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Inoculation of Sugar Beet Seed with Bacteria *P. fluorescens*, *B. subtilis* and *B. megaterium* – Chemical Fungicides Alternative

Suzana Kristek, Andrija Kristek and Dragana Kocevski
University of J. J. Strossmayer, Faculty of Agriculture
Croatia

1. Introduction

During the process of sugar beet cultivation, depending on weather conditions, the degree of soil infection with pathogenic microorganisms, and the application of soil management, the presence of sugar beet root rot is frequently observed. Pathogens are numerous, inflicting serious damage.

According to the occurrence time of root rot symptoms, the pathogens can be divided into three major groups: pathogens occurring during the emergence phase, pathogens occurring in the growing season, and ones that cause sugar beet root rot during the period of storage.

The most frequent decay pathogens on sugar beet seedlings are the fungi *Aphanomyces cochlioides*, *Pythium ultimum* and *Pythium debarianum*, as well as *Rhizoctonia solani*. In the growing season plant decay is most frequently caused by the fungi *Rhizoctonia solani*, *Fusarium* spp., and by the bacteria *Erwinia* spp. From the root rot pathogens most damage is inflicted by *Rhizoctonia solani*. Besides weather conditions that influence this type of root decay, the inadequate soil management – irregular soil cultivation resulting in damaged soil structure and rise in soil acidity – is a contributory factor.

Apart from the root pathogens occurring during cultivation in the field, certain pathogens cause sugar beet decay in the period of storage, and before processing. Storage conditions, as well as plant damages caused when digging are contributory factors in the occurrence of fungal diseases.

The most frequent pathogenic microorganisms observed are fungi, such as *Rhizoctonia solani*, *Botrytis cinerea, Penicillium* spp., *Aspergillus* spp. ... The pathogenic fungus *Rhizoctonia solani,* if compared with all other sugar beet root rot pathogens, is able to infect the plant from the emergence phase, during growing season, until the process of sugar beet storage.

Fungicidal treatment of seeds, use of tolerant hybrids, or proper soil management does not ensure full plant protection against this pathogen. Moreover, heavily infected soils and weather conditions favourable for development of the pathogen, contribute to the infection to higher or lower degree.

Agronomists are faced with a growing problem of evolving resistance of soil pathogens to chemical fungicides. Also, there is an increasing sensitivity to the importance of health food production and rising awareness for environmental protection. Namely, all pesticides which are not biodegraded in the soil are being washed out in deeper layers causing eutrophication of underground waters. Therefore, natural resources and processes in the soil are widely used as an alternative to chemical fungicides.

Seed inoculation with the bacteria showing antagonistic activity against pathogenic fungi such as *Rhizoctonia solani, Pythium ultimum, P. debarianum, Phoma betae* and *Aphanomyces cochlioides* account for an alternative to chemical fungicides and an option of solving problem of disease control, not only in sugar beet, but in other, mainly vegetable crops. Many authors (Koch et al., 2002; Sorensen et al., 2001; Thrane et al., 2000; Whipps, 2001) have studied control of these pathogens by the beneficial bacterium *Pseudomonas fluorescens* which shows pronounced antagonistic activity against the fungi. Similarly, results of their studies proved positive effect of the bacterium *Bacillus megaterium* against the same root decay agents of sugar beet (Asaka & Shoda, 1996; Choudhary & Johri, 2009; Thrane et al., 2000). However, some authors proved antagonistic effect of certain beneficial fungi and yeast (*Trichoderma harzianum, Candida valida, Rhodotula glutinis,* …) against these pathogenic fungi (Abada, 1994; El-Tarabily & Sivasithamparam, 2006).

Pseudomonas fluorescens belongs to rod-shaped, asporogenous, gram-negative bacteria that as saprophytes prevalent in soil and water belong to a group of soil microorganisms crucial to the process of soil denitrification.

Due to the production of antimicrobial agents, these bacteria also show a distinguished antibiosis against pathogens causing diseases on arable crops, by inactivating their growth and reproduction (Whipps, 2001). For the capacity to synthesize toxic cyclic lipopeptides (Andersen et al., 2003; Koch et al., 2002; Thrane et al., 2000) they are used as effective biological control agents (Sorensen et al., 2001). Many authors have reported that these purified lipopeptides show an antagonistic activity against certain fungi pathogenic on sugar beet roots such as *Rhizoctonia solani* (Andersen et al., 2003; Nielsen et al., 2000, 2002), *Aphanomyces cochlioides* (Raaijmakers et al., 2010; Sorensen et al., 2001), *Pythium ultimum* and *Pythium debarianum* (Andersen et al., 2003; Gorlach-Lira & Stefaniak, 2009; Lee et al., 2000; Nielsen et al., 2000; Thrane et al., 2000). This suggests that bacteria producing lipopeptides could have a potential role in the biocontrol of fungal diseases, which was approved in both laboratory and field trials (Thrane et al., 2000, 2001). Lipopeptides may also function as biosurfactants (Desai et al., 1997) which can facilitate bacterial growth on water – insoluble carbon sources (Koch et al., 1991; Ron & Rosenberg, 2001) or their interaction with hydrophobic surfaces (Neu, 1996), e. g., surface motility (Engelhardt et al., 2009; Lindum et al., 1998).

Bacillus subtilis and *Bacillus megaterium* form symbiotic relationship with the root system of field crops, and like *P. fluorescens* show antagonistic activity against these pathogens. In this study we have investigated synergistic effect of the bacteria in order to achieve as better results as possible.

Bacillus is a genus of Gram-positive rod-shaped bacteria and a member of the division *Firmicutes*. *Bacillus* species can be obligate aerobes or facultative anaerobes. The species *Bacillus subtilis* and *Bacillus megaterium* participate in nitrogen cycle, and in the soil processes

of organic matter humification and mineralization of humus, especially in mineralization of phosphorus and potassium converting them into plant-accessible forms. These bacteria belong to biological control agents, i. e. they produce antimicrobial substances that exhibit antagonistic activity against soilborne pathogens, agents of diseases in arable crops (Sorensen et al., 2001).

Therefore, seed inoculation with bacteria showing antagonism against pathogenic fungi is an acceptable alternative to chemical pesticide application (Andersen et al., 2003).

Moreover, considering heavily infected soils with the pathogen *R. solani* and the fact that beneficial bacteria *Pseudomonas fluorescens*, *B. subtilis* and *B. megaterium* are not sensitive to a low dose fungicide (Pedersen et al., 2002), it is possible to treat the seeds combining low doses of the chemical agents aiming to control growth and reproduction of the pathogenic fungi, which in consequence, produce positive effect on all the parameters of sugar beet yield and quality.

2. Materials and methods

The experiment was set up on two types of the soil: Mollic Gleysols (FAO, 1998) and Eutric Cambisols.

On Mollic Gleysols soil type in the period of 2005 – 2009 pathogenic fungus *R. solani* (FAO, 1998) was determined, whilst on Eutric Cambisols (Table 1) soil type in the same period *P. debarianum* was determined.

Investigated properties in a field	**Type of soil**	
Layer (0 – 0.3 m)	**Mollic Gleysols**	**Eutric Cambisols**
pH (H_2O)	7.58	7.33
pH (KCl)	6.90	6.62
Humus (%)	3.40	2.15
P (mg/ 100 g soil)	22.80	20.55
K (mg/ 100 g soil)	23.10	19.08

Table 1. Soil characteristics

In 2007, 2008 and 2009 the experiment was set up by completely randomised block design in 4 repetitions and 8 various seed treating variants: 1. control (untreated seed); 2. Thiram 42-S fungicide treated seed (48% Thiram, 600 ml/100 kg seed); 3. seed inoculated with *P. fluorescens* No 8569 (1.2 x 10^{10} bacteria/ha); 4. seed inoculated with *B. subtilis* No 2109 (1.2 x 10^{10} bacteria/ha); 5. seed inoculated with *B. megaterium* No 2894 (1.2 x 10^{10} bacteria/ha); 6. seed inoculated with *B. subtilis* No 2109 (0.6 x 10^{10} bacteria/ha) + seed inoculated with *B. megaterium* No 2894 (0.6 x 10^{10} bacteria/ha); 7. seed inoculated with *P. fluorescens* No 8569 (0.4 x 10^{10} bacteria/ha) + seed inoculated with *B. subtilis* No 2109 (0.4 x 10^{10} bacteria/ha) + seed inoculated with *B. megaterium* No 2894 (0.4 x 10^{10} bacteria/ha); 8. seed inoculated with *P. fluorescens* No 8569 (0.4 x 10^{10} bacteria/ha) + seed inoculated with *B. subtilis* No 2109 (0.4 x 10^{10} bacteria/ha) + seed inoculated with *B. megaterium* No 2894 (0.4 x 10^{10} bacteria/ha) + Thiram 42-S fungicide treated seed (48% Thiram, 200 ml/100 kg seed).

