and 25 kGy. *P. citricola* was the most sensitive species for irradiation. In greenhouse trials 15 kGy irradiation of peat protected chrysanthemum cuttings against *B. cinerea* and *P. ultimum* as well as rhododendron young plants against *P. citricola*. Irradiation of peat did not influence the growth and development of the tested plants.

Key words: chrysanthemum • electron beam (EB) irradiation • healthiness • rhododendron • soilborne pathogens

Introduction

Among soilborne pathogens *Pythium ultimum* Trow, *Phytophthora citricola* Sawada and *Botrytis cinerea* Pers. are highly dangerous for many plant species. All of them constitute serious and continuing problems for plant growers. *P. ultimum* and *B. cinerea* are known as pre- and postemerging seedlings damping off. Both pathogens are also the causal agents of rotting of cuttings. The last species is one of the most often occurring agent causing gray mold of leaves, stem parts and flowers of plants grown under covering and in open field [8]. *P. citricola*, known in Poland since the last decade of XX century, is the causal agent of stem base rot and top shoot blight of deciduous, coniferous and ericaceous plants [9–11]. For production of plants under covering and in hardy ornamental nurser stocks, the most often used substrates are peat or its mixture with composted pine bark or other components. Storing of substrate components near greenhouses or nurseries may cause their contamination with some pathogenic microorganisms spread with water or air. In such case substrates prepared for plant growing or already used ought to be disinfected. Steaming of substrates is expensive and acts destructively on peat structure. Application of pesticides, containing dazomet or metam sodium as the active ingredients, needs at least 4 weeks in the case of peat and composted pine bark for their disinfection, temperature at least 18°C and its efficacy is about 80% [3]. Usually, short time between production cycles and apprehensive about possible toxic residues of applied disinfectans, forced for searching for a new, simple and easy methods for substrates decontamination to eliminate the most common and dangerous microor-

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ganisms. The above mentioned requirements can be fulfilled by applying ionizing irradiation, commercially used for sterilization of single-use medical devices as well as microbiological decontamination of food products [1]. Studies of Gryczka *et al*. [2] indicated ionizing irradiation as a very effective method for the control of *Fusarium oxysporum* f. sp. *dianthi*, the most dangerous carnation pathogen causing Fusarium wilt.

In this study *in vitro* and *in vivo* effectiveness of different radiation doses in the control of *Botrytis cinerea*, *Phytopthora citricola* and *Pythium ultimum* were evaluated.

Materials and methods

Microorganisms used in laboratory and greenhouse trials

Botrytis cinerea, *Phytopthora citricola* and *Pythium ultimum* were used in all trials. Stock cultures were maintained on a PDA (potato dextrose agar) medium at 24°C in the dark. For inoculation of plant parts, 3 mm diam. plugs were cut out from the edge of cultures and transferred on the base of petioles and stem parts of the tested plants. For peat infestation, the method described by Orlikowski [5] was used. Three species were cultured for 2 weeks on rolled oats and after that time blended with a minimum of distilled water in a Waring Blender to form thick slurry. The homogenates were mixed with peat at a rate one Petri dish per 1 litre of substrate. After 10-day storage in plastic bags at 20°C, the infested substrates were ready for use [6].

Irradiation

A linear electron accelerator ELEKTRONIKA with 9 MeV energy was used for irradiation of Petri dishes, containing 3 microorganism species and peat substrates [4]. Microorganism's cultures growing on PDA in 90 mm Petri dishes were irradiated with doses 0 (control), 1.5, 3.0, 4.5 and 6.0 kGy. Peat substrates infested with *B. cinerea*, *P. citricola* and *P. ultimum* were treated with doses 10, 15 and 25 kGy using the technique described by Gryczka *et al*. [2].

In vitro growth of irradiated microorganisms

Four hours after irradiation of Petri dishes with 3 pathogens, 3 mm diam. discs of medium overgrown by microorganisms were taken from the edge of cultures and transferred on a clean PDA medium. After 2- and 4-day incubation at 24°C, the diameter of colonies was measured. Five days after culture irradiation, they were transferred for the second time on clean PDA using the same procedure.

