20. INFLUENCE OF BIOCHIKOL 020 PC USED AS SEED DRESSING OF BEAN ON HEALTHINESS AND YIELD OF PLANTS

Danuta Pięta, Elżbieta Patkowska, Alina Pastucha

Department of Plant Pathology,
University of Agriculture in Lublin, Poland,
e-mail: danuta.pieta@ar.lublin.pl

1. Introduction

Biopreparations which are used to protect the plants from pathogens and which are based on natural organic components or antagonistic microorganisms are safe for the environment and they do not cause any contamination. Biochikol 020 PC is a stimulator of plants' resistance. It is produced on the basis on chitosan and it is intended to protect vegetable and ornamental plants from virus, bacterial and fungal diseases.

It follows from earlier studies on the use of chitosan in seed dressing and plant spraying of soybean and bean that it had a positive effect on the numbers, healthiness and yielding [1 - 3]. According to Orlikowski et al. [4], Wojdyła [5], chitosan used for dressing the bulbs and onions of ornamental plants as well as for spraying them was effective in protecting those plants from pathogenic fungi from genus *Fusarium.*, especially its special forms *F. oxysporum.* as well as *Phytophthora. s*pp., *Sphaerotheca pannosa* var. *rosae* or *Peronospora sparsa* and *Botrytis* spp. Amborabe et al. [6], Bautista et al. [7] showed that chitosan limited the growth of *Botrytis cinerea* on the berries of grapes and *Colletotrichum gleosporioides* on papaya fruit. The studies carried out by Borkowski et al. [8, 9] found out that chitosan applied as an active substance of Biochikol 020 PC limited the growth of *Peronospora destructor* on onion. On the other hand, when it was used to spray tomato plants it caused an increase of the fruit yield.

The purpose of the present studies was to determine the effect of Biochikol 020 PC on the healthiness and yielding of pea, common bean and runner bean.

2. Material and methods

The studies were conducted in the years 2003-2004 in the area of the Experimental Station in Czesławice near Nałęczów, on the fields of monoculture of pea (cv. SześciotygodniowyTor) of common bean (cv. Narew) and runner bean (cv. Westa) with naturally accumulated infection material in the soil. It was established on the basis of earlier microbiological studies that the soil after the cultivation of particular plant species was colonized by *Ascochyta pisi, Botrytis cinerea,, Fusarium culmorum, Fusarium solani,* the special forms of *Fusarium oxysporum* f. sp. *phaseoli, Fusarium oxysporum* f. sp. *pisi, Phoma exigua, Pythium irregulare, Rhizoctonia solani* and *Sclerotinia sclerotiorum*. Before the sowing the seeds of particular plant species were dressed with a 2.5% solution of Biochikol 020 PC. The experiment also considered a control combination, i.e. without any seed dressing and a combination with chemical dressing of the seeds with Zaprawa Oxafun T. Each experimental combination considered 4 plots (four repetitions), where 100 seeds were sown on each. The second treatment, which was spraying the plants with a biopreparation or Bravo Plus 500 SC, was performed at the beginning of anthesis. Field observations were performed during the experiment, in the phase of seedlings and at anthesis. Those experiments established the numbers and healthiness of plants in particular combinations. Seedlings as well as older plants inhibited in their growth, with necrosis on the roots and the stem base, were subject to a mycological analysis according to Pięta [10].

After the harvest the size and quality of the seeds yield in particular species of *Fabaceae* plants were determined. For this purpose, 100 spot-free seeds and 100 seeds with spots on the seeds cover were subject to mycological analysis, which was done for each plant species and each experimantal variant.

The results concerning the numbers and healthiness of plants and the yield were statistically analyzed on the basis of Duncan's semi-intervals [11].

3. Results and discussion

Field observations in the phase of seedlings showed that in the case of pea the effect of Biochikol 020 PC matched that of Zaprawa Oxafun T, since the number of seedlings in those species was similar. On the other hand, on the plots sown with the seeds of common bean and runner bean the studies observed more seedlings in the combination with the chemical dressing of the seeds as compared with the application of the biopreparation (Table 1). The smallest number of seedlings, with the greatest number of the infected ones, was found in the control combination of all the examined species of plants (Table 1). The results concerning bean confirm earlier studies [3].