Bacteriological cultures applied in the experiment were from German Collection of Microorganisms and Cell Cultures (DSMZ). *P. fluorescens* No 8569 were transferred from lyophilised into vegetative form, and multiplied on Caso agar (Merck 105458) having following composition: peptone from casein 15.0 g, peptone from soymeal 5.0 g, NaCl 5.0 g, agar 15.0 g, distilled water 1000.0 ml. For *B. megaterium* No 2894 and *B. subtilis* No 2109 nutrient agar of the following composition was used: peptone 5.0 g, meat extract 3.0 g, agar 15.0 g, $MnSO_4 \times H_2O$ 10.0 mg, distilled water 1000.0 ml.

Month	Precipitation amounts					Air temperatures				
	Necessity	1901-91	2007	2008	2009	Necessity	1901-91	2007	2008	2009
April	40	56	3	50	19	-	11.2	13.3	12.5	12.4
May	50	63	56	67	39	14.2	16.8	18.3	18.1	16.5
June	50	88	33	76	63	18.0	19.4	22.2	21.4	20.3
July	80	66	27	79	12	18.5	21.2	23.9	21.8	23.2
August	65	61	46	46	61	18.2	20.4	22.2	21.8	21.7
September	35	46	65	86	10	14.0	16.8	14.5	15.6	15.6
October	40	56	94	30	55	-	11.1	10.3	13.0	9.1
Total	***360***	***436***	***324***	***434***	***259***	***-***	***-***	-	-	
Average	-	-	-	-	-	***-***	***16.7***	***17.8***	***17.7***	***17.0***

Table 2. Weather conditions in experimental years and years – long average in Osijek. (Meteorological and hydrological service of Croatia (DHMZ). Agrometeorological bureau in Osijek)

Hybrid of Belinda (KWS) sugar beet was used in the sowing. In the three trial years the sowing was conducted in the second decade of March. The row spacing was 50 cm, with 20 cm within row. Adequate plant stand was obtained with no corrections necessary.

Percentage of the plants infected with pathogenic fungi *R. solani* and *P. debarianum* as well as percentage of decayed plants was stipulated in 2 - 4 true leaves phase. The sugar beet digging conducted in the middle of October was followed by determination of root yield (t/ha), sugar content (%), sugar in molasses (%) and sugar yield (t/ha).

Weather conditions appeared to affect sugar beet growth. Data obtained from the Agrometeorological bureau in Osijek, Croatia (Table 2) were used in weather analysis. Weather conditions in 2007 were characterized by the increase in air temperature of more than 1^0C above multiyear average. Precipitation rates in the growing season measured 74% out of the average, with rainfall deficiency in June and July, and excessive rainfall in September and October which was unfavourable for sugar beet growth. 2008 and 2009 were also characterized by higher air temperatures in comparison with multiyear average, which was unfavourable for sugar beet growth due to the values significantly (2.5^{o}C in 2008; and 3.0^{o}C in 2009) above the optimum. Precipitation rates in the growing season 2008 were level with the multiyear average, with rainfall deficiency in August and excessive rainfall in September. Considering precipitation amount in the growing season (April – September), 2009 was dry. Only 59 % out of the average precipitation rates were measured, with rainfall deficiency throughout the year except for June. Such weather conditions were favourable for sugar beet growth until July, and then followed by the unfavourable conditions until the end of the growing season.

Results were processed by modern statistical methods (ANOVA) using the computer program StatSoft Inc. (2001) STATISTICA (data analysis software system), version 6.

3. Results and discussion

3.1 Percentage of infected and decayed plants

During the three - year research into the influence of beneficial bacteria and chemical fungicide Thiram 42-S on the intensity of infection and decay of sugar beet plants caused by the parasitic fungi *Rhizoctonia solani* on Mollic Gleysols, and by the parasitic fungi *Pythium debarianum* on Eutric Cambisols significant influence of the bacteria and the chemical fungicide was determined in all tested variants when compared with the control (Table 3).

On Mollic Gleysols in the three trial years the best results were recorded in the variant 7 - seed inoculated with *P. fluorescens* No 8569 (0.4 x 10^{10} bacteria/ha) + seed inoculated with *B. subtilis* No 2109 (0.4 x 10^{10} bacteria/ha) + seed inoculated with *B. megaterium* No 2894 (0.4 x 10^{10} bacteria/ha). All other variants of treated seeds obtained highly significant larger number ($p < 0.01$) of infected and decayed plants due to the attack of the parasitic fungi *Rhizoctonia solani* if compared with all other tested variants. Among the variants 2 - Thiram 42-S fungicide treated seed (48% Thiram, 600 ml/100 kg seed), 3 - seed inoculated with *P. fluorescens* No 8569 (1.2 x 10^{10} bacteria/ha) and 8 - seed inoculated with *P. fluorescens* No 8569 (0.4 x 10^{10} bacteria/ha) + seed inoculated with *B. subtilis* No 2109 (0.4 x 10^{10} bacteria/ha) + seed inoculated with *B. megaterium* No 2894 (0.4 x 10^{10} bacteria/ha) + Thiram 42-S fungicide treated seed (48% Thiram, 200 ml/100 kg seed) no significant differences were determined ($p > 0.01$), though the smallest number of the infected and decayed plants was recorded in the variant 8 in the three trial years. Average number of infected plants in treated variants in the three trial years was 10.86% or 58.17% lower than the three-year average in the control variants which was 25.96%. The difference in decayed plants was 74.70%.

The results obtained are in agreement with those of Whipps (2001) who reported that the plants inoculated with bacteria *P. fluorescens* were characterized with rapid initial growth that enabled fast undergoing through the most vulnerable phase when the damage of the pathogen affecting was the most pronounced. Due to the pronounced antagonism of the bacterium to the root rot agent of sugar beet – fungi *R. solani* high survival percentage of inoculated plants was obtained in comparison with the non-inoculated ones and decrease in the damage of infected plants, which consequently, influenced the sugar beet root yield. Kristek et al. (2007) in their research results of the effects of the beneficial bacterium *P. fluorescens* on the pathogenic fungus *R. solani* recorded obvious decrease in the number of infected and decayed plants in variants treated with the bacterium *P. fluorescens* when compared with the control variants. They also reported significant difference in the number of infected and decayed plants between tolerant hybrids compared with the hybrids sensitive to the pathogenic fungi. With tolerant hybrids in treated variants 4.10 % smaller number of infected plants was obtained than in the same variants of sensitive hybrids. The difference in the number of decayed plants was 3.86 %.

On Eutric Cambisols in the three trial years the best results were recorded in the variant 8 - seed inoculated with *P. fluorescens* No 8569 (0.4 x 10^{10} bacteria/ha) + seed inoculated with *B. subtilis* No 2109 (0.4 x 10^{10} bacteria/ha) + seed inoculated with *B. megaterium* No 2894

(0.4 x 10^{10} bacteria/ha) + Thiram 42-S fungicide treated seed (48% Thiram, 200 ml/100 kg seed), though no significant difference ($p > 0.01$) was determined between the above variant and variant 2 - Thiram 42-S fungicide treated seed (48% Thiram, 600 ml/100 kg seed). All other variants obtained highly significant ($p < 0.01$) larger number of infected and decayed plants due to the attack of the parasitic fungi *P. debarianum*. Average number of infected plants in the three trial years in treated variants was 11.70% or 56.16% lower than in the three-year average in the control variants which was 26.69%. The difference in decayed plants was 70.97%.

