Proceedings of ECOpole

Vol. 5, No. 2 2011

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ASPECTS OF DECREASE OF SOME SOIL PATHOGENS OF SUGAR BEET BY *Bacillus subtilis* AND STREPTOMYCETES

BADANIE MOŻLIWOŚCI OGRANICZANIA ROZWOJU WYBRANYCH GLEBOWYCH PATOGENÓW BURAKA CUKROWEGO PRZEZ *Bacillus subtilis* ORAZ PROMIENIOWCE

Abstract: Soil-borne pathogens of sugar beet (*Beta vulgaris* L.) are often responsible for its poor quality and yield loss. Protection against the group of pathogens is possible only in the early stage of plant development, later there is no possibility for application of chemicals into the soil. This is a reason of the importance of biological methods of plant protection as well as the natural occurrence in the soil of some potentially antagonistic microorganisms. In the laboratory experiments some interactions between pathogens and potentially antagonistic microorganisms were tested as well as their influence on the sugar beet seedlings. Pathogens (*Aphanomyces cochlioides*, *Rhizoctonia solani*, *Fusarium oxysporum* and *Streptomyces scabies*) were isolated from diseased sugar beet root tissues. The antagonists were *Bacillus subtilis*, *Streptomyces griseoviridis* and two isolate of streptomycetes A-2003 and II-2003. Antagonistic microorganisms could not or could only slightly limit development of pathogens. The best result was observed in the case of *R. solani* which was limited by *S. griseoviridis*. In vitro *B. subtilis* and *S. griseoviridis* could decrease also growth of *S. scabies*. *F. oxysporum* showed very low pathogenic ability against sugar beet seedlings, but isolates of A-2003 and II-2003 decreased the number of diseased seedlings in the soil inoculated by *F. oxysporum*. The microorganisms tested as the antagonists did not injury seedlings of sugar beets. The strongest pathogenicity showed *A. cochlioides* and any one of antagonistic bacteria could not protect the seedlings against it. They also could not protect seedlings against *R. solani*, but in the case of soil infected by *S. scabies* they showed the possibility to protect seedlings.

Keywords: sugar beet, biological methods of plant protection

The use of the antagonistic properties of microorganisms, including different *Streptomyces* spp. and other bacteria in the biological control of many plant diseases has been the subject of many studies. Actinomycetes represent 7% of the total number of bacteria in the wheat rhizosphere. Numbers of actinomycetes are higher in the rhizosphere and root-free soil than in the endorhizosphere [1]. Mayfield et al [2] studied the ecology of actinomycetes in soil and found that they exist in soil for much of the time as spores unevenly distributed throughout the soil. Only 4% of spores germinated in natural soil but in sterile and glucose supplemented soil germination increased. Spores were more stable than mycelia and more growth was observed in sterile than in non-sterile soil [1]. *Streptomyces griseoviridis*, the representative of actinomycetes has been reported as an antagonist to the plant pathogens *Alternaria brassicicola, Botrytis cinerea, Fusarium avenaceum, F. culmorum, F. oxysporum, Rhizoctonia solani, Sclerotinia sclerotiorum*. *In vitro* tests reveled that *S. griseoviridis* suppressed the growth of these fungi [3]. Moussa and Rizk [3] reported also that 70% culture filtrate of *S. aureofaciens* inhibited spore germination, mycelium development and spore production of *Fusarium solani*. They found that seed-coating was more effective than soil pre-inoculation.

Some strains of beneficial microorganisms are marketed as biopesticides: Kodiak (*Bacillus subtilis* GB03), Epic (*Bacillus subtilis* GB07), Dagger G (*Pseudomonas*

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fluorescens), Mycostop (*Streptomyces griseoviridis*), F-stop (*Trichoderma harzianum*), Deny (*Burgholderia cepacia*) [4]. Beneficial plant bacteria which are associated with the surfaces of plant roots may increase plant yield. Both *in vitro* and *in vivo* experiments with *Streptomyces griseoviridis*, the active ingredient of Mycostop, effectively inhibits the growth of a wide range of fungal pathogens. The antagonistic effect seems to be based on several modes of action: rhizosphere competence, lysis of the pathogen cell wall followed by hyperparasitism, antifungal metabolites, auxin excretion (IAA), competition for living space and nutrients and general growth stimulation of the plant [4].

