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USE OF CHEMICAL DRESSING AND POST-CULTURE LIQUIDS OF ANTAGONISTIC BACTERIA IN THE PROTECTION OF RUNNER BEAN (*Phaseolus coccineus* L.)

STOSOWANIE ZAPRAWY CHEMICZNEJ I PŁYNÓW POHODOWLANYCH BAKTERII ANTAGONISTYCZNYCH W OCHRONIE FASOLI WIELOKWIATOWEJ (*Phaseolus coccineus* L.)

Abstract: The purpose of the paper was to establish the protective effect of Zaprawa Oxafun T (active substances: carboxin 37.5 % + tiuram 37.5 %) and post-culture liquids of *Bacillus* sp. Bf 155 and *Pseudomonas* sp. Psf 47 against soil-borne fungi pathogenic towards *Phaseolus coccineus* L. Pre-sowing seed dressing with a chemical preparation or a post-culture liquid of bacteria considerably improved the emergences, healthiness and yielding of bean plants. Despite the pre-sowing seed dressing, plants and seeds obtained after the harvest were infected by *Alternaria alternata*, *Botrytis cinerea*, *Fusarium culmorum*, *Fusarium oxysporum* f. sp. *phaseoli*, *Fusarium solani*, *Phoma exigua*, *Pythium irregulare*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum.* The best protective effect against those plant pathogens was observed for the post-culture liquid of *Pseudomonas* sp. Psf 47.

Keywords: Zaprawa Oxafun T, runner bean, *Bacillus*, *Pseudomonas*, post-culture liquids

Excessive application of chemical preparations contaminates the environment and causes, for example, accumulation of harmful substances in the plants' yield. In the protection of crops, including leguminous plants, chemical methods are more and more frequently replaced by the biological method based on biopreparations [1, 2] or the microbiological material made of antagonistic microorganisms [3–6]. The literature provides information on the control of plant pathogens using antagonistic fungi (*Gliocladium* sp., *Trichoderma* sp.) and bacteria (*Bacillus* sp., *Pseudomonas* sp.), whose activity consists of antibiosis, competition or parasitism [3–6]. In practice, there is a possibility of using post-culture liquids of those antagonists, containing secondary

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metabolites. These metabolites inhibit the mycelium growth, germination of spores and their endospores, and they cause degradation of the cell walls of a number of fungi [7].

The purpose of the paper was to determine the protective effect of Zaprawa Oxafun T and post-culture liquids of *Bacillus* sp. Bf 155 and *Pseudomonas* sp. Psf 47 against soil-borne fungi pathogenic towards *Phaseolus coccineus*.

Material and methods

The object of the studies conducted in the years 2003–2005 on the field of the Experimental Station at Czeslawice near Naleczów were runner bean plants of 'Westa" cv. grown out of the seeds dressed directly before the sowing with post-culture liquids of *Bacillus* sp. Bf 155 and *Pseudomonas* sp. Psf 47 [8]. The bacteria used in the field experiment were from the soil environment of runner bean, and their antagonistic effect towards fungi pathogenic towards this plant was determined according to the method developed by Martyniuk et al [9]. The post-culture liquids of the used antagonistic bacteria was obtained as a result of the bacteria culture on a liquid medium PDB (Difco) at the temperature of 24 ^{o}C kept for 4 days [10]. The studies also considered a combination with chemical seed dressing with Zaprawa Oxafun T (active substances: carboxin 37.5 % + tiuram 37.5 %) in the quantity of 1 g · 100 g^{-1} seeds and a control combination, ie without any dressing. Each experimental combination included 4 plots (4 replications) with the area of 21 m^2, where 100 seeds were sown on each. In each year of studies observations were conducted twice – in the phase of seedlings and at plant anthesis – establishing the number of the grown plants and estimating their healthiness. Plants with distinct necrotic spots on the stem base and on the roots were sampled for a laboratory mycological analysis. After picking up the plants and drying up the seeds, the quantity of the seed yield and the proportion of seeds with spots were determined. The mycological analysis of the plant material and the seeds was conducted according to the method described by Pieta et al [11].