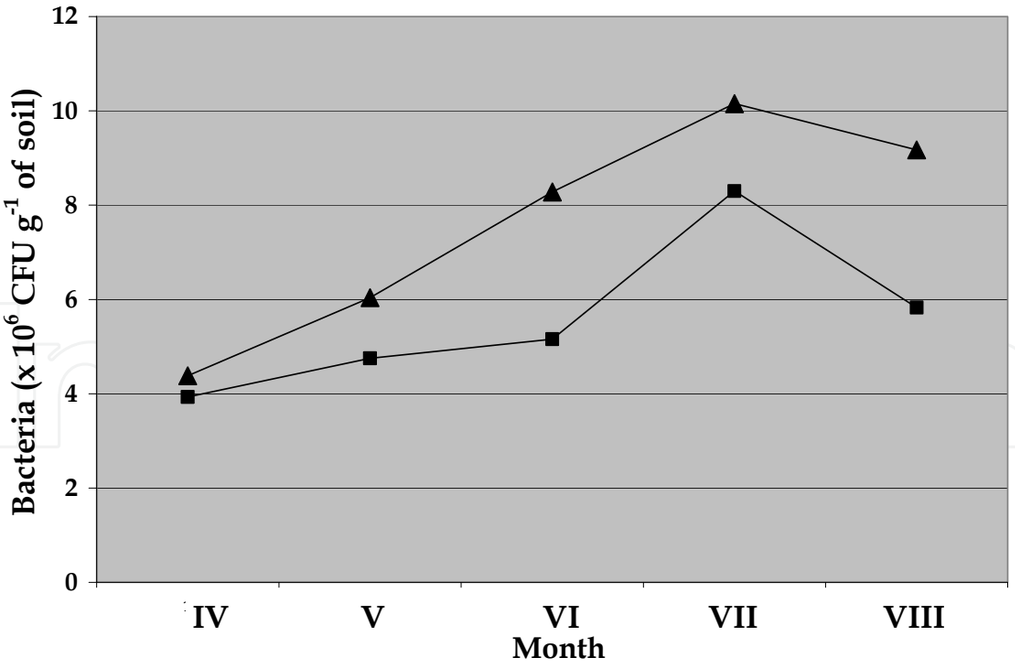
Investigated parameter	Variants	Mollic Gleysols (*Rhizoctonia solani*)				Eutric Cambisols (*Pythium debarianum*)			
		Year			*Average*	Year			*Average*
		2007	2008	2009		2007	2008	2009	
Infected plants (%)	1	29.46	20.78	27.65	***25.96***	28.46	23.51	28.10	***26.69***
	2	11.39	9.59	10.43	***10.47***	11.29	10.55	10.96	***10.93***
	3	11.35	9.66	10.39	***10.47***	11.71	10.96	11.44	***11.37***
	4	12.97	11.59	12.21	***12.26***	13.18	12.68	12.73	***12.86***
	5	12.85	11.53	12.29	***12.22***	12.99	12.43	12.65	***12.69***
	6	11.14	10.27	10.94	***10.78***	12.35	11.55	11.97	***11.96***
	7	10.25	8.90	9.66	***9.60***	11.64	10.89	11.39	***11.31***
	8	10.99	9.46	10.27	***10.24***	11.13	10.41	10.92	***10.82***
$LSD_{0.05}$		0.24	0.15	0.13	0.16	0.11	0.12	0.10	0.10
$LSD_{0.01}$		0.43	0.27	0.24	0.28	0.21	0.23	0.19	0.18
Decayed plants (%)	1	25.90	18.40	23.66	***22.65***	25.98	21.04	24.18	***23.73***
	2	5.61	5.27	5.28	***5.39***	6.70	5.99	6.24	***6.31***
	3	5.70	5.19	5.33	***5.41***	7.11	6.22	6.57	***6.63***
	4	7.12	6.33	6.90	***6.78***	7.98	7.08	7.85	***7.64***
	5	7.06	6.38	6.84	***6.76***	8.03	7.12	7.78	***7.64***
	6	6.19	5.65	5.92	***5.92***	7.65	6.79	7.20	***7.21***
	7	5.03	4.11	4.80	***4.65***	7.04	6.13	6.48	***6.55***
	8	5.48	4.95	5.11	***5.18***	6.56	5.96	6.16	***6.23***
$LSD_{0.05}$		0.14	0.18	0.13	0.15	0.09	0.08	0.06	0.07
$LSD_{0.01}$		0.26	0.34	0.25	0.27	0.17	0.15	0.13	0.14

1. control (untreated seed); 2. Thiram 42-S fungicide treated seed (48% Tiram, 600 ml/100 kg seed); 3. seed inoculated with *P. fluorescens* No 8569 (1.2 x 10^{10} bacteria/ha); 4. seed inoculated with *B. subtilis* No 2109 (1.2 x 10^{10} bacteria/ha); 5. seed inoculated with *B. megaterium* No 2894 (1.2 x 10^{10} bacteria/ha); 6. seed inoculated with *B. subtilis* No 2109 (0.6 x 10^{10} bacteria/ha) + seed inoculated with *B. megaterium* No 2894 (0.6 x 10^{10} bacteria/ha); 7. seed inoculated with *P. fluorescens* No 8569 (0.4 x 10^{10} bacteria/ha) + seed inoculated with *B. subtilis* No 2109 (0.4 x 10^{10} bacteria/ha) + seed inoculated with *B. megaterium* No 2894 (0.4 x 10^{10} bacteria/ha); 8. seed inoculated with *P. fluorescens* No 8569 (0.4 x 10^{10} bacteria/ha) + seed inoculated with *B. subtilis* No 2109 (0.4 x 10^{10} bacteria/ha) + seed inoculated with *B. megaterium* No 2894 (0.4 x 10^{10} bacteria/ha) + Thiram 42-S fungicide treated seed (48% Tiram, 200 ml/100 kg seed)

Table 3. Percentage of the infected and decayed plants as a consequence of *R. solani* (Mollic Gleysols), *P. debarianum* (Mollic Gleysols) infection in the 2–4 true leaves stage

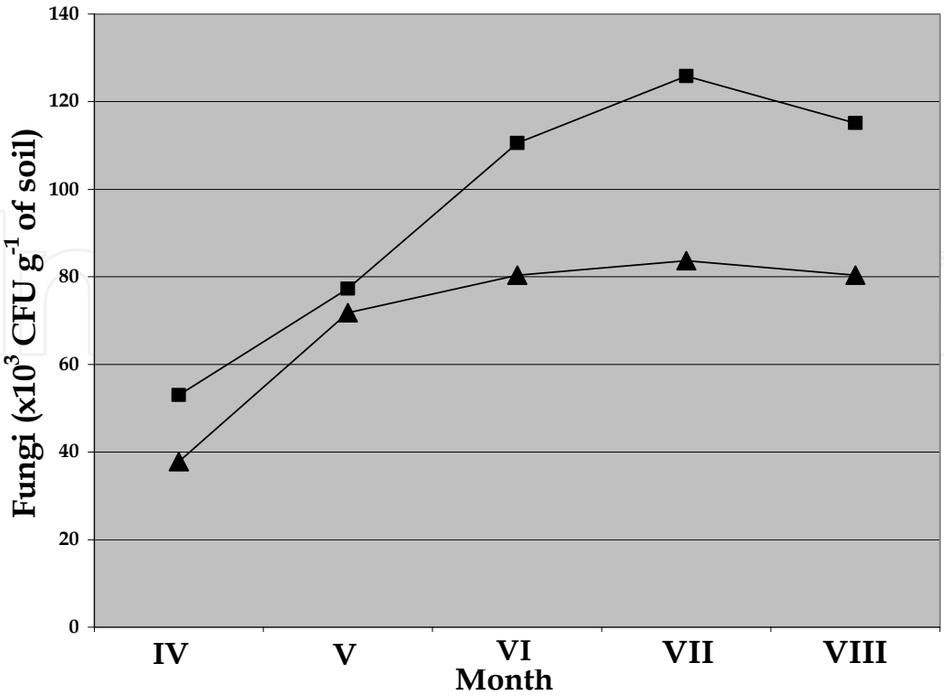
Similar results in examining the effect of the bacterium *B. megaterium* (a constituent of the applied microbiological preparation) in the control of pathogenic fungi *P. ultimum* and *P. debarianum* were reported in the studies on the same parameters by Evačić et al. (2008).

