P L A N T B R E E D I N G A N D S E E D S C I E N C E
Volume 52 2005

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ANTAGONISTIC ACTIVITY OF SOME FUNGI AND *ACTINOMYCETES* AGAINST PATHOGENS OF DAMPING-OFF OF SUGAR BEET SEEDLINGS

ABSTRACT

The antagonistic activity of some Biological Control Agents (BCA) isolates of *Trichoderma* sp., *Penicillium* sp., *Gliocladium* sp., and *Actinomycetes* - *Streptomyces* sp. against pathogens of sugar beet damping-off: *Aphanomyces cochlioides, Pythium debaryanum* and *Phoma betae* was tested *in vivo* and *in vitro*. Fourty-nine isolates of fungi and seventy four of *Actinomycetes* were isolated from compost and soil in which those pathogens did not occur. The microorganisms effective against at least two pathogens *in vitro* tests were incorporated into the soil or added to the seed as a treatment during pelleting process. The microorganisms used *in vivo* tests limited the number of infected seedlings. Some of them protected emerging plants on the same level as fungicides commercially used for seed treatment.

Key words: BCA, damping-off, pathogens, sugar beet

INTRODUCTION

The use of chemical means in plant protection has been the most effective method limiting losses caused by agrophages. It is well known that this method requires introducing new, more effective substances since pathogens and pests develope resistance to the applied chemical means. A common use of the chemicals for plant protection affects the degradation of environment and first of all, water and soil pollution and decreases food quality due to unfavourable phytotoxic effects. For this reason in some countries legal limitations were imposed to deminish their use up to 50-60% in plant protection (Sobótka 1996). Such decision without introduction of alternative methods of plant protection would result in yield decrease. An introduction of Integreated Pest Menagement (IPM) is the only solution of effective crop production in which all available ways and methods in controlling agrophages are applied. A part of IPM system are biological methods where live organisms or their metabolites are used to restrict or destroy the population of plant pests and pathogens. So far due mainly to low effectiveness and high costs, methods based on exploitation of microor-

Communicated by Edward Arseniuk

ganisms in contrrolling phytopathogenic fungi were not introduced into practice. It should not be ruled out the use of some microorganisms according to the strategy of integrated pest menagement for instance *Trichoderma harzianum* or *Pseudomonas* sp. (Pospieszny 2000). One of the requirements of sugar beet cultivation is an effective protection against damping-off caused by *Aphanomyces cochlioides* Dreschler and *Pytium debaryanum* Hesse- soil-born fungi and seed-born *Phoma betae* Frank. Chemical treatment with high rates of fungicides (Oxafun T 75 WS/DS (6 g per 1 kg seeds) and Tachigaren 70 WP (15 g per 1 kg seeds) for seedling protection are used. In Poland sugar beet is cultivated on the acreage of about 300.000 ha, which means about 20 t of fungicides are incorporated into the soil every year.

The aim of this work was the selection of microorganisms antagonistic to the pathogens of sugar beet damping-off, *A. cochlioides, P. betae* and *P. debaryanum* after including them into pellets.

MATERIAL AND METHODS

The microorganisms for tests were isolated by Warcap's method (after Kiraly *et al.1*977). Samples of soil from monoculture crops of maize, wheat, pea, sugar beet, and from compost were taken. All these soils were artificially inoculated with mycelium of damping-off pathogens of sugar beet seedlings. In spite of this no pathogens were detected in these soils.

The Potato Dextro Agar medium with antibiotics was used for isolating of fungi. *Actinomycetes* were isolated on selective Küster-Williams medium with antibiotics restricting the growth of fungi and Gram-negative bacteria. Fourty-nine isolates of fungi from *Trichoderma* sp, *Penicillium* sp., and *Gliocladium* sp, and seventy-four of *Actinomycetes* isolates - *Streptomyces* sp. were obtained. Isolates of sugar beet pathogens originated from the Plant Breeding and Acclimatization Institute Department in Bydgoszcz collection.

Estimation of antagonistic efficacy of fungal and bacterial isolates against *A. cochlioides*, *P. debaryanum* and *P. betae in vitro*.

