23. THE EFFECT OF CHITOSAN IN AMERICAN GINSENG (*PANAX QUINQUEFOLIUM* L.) PROTECTION

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1. Introduction

American ginseng (*Panax quinquefolium* L.) is a relative of very well known from almost 4000 years species - *Panax ginseng* C.A.Meyer, originated from south-eastern part of North America [10]. It is a perennial, long-lived plant (individuals aged above 100 years were found in natural state), in cultivation usually harvested after three or four years of vegetation [5]. Nowadays, American ginseng is cultivated in Canada, United States, and recently in China, Australia, Holland, England and Poland [7, 25]. Its roots as far as its raw material is concerned are taken as a medicine to regulate metabolism, control blood flow, sugar and cholesterol levels, stimulate the immune system, reduce stress, slow the degeneration of cells and increase longevity [5]. Ginseng has specific requirements (for example 75% of shading, mulching) and is susceptible to fungal diseases (they are the main reason for stand ruining or sever yields decrease). As Pięta and Berbeć (1995) and Pięta (1997) reported, in Polish conditions many plants on ginseng plantations were observed to have been infected by fungi (especially in the first years of vegetation). Traditionally, in Canada and the USA alternating spraying with fungicides are recommended [5, 6, 8, 9]. But they cause contamination of environment and ginseng raw material with fungicides residue. Therefore, it is very important to find other harmless methods of plant protection in ginseng cultivation. It seems to be worth examining methods and preparations recommended in ecological farming [8, 13, 23, 27]. For a few years many attempts have also been made also to use chitosan and it's derivatives in plant protection against diseases [3, 4, 11, 13, 14, 17-21, 26]. Among many interesting and useful properties it shows ability to inhibit the development of many pathogenic fungi and viral and bacterial infection [11, 20]. The objective of this study is to determine the effect of different plant protection methods in the control of fungal diseases of American ginseng.

2. Materials and methods

In the years 2000-2003 a field experiment, localised on sandy-loam soil in Krasnystaw (Lubelski region) was carried out. The effect of different methods of plant protection on growth, yielding and healthiness of American ginseng plants was investigated for four following years of vegetation.

The experiment comprised the following five objects: 1) traditionally recommended by OMAFRA in Canada spraying with fungicides (weekly alternating spraying with: Antracol 70 WP (0.6%), Baytan 17,5 WS (0.08%), Bravo 500 S.C. (0.2%), Ridomil MZ 72 WP (0.1%), Rowral Flo 255 S.C. (0.1%) [6, 15]; weekly alternating spraying with preparations recommended in ecological farming (Biochron (0.15%), Bioblatt EC (0.15%), Biosept 33 SL (0.2%), Miedzian 50 WP (0.25%); 3) every two weeks (sixfold in vegetation period) spraying with 0.1% solution of low-molecular-weight chitosan (in the form of microcrystalline gel containig 2.76% pure compound, obtained from Institute of Chemical Fibres in Łódź; 4) combined alternating spraying with preparations recommended in ecological farming (the same as in the third object) and sixfold treatment with chitosan (0.1%); 5) control – without any spraying.

Stratified seeds of American ginseng were sown in autumn 2000 on separate (1.2 metre-wide, 2 metre-long) raised (30-40 cm) beds (each objects contained four replications) in distance 15 × 3 cm and afterwards the soil was mulched with oat straw. During vegetation, shading with plastic fabric designed for a 75% reduction in light penetration as well as routine treatment of garden (weeding, fertilisation) were ensured. The methods of fungal diseases control mentioned above were performed yearly for three following vegetation periods with a hand-held sprayer. During particular vegetation periods the plant population, the height of 10 randomly chosen plants from each plot, as well as healthiness of plants (shown as a share of plants with pathogenic symptoms on plots; identification of the diseases was accomplished by specialists from Department of Plant Pathology, University of Agriculture in Lublin) were observed. In the autumn of each year of vegetation, above-ground parts and roots of 10 plants were measured. After three years all roots from each plot were dug, washed, measured and then dried at 35˚C. After that chemical analyses on particular ginsenosides content using HPLC method were performed at the Medical University in Łódź [7]. The results were analysed and the significance of differences was determined according to Tuckey's semi-intervals [12].

3. Results and discussion

In the experiment the significant effect of different plant protection methods on growth, development, quality parameters and yields of American ginseng plants was noted.