It is evident that weather conditions in the growing season of sugar beet influenced intensity of the infection by pathogenic fungi *Rhizoctonia solani* and *Pythium debarianum*. Namely, in the second trial year precipitation rates and air temperatures were favourable for crop development which enabled faster undergoing of young sugar beet plants through the most vulnerable phase of the growth. Consequently, intensity of the infection and the number of decayed plants as the result of the pathogen attack were both decreased which was recorded in the controls. Furthermore, there was obvious difference in the number of infected and decayed plants on different types of the soil. As it did not concern the same pathogen, it could be higher sensitivity of the hybrid to *Pythium debarianum* than to *Rhizoctonia solani*. Nevertheless, pathogenic fungi *Rhizoctonia solani* usually cause more economic damage in the middle and eastern European countries than pathogenic fungi *Pythium debarianum*. Belinda hybrid was chosen due to the high sensitivity to the fungi *Rhizoctonia solani*. Therefore chemical and microbiological soil properties could be presumed to have great importance in plant development and resistance against the infection caused by the pathogenic fungi. Namely, Mollic Gleysols has chemical (Table 1) and microbiological (Figure 1, 2, 3, 4, 5) properties of better quality.



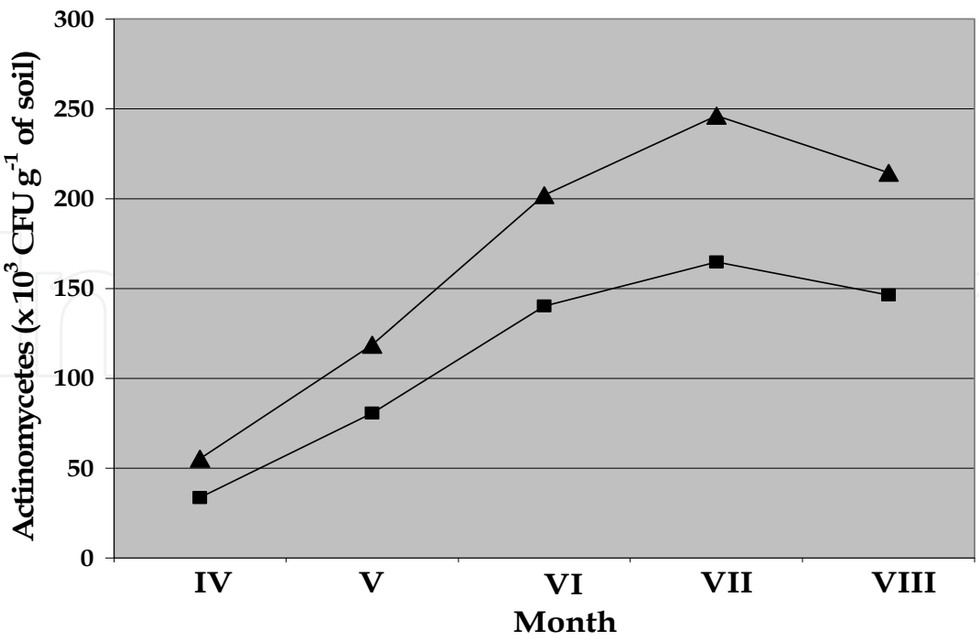
(▲ – Mollic Gleysols; ■ – Eutric Cambisols)

Fig. 1. Average number of total Bacteria in soil during three trial years



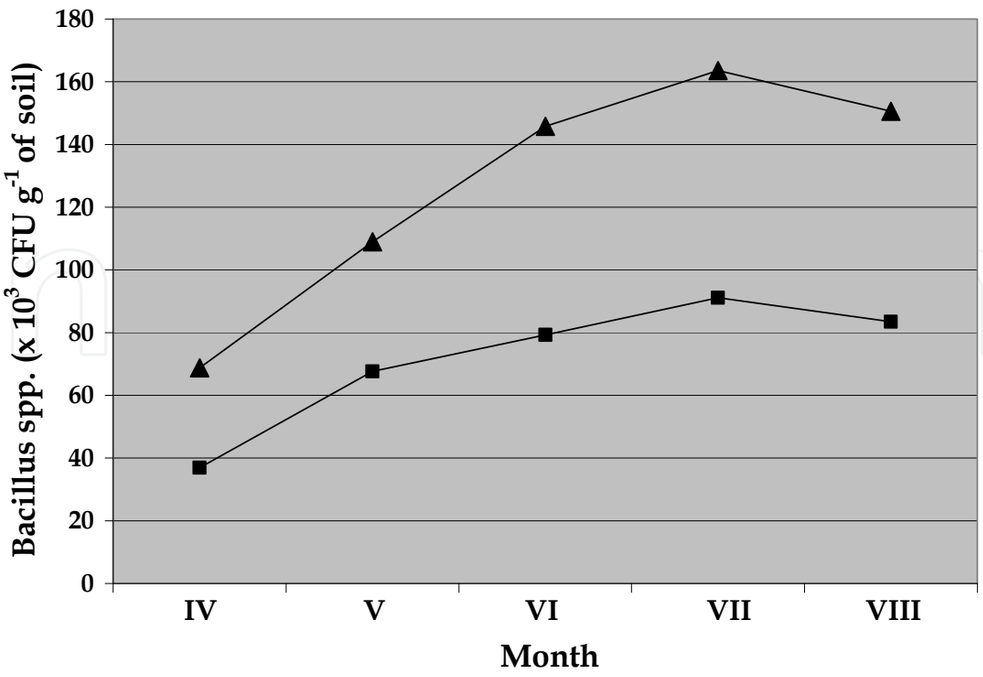
(▲ – Mollic Gleysols; ■ – Eutric Cambisols)

Fig. 2. Average number of total Fungi in soil during three trial years



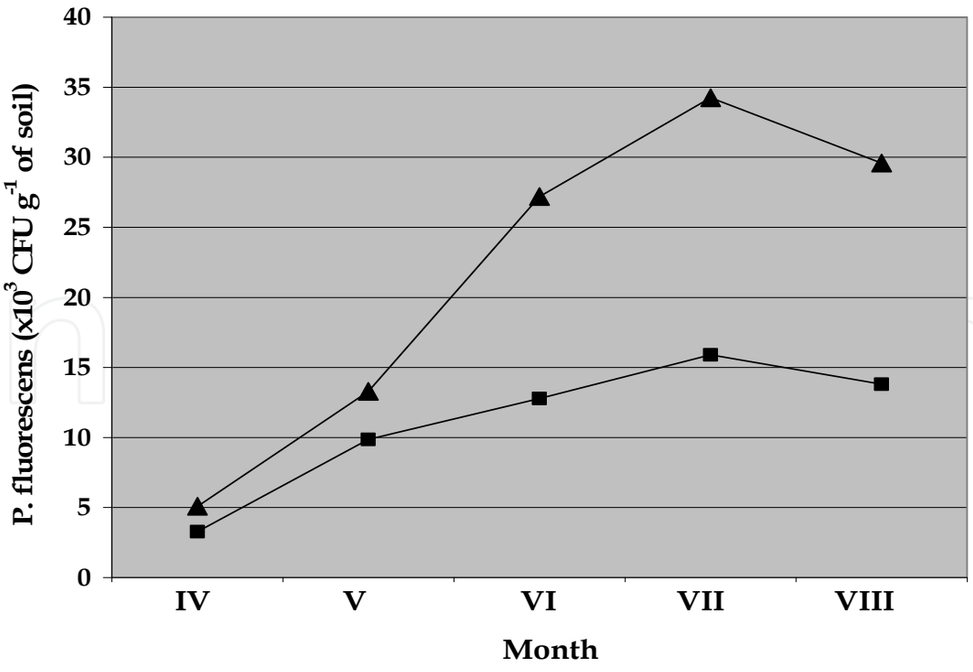
(▲ – Mollic Gleysols; ■ – Eutric Cambisols)

Fig. 3. Average number of total Actinomycetes in soil during three trial years



(▲ – Mollic Gleysols; ■ – Eutric Cambisols)

Fig. 4. Average number of *Bacillus* spp. in soil during three trial years



(▲ – Mollic Gleysols; ■ – Eutric Cambisols)

Fig. 5. Average number of *Pseudomonas fluorescens* in soil during three trial years

It is the soil of neutral reaction with high portion of organic matter and large supply of phosphorus and potassium. The soil is abundant in bacteria and actinomycetes that participate in the processes of soil organic matter humification and mineralization of humus. Large portion in microbial mass is comprised of non-symbiotic nitrogen fixers and cellulolytic microorganisms. The presence of the large number of the bacteria of the *Pseudomonas fluorescens* species, as well as *Bacillus* spp., showing antagonistic activity against the soilborne pathogenic fungi is a factor responsible for smaller number of infected plants in the controls if compared with the same on Eutric Cambisols, a soil of poorer chemical and microbiological properties. Average number of infected plants was in highly significant positive correlation with average number of decayed plants ($r=0.965$; $p<0.01$).