The activity of isolated microorganisms against *A. cochlioides*, *P. debaryanum* and *P. betae* was checked *in vitro* tests. *Actinomycetes* were inoculated on Petri dishes with PDA (pH=7.0) and incubated 7 days in 25°C. Then the discs of mycelium of pathogens were put on these plates. They were always put in the same distance from *Actinomycetes* colonies.

Fungi isolated from soil and pathogenic fungi were incubated on PDA medium in 25ºC for 9 days. Then a 5 mm in diameter discs of mycelium were cut-off from the edge of colony and put on PDA medium in Petri dishes. A constant distance was always kept between them. Pathogen growing alone on a dish was a control for both, Actinomycetes and fungi tests. Each test was done in 10 replications. All cultures were incubated in 25°C. The activity of *Actinomycetes* was estimated after 48 h, and activity of fungi after 3 days and 7 days of incubation.

The level of infection was recorded as a difference between diameter of colony of fungus growing in presence of tested BCA and fungus growing alone on

medium. This difference was counted in percent as an inhibition index. The antagonistic activity of BCA was estimated on a following scale (Gorlach 1995, Lewińska 1998):

1 - 0% - no activity
2 - 1-25% - weak activity
3 - 26-50% - middle activity
4 - 51- 75% strong activity
5 - 76-100% - very strong activity

Estimation of chosen BCA efficacy against *A. cochlioides*, *P. debaryanum* and *P. betae* in greenhouse.

The most effective BCA from *in vitro* tests were checked against *A. cochlioides*, *P. betae* and *P. debaryanum* in greenhouse. The BCA were introduced into the sterilised soil. Two days before sowing seeds antagonistic microorganisms were introduced into soil and at time of sowing, seeds of sugar beet were infected with certain amounts (2.9×10^8 of spore in one ml) of homogenised mycelium of *A. cochlioides* or *P. debaryanum*. Microorganisms for investigations were produced on liquid media (PDA for fungi and Küster's and Williams' for *Actinomycetes*) over 9 days. For planting untreated seeds variety Eureka were used. In experiments there were two check treatments; without pathogens, with soil inoculated pathogens. In a first check seeds were treated with Oxafun T+/Tachigaren at the rate of 6/15 g per 1 kg of seeds respectively, in the second treatment untreated seeds were used.

In other experiments performed in greenhouse with 7 isolates of fungi and 1 isolate of *Actinomycetes*, selected on previously results, were investigated. For experiments pelleted seed of variety Janina was used. Microorganisms were added to pelleting mass in two forms: two hundred ml of BCA in a liquid stage (2.9×10^8 of spore in one ml) was used per a unit of seeds (a unit = 100 000 seeds).BCA in dried form was also used for pelleting. Then 2 g of dried and powdered mycelium of BCA was added into pellet per one unit of seed. Treated with fungicides and untreated pelleted seeds were used as a check. The laboratory germination was estimated for all samples of seeds. The BCA was reisolated from pellets 1, 7, 15, and 30 days after pelleting process. Its activity against damping-off pathogens was examined every time. Pelleted seed were put on sterile PDA and after 9 days cultured microorganisms were determined.

RESULTS

Influence of tested BCA, Streptomyces and some *Trichoderma* sp., *Penicillium* sp., and *Gliocladium* sp. fungi on growth of *A. cochlioides*, *P. betae* and *P. debaryanum in vitro*

Out of the seventy-four isolates of tested *Actinomycetes*, three showed a very strong and one a strong inhibition activity against *A. cochlioides*. One isolate revealed a very strong and five strong activity against *P. betae*. A growth of *P. debaryanum* was inhibited in a high degree by three isolates of *Actinomycetes* (Table 1). One isolate (S14) showed a strong activity against *P. debaryanum* and very strong inhibition against *A. cochlioides* and *P. betae.*

Table 1

Antagonistic activity of tested isolates of *Streptomyces* on the growth of fungi pathogenic for sugar beet seedlings

Degree of scale	Inhibition	Number of tested isolates inhibited		
		Phoma betae	*Aphanomyces cochlioides*	*Pythium debaryanum*
1	lack	29	29	32
2	weak	17	38	40
3	middle	23	4	0
4	strong	5	1	3
5	very strong	1	3	0
Total		75	75	75

Forty-nine isolates of different fungi were tested. A very strong inhibition of growth of *A. cochlioides* was observed for two isolates and strong for four isolates after 3 days of incubation and the same isolates inhibited strongly *A. cochlioides* after 7 days post inoculation. Six isolates showed strong inhibition activity against *P. betae* after 3 days. Only one isolate showed a strong efficacy against *P. betae* but eighteen were strongly active against this pathogen after seven days of incubation. Four isolates exhibited a very strong activity inhibition against *P. debaryanum* 3 days and seven days of incubation (Table 2). All fungi, which showed positive activity against growth of pathogens mycelium, belong to *Trichoderma* sp. The most numerous group of fungi and *Actinomycetes* composed neutral isolates and isolates of a weak antagonistic activity.

