The role of mulch in the formation of microorganisms populations in the soil under the cultivation of American ginseng (*Panax quinquefolium* L.)

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S u m m a r y

Studies were conducted in 2005–2007 on an existing field plantation of American ginseng in Krasnystaw (Lubelski region). The object of the studies was the soil from the American ginseng cultivation where two kinds of mulch were used, i.e. oat straw and mineral mulch Agran. Microbiological analysis of the soil of particular experimental combinations showed differentiated populations of microorganisms. On average, the most colony-forming units (CFU) of bacteria in total were obtained from the soil taken from ginseng cultivation after using mineral mulch as compared to the combination with oat straw. Similar results of CFU were obtained in the case of *Bacillus* spp. and *Pseudomonas* spp. On the other hand, the most CFU of fungi were isolated from the soil samples taken from the combination after the use of oat straw. The most frequently isolated fungi regarded as pathogenic to ginseng plants were: *Fusarium* spp., *Cylindrocarpon* spp., *Phytophtora* sp., *Pythium irregulare, Rhizoctonia solani, Sclerotinia sclerotiorum* and *Altenaria alternata.*

Key words: *mulch, soil microorganisms, American ginseng*

INTRODUCTION

The proper functioning of eco-systems is affected not only by abiotic factors but also by biotic ones, including soil microorganisms. Quantitative and qualitative composition of the soil microorganisms is diverse and depends on soil conditions, ecological factors, the development stage the plant species [1-4]. Our research showed that soil environment, especially the rhizosphere, creates favourable conditions for the microorganisms development, mainly bacteria [5-9]. According to Richards [8], the population of soil microorganisms is influenced by organic compounds of easily oxidated carbon present in organic fertilizers and in the aftercrop residues of plants. The soil is a habitat of both microorganisms which are pathogenic to plants and of saprobiontic ones. Various reactions occur between them. Badura et al. [1] and Wielgosz et al. [3] found out that among all microorganisms in the soil, there is a great population of *Pseudomonas* spp. bacteria. The plant infection if this bacteria probably contributes to the pathogens inhibition.

The application of mulches in plant cultivation as cultivations' conservant also influences the physical, chemical and biological properties of the soil [10-12]. Such cultivations have a significant effect on the microbiological activity of the soil, including the composition of microorganisms populations [12].

The purpose of the present studies was to establish the quantitative and qualitative composition of microorganisms colonizing the soil after the application of mulch in the cultivation of American ginseng.

MATERIAL AND METHODS

The studies were conducted in 2005–2007 on a field plantation of American ginseng situated in Krasnystaw (Lubelski region). The soil where ginseng was cultivated had the mechanical composition of light loamy sand and was characterized with lightly acidic reaction (pH_{KCL} – 6.0), medium content of the organic matter (1.92%) and high potassium and phosphorus as well as medium magnesium content.The procedure of field preparation under the cultivation of ginseng and the experimental model were described in our earlier paper [13 a, b].

In 2005–2007 soil samples were taken at the depth of 5–6 cm of the plough layer from particular control plots (i.e. without any plant protection treatments) from a two-, three- and four-year-old ginseng plantation. The soil was submitted to a laboratory microbiological analysis by Martyniuk et al. [14]. In the laboratory, particular soil samples were carefully mixed, subsequently weighed into samples of 10 g and placed into 250 ml flasks, with 90 ml of sterile distilled water. Then the flasks were placed on a shaker and shaken for 30 minutes. Dilutions from 10^{-2} to 10^{-7} were prepared from so prepared soil solution.

Medium Nutrient Agar and the dilutions of the soil solution of 10^{-5}, 10^{-6}, 10^{-7} were used with the aim to obtain the total number of CFU of bacteria in the soil. In order to isolate CFU of *Bacillus* spp., the medium Tryptic Soy Agar was used and the dilutions of the soil solution of 10^{-4}, 10^{-5}, 10^{-6}. On the other hand, the medium Pseudomonas Agar F and the water dilutions of the soil solution 10^{-2}, 10^{-3}, 10^{-4} were used in order to establish the population of CFU of *Pseudomonas* spp. The total number of CFU of fungi in particular samples was determined using Martin's medium [15] and the dilutions of the soil solution of 10^{-2}, 10^{-3} and 10^{-4} were applied.

