

22. INFLUENCE OF MICROCRYSTALLINE CHITOSAN AND *TRICHODERMA VIRIDE* ON POPULATION DYNAMIC OF *PHYTOPHTHORA CRYPTOGEA* AND HEALTHINESS OF GERBERA

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1. Introduction

Phytophthora foot rot of gerbera (*Gerbera jamesonii*), caused by *P. cryptogea* Pethybr. et Laff., is one of the most dangerous disease of that plant grown under covering. The pathogen is also known as the causal agent of root and stem rot of pot flowers and field grown ornamental nursery plants (Orlikowski 1995). Losses caused by *P. cryptogea* varied from a few % sometimes even to 60%. Chemical compounds are mostly used for the disease control. Previous study of Orlikowski (1995) showed, that *Trichoderma* spp. and *Gliocladium roseum* may suppressed the development of *P. cryptogea*. In this study activity of *Trichoderma viride* isolates, obtained from diseased gerbera as well as furalaxyl and chitosan toward *P. cryptogea* and control of *Phytophthora* foot rot of gerbera were studied *in vitro* and in greenhouse trials.

2. Materials and methods

In vitro trials. Isolates of *T. viride* and *P. cryptogea* obtained from gerbera with foot rot symptoms were used in all trials. Stock cultures were maintained on PDA at 24 °C. In *in vitro* bioassays 3 mm diam plugs of both isolates, taken from the edge of 7-day-old cultures, were put on 90 mm Petri dishes 2 cm apart and incubated 6 days at 24 °C. Inhibition zone and overgrown of *P. cryptogea* colonies by *T. viride* was observed during 8 days. Additionally, penetration of *P. cryptogea* hyphae by the mycoparasite was observed under microscope.

Greenhouse trials. In greenhouse trials peat with pH 5.6 was infested with *P. cryptogea* (initial population density = 250 cfu/g of air dry substratum) was mixed with *T. viride* (1,5 x 10⁶ spores/g and 0,3 g of inoculum/g of air dry substratum).

The mycoparasite was applied 7 days before gerbera planting. In trials with chitosan (2% of the commercial product Biochikol 020 PC) the compound at conc. 500 and 1000 $\mu\text{g}/\text{cm}^3$ was applied 7 days before mixing of substratum together with *T. viride* spores. Additionally, the compound was applied singly as substratum drench 7 days before inoculation with *P. cryptogea*. Furalaxyl (a.i. of Fongarid 25 WP) at conc. 125 $\mu\text{g}/\text{cm}^3$ was applied singly as peat drench immediately after gerbera planting. One dm^3 pots filled with infested substratum with gerbera were incubated on greenhouse bench at temperature varying from 17 to 27 °C. Influence of the mycoparasite and 2 tested compounds on population dynamic of *P. cryptogea* (Orlikowski 1995, 1999) and development of *Phytophthora* foot rot of gerbera were studied. Experimental design was completely randomised with 4 replications (5 gerbera plants and 4 Petri dishes in each rep.) Trials were repeated 2 – 3 times.

3. Results

Trichoderma viride inhibited growth and overgrown colonies of *P. cryptogea* within 6 – day-incubation. Mycoparasitism mediated by intimate hyphal interaction was observed.

Application of *T. viride* alone, together with chitosan or this compound as single treatment 7 days before gerbera planting, resulted in the significant decrease of *P. cryptogea* population density (Table 1).

During 2 - 4 weeks of gerbera growth number of colony forming units of *P. cryptogea* in peat infested only with the pathogen (control) increased about 2 - 3 times. When the chitosan was applied 7 days before implementation of *T. viride* into substratum, the pathogen population density decreased within the first 2 weeks about 50-60%. During the next 4 weeks of gerbera growth number of *P. cryptogea* cfu oscillated on the level of 40% (Table 1). Application of chitosan alone was less effective in the suppression of the pathogen development than its mixture with the mycoparasite. Furalaxyl applied as substratum drench, immediately after gerbera planting was the most effective in the inhibition of *P. cryptogea* development (Table 1).