Plant population. Seeds germinated in spring (April-May) 2001. At the end of May the number of seedlings per plot in comparison with sown seeds was comparatively small (40-44%) – (Figure 1), while in Canada or the USA the number of germinating seeds

is much higher [22]. In our earlier experiments the number of seedling compared to seeds sown were sometimes much more lower [1]. In the following years of ginseng cultivation the decrease of plant population on particular plots caused mainly by fungal diseases (the same phenomenon observed Reeleder et al., 2000) was observed. In the first vegetation period fungal diseases reduced the number of plants, especially on the control plots and on the ones with combined spraying with preparations from ecological farming and chitosan (in autumn on average 34 - 35 plants per 1m^2 left). The highest number of plants was stated in the objects with preparations used in ecological farming or chitosan (Figure 1). The number of plants killed by diseases in the following years were smaller, caused however farther significant decrease in plant population (Table 1). After 3 years of vegetation, relatively (in comparison with the first year) the highest percentage of survived plants was stated on plots with ecological plant protection methods (preparations from ecological farming – 56.5% or chitosan applied alone – 43.7%). Similar effect in bean cultivation protected by chitosan was observed by Pięta et al. (2003).

Table 1. *Reduction of the number of plants (in %) caused by diseases in particular years of the experiment (the number of plants in the first year (I) – 100%).*

Treatment	Year		
	I	II	III
Fugicides	100	56.3	58.1
Preparations from ecological farming	100	41.7	43.5
Chitosan	100	52.0	56.3
Preparations from ecological farming and chitosan	100	62.4	69.2
Control	100	61.1	74.4

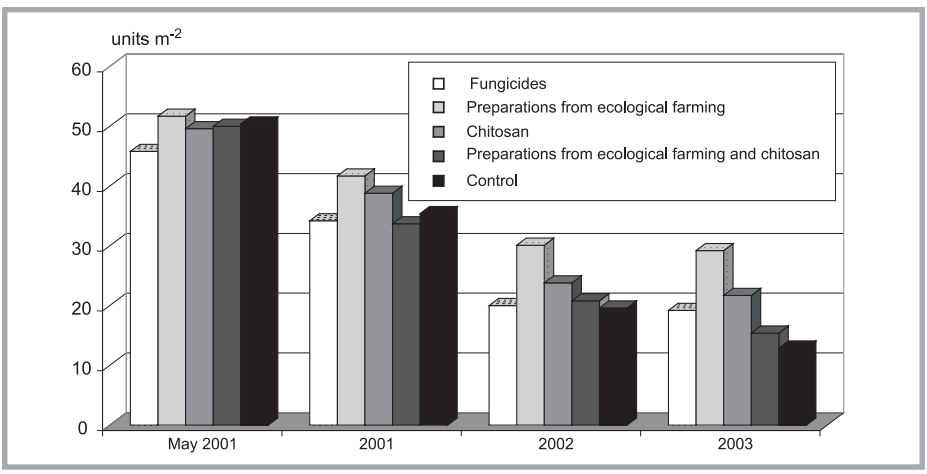

Figure 1. *American ginseng plant population (units . m-2) in following years of vegetation in particular objects.*

Disease infestation. Different plant protection methods during American ginseng cultivation did not completely protect against infection by pathogenic fungi. In the first year of vegetation dumping off caused by *Phytophthora* ssp., *Phytium*, *Fusarium* and *Rhizoctonia solani* infested plants. A considerable increase in the number of infected plants was observed on the control plots and on the ones where chitosan or combined method of preparations recommended in ecological farming and chitosan were applied. Similarly, in Li (1994) study there was stated that chitin addition to the soil did not affect the germination rate and the mortality rate of ginseng compared to the control. In the second year microbiological analyse showed appearance of *Fusarium oxysporum* and *Rhizoctonia solani*. Similar fungi appearance was reported on plantations in Poland by Pięta i Berbeć (1995) and in Canada by Li and Utkhede (1993) and Li (1994). Generally, the share of plants showing disease symptoms was lower than in the first year of cultivation, the highest one, however was in the case of control plots and those with traditional plant protection method (with fungicides). In the following year of vegetation phytophthora leaf and root blight (*Phytophthora cactorum*) was observed, especially in the sites, where water was permanently dropping from hollows in the shading fabric. There were no evidences of predilections of particular disease to any of the experimental objects. On average, in the period from 1st to 3rd year of experiment, the most infected plants were in the control object (Table 2). Chitosan and ecological preparations application (especially in the last two years of cultivation) resulted in decrease of the number of infected plants. Similar increase in resistance reaction on the plants after chitosan apllication was noted in the case of a bean [19], a carnation [14, 27], a top onion [2], mint and a lemon balm [11] or after preparation made from garlic [24] or Biosept [13, 28] application.