Tabela 2

Antagonistic activity of fungal BCA on the growth of fungi pathogenic for sugar beet seedlings

Degree of scale	Inhibition	Number of tasted isolates inhibited					
		Phoma betae		*Aphanomyces cochlioides*		*Pythium debaryanum*	
		after 3 days	after 7 days	after 3 days	after 7 days	after 3 days	after 7 days
1	lack	24	23	24	20	24	22
2	weak	9	5	18	4	18	20
3	middle	14	2	1	19	3	3
4	strong	2	18	4	6	4	4
5	very strong	0	1	2	0	0	0
Total		49	49	49	49	49	49

To the next experiments twenty-four isolates of fungi were chosen. Four of them were very effective against all three pathogens; six against *A. cochlioides* and *P. betae*. Four isolates of *Actinomycetes* were chosen. One of them was effective against all three pathogen, and three of them against *A. cochlioides* and *P. betae*.

Influence of selected BCA, *Streptomyces* and some *Trichoderma* fungi on the number of infected sugar beet seedlings by damping-off pathogens *in vivo*.

In the greenhouse experiments with sterile soil 26 of the tested fungi isolates restricted seedlings infection by *A. cochlioides*. Seven isolates protected seedlings on the same level as fungicides (T1, T8, T10, T11, T15, T18, T21), and only one isolate of *Actinomycetes*, S14 was as effective as these fungal BCA isolates. S14 limited the number of infected seedlings at the same level as a chemical seed treatment. No one of

Table 3

Effect of seed treatment with BCA on the level of sugar beet seedlings infection with *Aphanomyces cochlioide* (greenhouse experiment)

Isolate of microorganisms	Percent of infected seedlings
T1	0.9
T3	5.5
T7	2.5
T8	0.8
T10	1.7
T11	0.8
T12	5.7
T15	0.8
T16	6.1
T17	4.2
T18	1.6
T19	5.0
T21	1.7
T22	5.9
T24	6.5
T28	7.5
T29	10.7
T31	6.3
T42	5.1
T43	18.4
T44	10.3
T45	12.1
T46	12.1
T47	7.5
S48	16.8
S1	10.9
S7	11.9
S14	2.1
K OX+TACH *	1.7
control**	18.8
LSD p=0.05	1.5

*- Seeds treated with Oxafun T 75 WS/DS + Tachigaren 70 WP (6g+15g/ per kg of seeds)
** - untreated seeds

tested BCAs limited damping-off caused by *P. debaryanum*. For futher research T1, T8, T10, T11, T15, T18, T21 and S14 isolates were chosen (Table 3).

These eight BCA were incorporated into the seed pellet. A laboratory germination of pelleted with BCA seeds was as good as other treatment (Table 4). The number of

seedlings infected with *P. betae* was on the same level as treated with fungicides and lower than untreated seeds, but one variant (15 T/P). It was also found that

Table 4

Influence of seed treatment with BCA on germination and seedling health (laboratory test – sugar beet cultivar Janina)