Analyse of relationship between control agents application manner and healthiness of gerbera showed that furalaxyl applied alone was the most effective in the control of *Phytophthora* foot rot (Table 2). The chitosan applied in mixture with *T. viride* significantly decreased the development of the disease. Chitosan at concentration 500 $\mu\text{g}/\text{cm}^3$ decreased number of diseased plants but this effect was not satisfactory. Mixture of chitosan with *T. viride* was significantly more effective than peat application of the biocide alone (Table 2).

4. Conclusions

1. Chitosan applied 7 days before inoculation of substratum with *P. cryptogea* significantly decreased number of colony forming units of *P. cryptogea*. Better effect was obtained when mixture of *T. viride* with chitosan was applied

Table 1. Influence of microcrystalline chitosan and *Trichoderma viride* on population dynamic of *Phytophthora cryptogea*; number of colony forming units (cfu)/ g of air dry peat. Initial population density = 411 cfu/g of peat.

Treatments	Time of application	Reduction of colony forming units (in %) after weeks:		
		1	2	4
Control (peat)	-	480 = 0	720=0	1130 = 0
<i>Trichoderma viride</i>	7 days before gerbera planting	32 b	45 c	41 b
Chitosan 500 µg/cm ³ + <i>Trichoderma viride</i>	Chitosan applied 7 days before <i>T. viride</i> treatment	38 b	53 c	44 b
Chitosan 1000 µg/cm ³ + <i>Trichoderma viride</i>		46 bc	62 cd	42 b
Chitosan 500 µg/cm ³	7 days before substratum inoculation with <i>P. cryptogea</i>	8 a	14 a	11 a
Chitosan 1000 µg/cm ³		12 a	23 ab	14 a
Furalaxyl 125 µg/cm ³	Immediately after planting	75 c	80 d	67 c

Note: Means followed by the same letter do not differ with 5% of significance (Duncan's multiple range test).

Table 2. Influence of chitosan and *Trichoderma viride* on healthiness of gerbera plants.

Treatment	Time of application	Number of diseased plants (n=10) after weeks:		
		4	7	10
Control (peat)	-	1,3 b	6,8 d	9,5 e
<i>Trichoderma viride</i>	7 days before gerbera planting	0,5 a	4,0 bc	6,5 c
Chitosan 500 µg/cm ³ + <i>Trichoderma viride</i>	Chitosan applied 7 days before <i>T. viride</i> treatment	1,3 b	3,8 bc	5,8 c
Chitosan 1000 µg/cm ³ + <i>Trichoderma viride</i>		0,5 a	3,5 b	4,8 b
Chitosan 500 µg/cm ³	7 days before infestation of substratum with <i>P. cryptogea</i>	1,8 b	5,4 c	7,0 cd
Chitosan 1000 µg/cm ³		1,5 b	4,5 c	6,3 c
Furalaxyl 125 µg/cm ³	Immediately after planting	0,0 a	0,0 a	0,8 a

Note: see Table 1.

2. Furalaxyl alone was more effective in the control of *Phytophthora* foot rot than other treatments
3. Mixture of chitosan with *T. viride* applied 7 days before gerbera planting protected about 50% of gerbera.
4. Chitosan applied alone was less effective.

5. References

1. Orlikowski L. B., 1995: Studies on the biological control of *Phytophthora cryptogea*. II. Effectiveness of *Trichoderma* and *Gliocladium* spp. in the control of *Phytophthora* foot rot of gerbera. J. Phytopathology 143: 341 – 343.
2. Orlikowski L. B., 1999: Selective media for the evaluation of biocontrol agents efficacy in the control of soil-borne pathogens. Bull. Pol. Acad. Sci., Biol. Sci 47, 2 – 4: 167 – 172.