3.2 Root yield

Sugar beet root yield depended on soil types, or on chemical and microbiological soil properties, and on trial years.

On Mollic Gleysols in the three trial years the highest average sugar beet root yield was recorded in the variant 7 - seed inoculated with *P. fluorescens* No 8569 (0.4×10^{10} bacteria/ha) + seed inoculated with *B. subtilis* No 2109 (0.4×10^{10} bacteria/ha) + seed inoculated with *B. megaterium* No 2894 (0.4×10^{10} bacteria/ha). All other variants obtained statistically highly significant lower average root yield (Table 4). Average sugar beet root yield in treated variants was 80.63 t/ha, or 21.12% higher than average root yield in the control variant (66.57 t/ha). Since the variant with the strains of beneficial bacteria being applied together obtained significantly higher average root yield than the variant with the strains applied one at a time, it can be concluded that there was highly positive synergistic activity between them.

On Eutric Cambisols in the three trial years the highest average sugar beet root yield was recorded in the variant 8 - seed inoculated with *P. fluorescens* No 8569 (0.4×10^{10} bacteria/ha) + seed inoculated with *B. subtilis* No 2109 (0.4×10^{10} bacteria/ha) + seed inoculated with *B. megaterium* No 2894 (0.4×10^{10} bacteria/ha) + Thiram 42-S fungicide treated seed (48% Thiram, 200 ml/100 kg seed), though no significant difference ($p > 0.01$) was determined between this variant and variant 2 - Thiram 42-S fungicide treated seed (48% Thiram, 600 ml/100 kg seed). All other variants obtained highly significant ($p < 0.01$) lower sugar beet root yield. Average sugar beet root yield in treated variants was 69.45 t/ha or 21.08 % higher than the average root yield in the control variant (57.02 t/ha).

It is evident that on the soil type of poorer microbiological properties the best results were obtained with the application of chemical fungicides. It is also obvious that between variant 3 with application of the bacteria *P. fluorescens*, and variant 7 with application of

P. fluorescens, *B. subtilis* and *B. megaterium* no significant differences were determined. Therefore, it can be concluded that the bacteria *P. fluorescens* played a crucial role in the control of pathogenic fungi *P. debarianum*.

Significantly better results in sugar beet production by applying plant growth – promoting rhizobacteria *P. fluorescens* in the control of pathogenic fungi *R. solani* were reported by Esh & El-Kholi (2005), as well as by Kiewnick et al. (2001) in the control of fungi *Rhizoctonia crown* by combination of beneficial bacteria.

P. fluorescens shows distinguished biosurfactant properties (Raaijmakers et al., 2010; Ron & Rosenberg, 2001) and improves general plants condition resulting in arable crops yield and quality. As the results of their research studies some authors stated that *P. fluorescens* stimulate nutrients uptake (Duijff et al., 1993; Loper & Buyer, 1991), increases photosynthesis intensity (Zhang et al., 2002) as well as solubility of phosphorus inorganic forms. The aforesaid improves plants vigour and increases yield by 15-20% (Whipps, 2001).

3.3 Sugar content

This parameter was also influenced by weather conditions in the growing season of sugar beet. Namely, higher sugar content in sugar beet root (%) was recorded in the second trial year (Table 4).

On Mollic Gleysols in the three trial years the highest average sugar content in sugar beet was recorded in the variant 7 - seed inoculated with *P. fluorescens* No 8569 (0.4 x 10^{10} bacteria/ha) + seed inoculated with *B. subtilis* No 2109 (0.4 x 10^{10} bacteria/ha) + seed inoculated with *B. megaterium* No 2894 (0.4 x 10^{10} bacteria/ha). All other variants obtained statistically highly significant lower ($p<0.01$) average sugar content. Average sugar content in sugar beet in treated variants was 15.27% or 7.01% higher than sugar content in the control (14.27%).

No significant differences among the variants 2 - Thiram 42-S fungicide treated seed (48% Thiram, 600 ml/100 kg seed), 3 - seed inoculated with *P. fluorescens* No 8569 (1.2 x 10^{10} bacteria/ha) and 8 - seed inoculated with *P. fluorescens* No 8569 (0.4 x 10^{10} bacteria/ha) + seed inoculated with *B. subtilis* No 2109 (0.4 x 10^{10} bacteria/ha) + seed inoculated with *B. megaterium* No 2894 (0.4 x 10^{10} bacteria/ha) + Thiram 42-S fungicide treated seed (48% Thiram, 200 ml/100 kg seed) were determined. Due to the fact that variant 3, as well as variant 7- the one which obtained the best results, were not treated with a chemical fungicide, it can be left out from application in this case.

Fungicide treated seed on finishing is obviously neither sufficient nor safe measure since the fungi fast develop resistance to these pesticide active agents (Cooke et al., 1999; Durrant et al., 1998). Furthermore, fungicides are exposed to washing out, hence the possibility of underground waters eutrophication, i.e. direct danger for a human health and environment. For its physical characteristics, this beneficial bacterium represents an acceptable alternative to chemical fungicides application (Kristek et al., 2005, 2006; Thrane et al., 2000).

On Eutric Cambisols in the three trial years the highest average sugar content in sugar beet was recorded in the variant 8 - seed inoculated with *P. fluorescens* No 8569 (0.4 x 10^{10} bacteria/ha) + seed inoculated with *B. subtilis* No 2109 (0.4 x 10^{10} bacteria/ha) + seed inoculated with *B. megaterium* No 2894 (0.4 x 10^{10} bacteria/ha) + Thiram 42-S fungicide treated seed (48% Thiram, 200 ml/100 kg seed). Between this variant and variant 2 - Thiram 42-S fungicide treated seed (48% Thiram, 600 ml/100 kg seed) no significant difference was determined. High sugar content in sugar beet was obtained in variant 3 - seed inoculated with *P. fluorescens* No 8569 (1.2 x 10^{10} bacteria/ha) and variant 7 - seed inoculated with *P. fluorescens* No 8569 (0.4 x 10^{10} bacteria/ha) + seed inoculated with *B. subtilis* No 2109 (0.4 x 10^{10} bacteria/ha) + seed inoculated with *B. megaterium* No 2894 (0.4 x 10^{10} bacteria/ha) with no statistical significance determined. Average sugar content in sugar beet in treated variants was 14.44 % or 4.94 % higher than sugar content in the control (13.76 %).

Similar results were reported in the studies of Esh & El-Kholi (2005), Kristek et al. (2006, 2007) and Pedersen et al. (2002).

Investigated parameter	Variants	Mollic Gleysols (*Rhizoctonia solani*)				Eutric Cambisols (*Pythium debarianum*)			
		Year			*Average*	Year			*Average*
		2007	**2008**	**2009**		**2007**	**2008**	**2009**	
Root yield (t/ha)	1	63.40	70.21	66.10	***66.57***	50.65	68.30	52.10	***57.02***
	2	73.91	95.50	78.92	***82.77***	65.97	90.24	70.66	***75.62***
	3	72.96	94.63	79.45	***82.35***	58.79	87.10	64.98	***70.29***
	4	64.28	89.45	70.55	***74.76***	53.20	75.45	59.75	***62.80***
	5	65.96	88.16	69.18	***74.43***	54.88	76.21	60.30	***63.80***
	6	68.50	93.10	74.80	***78.80***	56.90	80.20	62.50	***66.53***
	7	78.10	98.35	86.54	***87.66***	60.25	86.47	65.10	***70.61***
	8	74.13	96.45	80.24	***83.61***	66.80	90.55	72.18	***76.51***
$LSD_{0.05}$		0.75	0.99	0.81	0.84	0.84	0.72	0.97	0.68
$LSD_{0.01}$		1.42	1.86	1.51	1.48	1.56	1.39	1.83	1.22
Sugar content (%)	1	14.08	14.53	14.21	***14.27***	13.46	14.28	13.55	***13.76***
	2	14.73	16.01	15.65	***15.46***	14.27	15.71	14.51	***14.83***
	3	14.68	15.84	15.59	***15.37***	13.99	15.29	14.09	***14.46***
	4	14.31	15.26	14.85	***14.81***	13.70	14.68	13.82	***14.07***
	5	14.37	15.32	14.62	***14.77***	13.69	14.70	13.90	***14.10***
	6	14.50	15.55	15.12	***15.06***	13.85	15.05	13.87	***14.26***
	7	15.26	16.45	16.08	***15.93***	13.82	15.36	14.11	***14.43***
	8	14.75	16.08	15.73	***15.52***	14.30	15.90	14.59	***14.93***
$LSD_{0.05}$		0.08	0.17	0.14	0.11	0.11	0.17	0.20	0.08
$LSD_{0.01}$		0.14	0.31	0.25	0.20	0.20	0.29	0.31	0.14