Variant	Emergence of seedlings after			Number of healthy seedling from 100 seeds	Number of seedling infected with *P. betae*	Weight of 100 seedling [g]	
	4 days	7 days	14 days			fresh	dry
1T/P.	89.3	93.3	94.5	92.8	0.8	4.03	0.29
1T/Z	89.0	93.3	93.8	93.3	0.0	4.25	0.27
8T/P.	91.3	94.0	94.5	94.3	0.0	4.13	0.28
8T/Z	90.0	93.8	95.0	95.0	0.0	4.03	0.26
10T/P.	90.0	93.5	94.0	94.0	0.0	4.17	0.26
10T/Z	89.0	93.5	94.5	94.5	0.0	4.20	0.27
11T/P.	89.8	95.0	95.5	95.0	0.0	4.29	0.29
11T/Z	92.5	95.5	96.8	96.8	0.0	4.18	0.27
15T/P.	89.5	91.5	93.5	91.5	2.0	4.00	0.26
15T/Z	89.5	91.5	93.4	93.1	0.3	3.97	0.28
18T/P.	90.8	92.5	94.0	94.0	0.0	3.97	0.28
18T/Z	86.3	90.3	93.3	93.0	0.3	3.96	0.27
21T/P.	89.8	92.5	93.5	93.0	0.5	4.04	0.27
21T/Z	89.8	93.5	94.8	94.8	0.0	3.95	0.27
S 14/P.	88.0	92.3	93.5	93.5	0.0	4.02	0.28
S 14/ Z	87.8	92.0	93.3	93.0	0.3	4.00	0.27
control OX+TACH *	91.3	93.5	95.8	94.0	0.0	3.45	0.27
control **	90.8	94.3	94.5	91.0	3.5	4.08	0.28
LSD p = 0.05	-	-	-	-	0.8	-	-

*- seeds treated with Oxafun T 75 WS/DS + Tachigaren 70 WP (6g+15g/ kg of seeds)
** - untreated seeds
T – Variant of test where selected of fungus isolates were added
S - Variant of test where selected of Actinomycetes isolates were added
P – Fungus of Actinomycetes added the pelleting material in dry powder
Z - Fungus of Actinomycetes added the pelleting material in suspension

the antagonistic activity of BCA isolated from pelleted seeds did not decrease after 1, 7, 14 and 30 days after pelleting in comparison to initial isolates.

The activity of BCA against *A. cochlioides* was tested in greenhouse and on the micro-plots. A lower number of infected seedlings by *A. cochlioides* was observed for variants with BCA than of untreated seeds in both experiments. The number of healthy seedlings was higher for seed samples treated with two BCA (10T/P and 18T/P) than for control seeds treated with fungicides. Seven of BCA isolates protected emerging plants on the same level as a treated control. Six of the tested BCA protected seedlings in the same degree as a fungicides on plots. The very high protection of seedlings in both greenhouse and plot experiments was recorded with isolates 1T, 18T and S14. These isolates protected sugar beet

Table 5

Influence of BCA on infection of sugar beet seedlings with *Aphanomyces cochlioides*

Isolates of microorganisms	Percent of plants infected with *Aphanomyces cochlioides*	
	greenhouse test	microplot test
1 T/Z	10.4	9.2
1 T/P.	9.2	9.7
8 T/Z	10.8	1.3
8 T/P.	11.7	12.4
10 T/Z	11.9	10.2
10 T/P.	5.3	12.4
11 T/Z	17.0	12.7
11 T/P.	14.5	12.3
15 T/Z	16.7	12.8
15 T/P.	24.4	11.7
18 T/Z	10.8	9.7
18 T/P.	2.9	9.5
21 T/Z	22.4	11.0
21 T/P.	24.0	11.2
S 14/Z	9.8	7.5
S 14/P.	10.4	8.0
control OX + TACH *	10.9	8.8
control**	26.4	14.4
LSD p = 0.05	1.8	1.5

*- seeds treated with 7 Oxafun T 75 WS/DS + Tachigaren 70 WP (6g+15g/per kg of seeds)
** - untreated seeds. Symbols as in Table 4

seedlings as effectively as fungicides (in form of dry powder or suspension) (Table 5).

DISCUSSION

Trichoderma sp. is one of the most intensive tested microorganisms for biological control since many years. It was used for biological control of plant pathogens in orchards, greenhouse crops, cereals, and sugar beet. These fungi can stunt growth of pathogen parasite and destroy spores or sclerotium (Ferrata and D'Ambra 1985) or cystosori (D'Ambra and Mutto 1987). It can stop germination of spores by exudation hydrolytic enzymes which are needed for this process (Elad 2000). Trichoderma sp. can very likely induce a plant resistance (Elad *et al. 2000).* This fungus produces auxins which stimulate the growth of young plants. (Mańka *et al.* 1997, Werner *et al.* 1998).