Field observations conducted at anthesis showed that the numbers of particular species were bigger in the case of the application of chemical dressing of seeds with Zaprawa Oxafun T than after using Biochikol 020 PC (Table 2). On the other hand, after the biopreparation was applied to common bean the studies found out fewer infected plants as

Table 1. *Number and healthiness of seedlings from Fabaceae family plants (means from the years 2003-2004).*

Experimental combination	Number of seedlings on the plot	Participation of infected seedlings
Pea		
Biochikol 020 PC	89*b	1.7a
Zaprawa Oxafun T	89b	3.1a
Control	69a	7.15b
Common bean		
Biochikol 020 PC	82.5b	3.5a
Zaprawa Oxafun T	84b	3.5a
Control	58.5a	8.3b
Runner bean		
Biochikol 020 PC	80b	3.1a
Zaprawa Oxafun T	88.5b	3.5a
Control	70.5a	5.9b

compared to Zaprawa Oxafun T. The latter proved more effective in its protective effect in the other plants. The greatest number of infected plants was observed on the plots of the control combination (Table 2).

The mycological analysis of the infected seedlings of the studied species of *Fabaceae* plants showed that *Fusarium oxysporum, F. culmorum, F. solani, Pythium irregulare* and *Rhizoctonia solani* were most frequently isolated among the pathogenic fungi (Table 3). The greatest number of these fungi was isolated from the seedlings taken from the control combination, while fewer from the combination after dressing the seeds with Biochikol 020 PC and Zaprawa Oxafun T (Table 3). This fact can be explained by the protective effect of chitosan, which is an element of Biochikol 020 PC, before

Table 2. *Number and healthiness of Fabaceae family plants at anthesis (means from the years 2003-2004).*

Experimental combination	Number of plants on the plot	Participation of infected plants
Pea		
Biochikol 020 PC	80.5*b	4.0a
Zaprawa Oxafun T	84.5b	3.75a
Control	66.5a	8.5b
Common bean		
Biochikol 020 PC	76.5b	6.5a
Zaprawa Oxafun T	79.5b	7.2a
Control	55a	9.8b
Runner bean		
Biochikol 020 PC	80b	4.3ab
Zaprawa Oxafun T	85b	3.95a
Control	67.5a	7.0b

** mean in columns fellowed by the same letter do not differ significantly at $P \leq 0.05$*

Table 3. *Fungi isolated from infected seedlings of the investigated Fabaceae family plants (sums of isolates from the years 2003-2004).*

Fungus species	Experimental combination / Number of isolates									Total
	Pea			Common bean			Runner bean			
	B	Z	K	B	Z	K	B	Z	K	
Acremonium roseum (Oud.)	7	8	14	3	13	15	8	6	6	80
Alternaria alternata (Fr.) Keissler	11	9	22	7	20	23	20	14	23	149
Alternaria tenuissima (Fres.) Wiltshire	1	2	12	-	-	-	-	-	-	15
Aspergillus flavus Link	-	-	-	3	3	4	2	9	12	33
Aspergillus niger van Tiegh	6	10	17	-	-	-	2	-	6	41
Botrytis cinerea Pers.	-	-	-	-	-	-	2	7	13	22
Cladosporium cladosporioides (Fres.) de Vries	4	11	5	1	10	7	-	4	-	42
Cladosporium herbarum (Pers.) Link	-	-	-	-	-	-	5	1	-	6
Epicoccum purpurascens (Ehr. ex. Schl.)	1	-	2	1	14	11	2	5	15	51
Fusarium culmorum (W. G. Sm.) Sacc.	6	15	35	6	25	21	6	6	11	131
Fusarium oxysporum Schl.	22	20	90	25	22	94	35	36	73	417
Fusarium solani (Mart.) Sacc.	12	12	43	5	5	28	8	11	31	155
Gliocladium catenulatum Gilman Abbott	-	-	-	4	3	8	29	6	3	53
Gliocladium roseum Bainier	-	-	-	23	3	3	3	3	2	37
Humicola grisea Domsch	-	-	-	-	-	-	2	5	-	7
Mucor hiemalis Wehmer	12	14	-	4	20	18	3	12	-	83
Mucor mucedo Fresenius	-	-	-	-	-	-	7	6	2	15
Papulaspora irregularis Hotson	-	-	-	-	-	-	2	4	-	6
Penicillium canescens Thom	-	-	-	3	2	4	3	5	1	18
Penicillium chrysogenum Thom	-	-	-	3	2	4	1	11	1	22
Penicillium notatum Westling	-	-	-	5	1	16	7	13	-	42
Penicillium nigricans (Bain.) Thom	2	4	4	12	9	12	-	-	-	43
Penicillium purpurogenum Stoll	11	4	17	2	4	8	-	-	-	46