Laboratory and greenhouse trials with plants

Chrysanthemum cv. Froggy unrooted cuttings and rhododendron cv. Nova Zembla plants in stage of 4–6 leaves were used. In laboratory trial bases of leaf petioles and stem parts were put into photographic trays covered with 2 layers of a sterile blotting paper and a plastic net and inoculated with 3 mm diam. medium plugs overgrown by the tested microorganisms. Trays were covered with a foil and incubated at 22–24°C in the dark. Length of necroses was measured 2 and 5 days after inoculation. In the case of *P. citricola* rhododendron, leaf blades were inoculated and the diameter of necroses was measured after 5 and 9 days of incubation.

In greenhouse trials unrooted chrysanthemum cuttings were planted into peat infested by *B. cinerea* and *P. ultimum*, whereas rhododendron young plants into peat infested by *P. citricola*. After 7- and 10-day growth on greenhouse bunch at 20–25°C, the number of diseased plants was evaluated. Additionally, the number of chrysanthemum rooted cuttings was estimated.

Experimental design was completely randomized with four replications and five plants in each experimental unit. Trials were repeated twice.

Estimation of *Phytophthora citricola* and *Pythium ultimum* occurrence in untreated and irradiated peat

Rhododendron leaf blade baits were used for detection of both species from infested peat using the procedure described by Orlikowski and Ptaszek [7, 12]. Non-treated and irradiated peat was checked for the presence of *P. citricola* and *P. ultimum* 24 h after treatment and after 10 day growth of chrysanthemum cuttings and rhododendron young plants. About 0.5 l of substratum samples from each treatment were put into trays and flooded to a depth of 3 cm with tap water and baiting leaves were put on the surface. The trays were covered with a foil and incubated at 22–24°C in the dark. After four days, the leaves were removed, washed under tap and distilled water, blotted dried and about 3 mm diam. necrotic parts were transplanted on a PDA medium. Within 48 h of incubation, the number of *P. citricola* and *P. ultimum* colonies growing around of inocula were counted.

Results

Influence of irradiation culture of *Botrytis cinerea* and infested peat on the species growth and healthiness of chrysanthemum

First transplanting of the species from irradiated Petri dishes on clean PDA medium 4 h after treatment resulted in the radial growth independently of irradiation doses. After two days, the species grown on the medium seeded by culture treated with doses 0, 1.5 and 3 kGy with a significantly faster development on control plates. Two days later the tested species developed on all Petri dishes with the significantly quickest growth on control plates, whereas the slowest on the medium seeded with plugs taken from culture irradiated with 4.5 and 6 kGy. The second transplanting (5 days after the first) of inocula, taken from irradiated cultures on a clean PDA medium, resulted in the lack of the species

Table 1. Growth (mm) of irradiated *Botrytis cinerea* transplanted on clean PDA medium in relation to irradiation doses

Irradiation dose (kGy)	1st transplanting after days		2nd transplanting after days	
	2	4	3	5
0	36.5 c	80.1 d	34.1 d	52.4 c
1.5	6.8 b	28.9 c	28.0 c	44.5 bc
3.0	5.5 b	29.8 c	21.8 b	40.8 b
4.5	0 a	15.5 b	21.1 b	38.8 b
6.0	0 a	5.5 a	0 a	0 a

Note: means in columns, followed by the same letter, do not differ at 5% of significance acc. to Duncan's multiple range test. Statistical analyses separate for each observation.