Actinomycetes are the next very interesting group of microorganisms which can be used as the BCA. They can produce antibiotics (Broadbent *et al.* 1971, Gorlach 1995), cellulolitic and pectinolitic enzymes, and sometimes chitinolitic enzymes

which destroy wall structures of fungal mycelium (Bayer and Dickmann 1984, Strzelczyk *et al*. 1991, Lewińska 1998).

In our investigations conducted *in vitro* twenty-five of *Trichoderma* sp. isolates were found which stunted a growth of *A. cochlioides*, *P. betae* and *P. debaryanum mycelium*. Among them four showed a strong antagonistic activity against all three pathogens and six against A. cochlioides and *P. betae* and the remaining ones were effective only against one pathogen. The similar activity revealed four isolates of *Actinomycetes,* one being effective against all three pathogens, and three against *A.cochlioides* and *P. betae.* As a result of our investigations in vivo two isolates of *Trichoderma sp.* and one of *Streptomyces sp.* were identified, which restricted infection of sugar beet seedlings by *A. cochlioides* and *P. betae.* The obtained results *in vivo* and *in vitro* are not consistent which was also demonstrated by other authors (Weber *et al.1*996, Williams and Asher 1996) indicating that in natural conditions there may occur modyfying biotic and abiotic factors which are not present *in vitro*.

One of basic problem in biological control is working out of effective methods to transfer the antagonistic activity of microorganisms into practice. Direct introduction of BCA into the soil has given positive results (Kowalik 1982, Camparota *et al. 1988,* Nowakowska 1994, Werner *et al.* 1997, Saniewska *et al.* 1998). This has been confirmed by our results where the infection of sugar beet seedlings by *A. cochlioides* was clearly reduced. For practical use of BCA there is necessary to work with biopreparations in a form enabling storage and maintaining the activity incorporated in these microorganisms. A lot of work has been done to evaluate different matrix substances which can be used for that purpose (Herman and Nelson 1994, Weber *et al.* 2000).

Direct methods of coating seeds with BCA has been tested for many years. Interesting results with *T. koningii* against *Gaeumannomyces graminis* obtained Brion *et al.* (1996) coating directly wheat grains with BCA. Tests with sugar beet seeds coating with *Trichoderma harzianum* was done by Ruppel *et al.* (1983). The level of plant protection by this fungus was comparable to chemical seed treatment. Walther and Gindrart (1988) observed much reduction of damaged seedlings by *P. ultimum, P. betae,* and *Rhizoctonia solani* after seed coating with *Chaetomium globosum* ascospores. Similar results were recorded by Pietro *et al.* (1991) against *P. ultimum*.

The pelleting of sugar beet seed is now commonly used and BCA against damping-off pathogens can be easy introduced in this technology. This kind of research was done in Broom's Barn. Bacteria from sugar beet seedlings were isolated and their activity was tested against *P. ultimum* and *A. cochlioides in vitro*. Chosen isolates were added into seed pellet. A substantial reduction of infected seedlings was achieved, but the effect was, however, lower as compared to seeds treated with fungicides (Williams and Asher 1996).

In our investigations seven isolates of *Trichoderma* sp. and one of *Streptomyces* sp.were added into seed pellet. They were chosen based on results obtained *in vitro* and in greenhouse tests, where BCA were directly incoporated into sterilised soil. The greenhouse and microplots tests showed that two isolates of tested fungi and

one of *Actinomyces* restricted the number of seedlings demaged by damping-off pathogens on the same level as chemical treatment.

The use of BCA for sugar beet seedling protection against damping-off pathogens seems to be very difficult for chemical reasons now when effective and common chemical treatments are used. It does not exclude, however, futher investigations in this field.

CONCLUSIONS

1. The results obtained so far sugest a continuation of this research work. It would be desirable to test more isolates showing an antagonistic activity against damping- off pathogens and checking the possibility of comon use for seed treatment. This would, no doubt, increase the efficiency and reliability of this method.
2. Working out of effective method of BCA and their adding into seed pellet is a very important element in the biological method of sugar beet protection. It would give reasons for continuation the research work and to compare different methods of incorporating microorganisms into the pellet.

ACKNOWLEDGEMENTS

The author wish to thank to Professor Z. Szota for help in preparing the manuscript and to Kutnowska Hodowla Buraka Cukrowego Sp. z o.o. and Mr. J. Stec for sugar beet seeds and help in seed treatment and pelleting.

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