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ORIGINAL PAPER

Effectiveness of electron beam irradiation in the control of *Rhizoctonia solani*

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Abstract. Effectiveness of electron beam irradiation was evaluated against *Rhizoctonia solani*, which is one of the most dangerous soil-borne pathogen, causing stem base and root rot of many horticultural plants. Treatment of *in vitro* cultures with 0.5 kGy significantly inhibited the pathogen's growth and the spread of necrosis on chrysanthemum leaf blades inoculated with the irradiated cultures. Application of e-beam irradiation for peat disinfection resulted in a two-fold decrease rot development in chrysanthemum stem at a dose of 5 kGy and complete inhibition at 10 kGy. For the elimination of *R. solani* from composted pine bark and its mixture with peat, irradiation of both substrates with 15 kGy was necessary.

Key words: effectiveness • electron beam irradiation • *Rhizoctonia solani* • chrysanthemum • substrates

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Introduction

Stem base and root rot is one of the most destructive plant diseases caused by various pathogens including *Rhizoctonia solani* Kühn [3]. The opening of the Polish borders in the last decade of the 20th century resulted in a dramatic increase in the import of young plants. This trend was also noticed by Brasier [1], who pointed out about a two-fold increase in the international plant trade within the last 10 years and the entering of a stream of invasive pathogens. In the author's opinion "major problems may arise if a pathogen escapes or is introduced to another region of the world where the native plants have little resistance and the pathogen has eluded its natural enemies. Such events can trigger damaging disease episodes that may also have long-term negative impacts on the environment, economy and cultural heritage". Among the species entering the hardy Polish nursery stocks, *Rhizoctonia solani* Kühn has spread very fast on a still not known plant host. On the other hand, the species is a known pathogen of many greenhouse plants and has been isolated from them and from growth substrates within the last 5 years as the causal agent of stem base rot in cuttings and older plants. Among the host plants such as pelargonium, poinsettia, begonia and kalanchoe, widely grown under cover, *R. solani* has caused losses as high as 20%. In the production of perennial plants, the species often occurs together with *Phytophthora* spp. causing losses of up to 50%. Among the different sources of the pathogen, infected substrates, especially the peat that is widely used in the production of horticultural plants, cause the pathogen to spread easily during plant cultivation, by water, machine tires and people, as well as when selling plants [10]. Also, storing fresh substrates near greenhouses and in nurseries may cause them to become contaminated with pathogens spread with water, on shoes, tires and by insects. In such a situation, disinfection of the already used substrates or infected soils to minimize the spread of soil-borne pathogens is a promising strategy in integrated disease and pest management [11]. Usually, short intervals between production cycles and apprehension about toxic residues of the applied disinfectants drive the search for new, simple and easy methods of decontaminating substrates to eliminate the most common and dangerous microorganisms. The above-mentioned requirements can be fulfilled by applying ionizing radiation, used commercially for the sterilization of medical devices as well as microbiological decontamination of food products [2]. Ionizing irradiation has found to be a very effective method in the control of *Botrytis cinerea*, *Fusarium oxysporum* f. sp. *dianthi*, *Pythium ultimum* and *Phytophthora* spp. [5, 7, 8, 13]. Applications of dazomet or metham sodium as soil and substrate disinfectants need at least a 3-week waiting period, a temperature of at least 18°C, and their effectiveness is often unsatisfactory [6]. The aim of this study was to use irradiation with an electron beam with an energy of 10 MeV in the *in vitro* and *in vivo* control of *Rhizoctonia solani*.

Materials and methods

Irradiation

A linear electron accelerator ELEKTRONIKA 10/10 was used for irradiation with an electron beam of energy 10 MeV. Doses of radiation were measured with PVC dosimeters using a dose reader CD-07 [4].

Radiation treatment of *Rhizoctonia solani* in Petri dishes

The cultures were grown for 72 h on a PDA (potato dextrose agar) medium and then were treated with doses of 0 (control), 0.5, 1.0, 1.5, 3.0 and 4.5 kGy. The effectiveness of irradiation was assessed by transplanting the treated cultures onto a clean PDA medium 3 h after treatment and incubating them for 4 days at 24°C. Additionally, stem parts of chrysanthemum cv. 'Froggy' were inoculated with the irradiated cultures using the procedure of Orlikowski and Szkuta [9]. The extent of necrosis on the infected stem parts was measured after 4-day incubation of chrysanthemum cuttings in plastic ash trays covered with wet sterile blotting paper covered with a plastic net.