1. control (untreated seed); 2. Thiram 42-S fungicide treated seed (48% Tiram, 600 ml/100 kg seed); 3. seed inoculated with *P. fluorescens* No 8569 (1.2 x 10^{10} bacteria/ha); 4. seed inoculated with *B. subtilis* No 2109 (1.2 x 10^{10} bacteria/ha); 5. seed inoculated with *B. megaterium* No 2894 (1.2 x 10^{10} bacteria/ha); 6. seed inoculated with *B. subtilis* No 2109 (0.6 x 10^{10} bacteria/ha) + seed inoculated with *B. megaterium* No 2894 (0.6 x 10^{10} bacteria/ha); 7. seed inoculated with *P. fluorescens* No 8569 (0.4 x 10^{10} bacteria/ha) + seed inoculated with *B. subtilis* No 2109 (0.4 x 10^{10} bacteria/ha) + seed inoculated with *B. megaterium* No 2894 (0.4 x 10^{10} bacteria/ha); 8. seed inoculated with *P. fluorescens* No 8569 (0.4 x 10^{10} bacteria/ha) + seed inoculated with *B. subtilis* No 2109 (0.4 x 10^{10} bacteria/ha) + seed inoculated with *B. megaterium* No 2894 (0.4 x 10^{10} bacteria/ha) + Thiram 42-S fungicide treated seed (48% Tiram, 200 ml/100 kg seed)

Table 4. Root yield (t/ha) and sugar content (%) on two soil types during three trial years

3.4 Sugar in molasses

Sugar content in molasses depended, respectively, on seed treated variant, hybrid properties (tolerant, sensitive), soil type tested, and trial year.

The lowest average sugar content in molasses in the three year trial (Table 5) was recorded on Mollic Gleysols in the variant 7 - seed inoculated with *P. fluorescens* No 8569 (0.4 x 10^{10} bacteria/ha) + seed inoculated with *B. subtilis* No 2109 (0.4 x 10^{10} bacteria/ha) + seed inoculated with *B. megaterium* No 2894 (0.4 x 10^{10} bacteria/ha). Average sugar content in molasses in treated variants was 2.60% or 14.75% lower than the average sugar content in molasses in the control variants (3.05%).

Investigated parameter	Variants	Mollic Gleysols (*Rhizoctonia solani*)				Eutric Cambisols (*Pythium debarianum*)			
		Year			*Average*	Year			*Average*
		2007	2008	2009		2007	2008	2009	
Sugar in molassess (%)	1	3.15	2.95	3.04	***3.05***	4.26	3.58	4.06	***3.97***
	2	2.64	2.37	2.59	***2.53***	3.41	2.75	3.22	***3.13***
	3	2.62	2.38	2.61	***2.54***	3.69	2.99	3.44	***3.37***
	4	2.92	2.65	2.82	***2.80***	3.81	3.28	3.80	***3.63***
	5	2.85	2.61	2.88	***2.78***	3.80	3.36	3.80	***3.65***
	6	2.78	2.42	2.74	***2.65***	3.87	3.12	3.60	***3.53***
	7	2.54	2.26	2.38	***2.40***	3.66	2.94	3.38	***3.33***
	8	2.65	2.35	2.55	***2.52***	3.38	2.75	3.15	***3.09***
$LSD_{0.05}$		0.028	0.039	0.050	0.025	0.034	0.030	0.043	0.037
$LSD_{0.01}$		0.051	0.072	0.086	0.043	0.062	0.058	0.081	0.066
Sugar yield (t/ha)	1	6.92	8.13	7.38	***7.48***	4.66	7.30	4.94	***5.63***
	2	8.93	13.03	10.30	***10.75***	7.16	11.69	7.98	***8.94***
	3	8.79	12.73	10.31	***10.61***	6.06	10.71	6.92	***7.90***
	4	7.32	11.27	8.48	***9.02***	5.26	8.60	5.99	***6.62***
	5	7.59	11.20	8.28	***9.02***	5.43	8.64	6.09	***6.72***
	6	8.03	12.22	9.26	***9.84***	5.66	9.57	6.42	***7.22***
	7	9.93	13.95	11.86	***11.90***	6.12	10.74	6.99	***7.95***
	8	9.22	13.24	10.57	***11.01***	7.29	11.90	8.26	***9.15***
$LSD_{0.05}$		0.28	0.30	0.21	0.24	0.13	0.16	0.17	0.15
$LSD_{0.01}$		0.50	0.56	0.38	0.46	0.24	0.29	0.31	0.27

1. control (untreated seed); 2. Thiram 42-S fungicide treated seed (48% Tiram, 600 ml/100 kg seed); 3. seed inoculated with *P. fluorescens* No 8569 (1.2 x 10^{10} bacteria/ha); 4. seed inoculated with *B. subtilis* No 2109 (1.2 x 10^{10} bacteria/ha); 5. seed inoculated with *B. megaterium* No 2894 (1.2 x 10^{10} bacteria/ha); 6. seed inoculated with *B. subtilis* No 2109 (0.6 x 10^{10} bacteria/ha) + seed inoculated with *B. megaterium* No 2894 (0.6 x 10^{10} bacteria/ha); 7. seed inoculated with *P. fluorescens* No 8569 (0.4 x 10^{10} bacteria/ha) + seed inoculated with *B. subtilis* No 2109 (0.4 x 10^{10} bacteria/ha) + seed inoculated with *B. megaterium* No 2894 (0.4 x 10^{10} bacteria/ha); 8. seed inoculated with *P. fluorescens* No 8569 (0.4 x 10^{10} bacteria/ha) + seed inoculated with *B. subtilis* No 2109 (0.4 x 10^{10} bacteria/ha) + seed inoculated with *B. megaterium* No 2894 (0.4 x 10^{10} bacteria/ha) + Thiram 42-S fungicide treated seed (48% Tiram, 200 ml/100 kg seed)

Table 5. Sugar in molasses (%) and sugar yield (t/ha) on two soil types during three trial years

By drawing comparison among the variants with the strains of the beneficial bacteria applied one at a time, the best results in this parameter as well were recorded with the bacteria *P. fluorescens.* The variants comprising the bacteria *B. subtilis* and *B. megaterium*, being applied one at a time, or together, obtained highly significant ($p<0.01$) lower values in all tested parameters.

This is in agreement with the results of Lifshitz et al. (1987) and Whipps (2001) who stated that *P. fluorescens* as plant growth – promoting bacteria increased the solubility of phosphorus in inorganic forms around the active root zone. By mobilizing soil phosphorus plant vigour improves and yield increases by 15–20% (great root availability reduce negative nitrogen effect on sugar beet achieving balanced plant nutrition and reduced production of alpha - amino nitrogen, potassium and sodium i.e. decrease in the amount of sugar which cannot be isolated in the production process but turns into molasses).

The lowest average sugar content in molasses in the three year trial was recorded on Eutric Cambisols with the variant 8 - seed inoculated with *P. fluorescens* No 8569 (0.4×10^{10} bacteria/ha) + seed inoculated with *B. subtilis* No 2109 (0.4×10^{10} bacteria/ha) + seed inoculated with *B. megaterium* No 2894 (0.4×10^{10} bacteria/ha) + Thiram 42-S fungicide treated seed (48% Thiram, 200 ml/100 kg seed). Between this variant and variant 2 - Thiram 42-S fungicide treated seed (48% Thiram, 600 ml/100 kg seed) no statistical significance ($p>0.05$) was determined. All other variants obtained highly significant ($p<0.01$) higher values of sugar in molasses. Average sugar content in molasses in treated variants was 3.39% or 14.61% lower than average sugar content in molasses in the control variants (3.97%).