Table 3 a. *Fungi isolated from infected seedlings of the investigated Fabaceae family plants (sums of isolates from the years 2003-2004).*

Fungus species	Experimental combination / Number of isolates									Total
	Pea			Common bean			Runner bean			
	B	Z	K	B	Z	K	B	Z	K	
Penicillium verrucosum Dierckx var. cyclopium (West.) Samson, Stolk et Hadlok	13	17	27	26	15	25	19	12	20	174
Penicillium verrucosum Dierckx var. verrucosum Samson, Stolk et Hadlok	-	-	-	4	2	22	-	-	-	28
Phoma eupyrena Sacc.	-	-	-	-	-	-	2	6	1	9
Pythium irregulare Buisman	12	10	27	21	9	13	4	28	27	151
Rhizoctonia solani Kühn	10	7	29	4	1	22	9	11	23	116
Rhizopus nigricans Ehrenberg	-	-	-	-	5	6	5	15	19	50
Trichoderma hamatum (Bonord.) Bain.	-	-	-	-	-	-	10	2	1	13
Trichoderma harzianum Rifai	9	3	1	16	13	1	17	8	-	68
Trichoderma koningii Oud.	15	8	5	19	5	1	14	7	-	74
Trichoderma polysporum (Link ex. Pers.) Rifai	-	-	-	2	4	4	-	-	-	10
Trichoderma viride Pers. ex. S. F. Gray	6	6	1	-	-	-	12	5	4	34
Verticillium albo-atrum Reinke & Berth	7	4	2	-	-	-	-	-	-	13
Total	167	164	353	199	210	370	239	258	294	2254

B – Biochikol 020 PC; Z – Zaprawa Oxafun T; K –Control

the infection of the studied *Fabaceae* plants. This compound belongs to the elicitors inducing plants' resistance to infection by pathogens. This type of effect of chitosan was pointed at by Orlikowski et al. [12, 13], Pospieszny [14]. Besides, fungi from the genera *Alternaria, Aspergillus, Cladosporium, Mucor, Penicillium* and *Acremonium roseum, Epicoccum purpurascens, Humicola grisea, Rhizopus nigricans* were obtained from the seedlings. The above-mentioned fungi were not always isolated in all the samples of the studied plant material (Table 3). Among saprophytic fungi the fungi of antagonistic effect *Gliocladium s*pp. and *Trichoderma* spp. were also isolated. Those fungi were most frequently isolated in the combination after the application of Biochikol 020 PC as compared to the other experimental combinations (Table 3).

The mycological analysis of the infected parts of plants at anthesis showed that they were infected by *Botrytis cinerea, Fusarium culmorum, F. oxysporum, F. solani, Phoma exigua, Rhizoctonia solani* and *Sclerotinia sclerotiorum* (Table 4). *Fusarium avenaceum* was also isolated from the infected plants, but only in case of pea, while *Fusarium poae* was isolated from the infected plants of pea and common bean (Table 4). *Gliocladium* spp. and *Trichoderma* spp. were isolated from all the species of *Fabaceae* plants at anthesis. The smallest number of those fungi was isolated from the control combination (Table 4). The increase of the number of the above-mentioned fungi isolated from *Fabaceae* plants after the application of Biochikol 020 PC can be explained by the stimulating effect of this compound (chitosan) on the growth of the biomass of chitinolitic fungi [15]. Besides, *Penicillium* spp., *Cladosporium s*pp. and *Aspergillus* spp. were isolated from the infected parts of *Fabaceae* plants at anthesis.