The obtained results concerning the number, healthiness and yielding of plants were analyzed statistically, and the significance of differences was established on the basis of Tukey's confidence intervals [12].

Results and discussion

Post-culture liquids of the applied antagonistic bacteria had a more positive effect on the emergences, healthiness and yielding of runner bean than Zaprawa Oxafun T. The most number of seedlings grew on the plots sown with the seeds soaked in a post-culture liquid of *Pseudomonas* sp. Psf 47 and *Bacillus* sp. Bf 155 (mean 89 and 87 seedlings, respectively) (Table 1). Slightly worse emergences were observed after the application of the chemical preparation (mean 79 seedlings).

The smallest number of seedlings grew on the plot of the control combination, without any seed dressing (mean 60 seedlings). Seedlings with inhibited growth and yellowing leaves occurred on all plots. The proportion of infected seedlings after dressing with Zaprawa Oxafun T or the post-culture liquids of *Bacillus* sp. Bf 155 and

Pseudomonas sp. Psf 47 was relatively small and it ranged from 3.0 % to 3.9 %, on average. A much higher proportion of infected seedlings occurred in the control (10.5 %, on average). In the period of bean anthesis, only small losses of plants and a slight increase of the proportion of infected plants with distinct necrotic spots on the stem base and the roots were noticed (Table 1).

Table 1

Number and healthiness of runner bean plants (mean from 2003–2005)

Experimental combination	Seedlings		Plants at anthesis	
	number of runner bean plants	mean share of infected runner bean plants [%]	number of runner bean plants	mean share of infected runner bean plants [%]
Seeds soaked in post-culture liquids of *Bacillus* sp. Bf 155	87^{bc}*	3.4^{a}	85^{bc}	4.0^{ab}
Seeds soaked in post-culture liquids of *Pseudomonas* sp. Psf 47	89^{c}	3.0^{a}	88^{c}	3.7^{a}
Seeds dresed with Zaprawa Oxafun T	79^{b}	3.9^{a}	77^{b}	5.0^{b}
Control	60^{a}	10.5^{b}	58^{a}	14.6^{c}

* Means in columns differ significantly ($p \leq 0.05$) if they are not marked with the same letter.

The quantity of the obtained seed yield was proportional to the number and healthiness of plants on the plots of individual experimental combinations (Table 2). The highest seed yield was collected from plants after the application of the post-culture liquid of *Pseudomonas* sp. Psf 47, slightly lower – in the combinations with the post-culture liquid of *Bacillus* sp. Bf 155 or Zaprawa Oxafun T. The smallest number of seeds was collected from the plants of the control combination. Small seeds, with spots on the seed cover occurred in the yield. The proportion of such seeds ranged from 4.5 % (in the combination with the post-culture liquid of *Pseudomonas* sp. Psf 47) to 12.0 %, on average (in the control) (Table 2).

Table 2

Yield and healthiness of runner bean seeds (mean from 2003–2005)

Experimental combination	Mean yield of runner bean seeds [g on the plot]	Mean share of infected seeds [%]
Seeds soaked in post-culture liquids of *Bacillus* sp. Bf 155	3320^{bc}*	4.75^{a}
Seeds soaked in post-culture liquids of *Pseudomonas* sp. Psf 47	3568^{c}	4.50^{a}
Seeds dresed with Zaprawa Oxafun T	2956^{b}	5.50^{a}
Control	2017^{a}	12.00^{b}

* Means in columns differ significantly ($p \leq 0.05$) if they are not marked with the same letter.