Among the bacteria colonies isolated from the soil, 100 isolates of each *Bacillus* spp. and *Pseudomonas* spp. as well as all isolated colonies of *Gliocladium* spp. and *Trichoderma* spp. were used in order to determine their antagonistic effect on the fungi pathogenic to ginseng. In the laboratory tests the colonies of following plant pathogens were used: *Altenaria alternata, Botrytis cinerea, Cylindrocarpon destructans, Fusarium oxysporum, Fusarium solani, Pythium irregulare, Rhizoctonia solani* and *Sclerotinia sclerotiorum*. The antagonistic effect of the studied bacteria was established with the use of the method described by Martyniuk et al. [14] and Pięta [16], whereas biotic tests described by Mańka [17] were used for the fungi.

In *Bacillus* spp. and *Pseudomonas* spp. bacteria the criteria of antagonistic effect were the size of the inhibition zone and the percentage of growth inhibition of the tested pathogenic fungus. On the other hand, while establishing the antagonistic effect of *Gliocladium* spp. and *Trichoderma* spp., the same parameters were considered as in bacteria. In addition, the growth of the saprobionts onto the plant pathogens was found out. The latter criterion was checked by means of a microscope with the aim of establishing the parasitism of *Gliocladium* spp. and *Trichoderma* spp. on pathogenic fungi.

The obtained results concerning the total number of CFU of bacteria, *Bacillus* spp., *Pseudomonas* spp. and fungi were statistically analyzed using Duncan's semi-intervals [18].

RESULTS

The microbiological analysis showed differentiated populations of soil microorganisms in particular soil samples. A higher average total number of CFU of bacteria was characteristic for the soil from the plots after the application of mineral mulch as compared to the soil taken from the combination with oat straw (tab. 1). Besides, the average number of CFU of all bacteria was the highest in the soil samples taken from a two-year-old plantation of ginseng after using both Agran as a mineral mulch and oat straw as compared to the soil samples taken from older plantations (tab. 1).

Table 1.

Number of bacteria and fungi isolated from the soil after using mulch in the cultivation of American ginseng

the combination of the experiment	total number CFU of bacteria (mln x g^{-1} d.w. of soil)				total number CFU *Bacillus* spp. (mln x g^{-1} d.w. of soil)			
	2005	2006	2007	mean	2005	2006	2007	mean
	II	III	IV		II	III	IV	
straw	2.63^{a*}	2.36^{a}	1.24^{a}	2.07^{a}	1.71^{a}	0.82^{a}	0.59^{a}	1.04^{a}
Agran	4.16^{b}	3.24^{b}	2.41^{b}	3.27^{b}	1.80^{b}	1.32^{b}	1.25^{b}	1.46^{b}

the combination of the experiment	total number CFU *Pseudomonas* spp. (mln x g^{-1} d.w. of soil)				total number CFU of fungi (thous x g^{-1} d.w. of soil)			
	2005	2006	2007	mean	2005	2006	2007	mean
	II	III	IV		II	III	IV	
straw	0.28^{a}	0.45^{a}	0.47^{a}	0.40^{a}	13.30^{b}	16.20^{b}	18.55^{b}	16.00^{b}
Agran	1.27^{b}	1.50^{b}	0.76^{b}	1.18^{b}	5.16^{a}	5.17^{a}	12.35^{a}	7.56^{a}

* mean in columns followed by the same letter do not differ significantly at $p \leq 0.05$
II – two-year-old ginseng plantation
III – three-year-old ginseng plantation
IV – four-year-old ginseng plantation

The population of *Bacillus* spp. ranged from 1.46 million · g^{-1} d.m. of the soil taken after using mineral mulch to 1.04 million · g^{-1} d.m. of the soil in the case of mulching with oat straw (tab. 1). The biggest colonies of *Bacillus* spp. number was obtained from the soil taken from two-year-old plantation, while the fewest from the soil taken from a four-year-old one. The population of *Pseudomonas* spp. ranged from 1.18 million · g^{-1} d.m. to 0.40 million · g^{-1} d.m. Much more *Pseudomonas* colonies of this kind were found in the soil taken from the plantation after using Agran as compared to oat straw.

Fungi were also isolated from the soil samples. On average, the highest total number of CFU of fungi colonies totally occurred in the soil samples taken from the plots mulched with oat straw as compared to the soil taken from the combinations mulched with Agran (tab. 1). Besides, it was found out that in the soil taken from the oldest (four-year-old) plantations, regardless of the kind of mulch, the population of those microorganisms was the highest one.