After the harvest the studies established the yield and healthiness of plants on particular plots. The highest yield was observed in all the plant species growing in the control combination, with the greatest proportion of infected seeds (Table 5). On the other

Table 5. *Yield and healthiness of seeds from Fabaceae family plants (means from the years 2003-2004).*

Experimental combination	Yield of seeds in g on the plot	Participation of infected seeds
Pea		
Biochikol 020 PC	297.5*^{ab}	5.1^{ab}
Zaprawa Oxafun T	305.0^{b}	4.2^{a}
Control	211.5^{a}	9.3^{b}
Common bean		
Biochikol 020 PC	597.5^{b}	4.2^{a}
Zaprawa Oxafun T	645.0^{b}	3.0^{a}
Control	384.0^{a}	6.5^{b}
Runner bean		
Biochikol 020 PC	3592.5^{b}	6.4^{ab}
Zaprawa Oxafun T	3649.5^{b}	3.9^{a}
Control	2214.5^{a}	8.75^{b}

* *mean in columns fellowed by the same letter do not differ significantly at* $P \leq 0.05$.

Table 4. *Fungi isolated from the investigated plants of Fabaceae family at anthesis (sums of isolates from the years 2003-2004).*

Fungus species	Experimental combination / Number of isolates									Total
	Pea			Common bean			Runner bean			
	B	Z	K	B	Z	K	B	Z	K	
Acremonium roseum (Oud.)	2	5	7	5	5	-	2	5	-	31
Alternaria alternata (Fr.) Keissler	2	1	2	2	8	7	5	16	13	56
Aspergillus flavus Link	-	-	-	-	-	-	-	5	4	9
Aspergillus niger van Tiegh	-	-	-	12	7	-	2	6	-	27
Botrytis cinerea Pers.	-	7	11	4	11	10	-	6	15	64
Cladosporium cladosporioides (Fres.) de Vries	2	6	3	7	1	-	8	1	3	31
Cladosporium herbarum (Pers.) Link	3	5	10	-	-	-	-	-	-	18
Epicoccum purpurascens (Ehr. ex. Schl.)	-	3	-	3	1	-	1	1	-	9
Fusarium avenaceum (Corda ex. Fr. Sacc.)	5	6	7	-	-	-	-	-	-	18
Fusarium culmorum (W. G. Sm.) Sacc.	5	2	4	9	22	20	5	10	16	93
Fusarium oxysporum Schl.	49	54	44	42	40	64	36	43	59	431
Fusarium poae (Peck.) Wollenw.	1	-	9	1	4	6	-	-	-	21
Fusarium solani (Mart.) Sacc.	4	11	15	7	8	9	4	2	4	64
Gliocladium catenulatum Gilman Abbott	2	-	-	8	1	-	3	3	-	17
Gliocladium roseum Bainier	8	1	2	9	4	5	8	-	1	38
Humicola grisea Domsch	3	-	-	3	1	-	1	-	-	8
Mucor hiemalis Wehmer	3	2	1	8	1	1	5	1	-	22
Penicillium canescens Thom	1	5	4	3	5	5	-	-	-	23
Penicillium chrysogenum Thom	6	4	-	-	1	-	-	1	-	12
Penicillium expansum Link ex S. F. Gray	2	-	1	3	1	-	-	-	1	8

Table 4a. *Fungi isolated from the investigated plants of Fabaceae family at anthesis (sums of isolates from the years 2003-2004).*