Table 3

Fungi isolated from infected of runner bean plants (total from 2003–2005)

Fungus species	Number of isolates										
	Seedlings					Plants at anthesis					Total
	Seeds soaked in post-culture liquids		Seeds dresed with Zaprawa Oxafun T	Control	Total	Seeds soaked in post-culture liquids		Seeds dresed with Zaprawa Oxafun T	Control	Total	
	of *Bacillus* sp. Bf 155	of *Pseudomonas* sp. Psf 47				of *Bacillus* sp. Bf 155	of *Pseudomonas* sp. Psf 47				
Acremonium roseum (Oud.) W. Gams	—	—	2	6	8	2	1	5	7	15	23
Alternaria alternata (Fr.) Keissler	3	3	5	8	19	6	5	7	10	28	47
Aspergillus niger van Tiegh	1	1	3	5	10	3	2	5	8	18	28
Botrytis cinerea Pers.	—	—	1	2	3	2	2	3	4	11	14
Cladosporium cladosporioides (Fres) de Vries	3	2	5	8	18	4	3	7	10	24	42
Epicoccum purpurascens Ehr. ex. Schl.	4	3	7	9	23	7	5	8	9	29	52
Fusarium culmorum (W.G. Sm.) Sacc.	3	2	6	7	18	4	3	7	9	23	41
Fusarium oxysporum Schl. f. sp. *phaseoli* Kend. Snyd.	5	4	9	12	30	6	5	10	17	38	68
Fusarium solani (Mart.) Sacc.	3	2	6	9	20	3	3	8	11	25	45
Gliocladium catenulatum Gilman Abbott	2	3	1	—	6	5	8	3	1	17	23

Table 3 contd.

Fungus species	Number of isolates										
	Seedlings					Plants at anthesis					Total
	Seeds soaked in post-culture liquids		Seeds dresed with Zaprawa Oxafun T	Control	Total	Seeds soaked in post-culture liquids		Seeds dresed with Zaprawa Oxafun T	Control	Total	
	of *Bacillus* sp. Bf 155	of *Pseudo-monas* sp. Psf 47				of *Bacillus* sp. Bf 155	of *Pseudo-monas* sp. Psf 47				
Gliocladium roseum Bainier	2	2	1	—	5	4	4	3	1	12	17
Mucor mucedo Fresenius	1	1	3	4	9	2	2	5	7	16	25
Penicillium nigricans (Bain.) Thom.	4	5	3	1	13	5	6	3	1	15	28
Penicillium verrucosum Dierckx var. *cyclopium* (West.) Samson, Stolk et Hadlok	5	6	3	—	14	7	8	5	2	22	36
Phoma exigua Desm.	3	3	5	6	17	4	3	6	7	20	37
Pythium irregulare Buisman	2	2	7	11	22	—	—	—	—	—	22
Rhizoctonia solani Kühn	4	4	6	10	24	4	5	8	12	29	53
Sclerotinia sclerotiorum (Lib.) de Bary	3	2	5	7	17	3	4	7	10	24	41
Trichoderma harzianum Rifai	6	6	3	—	15	7	8	4	2	21	36
Trichoderma koningii Oud.	8	9	5	1	23	11	11	8	4	34	57
Trichoderma viride (Link ex Pers.) Rifai	8	8	4	—	20	9	10	6	2	27	47
Total	70	68	90	106	334	98	98	118	134	448	782

Table 4

Fungi isolated from infected seeds of runner bean (total from 2003–2005)

Fungus species	Number of isolates				
	Seeds soaked in post-culture liquids of *Bacillus* sp. Bf 155	Seeds soaked in post-culture liquids of *Pseudomonas* sp. Psf 47	Seeds dresed with Zaprawa Oxafun T	Control	Total
Acremonium roseum (Oud.) W. Gams	4	2	7	9	22
Alternaria alternata (Fr.) Keissler	6	5	8	14	33
Aureobasidium pullulans (de Bary) Arnaud.	3	3	5	8	19
Botrytis cinerea Pers.	2	2	6	15	25
Cladosporium cladosporioides (Fres) de Vries	4	3	7	11	25
Epicoccum purpurascens Ehr. ex. Schl.	2	—	4	7	13
Fusarium culmorum (W.G. Sm.) Sacc.	6	4	10	12	32
Fusarium oxysporum Schl.	11	10	16	24	61
Fusarium solani (Mart.) Sacc.	4	2	5	9	20
Gliocladium catenulatum Gilman Abbott	6	7	4	2	19
Gliocladium fimbriatum Gilman Abbott	6	4	3	—	13
Mucor hiemalis Wehmer	—	—	3	8	11
Mucor mucedo Fresenius	2	1	5	9	17
Penicillium expansum Link ex S.F.Gray	3	4	2	1	10
Phoma exigua Desm.	8	7	12	19	46
Rhizoctonia solani Kühn	7	6	11	16	40
Rhizopus nigricans Ehrenberg	3	2	5	8	18
Sclerotinia sclerotiorum (Lib.) de Bary	10	8	14	20	52
Trichoderma harzianum Rifai	9	10	5	1	25
Trichoderma koningii Oud.	10	11	4	2	27
Total	106	91	136	195	528