Among the fungi isolated from particular soil samples, the greatest number of colonies regarded as pathogenic to ginseng plants was obtained from the combination after using oat straw as compared to mineral mulch (tab. 2). The most frequently isolated fungi were those of *Fusarium* spp.: *F. culmorum, F. equiseti, F. oxysporum, F. solani* and *F. sporotrichioides*. Although, these species were not isolated from all plantations and not in all years (tab. 2). Besides, the following species were isolated from soil samples taken under the cultivation of ginseng: *Cylindrocarpon* spp., *Phytophthora* sp., *Pythium irregulare, Rhizoctonia solani, Sclerotinia sclerotiorum* and *Altenaria alternata* (tab. 2).

Table 2

Fungi frequently isolated from the soil after using mulch in the cultivation of American ginseng

fungus species	experimental combination/number of isolates								total
	straw				Agran				
	2005	2006	2007	total	2005	2006	2007	total	
	II	III	IV		II	III	IV		
Acremonium murorum (Corda) W. Gams	-	13	9	**22**	-	8	31	**39**	**61**
Alternaria alternata (Fr.) Keissler	3	7	13	**23**	1	7	8	**16**	**39**
Aspergillus flavus Link	-	4	2	**6**	-	5	6	**11**	**17**
Cladosporium cladosporioides (Fres.) de Vries	7	1	11	**19**	-	4	8	**12**	**31**
***Cylindrocarpon* spp.**	-	**8**	**6**	**14**	-	**3**	**8**	**11**	**25**
Cylindrocarpon decumbens Corda	-	3	2	**5**	-	-	2	**2**	**7**
Cylindrocarpon destructans (Zins.) Scholt.	-	5	4	**9**	-	3	6	**9**	**18**
***Fusarium* spp.**	**19**	**28**	**21**	**68**	**21**	**13**	**20**	**54**	**122**
Fusarium culmorum (W. G. Sm.) Sacc.	-	4	10	**14**	-	2	-	**2**	**16**
Fusarium equiseti (Corda) Sacc.	1	-	-	**1**	4	-	-	**4**	**5**
Fusarium oxysporum Schl.	18	14	8	**40**	2	6	12	**20**	**60**
Fusarium solani (Mart.) Sacc.	-	10	2	**12**	15	5	5	**25**	**37**
Fusarium sporotrichioides Sherb.		-	1	**1**	-	-	3	**3**	**4**
Gliocladium roseum (Link) Bainier	-	1	-	**1**	-	3	2	**5**	**6**
***Mucor* spp.**	**12**	**4**	-	**16**	-	-	-	-	**16**
Mucor globosus Fischer	-	4	-	**4**	-	-	-	-	**4**
Mucor racemosus Fresenius	**12**	-	-	**12**	-	-	-	-	**12**
Papulaspora irregularis Hotson	-	-	3	**3**		-	-	-	**3**
***Penicillium* spp.**	**54**	**11**	**8**	**73**	**7**	**7**	**2**	**16**	**89**
Penicillium canescens Scopp.	23	-	-	**23**	-	-	-	-	**23**
Penicillium chrysogenum Thom	-	-	-	-	-	3	-	**3**	**3**
Penicillium expansum Link ex S. F. Gray	14	-	-	**14**	6	-	-	**6**	**20**
Penicillium frequentans Westling	-	9	-	**9**	-	4	2	**6**	**15**
Penicillium janthinellum Biourge	-	2	8	**10**	-	-	-	-	**10**
Penicillium verrucosum Dierckx var. *cyclopium* (West.) Samson et al.	17	-	-	**17**	1	-	-	**1**	**18**
Phytophthora sp.	-	8	-	**8**	-	-	2	**2**	**10**
Pythium irregulare Buisman	11	-	-	**11**	7	-	-	**7**	**18**
Rhizoctonia solani Kühn	-	4	-	**4**	-	-	5	**5**	**9**
Rhizopus nigricans Ehrenberg	18	3	4	**25**	6	7	-	**13**	**38**
Sclerotinia sclerotiorum (Lib.) de Bary	6	7	-	**13**	2	3	4	**9**	**22**
***Trichoderma* spp.**	**4**	**2**	-	**6**	**2**	**4**	**3**	**9**	**15**
Trichoderma aureoviride Rifai	4	-	-	**4**	2	-	-	**2**	**6**
Trichoderma koningii Oud.	-	2	-	**2**	-	4	3	**7**	**9**
total	**134**	**101**	**77**	**312**	**46**	**64**	**99**	**209**	**521**

In the all soil samples, saprophytic fungi from *Penicillium* genus were the most frequently and numerously isolated. Moreover colonies of *Mucor* spp. and *Trichoderma* spp., as well as *Acremonium murorum, Aspergillus flavus, Cladosporium cladosporioides, Gliocladium roseum* and *Rhizopus nigricans* were identified (tab. 2).