Soil-borne pathogens of sugar beet (*Beta vulgaris* L.) are often responsible for its poor quality and yield loss. Protection against the group of pathogens is possible only in the early stage of plant development, later there is no possibility for application of chemicals into the soil. This is a reason of the importance of examination of biological methods for plant protection as well as natural occurrence of some potentially antagonistic microorganisms in the soil.

Materials and methods

In the laboratory experiments, some interactions between pathogens and potentially antagonistic microorganisms were tested *in vitro* as well as their influence on the sugar beet seedlings in the pot test. Pathogens used in the experiments were originally isolated from diseased sugar beet root tissues. They were: *Aphanomyces cochlioides*, *Rhizoctonia solani*, *Fusarium oxysporum* and *Streptomyces scabies*. As the antagonists four isolates of bacteria were used: *Bacillus subtilis*, *Streptomyces griseoviridis* and two isolate of streptomycetes coded as A-2003 and II-2003. The last two isolates were obtained from sugar beet root tissues with the symptoms of scab.

The interactions between pathogens and the antagonistic bacteria were tested on Petri dishes filled with PDA medium. Each dish was inoculated in the centre with 5 mm disc of the medium overgrown by one-week old mycelium of appropriate microorganism and than bacteria were transferred on the two opposite lines situated on the margin of the PDA medium. For *Streptomyces scabies* liquid medium with the pathogen was used. The medium was transferred to the crack in the solid medium, and the antagonists were inoculated on the medium surface. The combinations without antagonists were used as controls. The experiment was done in five replications, dishes were kept in a room temperature, in the dark. The inhibition of growth of tested pathogens was observed.

The influence of the same microorganisms on the sugar beet seedlings was examined in the laboratory pot test. Fungal pathogens were grown on corn-sand medium during three weeks. Pots (500 cm^3) were filled with the sterilized soil, and after that inoculated with a portion (5 cm^3) of corn-sand medium overgrown by pathogen mycelium. The medium was covered with 1 cm (depth) of the soil and after two days ten non-pelleted sugar beet seeds were sown in each pot. Before sowing, seeds were surface sterilized, dried and soaked in the liquid culture of antagonists (15 min). For this experiment actinomycetes were grown on the liquid *oat-malt broth* (OMB) and *Bacillus subtilis* was grown on the liquid malt medium. The controls were not infected pots as well as pots inoculated only by one type of microorganism (pathogen or antagonist). The experiment was done twice in three replications and it was finished when plants developed the first pair of true leaves. In this experiment the disease development was observed. Each diseased seedling was transferred to the sterilized water, incubated for 24 hours and microscopically examined to confirm the appropriate pathogen presence. In the end of the experiment the dry mass of seedlings grown in each pathogen/antagonist combination was measured.

Results

Antagonistic microorganisms could not or could slightly limit development of pathogens. The best result was observed in the case of *R. solani* which was limited by *S. griseoviridis*. In vitro *B. subtilis* and *S. griseoviridis* could decrease also growth of *S. scabies*. The growth of *Aphanomyces cochlioides* was even stimulated slightly (Fig. 1).

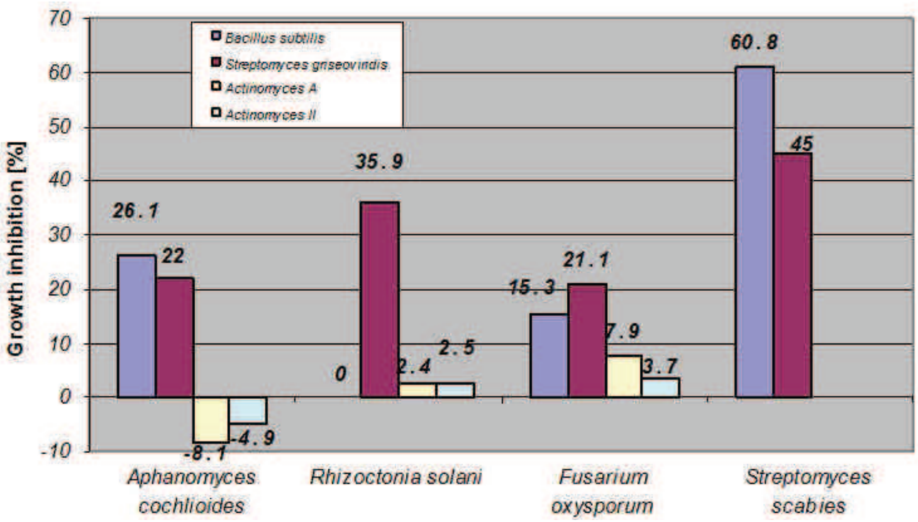

Fig. 1. Interactions between pathogens and antagonists *in vitro* ("–" means growth stimulation)