Effect of irradiation of infected substrates on the health and rooting of chrysanthemum cuttings

In the first greenhouse trial, peat infected with the pathogenic species and irradiated with doses from 5 to 20 kGy was used for rooting cuttings of chrysanthemum cv. 'Froggy'. Nemazin 97 FG (97% of dazomet) at an amount of 300 g/m^3 was used as the standard disinfectant. Non-infested and infested, but not treated, peat was used as the control. After planting cuttings in the treated substrate in 1 litre pots, the plants were covered with plastic foil and incubated on a greenhouse bench at a temperature of 19–25°C for 2 weeks. After 7 and 10 days, the number of diseased cuttings with stem base rot symptoms was recorded, and after 2 weeks the number of rooted cuttings was evaluated. In the next trial, peat, composted pine bark (cpb), a mixture of cpb with peat (1:1) and perlite infested with *R. solani* were treated with doses from 0 (control) to 20 kGy, with dazomet, as in the previous experiment, being used as the standard product. Five days after treatment, chrysanthemum cuttings cv. 'Intrepid' were planted in the treated substrates and grown on a greenhouse bench. The number of diseased plants in relation to the substrate and irradiation dose was assessed. In both laboratory and greenhouse trials, the experimental design was completely randomized with 4 replications, each consisting of 4 Petri dishes or 5 chrysanthemum cuttings.

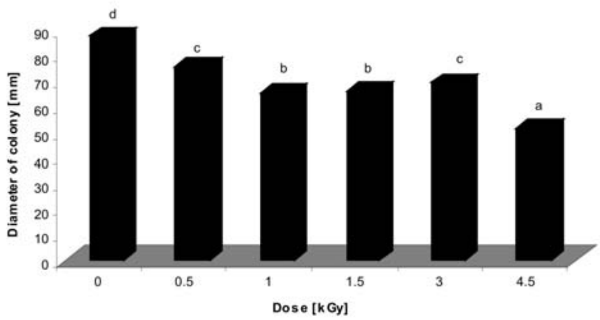

Fig. 1. Growth of *Rhizoctonia solani* in relation to irradiation doses.
Note: means on columns followed by the same letter do not differ at the 5% level of significance according to Duncan's multiple range test.

Results

Effect of irradiation of *R. solani* cultures on their growth and colonization of chrysanthemum stem parts

Growth of the pathogen was observed on both untreated and irradiated PDA medium (Fig. 1). The fastest development of the tested species was observed on clean, untreated plates, with a significant decrease in the colonies' diameter with increasing irradiation dose. The effect of irradiating *R. solani* was especially noticeable when the cultures were treated with 4.5 kGy (Fig. 1). Inoculation of chrysanthemum stem parts with *R. solani* cultures resulted in the development of stem base rot (Fig. 2). After 2 days, the fastest development of necrosis was observed on the chrysanthemums inoculated with an untreated culture, and the slowest on the stem parts inoculated with *R. solani* irradiated with 1.5 kGy. After another 2 days, the fastest spread of necrosis was observed on the plant parts inoculated with a non-irradiated culture, but its development was significantly slower on the stem bases treated with an irradiated inoculum (Fig. 2).

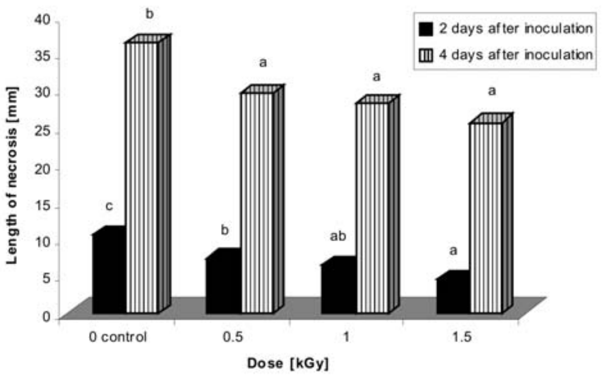

Fig. 2. Effect of e-beam irradiation of Petri dishes with *Rhizoctonia solani* on the activity of the pathogen in the colonization of chrysanthemum stem parts 2 and 4 days after inoculation.
Note: see Fig. 1; statistical analysis separately for observation period.