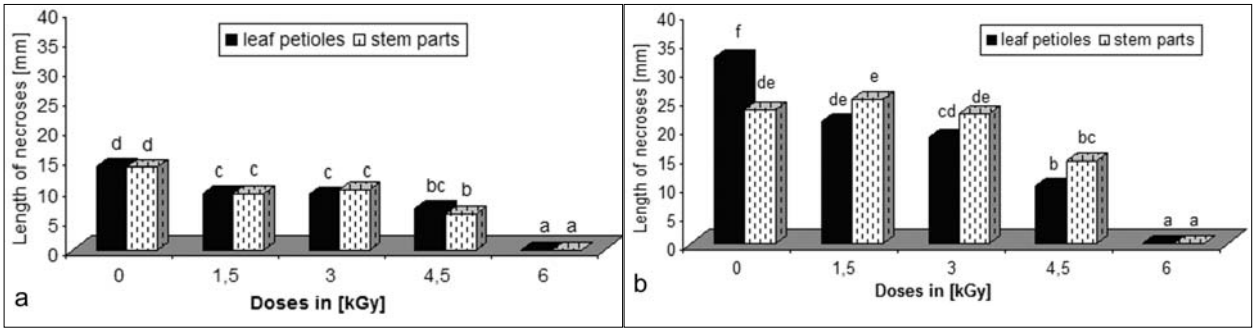

Fig. 1. Colonization of chrysanthemum parts by radiated *B. cinerea* culture in relation to irradiation doses and incubation time. a – 2 days after inoculation; b – 5 days after inoculation. Note: means in columns marked by the same letter, do not differ at 5% of significance acc. to Duncan's multiple range test.

growth from plugs irradiated with 6 kGy, both, after 3- and 5-day incubation (Table 1).

Inoculation of chrysanthemum leaf petioles and stem parts by irradiated and non-treated cultures of *B. cinerea* caused the colonization of plant parts by the fungus already after 2-day incubation (Fig. 1a). The spread of necrosis was similar on leaf petioles and stem parts, but the disease development was the quickest on plant tissues inoculated with plugs from the non-treated culture. The slowest necrosis development was noticed on plant parts inoculated with *B. cinerea* culture irradiated to dose 4.5 kGy. A similar tendency in chrysanthemum colonization was observed 3 days later (Fig. 1b). Development of necrosis was not observed on chrysanthemum parts inoculated with *B. cinerea* culture treated with 6 kGy (Figs. 1a and 1b).

In greenhouse trial 1/5 of chrysanthemum cuttings showed base rot and wilting already after 7 days of growth and 3 days later the majority of plants died. On cuttings grown in the irradiated peat, previously infested with *B. cinerea*, stem base rot was observed on about 1/5 of the plants growing in the substrate treated with 10 kGy and only sporadically in the substrate irradiated with 15 kGy. More than 4/5 of the cuttings produced roots in peat irradiated with 15 and 25 kGy without any phytotoxic symptoms on leaves (Table 2).

Influence of irradiation of *Pythium ultimum* culture and infested peat on the species growth and healthiness of chrysanthemum

First transplanting of the species plugs from irradiated cultures (doses from 1.5 to 6 kGy) 4 h after treatment resulted in the species growth with a significant decrease of development in relation to the increasing doses. The second transplanting of plugs on a clean PDA medium resulted in the lack of growth of irradiated cultures. Non-treated culture (control) grown about 30 mm/24 h (Table 3).

Inoculation of leaf petioles and stem parts of chrysanthemum by *P. ultimum* cultures caused the colonization of them after 5-day incubation independently of the irradiation doses. The quickest necrosis spread was observed on plant parts inoculated with non-irradiated cultures. Plugs taken from culture treated with 6 kGy for inoculation of stem parts did not cause any disease symptoms. Irradiation of *P. ultimum* cultures caused

Table 2. Relationship between irradiation doses, growing time and healthiness of chrysanthemum cuttings cv. Froggy and their rooting in peat infested by *Botrytis cinerea*

Irradiation doses (kGy)	Number of diseased cuttings (n = 5) after days of cultivation		Number of rooted cuttings (n = 5)
	7	10	
0 (control noninfested)	0 a	0.3 a	4.8 b
0 (control infested)	1.3 b	4.8 c	0 a
10	0.3 a	0.8 b	4.2 b
15	0.3 a	0.3 a	4.8 b
25	0 a	0 a	5.0 a

Note: means in columns, followed by the same letter, do not differ at 5% of significance acc. to Duncan's multiple range test. Statistical analyses separate for each observation.