A differentiation in the populations of antagonistic bacteria and fungi in particular samples of the examined soil was stated. It was shown that more antagonistic microorganisms colonized the soil taken from the plots after the application of mineral mulch in comparison to the combination with oat straw (tab. 3, fig. 1). Laboratory tests showed that regardless of the experimental combination much more colonies of antagonistic bacteria were obtained from the samples taken from younger plantations than from four-year-old ginseng plantation. In contrary, in the antagonistic fungi the situation was reverse since much more colonies of antagonistic fungi were obtained from the soil taken from the older plantations (tab. 3).

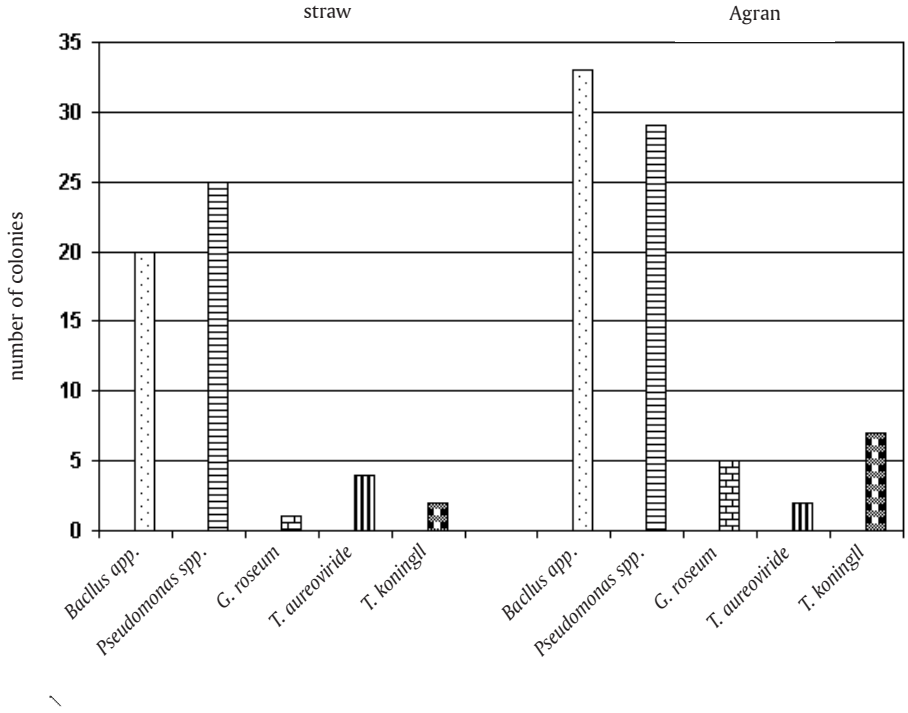

Figure 1. The effect of the kind of mulch on the occurrence of antagonistic microorganisms in the soil after the American ginseng cultivation (total from 2005-2007)

Table 3

Antagonistic bacteria and fungi isolated from the soil under American ginseng cultivation

antagonistic bacteria and fungi	number of colonies/experimental combination						total
	straw			Agran			
	2005	2006	2007	2005	2006	2007	
	II	III	IV	II	III	IV	
Bacillus spp.	7	4	9	11	11	11	**53**
Pseudomonas spp.	13	8	4	13	10	6	**54**
total	**20**	**12**	**13**	**24**	**21**	**17**	**107**
Gliocladium roseum	-	1	-	-	3	2	**6**
Trichoderma aureoviride	4	-	-	2	-	-	**6**
Trichoderma koningii	-	2	-	-	4	3	**9**
total	**4**	**3**	-	**2**	**7**	**5**	**21**

II – two-year-old ginseng plantation;
III – three-year-old ginseng plantation
IV – four-year-old ginseng plantation

DISCUSSION

The present studies showed relations between the kind of mulch and the quantitative and qualitative composition of microorganisms populations in the soil. In the soil environment after the application of oat straw as mulch – regardless of the age of the plantation – there were significantly fewer CFU of total bacteria, including *Bacillus* spp. and *Pseudomonas* spp. in comparison to the experimental combination with Agran. On the other hand, a significant increase of CFU of fungi was found out after the use of oat straw. It should be supposed that oat straw proved to be a worse stimulator for the bacteria development, whereas as the organic matter it contributes to the development of certain populations of saprobionts such as *Mucor* spp., *Penicillium* spp., and *Acremonium murorum*, *Papulaspora irregularis* and *Rhizopus nigricans*. It can be stated that the soil – regardless of the applied mulch – was poor in *Gliocladium* spp. and *Trichoderma* spp., commonly regarded as antagonistic fungi [19].