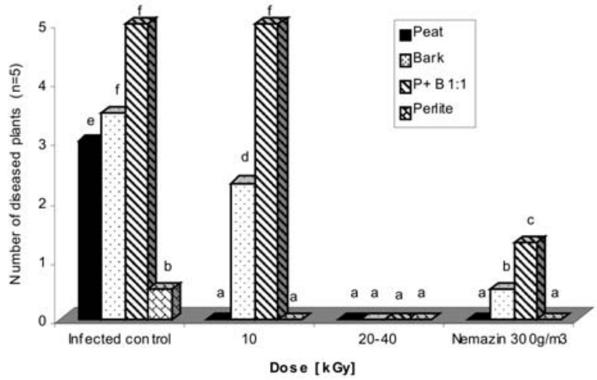

Fig. 3. Effect of e-beam irradiation of 4 substrates infested with *Rhizoctonia solani* on the health of chrysanthemum cuttings 14 days after planting.
Note: see Fig. 1.

Effect of substrate irradiation on the health and rooting of chrysanthemum cuttings in a greenhouse

In the first trial, transplanting cuttings into infested, untreated peat resulted in the development of necrosis on the leaves already during 7-day incubation and after the next 3 days all the plants were destroyed (Table 1). Application of irradiation at a dose of 5 kGy caused a two-fold reduction in the extent of disease development and half of the cuttings produced roots. Increasing irradiation dose to 10 kGy completely inhibited infection of chrysanthemum stem base and leaves, and all the cuttings produced a root system. This effect was also obtained when higher irradiation doses had been used (Table 1).

In the next trial, 4 substrates were used to study the relationship between irradiation dose, the kind of substrate and the health of chrysanthemum cuttings (Fig. 3).

After 10-day growth, stem base and leaf blade rot was observed mainly on the chrysanthemums cultivated in the infested but not irradiated substrates. Four days later, at least 3/5ths of the plants were found dead after they were grown in infested peat, composted pine bark and a mixture of the two components. The disease occurred only sporadically on the chrysanthemums grown in perlite (Fig. 3). Treating composted pine bark mixed with peat with 10 kGy did not inhibit the development of stem base and leaf rot because all the plants showed symptoms of the disease. This dose, however, caused about a two-fold decrease in the development of *Rhizoctonia* rot in the chrysanthemums grown in composted pine bark and complete inhibition in the plants rooting in peat and perlite (Fig. 3). No development of the disease was observed after e-beam irradiation at doses of 20 and 40 kGy. Dazomet used as the standard did not completely protect chrysanthemums grown in composted pine bark and its mixture with peat (Fig. 3).

Conclusions

The results of the irradiation treatments of *R. solani* indicate a lower susceptibility of this species to irradiation than other plant pathogens. Growth of the pathogen was still observed when cultures had been treated with 4.5 kGy, whereas *Phytophthora cinnamomi* [7] and *Phytophthora citricola* did not grow after treatment with 1 kGy [8]. However, a response to e-beam irradiation similar to that of *R. solani* was observed after *Botrytis cinerea* had been treated with 6 kGy [8]. Application of irradiation as a treatment for horticultural substrates has given different results in relation to the tested pathogens and types of substrates. In this study, irradiation of peat and perlite with 10 kGy completely eliminated *R. solani*, whereas a dose of 20 kGy was necessary to kill the pathogen in composted pine bark and its mixture with peat. In the studies by Ptaszek *et al*. [12] and Orlikowski *et al*. [8], the treatment of peat infested with *Sclerotinia sclerotiorum* or *P. citricola* with 10 kGy resulted in almost 100% survival of chrysanthemum plants and about 90% of rhododendron plants growing in this

Table 1. Effect of e-beam irradiation of peat infected with *Rhizoctonia solani* on the health and rooting of chrysanthemum cuttings cv. 'Froggy'

Irradiation dose (kGy)	Number of cuttings (n = 5) with dying leaves after days from planting		Number of rooted cuttings (n = 5) after 14 days
	7 days	10 days	
Control, non-infested	0 a	0 a	5.0 c
Control, infested	3.5 c	5.0 d	0 a
5	0 a	2.5 b	2.5 b
10	0 a	0 a	5.0 c
15	0 a	0 a	5.0 c
20	0 a	0 a	5.0 c

Note: The means in columns followed by the same letter do not differ at the 5% level of significance, according to Duncan's multiple range test.

substrate. Peat is most often used for growing plants under cover and in open field nurseries, and both the present and previous results indicate that subjecting it to irradiation with 10–15 kGy results in 100% survival of the plants transplanted into this substrate after such a treatment. The results obtained confirm the previous data collected by Gryczka *et al*. [5], Orlikowski *et al*. [8] and Ptaszek *et al*. [12] which indicated that irradiation is highly effective in the control of the most dangerous soil-borne pathogens and an environmentally safe method of disinfecting substrates compared to other techniques.

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