Height of plants and leaves dimensions. In the first year of vegetation American ginseng creates 6 - 10 cm. high petiole with three leaflets. As far as height of plants and leaves dimensions (an average length and width of petioles) are concerned there were not differences between particular experimental objects (Figure 2, Table 3). In the second year plants had 11 - 13 cm-long stem with two compound leaves with 3 - 5 leaflets and short 1 - 3 cm-long peduncle growing from the top of the same node as the leaf petioles [5, 7]. Plants from the object with traditional chemical control were characterised by significantly higher stems, whereas the ones obtained from the plots sprayed with preparations recommended in ecological farming had longer and wider leaves. In

Table 2. *Share of plants infected by fungi (in %) in following years of vegetation in particular objects.*

Treatment	2001		2002		2003	
	12.VI	24.VII	15.VI	26.VII	14.VI	25.VII
Fugicides	13.8	35.0	6.7	19.5	5.3	13.2
Preparations from ecological farming	8.7	27.8	5.6	11.0	2.2	11.0
Chitosan	16.1	36.3	5.7	5.7	2.8	12.0
Preparations from ecological farming and chitosan	13.3	40.5	7.0	7.1	3.7	15.0
Control	18.5	43.3	8.9	28.9	6.9	17.2

the following year of cultivation plants consisted of one stem, 13 - 17 cm heigh with a whorl of 3 leaves of 5 leaflets each, attached to the node by petioles. Peduncles were longer than in the last year of vegetation by 7 - 10 cm. Significantly better developed leaves and plants with the highest stems and peduncels were noted on the plots where ecological control and chitosan were used (Table 3, Figure 2). Similar effects were also noted on lettuce [2], rice and soybean [25].

Yields of fruit. In the first year of vegetation American ginseng does not bloom and produce any fruits or seeds. Seeds are obtained mainly from three or four year-old plantations, however, in the second year plants create only a small amount of fruits on the top of the peduncle [5, 7]. In the second year American ginseng plants from traditionally controlled object (with fungicides) were characterised by the highest fruits and seeds yields (per plant), whereas the lowest yields were obtained on the control plots (both per plant and per 1 m^2). Although the weight of fruits and seeds produced by a single plant was not high, probably due to the higher number of plants, the highest yields of fruits and seeds were noted on the plots with ecological preparations application. Application of 0.1% chitosan was connected with lowering both: yield of fruits and seeds in the second year of vegetation, but they were still twice as high as on the control one (Table 4).

In the following, the third year of ginseng vegetation decrease of fruit and seeds production on plots with chemical control was noted (due to phytophthora leaf and root blight), but still significantly higher than on the control plots. The highest yields of fruit and seeds were obtained from the objects with preparations used in ecological farming (respectively by 90 and 59% - per plant and almost five-fold – per 1 m^2 in comparison with the control ones). Every two weeks spraying with 0.1% solution of low-molecular-

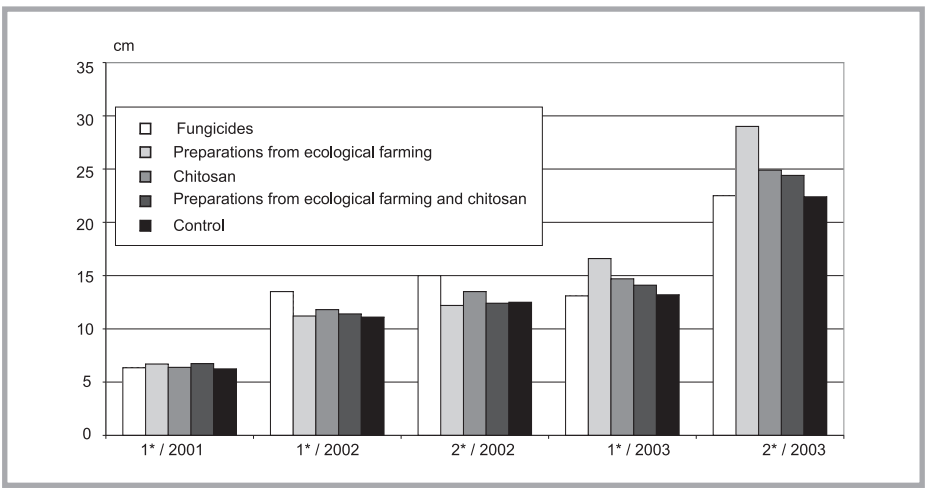

Figure 2. *Average height of plants (cm) measured at the end of vegetation period in following years of vegetation (1* - height of stem, 2* - height of stem with inflorescence).*