Fungus species	Experimental combination / Number of isolates									Total
	Pea			Common bean			Runner bean			
	B	Z	K	B	Z	K	B	Z	K	
Penicillium verrucosum Dierckx var. cyclopium (West.) Samson, Stolk et Hadlok	4	1	1	3	-	1	7	1	3	21
Penicillium verrucosum Dierckx var. verrucosum Samson, Stolk et Hadlok	2	6	4	3	5	1	-	-	-	21
Phoma eupyrena Sacc.	4	3	6	-	-	-	-	4	8	25
Phoma exigua Desm.	-	-	-	2	6	9	1	8	8	34
Rhizoctonia solani Kühn	1	9	5	4	7	12	4	11	17	70
Rhizopus nigricans Ehrenberg	1	7	-	1	1	-	4	2	3	19
Sclerotinia sclerotiorum (Lib.) de Bary	4	6	7	4	7	8	2	5	4	47
Trichoderma hamatum (Bonord.) Bain.	4	-	-	5	1	1	-	1	-	12
Trichoderma harzianum Rifai	6	3	1	6	4	2	7	5	4	38
Trichoderma koningii Oud.	8	3		4	4	3	9	4	4	39
Trichoderma viride Pers. ex. S. F. Gray	-	2	-	3	4	4	1	-	2	16
Total	138	159	149	164	164	169	118	149	178	1388

Biochikol 020 PC; Z – Zaprawa Oxafun T; K –Control

Table 6. *Fungi isolated from the seeds of Fabaceae family plants (sums of isolates from the years 2003-2004)*

Fungus species	Experimental combination / Number of isolates																		Total
	Pea						Common bean						Runner bean						
	B		Z		K		B		Z		K		B		Z		K		
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
Acremonium roseum (Oud.)	-	-	-	-	-	-	-	-	2	1	3	1	-	-	-	-	2	-	9
Alternaria alternata (Fr.) Keissler	4	2	4	1	5	2	3	2	3	4	3	1	10	6	10	4	19	9	92
Ascochyta pisi Libert	2	-	4	-	4	1	-	-	-	-	-	-	-	-	-	-	-	-	11
Aspergillus niger van Tiegh	-	-	-	-	-	-	1	-	3	1	4	2	4	1	-	-	3	2	21
Botrytis cinerea Pers.	4	1	7	3	10	6	3	1	6	2	8	4	13	5	14	6	18	8	119
Cladosporium cladosporioides (Fres.) de Vries	2	-	4	1	4	2	2	2	4	2	3	1	-	-	2	1	1	-	31
Cladosporium herbarum (Pers.) Link	-	-	2	-	1	-	-	-	2	1	-	-	-	-	-	-	-	-	6
Epicoccum purpurascens (Ehr. ex. Schl.)	3	-	1	-	3	1	1	-	2	1	2	2	13	3	8	4	14	1	59
Fusarium avenaceum (Corda ex. Fr. Sacc.)	-	-	-	-	-	-	-	-	-	-	-	-	2	1	5	3	8	4	23
Fusarium culmorum (W. G. Sm.) Sacc.	2	2	6	4	9	4	4	2	7	5	11	5	14	5	16	6	19	8	129
Fusarium oxysporum Schl.	1	1	7	1	12	2	5	2	9	3	13	5	23	5	25	10	33	11	168
Fusarium solani (Mart.) Sacc.	-	-	-	-	-	-	-	-	2	1	4	2	7	2	9	5	12	8	52
Fusarium sporotrichioides Sherb.	-	-	-	-	-	-	-	-	-	-	5	2	-	-	-	-	-	-	7
Gliocladium catenulatum Gilman Abbott	2	4	-	1	-	-	2	3	1	-	-	1	1	4	-	1	-	-	20
Humicola grisea Domsch	-	-	-	-	-	-	2	-	2	1	-	-	2	-	4	1	11	3	26
Mucor hiemalis Wehmer	-	-	5	-	2	3	2	1	1	-	2	-	9	1	9	-	11	5	51
Papulaspora sphaerosperma (Persoon) von Höhnel	3	1	3	1	4	1	2	-	-	-	2	-	-	-	-	-	-	-	17
Penicillium canescens Thom	-	-	-	-	-	-	-	-	-	-	3	1	2	1	4	2	6	1	20

Table 6a. *Fungi isolated from the seeds of Fabaceae family plants (sums of isolates from the years 2003-2004)*