Kiecana and Mielniczuk [20] found out that oats was infected by *Fusarium* spp., *Pythium* spp., *Botrytis cinerea, Cylindrocarpon destructans, Rhizoctonia solani.* Therefore, the use of mulch of oat straw probably contributed to the increase of the number of phytopathogens in the soil, which was confirmed in the microbiological analysis of the soil.

After the use of Agran, on average twice less total fungi were obtained. Contrary to the combination with oat straw, Agran had a positive effect on the presence of bacteria in the soil, especially of *Pseudomonas* spp. It should be supposed that

the presence of fly ash, betonite and coal dust in mineral mulch probably stimulates the increase and development of total bacteria, especially of *Pseudomonas* spp. These bacteria inhibit the development of soil plant pathogens and plant infection [1, 3, 21]. The studies results showed that bacteria occurred more numerously in the soil from younger plantations of ginseng as compared to older ones. According to Wielgosz and Szember [22], both the plants' age and developmental stage had a big influence on the quantity and quality of root exudates. This is closely connected with the composition of soil microorganisms for which the exudates are the main source of nutrition. Such numerous populations of bacteria, mainly of *Pseudomonas* spp., especially in the initial period of ginseng growth is fairly important since they produce the substances supporting the plants' growth as well as antibiotics which inhibit the development of soil pathogens [23]. Ginseng is a perennial plant and achieves the proper yield useful for the herb industry only in the fourth year of the cultivation. Hence, it is very important that young plantations should not be threatened with pathogenic fungi.

According to Myśkow and Zięba [24], there are definite proportions between the population of soil microorganisms. The environment with more bacteria contains much fewer colonies of fungi and vice versa, where there are less bacteria, the population of fungi increases.

In order to protect plants from infection and to make the plant pathogens development in the soil difficult or impossible it is important to create the conditions for the antagonists development. Laboratory tests showed that Agran had a better effect on the populations of both antagonistic bacteria and fungi. Numerous presence of antagonists reduces the CFU of plant pathogens which makes the soil more resistant [21].

Results from the studies conducted by Pięta and Berbeć [25] as well as by Pastucha and Kołodziej [13 a, b] show that ginseng plants were infected by pathogenic plants throughout the vegetation period. Those fungi caused first necrosis before and after plants emergence, and subsequently dying out of older plants, which destroyed the whole plantation. That is the reason why it is so important to carry out microbiological analyses of the soil in order to get to know the composition of microorganisms populations. This will enable us to predict the occurrence of diseases, and at the same time to prepare the methods in reducing the losses in the field. It is crucial to continue such studies, especially in order to set and apply several pro-ecological cultivation technologies of all herbs, including ginseng.

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ROLA MULCZU W KSZTAŁTOWANIU POPULACJI MIKROORGANIZMÓW W GLEBIE SPOD UPRAWY ŻEŃ-SZENIA PIĘCIOLISTNEGO (*PANAX QUINQUEFOLIUM* L.)

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S t r e s z c z e n i e

Badania prowadzono w latach 2005–2007 na istniejącej plantacji żeń-szenia pięciolistnego zlokalizowanej w Krasnymstawie (woj lubelskie). Przedmiotem badań była gleba pobierana spod uprawy żeń-szenia amerykńskiego, gdzie zastosowano dwa rodzaje mulczu tj. słomę owsianą oraz mulcz mineralny Agran. Wykonana analiza mikrobiologiczna gleby pochodzącej z poszczególnych kombinacji wykazała zróżnicowaną liczebność mikroorganizmów. Średnio najwięcej jednostek tworzących kolonie (JTK) bakterii ogółem uzyskano z gleby pobranej spod uprawy żeń-szenia pięciolistnego po zastosowaniu mulczu mineralnego w porównaniu z kombinacją ze słomą owsianą. Podobne wyniki JTK uzyskano w przypadku *Bacillus* spp. oraz *Pseudomonas* spp. Natomiast najwięcej JTK grzybów wyizolowano z prób gleby pobranych z kombinacji po zastosowaniu słomy owsianej. Najczęściej izolowano *Fusarium* spp., *Cylindrocarpon* spp., *Phytophthora* sp. oraz *Pythium irregulare, Rhizoctonia solani, Sclerotinia sclerotiorum* i *Alternaria alternata*.

Słowa kluczowe: *mulcz, mikroorganizmy glebowe, żeń-szeń amerykański*