Table 3. *Average length of petioles and length and width of leaves (cm) in following years of vegetation in particular objects; A* - length of leaves; B*- width of leaves; C* - length of petioles.*

Treatment	2001		2002			2003		
	A*	B*	A*	B*	C*	A*	B*	C*
Fugicides	4.12	2.08	6.58	3.16	5.15	7.69	3.65	6.08
Preparations from ecological farming	4.63	2.34	7.07	3.39	5.34	9.61	4.41	7.76
Chitosan	4.15	2.07	6.64	2.94	4.88	9.07	4.35	6.87
Preparations from ecological farming and chitosan	4.28	2.08	6.48	3.05	4.92	8.64	4.15	6.56
Control	4.43	2.19	6.61	3.04	4.71	7.94	3.81	5.97

weight chitosan were connected with almost three-fold increase of fruit and seeds yields per 1 m^2 compared to the control ones. Chitosan spraying in the culture of runner bean and bean gave similar effects [18, 19]. Combined ecological preparations and chitosan spraying resulted in two-fold increase of fruit and seeds yields (Table 4).

Table 4. *Yields of fruit and seeds in the second and third year of vegetation in particular objects. D* - yields of fruit (in g per plant); F* - yields of fruit (in g · $m.^{-2}$); G* - yields of seeds (in g per plant); H* - yields of seeds (in g · $m.^{-2}$).*

Treatment	2002				2003			
	D*	F*	G*	H*	D*	F*	G*	H*
Fugicides	1.20	3.42	0.34	4.777	4.81	54.29	6.70	15.46
Preparations from ecological farming	0.72	7.08	0.24	9.117	7.09	118.2	9.24	33.70
Chitosan	0.71	1.38	0.15	1.617	5.1	68.23	7.15	19.53
Preparations from ecological farming and chitosan	0.44	2.46	0.14	3.087	5.78	49.72	8.52	14.62
Control	0.23	0.79	0.08	0.853	3.72	24.02	5.79	7.12
$LSD_{0,05}$	0.631	2.471	0.190	1.567	0.972	56.82	n.s.	7.08

Yields and characteristics of roots and above ground parts of plants. In the first year of vegetation ginseng produced small (below 1 g of dry matter) roots, looking as a pencil. At this stage there were not any differences in roots weight and dimensions between experimental objects described (Table 5). In the second and third year of cultivation plants produced better-developed and heavier roots with several lateral roots. Average dry matter of single root however, didn't significantly depend on experimental factors used and were similar to Li and Wardle (2002) and Berbeć, Kołodziej (1998) and Kołodziej (2003) reports. In all years examined plants from plots with ecological preparations and combined with chitosan application produced slightly heavier and longer roots with wider diameter, while roots obtained from the control plots were characterized by lower quality of parameters (Table 5).

Table 5. *American ginseng roots characteristic in following years of vegetation in particular objects; K* - average dry matter og single root (in g per plant); L* - average length of single root (cm); M* - average diameter of single root (mm).*

Treatment	2001			2002			2003		
	K*	L*	M*	K*	L*	M*	K*	L*	M*
Fugicides	0.251	10.7	0.676	2.220	16.3	14.12	6.009	21.5	20.40
Preparations from ecological farming	0.276	9.6	0.605	2.136	17.9	13.37	8.496	22.5	20.54
Chitosan	0.284	9.9	0.609	1.878	15.9	12.37	6.811	22.1	19.07
Preparations from ecological farming and chitosan	0.276	8.6	0.636	2.009	16.6	13.56	7.232	22.4	19.69
Control	0.305	10.1	0.627	1.644	13.7	13.14	6.344	21.2	18.71
$LSD_{0,05}$	n.s.	n.s.	n.s.	n.s.	1.35	1.114	0.422	n.s.	n.s.

Raw material yields were proportional to the number and healthiness of plants on the plots (Figure 1, Table, 2, 6). The highest yields of roots and above-ground parts of plants were obtained on the plots with preparations recommended in ecological farming (respectively almost five times and four times as high as on the control ones). Chitosan application resulted in three-fold increase both of stems and leaves and raw material yields in comparison with the control plots. Traditional, chemical control of American ginseng plantation as well as combined application of ecological preparations and chitosan caused almost two-fold increase in root yields and 63% increase of above ground parts yields compared to the control object (Table 6). Roots classification seems to be a very important factor in American ginseng marketing. A method for the classification by shape of dried roots of ginseng was described in Canada [23]. The method is based on traditional preferences in the market and allows for the separation of desirable roots (chunk or forked) grades. As far as Polish conditions are concerned the share of desirable fraction was rather high (from 25 to 44%). There was observed a tendency of decrease of chunk and forked roots share in the case of control plots and plots with combined ecological method of plant protection (Table 6). Extremely high share of pencil (carrot) fraction (less desirable) was observed on the control plots, whereas spider grade was the most important on the plots with combined (ecological and with chitosan application) method of plant protection (Table 6).