Fusarium oxysporum showed very low pathogenic ability against sugar beet seedlings, but isolates of A-2003 and II-2003, as well as *B. subtilis* decreased the number of diseased seedlings in the soil inoculated by *F. oxysporum*. The same effect was observed in the case of pots infected with *S. scabies* (Fig. 2). The microorganisms tested as the antagonists did not injury seedlings of sugar beets. The strongest pathogenicity showed *A. cochlioides* and any one of antagonistic bacteria could not protect the seedlings against it (Fig. 2). They also could not protect seedlings against *R. solani*, but in the case of soil infected by *S. scabies* they showed the possibility to protect seedlings (Fig. 2).

The dry mass content of sugar beet seedlings is the inverse of the tissue hydration. Plants with more hydrated tissues have better resistance possibility against pathogens [5]. Tested antagonists showed the possibility to decrease of dry mass content in sugar beet seedlings, in some cases, especially in case of pots infected with *A. cochlioides*, but this effect do not correlated with disease development (Figs. 1 and 3). Antagonists added alone to the soil without pathogens increased the dry mass content in sugar beet seedlings (Fig. 3).

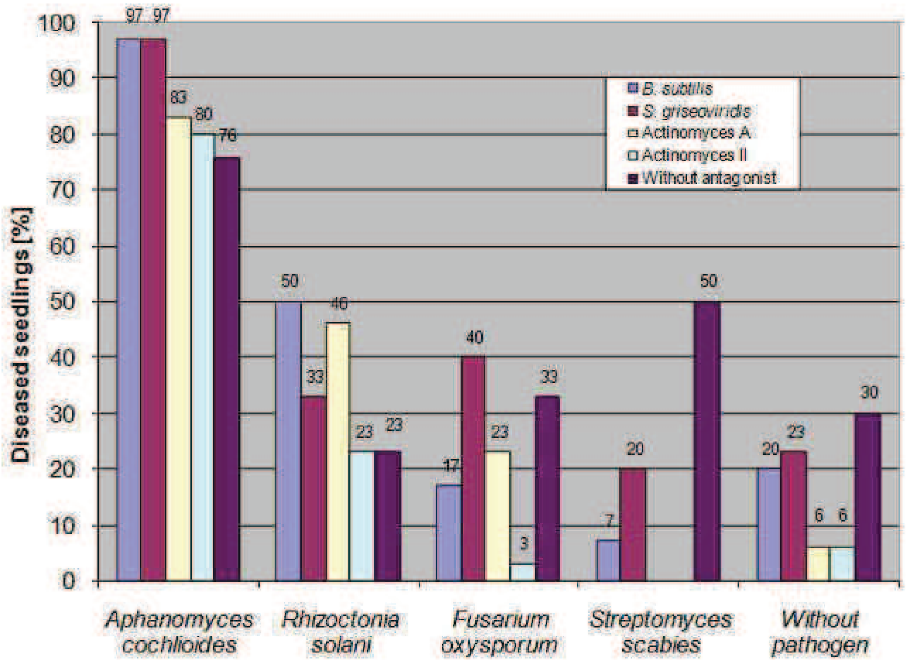

Fig. 2. The influence of antagonists on the disease development on sugar beet seedlings