Fungus species	Experimental combination / Number of isolates																		Total
	Pea						Common bean						Runner bean						
	B		Z		K		B		Z		K		B		Z		K		
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
Penicillium canescens Thom	-	-	-	-	-	-	-	-	-	-	3	1	2	1	4	2	6	1	20
Penicillium chrysogenum Thom	-	-	-	-	-	-	1	-	3	1	-	-	-	-	-	-	-	-	5
Penicillium expansum Link ex S. F. Gray	-	2	-	-	3	-	1	-	4	-	3	1	3	1	3	2	4	3	30
Penicillium notatum Westling	-	-	2	-	-	-	2	-	3	-	4	-	2	-	2	-	3	1	19
Penicillium nigricans (Bain.) Thom	-	-	-	-	-	-	1	-	3	1	4	-	-	-	4	2	5	3	23
Penicillium verrucosum Dierckx var. cyclopium (West.) Samson, Stolk et Hadlok	1	-	3	1	3	2	-	-	3	-	3	1	-	-	-	-	-	-	17
Phoma exigua Desm.	-	1	3	1	4	-	-	-	3	1	4	2	-	-	-	-	3	1	23
Rhizoctonia solani Kühn	12	1	15	4	17	5	1	-	6	3	8	1	3	1	5	1	7	4	94
Rhizopus nigricans Ehrenberg	-	-	2	1	1	1	-	-	3	1	-	-	-	-	-	-	-	-	9
Sclerotinia sclerotiorum (Lib.) de Bary	-	-	3	1	5	2	2	-	3	2	6	2	9	2	15	4	21	7	84
Trichoderma hamatum (Bonord.) Bain.	2	4	-	1	-	-	-	-	-	-	-	-	-	4	-	-	1	-	12
Trichoderma koningii Oud.	3	3	1	-	-	1	4	5	-	1	-	1	2	5	1	2	-	2	31
Torula herbarum Link	1	-	-	-	3	1	-	-	-	-	-	-	-	-	3	1	-	-	9
Razem	42	22	72	21	90	34	39	18	75	32	95	35	119	47	139	55	201	81	1217

B – Biochikol 020 PC; Z – Zaprawa Oxafun T; K – Control, 1-seeds with spots, 2-seeds without spots

hand, the best yield, with the lowest proportion of infected seeds, was obtained from the plants of pea, common bean and runner bean after the application of Zaprawa Oxafun T. The size and quality of the yield from the plants grown from the seeds dressed with Biochikol 020 PC were slightly smaller as compared to the combination with Zaprawa Oxafun, the difference being statistically insignificant (Table 5).

The mycological analysis of the seed samples obtained from particular plots isolated 1217 fungi colonies belonging to 31 species (Table 6). Approximately three times as many fungi colonies were obtained from the spotted seeds as from the seeds without the spots. *Fusarium oxysporum* was most frequently isolated from the examined seeds. Besides, *Fusarium culmorum, Botrytis cinerea, Alternaria alternata* and *Sclerotinia sclerotiorum* were also isolated (Table 6). These fungi not only decrease the size and quality of the yield but they also decrease their vitality, besides causing contamination with mycotoxins [16]. Those species were isolated much more frequently from the seeds with spots as compared to the seeds without spots (Table 6).

The results obtained from the plots of a small area showed a positive protective effect of Biochikol 020PC against the fungi colonizing the soil environment. It is recommended that such an experiment should be repeated on large area plantations of particular plant species. Results obtained by other researchers and the ones achieved in the present studies pointed to a long-term protective effect of chitosan. According to Wojdyła and Orlikowski [17], chitosan effectively protected carnation plants from the soil-borne pathogens, and especially from *F. oxysporum* f. sp. *dianthi* even after ten weeks. A similar effect of chitosan was found out in the case of gerbera protection from *Phytophthora cryptogea* Pethybr. et. Laff [18]. It should be supposed that on the one hand, chitosan acts as an elicitor stimulating the plants' resistance to the infection by phytopathogens, and on the other hand, it has a stimulating effect on the development of antagonists, for example *Trichoderma* spp. [14, 18, 19].

4. References

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Acknowledgments

The studies in the year 2004 were financed by the Ministry of Science and Information within the project No 3P06 034 25.