Table 3. Radial growth (mm) of *Pythium ultimum* culture transplanting on clean PDA medium in relation to irradiation doses

Irradiation dose (kGy)	1st transplanting after days		2nd transplanting after days	
	2	3	3	5
0 (control)	79.8 d	90.0 e	85 b	90 b
1.5	9.8 c	14.8 d	0 a	0 a
3.0	6.0 b	8 c	0 a	0 a
4.5	5.0 b	5.5 b	0 a	0 a
6.0	0 a	0 a	–	–

Note: means in columns, followed by the same letter, do not differ at 5% of significance acc. to Duncan's multiple range test. Statistical analyses separate for each observation.

Table 4. Colonization of chrysanthemum parts cv. Froggy by *Pythium ultimum* cultures in relation to irradiation doses and incubation time; length of necrosis (mm) 2 (a) and 5 (b) days after inoculation

Irradiation doses (kGy)	Leaf petioles		Stem parts	
	a	b	a	b
0 (control)	23.4 d	38.5 d	24.1 d	28.8 d
1.5	0 a	8.0 b	2.1 a	11.9 b
3.0	0 a	6.1 a	9.6 b	22.5 c
4.5	0 a	13.6 c	14.3 c	19.4 c
6.0	0 a	10.4 bc	0 a	0 a

Note: means in columns, followed by the same letter, do not differ with 5% of significance acc. to Duncan's multiple range test. Statistical analyses separate for each observation.

a decrease at least 3 times of necrosis development in comparison to control, non-treated species (Table 4).

In greenhouse trials growing of chrysanthemum cuttings in peat infested by *P. ultimum* and irradiated with doses 15 and 25 kGy resulted in their protection. In non-treated substrate more than 3/5 of the plants showed stem base rot of about 2 cm in length after 7-day cultivation and 3 days later most of them died (Table 5). In peat irradiated with 10 kGy only about 1/5 of the cuttings died within 10-day growth.

Cultivation of chrysanthemum in non-infested peat and infested but irradiated with 3 tested doses caused that at least 4/5 of the cuttings formed from 4 to 7 roots within 10-day growth (Table 5).

The use of rhododendron baiting leaves for the detection of *P. ultimum* from infested peat, resulted in recovery of about 25 *Pythium* colonies from control, untreated substratum. The pathogen was not detected from baiting leaves placed in the peat irradiated with 10, 15 and 25 kGy.

Influence of irradiation of *Phytophthora citricola* culture and infested peat on the species growth and healthiness of rhododendron

Transplanting of medium plugs overgrown by *P. citricola* irradiated with doses from 1.5 to 6.0 kGy on a

Table 5. Relationship between irradiation doses, growing time and healthiness of chrysanthemum cuttings cv. Froggy and their rooting in peat infested with *Pythium ultimum*

Irradiation doses (kGy)	Number of diseased plants (n = 5) after days of cultivation		Number of rooted cutting (n = 5)
	7	10	
Control noninfested	0 a	0 a	5.0 b
0 (control infested)	3.5 b	4.3 c	0 a
10	0.5 a	0.8 b	4.8 b
15	0 a	0 a	4.5 b
25	0 a	0 a	5.0 b

Note: means in columns, followed by the same letter, do not differ with 5% of significance acc. to Duncan's multiple range test. Statistical analyses separate for each observation.

Table 6. Radial growth (mm) of *Phytophthora citricola* culture transplanting on clean PDA medium in relation to irradiation doses

Irradiation dose (kGy)	1st transplanting after days		2nd transplanting after days	
	2	4	3	5
0 (control)	6 b	20.9 b	6.3 b	22.0 b
1.5	0 a	0 a	0 a	0 a
3.0	0 a	0 a	0 a	0 a
4.5	0 a	0 a	0 a	0 a
6.0	0 a	0 a	0 a	0 a

Note: means in columns, followed by the same letter, do not differ at 5% of significance acc. to Duncan's multiple range test. Statistical analyses separate for each observation.