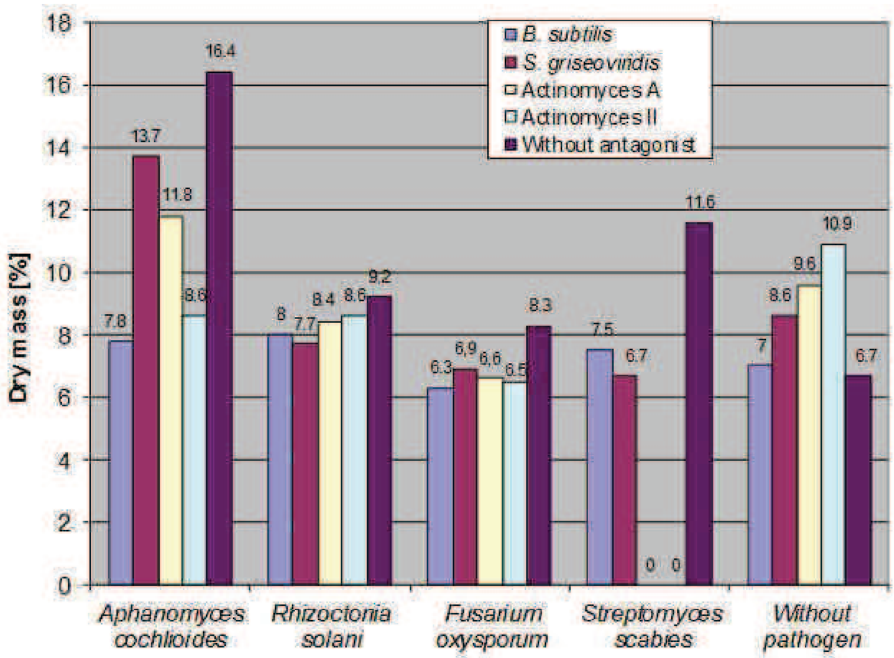

Fig. 3. The influence of antagonists on the dry mass content in sugar beet seedlings

Conclusions

Tested antagonists showed no destructive abilities against sugar beet seedlings, they were also not sufficiently effective against most of tested pathogens. *B. subtilis* as well as *S. griseoviridis* have protected sugar beet seedlings against *S. scabies*. Antagonistic isolate of *B. subtilis*, Actinomyces A and Actinomyces II could also decrease the seedlings injury made by *F. oxysporum*. The mode of action of antagonists is due to some extent with the tissue hydration.

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Abstrakt: Choroby korzeni buraka cukrowego (*Beta vulgaris* L.) powodują znaczne obniżenie ilości oraz jakości plonu tej rośliny. Zwalczanie patogenów glebowych ograniczone jest tylko do fazy siewek buraka, w późniejszym czasie nie ma możliwości chemicznej walki z patogenami glebowymi. Dlatego bardzo ważna jest obecność w glebie naturalnych antagonistów mikroorganizmów patogennych. Organizmy antagonistyczne można także wprowadzać sztucznie do środowiska glebowego, co umożliwia rozwój metod biologicznej ochrony roślin. W przeprowadzonych doświadczeniach, w warunkach laboratoryjnych, zbadano wzajemne oddziaływanie, w warunkach *in vitro*, pomiędzy patogenami otrzymanymi z zainfekowanych korzeni buraka cukrowego: *Aphanomyces cochlioides*, *Rhizoctonia solani*, *Fusarium oxysporum* oraz *Streptomyces scabies* a potencjalnymi antagonistami: *Bacillus subtilis*, *Streptomyces griseoviridis* oraz dwoma wybranymi izolatami promieniowców (A-2003 i II-2003) otrzymanymi z ranek parchowych na korzeniach buraka. Zbadano także ich wpływ na rośliny buraka w infekcyjnym doświadczeniu wazonowym. Stwierdzono, że wpływ antagonistów na rozwój patogenów był niejednakowy. Obserwowano niewielki stopień ograniczenia wzrostu patogenów przez *S. griseoviridis* i przez *B. subtilis*, szczególnie w odniesieniu do *R. solani*. *F. oxysporum* wykazywał bardzo nikłe właściwości patogenne wobec siewek buraka, a badane promieniowce A-2003 i II-2003 w niewielkim stopniu ograniczały rozwój jego grzybni oraz w odniesieniu do kombinacji kontrolnej zmniejszały liczbę chorych roślin w teście wazonowym. Wykazano, że żaden z potencjalnych antagonistów nie powodował uszkodzeń siewek buraka. Siewki hodowane w wazonach zainokulowanych antagonistami oraz *A. cochlioides* nie były chronione przed tym patogenem, podobnie jak w przypadku wazonów infekowanych *R. solani*, natomiast *B. subtilis* i *S. griseoviridis* dobrze chroniły siewki przed *S. scabies*.

Słowa kluczowe: burak cukrowy, biologiczna ochrona roślin