Three years old ginseng roots contained relatively high amount of active compounds (Table 7). Total amount of five ginsenosides determined ranged from 4.51% to 7.98% and were higher than reported by Kołodziej, 2003, Wills et al. (2002) or Li and Wardle (2002). The sum of ginsenosides was the highest on the plots with chemical control, while on the plots without any spraying was the lowest. Three year-old ginseng roots contained mainly ginsenoside Rb1 (from 19.873 on the control to 56.8 mg·g of d m^{-1} in the case of fungicides application) and Re (from 7.733 on combined ecological plant protection method to 13.757 mg · g of d m^{-1} on the control plots). The minor ginsenosides were Rc and Rg1. Similar relations in particular ginsenosides content were observed by Roy et al.(2003), Kołodziej (2003), Reeleder et al. (2000) and Li and

Table 6. *Yields of above-ground parts and roots of American ginseng (in g of d m. · m.$^{-2}$) and roots in following years of vegetation in particular objects.*

Treatment	Yields of roots	Yields of above-ground parts	Share of grade (%)				
			Pencil	Chunk	Spider	Forked	Fibre
Fugicides	44.5	12.1	23.5	19.3	29.8	24.9	2.5
Preparations from ecological farming	113.7	31.5	31.2	20.9	27.3	17.4	3.2
Chitosan	72.5	21.8	24.9	15.8	28.7	25.8	4.8
Preparations from ecological farming and chitosan	41.8	12.1	33.1	6.0	37.8	19.7	3.4
Control	22.8	7.4	44.3	10.4	26.9	16.9	1.5
$LSD_{0,05}$	1,02	n.s.					

Wardle (2002). As far as theoretical yields of ginsenosides are concerned the higher yields were obtained on the plots with preparations recommended in ecological farming (seven times as high as on the control ones) and after chitosan and fungicides using (four-fold in comparison with the control object).

Table 7. *Total and particular ginsenosides content (in mg · g of d m $^{-1}$) in roots and theoretical yields of ginsenosides (mg · m.$^{-2}$) from particular objects.*

Treatment	Rb1	Rc	Rd	Rg1	Re	Total	Yields of ginsenosides
Fugicides	56.8	3.16	4.907	3.533	11.353	79.753	3.55
Preparations from ecological farming	41.2	1.28	5.547	3.267	11.607	62.900	7.11
Chitosan	37.0	4.22	4.020	2.207	7.953	55.403	4.01
Preparations from ecological farming and chitosan	30.89	1.84	2.953	3.280	7.733	46.293	1.94
Control	19.87	1.86	4.293	5.320	13.757	45.110	1.03

4. Conclusion

1. Using traditional, chemical plant protection method was not sufficient. Though plant density was significantly better, share of infected plants in the last years of vegetation was similar to the control plots. At the same time almost two-fold increase of root and seeds yields, improving raw material quality (the highest share of desirable roots grade and ginsenosides content) was noted compared to the control object.
2. Every two weeks spraying with 0.1% solution of low-molecular-weight chitosan resulted in the increase of the number of plants and yields of fruit, seeds, above-ground parts and roots in comparison with the control object. The lowest number of infected plants and high content of ginsenosides in American ginseng roots was also noted.
3. Yearly alternating spraying with preparations used in ecological farming seemed to be the successful method of ginseng protection, significantly increasing yields

both of above-ground and under-ground parts of plants, increasing plant population, improving quality parameters of plants and raw material. High active substances content and the highest theoretical ginsenosides yields (seven-fold higher than on control) on the described objects was noted.

4. Combined alternating spraying with preparations recommended in ecological farming and six-fold treatment with chitosan (0.1%) caused increase in raw material yields and to a slight extend limited plants infestation.
5. The worst effects concerning healthiness, plants parameters and yielding were obtained from the control plots. Hence, cultivation without any plant protection method shouldn't be used on American ginseng plantations.
6. Therefore, spraying ginseng plantations with chitosan and particularly preparations used in ecological farming could be a recommendable option to chemical protection of American ginseng.

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