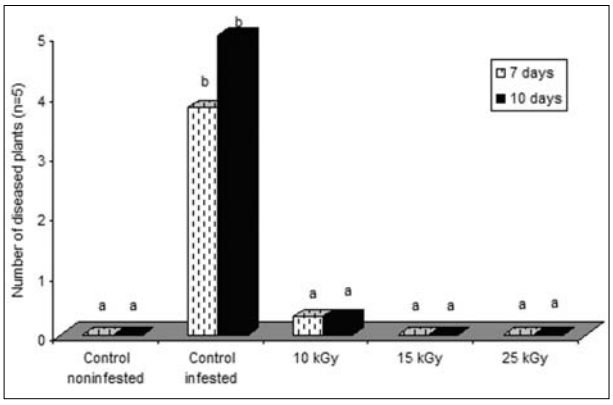

Fig. 2. Relationship between irradiation doses, growing time and healthiness of rhododendron cv. Nova Zembla growing in peat infested with *P. citricola*. Note: means in columns, marked by the same letter, do not differ at 5% of significance acc. to Duncan's multiple range test.

clean PDA medium 4 h after treatment and 5 days later resulted in the lack of culture development. On clean PDA seeded with control, non-treated culture plugs, the species grown about 5 mm/24 h (Table 6).

The use of irradiated *P. citricola* cultures for inoculation of rhododendron leaf blades resulted in the lack of necroses development. Only non-irradiated culture colonized rhododendron leaves and necrosis spread about 2 mm/24 h.

In greenhouse trials stem and root rot was observed on 4/5 of rhododendron after 7-day growth and 3 days later all of them died, whereas on plants cultivated in peat treated with 10 kGy disease symptoms occurred only on 1/5 of cuttings (Fig. 2). Within the next 30-day growth the development of stem and root rot was not noticed on rhododendron grown in a substrate treated with 15 and 25 kGy.

On rhododendron leaf baits, used for detection of *P. citricola* from the irradiated substrate, lack of necrotic spots were observed after 10-day growth of rhododendron, whereas in non-treated, control peat 14 necrotic spots/leaf bait (mean number from 8 leaves) was noticed.

Conclusions

Previous study of Gryczka *et al*. [2] indicated that irradiation of *Fusarium oxysporum* F. sp. *dianthi* cultures, grown *in vitro* on a PDA medium, inhibited mycelium development and pathogen sporulation. The effectiveness of the process increased with irradiation dose. The cultures irradiated with 6 kGy did not developed after their transplanting which indicate that this dose was lethal for tested pathogen.

The carried out studies showed differentiated reaction of 3 tested microorganisms on irradiation. *Phytophthora citricola* was the most sensitive because the lack of *in vitro* growth observed after irradiation with 1.5 kGy, whereas *Botrytis cinerea* colonies developed even after their treatment with 6 kGy and *Pythium ultimum* with 4.5 kGy. The use of irradiated cultures for inoculation of chrysanthemum and rhododendron parts confirmed different reaction of 3 pathogens on irradiation. In greenhouse trials, however, application of irradiation at a dose of 15 kGy for disinfection of peat, infested by 3 pathogens, resulted in the decrease of *P. citricola* and *P. ultimum* population to uncovered level using rhododendron baiting leaves. The lack of diseased chrysanthemum and rhododendron plants growing in irradiated peat confirmed results obtained with baiting leaves. In Yun *et al*. [13] studies most of aerobic bacteria were eliminated from organic composts by irradiation at the dose of 10 kGy. In the case of *B. cinerea* irradiation of infested peat with the dose of 15 kGy caused almost complete protection of cuttings against gray mold.

Obtained results confirmed the high efficacy of the substrate decontamination by irradiation showed in Gryczka *et al*. [2] study with Fusarium wilt disease of carnation. Application of EB irradiation already at a dose of 10 kGy decreased the number of colony forming units of *F. oxysporum* f. sp. *dianthi* about 90 times. Lack of *Fusarium* wilt symptoms on cultivated plants was observed in such treated peat during 12-week growth. Irradiation of peat even with a dose of 25 kGy did not influence the growth and development of the tested plants. Rooting of chrysanthemum cuttings cultivated in irradiated peat was similar like in non-treated substrate with lack of any phytotoxic symptoms on leaves. EB irradiation did not effect also the development of rhododendron young, quickly growing plants.

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