The obtained results confirmed the information on the protective effect of antagonistic microorganisms against infection by plant pathogens [3, 5, 6, 11]. Post-culture liquids of the applied bacteria pointed to the positive effect on the emergences, healthiness and yielding of runner bean, and similar results were also obtained while studying other microorganisms in the biological protection of soybean [8, 11].

The species composition of fungi isolated from the infected seedlings, older plants and the collected seeds of bean as a result of the mycological analysis were similar. However, differences were observed in the quantitative composition of the isolated fungi (Tables 3 and 4). Totally, 334 fungi isolates were obtained from the infected seedlings, 782 – from plants at anthesis and 528 – from bean seeds. The least fungi were isolated after the application of post-culture liquids of *Pseudomonas* sp. Psf 47 or *Bacillus* sp. Bf 155. A little more fungi were obtained using Zaprawa Oxafun T, and the most – from plants of the control combination (Tables 3 and 4). Despite the pre-sowing dressing of the seeds, plants and seeds obtained after the harvest were infected by *Alternaria alternata*, *Botrytis cinerea*, *Fusarium culmorum*, *Fusarium oxysporum* f. sp. *phaseoli*, *Fusarium solani*, *Phoma exigua*, *Pythium irregulare*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum.* The protective effect against those plant pathogens was shown not only by Zaprawa Oxafun T but also by the post-culture liquids of the used antagonistic bacteria, with *Pseudomonas* sp. Psf 47 being the most effective. It should be supposed that the high effect of the post-culture liquid of those bacteria consisted in the activity of secondary metabolites such as antibiotics, enzymes, siderophores and many other substances inducing plants' resistance to infection by plant pathogens [4–7, 10]. In practice, therefore, the application of chemical preparations can be reduced for the benefit of antagonistic microorganisms and their post-culture liquids.

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STOSOWANIE ZAPRAWY CHEMICZNEJ I PŁYNÓW POHODOWLANYCH BAKTERII ANTAGONISTYCZNYCH W OCHRONIE FASOLI WIELOKWIATOWEJ (*Phaseolus coccineus* L.)

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Abstrakt: Celem pracy było określenie skuteczności ochronnego działania Zaprawy Oxafun T (substancja aktywna: karboksyna 37.5 % + tiuram 37.5 %) oraz płynów pohodowlanych *Bacillus* sp. Bf 155 i *Pseudomonas* sp. Psf 47 przeciwko patogenicznym dla *Phaseolus coccineus* grzybom przeżywającym w glebie.

Przedsiewne zaprawianie nasion, preparatem chemicznym lub płynem pohodowlanym bakterii, znacznie poprawiło wschody, zdrowotność oraz plonowanie roślin fasoli. Mimo przedsiewnego zaprawiania nasion, rośliny oraz uzyskane po zbiorze nasiona fasoli były porażane przez *Alternaria alternata*, *Botrytis cinerea*, *Fusarium culmorum*, *Fusarium oxysporum* f. sp. *phaseoli*, *Fusarium solani*, *Phoma exigua*, *Pythium irregulare*, *Rhizoctonia solani* i *Sclerotinia sclerotiorum.* Najskuteczniejszym ochronnym działaniem przed tymi fitopatogenami wyróżnił się płyn pohodowlany *Pseudomonas* sp. Psf 47.

Słowa kluczowe: Zaprawa Oxafun T, runner bean, *Bacillus*, *Pseudomonas*, post-culture liquids