3.5 Sugar yield

On Mollic Gleysols the highest average sugar yield in the three trial years was recorded in the variant 7 - seed inoculated with *P. fluorescens* No 8569 (0.4×10^{10} bacteria/ha) + seed inoculated with *B. subtilis* No 2109 (0.4×10^{10} bacteria/ha) + seed inoculated with *B. megaterium* No 2894 (0.4×10^{10} bacteria/ha). All other variants obtained statistically highly significant lower average sugar yield. Average sugar yield in treated variants was 10.31 t/ha or 37.83% higher than average sugar yield in the control (7.48 t/ha). It is evident that in this parameter as well, the best results were obtained in the second year of the trial (Table 5).

On Eutric Cambisols the highest average sugar yield in the three trial years was recorded in the variant 8 - seed inoculated with *P. fluorescens* No 8569 (0.4×10^{10} bacteria/ha) + seed inoculated with *B. subtilis* No 2109 (0.4×10^{10} bacteria/ha) + seed inoculated with *B. megaterium* No 2894 (0.4×10^{10} bacteria/ha) + Thiram 42-S fungicide treated seed (48% Thiram, 200 ml/100 kg seed), though no statistical significant difference ($p > 0.05$) was determined between the above variant and variant 2 - Thiram 42-S fungicide treated seed (48% Thiram, 600 ml/100 kg seed). All other variants obtained highly significant ($p < 0.01$) larger number of infected and decayed plants. Average sugar yield in treated variants was 7.78 t/ha or 38.18% higher than average sugar yield in the control variant (5.63 t/ha).

Sugar yield was in very significant positive correlation with root yield ($r=0.975$; $p<0.01$) and sugar content ($r=0.946$; $p<0.01$).

4. Conclusion

Inoculation of sugar beet seed with the bacteria *Pseudomonas fluorescens*, *Bacillus subtilis* and *Bacillus megaterium* affected root decay agents *Rhizoctonia solani* and *Pythium debarianum* and showed highly significant influence on all the elements of sugar beet yield and quality in the three trial years.

The best results in all elements of the research on both soil types were recorded in the second trial year (2008) due to the more favourable weather conditions (air temperatures, precipitation values) in the growing season 2008 than in the dry 2007 and 2009.

Better results in all tested parameters in the three trial years were obtained on Mollic Gleysols. The reason is in chemical and microbiological properties of much better quality than on Eutric Cambisols. In Mollic Gleysols total number of the bacteria and actinomycetes is higher enabling more intensive humification of organic matter and mineralization of humus in the plant-accessible mineral compounds. Significantly higher number of beneficial bacteria in the soil (*P. fluorescens*, *Bacillus* spp.) that show antagonism against soilborne pathogens is evident.

On Mollic Gleysols the best results in all tested parameters were recorded in the variant treated with the beneficial bacteria (*P. fluorescens*, *B. subtilis*, *B. megaterium*), whereas all other variants obtained significantly ($p<0.01$) lower values. Due to the beneficial bacteria in the soil, population of pathogenic microorganisms was reduced, making application of the beneficial bacteria in the sowing period sufficient to reduce or control the infection with pathogenic fungi *R. solani*. Among the beneficial bacteria *P. fluorescens*, *B. subtilis* and *B. megaterium* highly positive synergism is evident.

On Eutric Cambisols the best results in all tested parameters were reached in the variant treated with chemical fungicide and the beneficial bacteria (*P. fluorescens*, *B. subtilis*, *B. megaterium*). Nevertheless, in most cases no statistically significant difference ($p>0.05$) was determined between this variant and the variant being treated solely with chemical fungicide. The reason is in poorer quality of chemical and microbiological soil properties which is favourable for reproduction of pathogenic fungi *P. debarianum* in the soil.

If necessary measures of improving chemical soil properties are to be carried out, neutralization of soil acidity in the first place, microbiological soil properties will be improved, and the beneficial soil microorganisms will increase in number. Due to the antagonism of beneficial microorganisms against soilborne plant pathogens the number of pathogenic microorganisms will be reduced as well as the infection of arable crops. In this case the application of beneficial microorganisms will reach satisfactory level without application of chemical pesticides. By introducing beneficial microorganisms microbiological soil properties will be consequently improved and number of pathogens in the forthcoming growing season will be decreasing. The reduction in the application of chemical pesticides is the matter of great importance from the both economic and ecological point of view.

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6. References

Andersen, J.B.; Koch, B.; Nielsen, T.H.; Sørensen, D.; Hansen, M.; Nybroe, O.; Christophersen, C.; Sørensen, J.; Molin, S. & Giskov, M. (2003). Surface motility in *Pseudomonas sp*. DSS73 is required for efficient biological containment of the root – pathogenic microfungi *Rhizoctonia solani* and *Pythium ultimum*. *Microbiology*, Vol.149, No. 1, (January 2003), pp. 37-46, ISSN 1350-0872

Asaka, O. & Shoda, M. (1996). Biocontrol of *Rhizoctonia solani* damping-off of tomato with *Bacillus subtilis* RB14. *Applied and Environmental Microbiology*, Vol.62, No.11, (November 1996), pp. 4081-4085, ISSN 0099-2240

Choudhary, D.K. & Johri, B.N. (2009). Interactions of *Bacillus* spp. and plants-with special reference to induced systemic resistance (ISR). *Mirobiological research,* Vol.164, No.5, (September 2009), pp. 493-513, ISSN 0944-5013

Cooke, D.A.; Dewar, A.M. & Asher, M.J.C. (1989). Pests and diseases of sugar beet, In: *Pest and Disease Control Handbook,* N. Scopes & L. Stables, (Ed.), 241-259, British Crop Protection Council, ISBN 0-948404-28-0, Thornton Heath, UK

Desai, J.D. & Banat, I. M. (1997). Microbial production of surfactants and their commercial potential. *Micr. Mol. Biol. Rev.* Vol.61, No.1, (March 1997), pp. 47-64, ISSN 1092-2172

Duijff, B.J.; Meijer, J.W.; Bakker, P.A.H.M. & Schippers, B. (1993). Siderophore mediated competition for iron and induced disease resistance in the suppression of *Fusarium wilt* of carnation by fluorescent *Pseudomonas* spp. *Neth. J. Plant Pathol.,* Vol.99, No.5-6, (September 1993), pp. 277-289, ISSN 0028-2944

Durrant, M.J.; Payne, P.A.; Prince, J.W.F. & Fletcher, R. (1988). Thiram steep seed treatment to control *Phoma betae* and improve the establishment of the sugar beet plant stand. *Crop protection,* Vol.7, No.5, (October 1988), pp. 319-326, ISSN 0261-2194

El-Tarabily, K.A. & Sivasithamparam, K. (2006). Potential of yeasts as biocontrol agents of soil-borne fungal plant pathogens and as plant growth promoters. *Mycoscience*, Vol.47, No. 1, (February 2006), pp. 25-35, ISSN 1340-3540

Engelhardt, S.; Lee, J.; Gäbler, Y.; Kemmerling, B.; Haapalainen, M.L.; Li, C.M.; Wei, Z.; Keller, H.; Joosten, M. & Taira, S. (2009). Separable roles of the *Pseudomonas syringae* pv. *phaseolicola* accessory protein HrpZ1 in ion-conducting pore formation and activation of plant immunity. *Plant J.*, Vol.57, No.4, (February 2009), pp. 706-717, ISSN 0960-7412

Esh, A.M.H. & El-Kholi, M.M. (2005). Effect of *Pseudomonas fluorescens* extra cellular enzymes and secondary metabolites on *Rhizoctonia solani* the causal of sugar beet damping off disease. *Zagazig Journal of Agricultural Research,* Vol.32, pp. 1537-1557, ISSN 1110-0338

Evačić, M.; Kristek, S. & Stanisavljević, A. (2008). Use of the bacteria *Bacillus megaterium* in the control of sugar beet root decay agents – *Pythium ultimum* and *Pythium debarianum. Cereal Resear.Comm.*, Vol.36, Suppl.5, Part1, pp. 383-386, ISSN 0133-3720

FAO. World Reference Base for Soil Resources, (1998). FAO Roma

Gorlach-Lira, K. & Stefaniak, O. (2009): Antagonistic activity of bacteria isolated from crops cultivated in a rotation system and a monoculture against *Pythium debaryanum* and *Fusarium oxysporum*. *Folia microb.*, Vol.54, No.5, (July 2009), pp. 447-450, ISSN 0015-5632

