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Effect of seed pickling without pesticides on the growth of *Penicillium expansum* and patulin biosynthesis

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The effect of seed pickling without pesticides recommended by the Research Institute of Vegetable Crops in Skierniewice in the method of intensive environment-friendly cultivation on the growth and patulin biosynthesis of patulinogenous microfungus *Penicillium expansum*, was investigated. A complete inhibition of patulin biosynthesis at 6% and no growth at 8% concentration of the dressing in in vitro conditions was revealed.

Key words: *Penicillium expansum*, patulin biosynthesis.

INTRODUCTION

Penicillium expansum Link ex Gray like its metabolite, patulin contaminates both soil and food. The growth of the pathogen as well as patulin biosynthesis is partly or completely inhibited by a number of fungicides commonly used during agrotechnical procedures as well as in storage fruits and vegetables (F l o r i a n o w i c z 1991, 1994, 1995). Due to the irrational usage of fungicides, development of the pathogens resistant to the applied agents as well as soil fatique are observed. Residues of fungicides – even when relatively unstable in the environment (e. g. compounds from the group of dithiocarbaminians) are detected in some of the samples e.g. mainly of greenhouse vegetables (L u d w i c k i 1988). In many cases the products of their disintegration (e.g. cancerogenic ethylenothiourea) pose a serious threat to man and the environment. For this reason, research on introducing restrictions of the usage of fungicides, and replacing them with other effective and less toxic to the consumers and the environment antipathogenic agents is being conducted. In the method of intensive environment-friendly cultivation

(Nowosielski and Macias 1995) synthetic pesticides were replaced with the seed picklings without pesticides. The active substance in seed picklings is active chlorine in statu nascendi in conjunction with garlic and properly selected salts of the nutrient products in lactic acid solution. Their protective role stems from the selection of alimentary ingredients at the concentrations toxic to pathogens but not phytotoxic (Macias and Nowosielski 1993). The aim of the following study was to evaluate the effects of "seed pickling without pesticides – bz" on the growth of patulinogenous microfungus *Penicillium expansum* and its ability to synthesize patulin.

MATERIALS AND METHODS

Penicillium expansum strain No. 729 was obtained from the collection of the Research Institute of Pomology and Floriculture in Skierniewice. The strain was stored on Czapek's agar medium supplemented with yeasts extract (0,2%) and glucose (0,8%) (Burbianka et al. 1983). The "seed pickling without pesticides – bz" preparation produced by "Basma" (Żychlin) was obtained from the Research Institute of Vegetable Crops in Skierniewice.

The fungicide activity of the tested preparation was determined in in vitro conditions based on the degree of inhibition of the fungus linear growth on supplemented Czapek's agar medium with the addition of 1 to 10% of the agent. The inhibition of the increase of the mycelium weight was carried out in the liquid medium. After the ten days, culture of the fungus spores were washed off with 100 cm^3 of 0,9% NaCl supplemented with 0.05% Tween and transferred to liquid medium. The control was a medium without the seed pickling. The culture was grown during 9 days in a moist chamber at room temperature. The mycelium was then separated from the breeding-ground rinsed with ethyl acetate and dried to a constant weight at 105°C. Subsequently the biomass content of fungi was estimated.

The content of patulin in the breeding liquid was detrmined by Sommer's method (Sommer et al. 1974). The linear growth of the fungus in Petri dishes on supplemented Czapek's agar medium containing the seed pickling was analysed. Inoculation was performed by transferring a ring of breeding ground covered by 3-day-old culture of the fungus onto the middle of the medium plate. The rings constituting the inoculum were situated with their surface up. The culture was grown at room temperature. Linear growth was assessed at 24 hour intervals by measuring the diameter of the culture till the whole area was cavered by a control fungus culture. The experiment was repeated three times.

RESULTS AND DISSCUSSION

The linear growth of *P. expansum* in 6-day-old culture on Czapek's agar medium with the seed pickling without pesticides in relation to the control group was investigated (Tab. 1). Accompanying increase of the dressing concentration in the breeding ground below 7%, slowing of the fungus growth was noted. At 7% dressing concentration after 6 days the mean increase of culture diameter was 3–5% of the control cultures. 8% concentration totally inhibited the growth of the fungus culture. Similarly in 9-day-old culture in the liquid medium a gradual slowing of the mycelium weight increase to its total inhibition at 8% concentration of the dressing was observed (Tab. 2).

Table 1

Linear fungus growth on supplemented Czapek's agar medium containing seed pickling without pesticides (expressed in % in comparison to the control)

Concentration of seed pickling without pesticides (%)	Time of growth (days)					
	1	2	3	4	5	6
1	80 100 100	75 80 75	71 100 71	90 93 83	95 93 91	93 100 93
2	50 66 50	75 60 75	71 100 92	90 85 90	95 90 91	77 96 85
3	50 66 66	50 40 50	42 57 52	45 28 45	32 33 35	35 48 50
4	50 66 60	50 60 55	42 50 57	27 35 40	18 23 25	25 29 25
5	50 33 50	50 40 50	28 42 42	27 28 40	13 16 20	12 19 24
6	0 0 0	25 40 40	14 28 28	9 14 16	6 6 7	4 8 7
7	0 0 0	25 20 25	14 14 14	9 7 12	6 3 4	4 3 5
8, 9 or 10	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0

Table 2

The effect of "seed pickling without pesticides – bz" on the biomass and patulin production by *Penicillium expansum* after 9 days of incubation at 21–23°C on the Czapek's medium (mean values after 3 replications)

Concentration of seed pickling without pesticides (%)	Mycelial dry weight		Patulin	
	mg dry wt/100 cm^3 medium	% control	μg/mg mycelial dry wt	% control
0	955		6,5	
1	907	95	8,5	130
2	867	90	7,8	120
3	677	71	3,9	60
4	668	70	2,0	30
5	631	66	1,6	25
6	503	53	0	0
7	458	48	0	0
8	0	0	–	–
9 or 10	0	0	–	–

In the concentrations below 3% stimulation of patulin concentration, while at 3–5% the decrease of toxic accumulation to its total inhibition at 6% were observed. Increased spore production was noted in these concentrations.

The obtained results indicate that 10% solution of the "seed pickling without pesticides – bz" recommended by the Research Institute of Vegetable Crops is an effective agent against *Penicillium expansum*.

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Wpływ „zaprawy bezpestycydowej – bz" na wzrost *Penicillium expansum* i biosyntezę patuliny

Streszczenie

Badano skuteczność działania „zaprawy bezpestycydowej – bz" zalecanej przez Instytut Warzywnictwa w Skierniewicach w intensywnej uprawie przyjaznej środowisku w odniesieniu do patulinogennego grzyba *Penicillium expansum*. W badaniach in vitro wykazano całkowite hamowanie biosyntezy patuliny przy 6%, a brak wzrostu patogena przy 8% stężeniu preparatu.