Kiewnick, S.; Barry, J.J.; Braun-Kiewnick, A.; Eckhoff, L.A. & Bergman, J.W. (2001). Integrated control of *Rhizoctonia crown* and root rot of sugar beet with fungicides

and antagonistic bacteria. *Plant Disease*, Vol.85, No.7, (July 2001), pp. 718-722, ISSN 0191-2917

Koch, A.K.; Kappeli, O.; Fiechter, A. & Reiser, J. (1991). Hydrocarbon assimilation and biosurfactant production in *Pseudomonas aeruginosa* mutants. *Journal of Bacteriology,* Vol.173, No.13, (July 1991), pp. 4212-4219, ISSN 0021-9193

Koch, B.; Nielsen, T.H.; Sørensen, D.; Andersen, J.B.; Christophersen, C.; Molin, S.; Giskov, M.; Sørensen, J. & Nybroe, O. (2002). Lipopeptide production in *Pseudomonas* sp. strain Dss73 is regulated by components of sugar beet seed exudates via the Gac two – component regulatory system. *Applied and Environmental Microbiology*, Vol.68, No.9, (September 2002), pp. 4509-4516, ISSN 0099-2240

Kristek, S. & Kristek, A. (2005). Inoculation of sugar beet seed by the bacterium *Pseudomonas fluorescens* - chemical fungicides alternative. *Listy Cukrovarnicke a Reparske*, Vol.121, No.4, pp. 49-53, ISSN 0024-4449

Kristek, S.; Kristek, A.; Guberac, V. & Stanisavljević, A. (2006). Effect of bacterium *Pseudomonas fluorescens* and low fungicide dose seed treatments on parasite fungus *Aphanomyces cochlioides* and sugar beet yield and quality. *Plant, soil and environment*, Vol.52, No.7, (July 2006), pp. 314-320, ISSN 1214-1178

Kristek, S.; Kristek, A.; Pospišil, M.; Glavaš Tokić, R. & Ćosić, J. (2007). Influence of bacterium *Pseudomonas fluorescens* on the pathogen of root rot *Rhizoctonia solani*, storage period and elements of sugarbeet yield and quality. *Zuckerindustrie*, Vol.132, No.7, (July 2007), pp. 568-575, ISSN 0344-8657

Lee, C.H.; Kempf, H.J.; Lim, Y. & Cho, H. (2000). Biocontrol activity of *Pseudomonas cepacia* AF2001 and anthelmintic activity of its novel metabolite, cepacidine A. *Journal of Microb. and Biotechnology,* Vol.10, No.4, (August 2000), pp. 568-571, ISSN 1017-7825

Lifshitz, R.; Kloepper, J.W. & Kozlowski, M. (1987). Growth promotion of canola (repeseed) seedlings by a strain of *Pseudomonas putida* under gnotobiotic conditions. *Can. J. Microbiol*., Vol.33, No.5, (May 1987), pp. 390-395, ISSN 0008-4166

Lindum, P.W., Anthoni, U., Christophersen, C., Eberl, L., Molin, S. & Givskov, M. (1998). N – Acyl – L – homoserine lactone autoinducers control production of an extracellular lipopeptide biosurfactant required for swarming motility of *Serratia liquefaciens* MG1. *J. Bacteriol*, Vol. 180, No. 23, pp. 6384-6388, ISSN 0021-9193

Loper, J.E. & Buyer, J.S. (1991). Siderophores in microbial interactions on plant surfaces. *Mol. Plant - Microbe Interact.*, Vol.4, No.1, (Jan.-February 1991), pp. 5-13, ISSN 0894-0282

Neu, T.R. (1996). Significance of bacterial surface – active compounds in interaction of bacteria with interfaces. *Microbiol. Rev.*, Vol.60, No.1, (March 1996), pp. 151-166, ISSN 0146-0749

Nielsen, T.H.; Thrane, C.; Christophersen, C.; Anthoni, U. & Sørensen, J. (2000). Structure, production characteristics and fungal antagonism of tensin – a new antifungal cyclic lipopeptide from *Pseudomonas fluorescens* strain 96.578. *Journal of Applied Microbiology*, Vol.89, No.6, (December 2000), pp. 992-1001, ISSN 1364-5072

Nielsen, T.H.; Sørensen, D.; Tobiasen, C.; Andersen, J.B.; Christophersen, C.; Giskov, M. & Sørensen, J. (2002). Antibiotic and biosurfactant properties of cyclic lipopeptides produced by fluorescent *Pseudomonas* spp. from the sugar beet rhizosphere. *Applied and Envir. Microbiology*, Vol.68, No.7, (July 2002), pp. 3416-3423, ISSN 0099-2240

Pedersen, H.C.; Weiergang, I.; Pontoppidan, M.M.; Jørgensen, L. & Svingel, A. (2002). Danisco Seed, Seed Technology Dept., Hoejbygaardvej 31, DK – 4960 Holeby, Denmark

Raaijmakers, J.M.; De Brujin, I.; Nybroe, O. & Ongena, M. (2010). Natural functions of lipopeptides from *Bacillus* and *Pseudomonas*: more than surfactants and antibiotics. FEMS *Microb. Reviews,* Vol.34, No.6, pp.1037-1062, ISSN 1574-6976

Ron, E.Z. & Rosenberg, E. (2001). Natural roles of biosurfactants. *Environmental Microbiol*ogy, Vol.3, No.4, (April 2001), pp. 229-236, ISSN 1462-2912

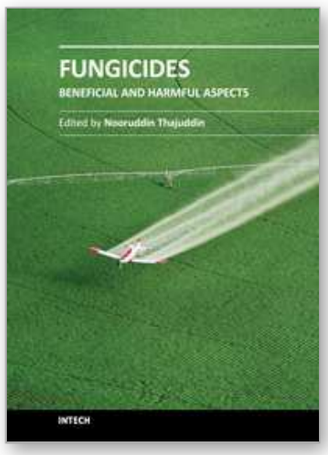
Sørensen, D.; Nielsen, T.H.; Christophersen, C.; Sørensen, J. & Gajhede, M. (2001). Cyclic lipoundecapeptide amphisin from *Pseudomonas* sp. Strain Dss73. *Acta Crystallographica Section C Crystal Structure Communications,* Vol.57, No.9, (September 2001), pp. 1123 – 1124, ISSN 0108-2701

Thrane, C.; Nielsen, T.H.; Nielsen, M.N.; Sørensen, J. & Olsson, S. (2000). Viscosinamide – producing *Pseudomonas fluorescens* DR54 exerts a biocontrol effect on *Pythium ultimum* in sugar beet rhizosphere. *FEMS Microbiology Ecology,* Vol.33, No.2, (August 2000), pp. 139-146, ISSN 0168-6496

Zhang, S.; Reddy, M.S. & Kloepper, J.W. (2002). Development of assays for assessing induced systemic resistance by plant growth-promoting rhizobacteria against blue mold of tobacco. *Biological Control*, Vol.23, No.1, (January 2002), pp. 79-86, ISSN 1049-9644

Whipps, J.M. (2001). Microbial interactions and biocontrol in the rhizosphere. *Journal of Experimental Botany*, Vol.52, Suppl.1, (March 2001), pp. 487-511, ISSN 0022-0957

www.statsoft.com



Fungicides - Beneficial and Harmful Aspects
Edited by Dr. Nooruddin Thajuddin

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Fungicides are a class of pesticides used for killing or inhibiting the growth of fungus. They are extensively used in pharmaceutical industry, agriculture, in protection of seed during storage and in preventing the growth of fungi that produce toxins. Hence, fungicides production is constantly increasing as a result of their great importance to agriculture. Some fungicides affect humans and beneficial microorganisms including insects, birds and fish thus public concern about their effects is increasing day by day. In order to enrich the knowledge on beneficial and adverse effects of fungicides this book encompasses various aspects of the fungicides including fungicide resistance, mode of action, management fungal pathogens and defense mechanisms, ill effects of fungicides interfering the endocrine system, combined application of various fungicides and the need of GRAS (generally recognized as safe) fungicides. This volume will be useful source of information on fungicides for post graduate students, researchers, agriculturists, environmentalists and decision makers.

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InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

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