Katarzyna GLEŃ-KAROLCZYK
University of Agriculture in Krakow, Department of Agricultural Environment Protection
Al. Mickiewicza 21, 31-120 Kraków, Poland
e-mail: rrglen@cyf-kr.edu.pl

THE EFFECT OF CHITOSAN ON YIELDING, HEALTH OF THE HORSERADISH (*ARMORACIA RUSTICANA* GAERTN.) ROOTS AND PATHOGENIC FUNGI

Summary

The study aimed at the evaluation of the efficacy of horseradish seedling treatment with chitosan (Biochikol 020 PC) for the protection of the roots against Verticillium wilt and influence on the size and root yield structure. The direct effect of chitosan on the fungi which contribute to the degradation of root health was tested in laboratory conditions. The horseradish root yield and contribution of individual root fractions and the intensity of Verticillium wilt was significantly dependent on the year of cultivation. Soaking the horseradish seedlings in a 2.5% chitosan solution contributed to the mean increase of 1.7 t·ha^{-1} of the total yield of roots and the seedling fraction with the length of 30–35 cm by 1.6 t·ha^{-1}, as well as to the decrease of the amount of the fraction of roots shorter than 25 cm and waste material. The used chitosan limited the occurrence of horseradish root Verticillium wilt in every year. In the in vitro study, no fungistatic effect of chitosan was reported only in reference to S. sclerotiorium. On the other hand, the growth of the mycelium of the remaining Verticillium wilt causing fungi depended on the chitosan concentration. Normally, the fungistatic activity of chitosan increased with the increase of the dose and its highest level occurred at 2.5%. The mean surface growth inhibition coefficient enabled raking the fungi according to their decreasing sensitivity to chitosan: F. culmorum 64.9%, P. exiqua 58.1%, V. dahliae 44%, R. solani 35.4%, F. oxysporum 34.3%, F. solani 32.4%.
***Key words**: chitosan, antifungal activity, horseradish, root yield, Verticillium wilt, pathogenic fungi*

WPŁYW CHITOZANU NA PLONOWANIE, ZDROWOTNOŚĆ KORZENI CHRZANU (*ARMORACIA RUSTICANA* GAERTN.) ORAZ GRZYBY PATOGENICZNE

Streszczenie

Celem pracy była ocena skuteczności zaprawiania sadzonek chrzanu chitozanem (Biochikol 020 PC) w ochronie korzeni przed czernieniem pierścieniowym oraz wpływu na wielkość i strukturę plonu korzeni. Bezpośrednie oddziaływanie chitozanu na grzyby przyczyniające się do pogorszenia zdrowotności korzeni badano w warunkach laboratoryjnych. Plon korzeni chrzanu oraz udział w nim poszczególnych frakcji oraz nasilenie czernienia pierścieniowego istotnie zależało od lat uprawy. Moczenie sadzonek chrzanu w 2,5% roztworze chitozanu zwiększało średnio o 1,7 t·ha^{-1} plon całkowity korzeni i frakcji sadzonek o długości 30-35 cm o 1,6 t·ha^{-1} oraz zmniejszało ilość frakcji korzeni krótszych niż 25 cm i odpadów. Zastosowany chitozan w każdym roku ograniczał występowanie czernienia pierścieniowego korzeni chrzanu. W badaniach in vitro tylko w odniesieniu do S.sclerotiorum nie stwierdzono fungistatycznego oddziaływania. Z kolei wzrost grzybni pozostałych grzybów powodujących czernienie pierścieniowe zależał od stężenia chitozanu. Na ogół aktywność fungistatyczna chitozanu wzrastała wraz ze zwiększeniem dawki i była największa dla 2,5%. Średnie współczynniki zahamowania rozrostu powierzchniowego pozwoliły na uszeregowanie grzybów według ich malejącej wrażliwości na chitozan: F. culmorum 64,9%, P. exiqua 58,1%, V. dahliae 44%, R. solani 35,4%, F. oxysporum 34,3%, F. solani 32,4%.
***Słowa kluczowe**: chitozan, aktywność fungistatyczna, chrzan, plon korzeni, czernienie pierścieniowe, grzyby patogeniczne*

1. Introduction

Horseradish roots constitute valuable raw material for the food and cosmetics industry and medicine as the main source of peroxidase [1, 27]. However, the root cross section often exhibits discolorations within the vascular bundles. The main causing factor for these changes is the fungi *Verticillium dahliae*, which is accompanied by a complex of soil pathogens: Fusarium, Phoma, Sclerotinia, Rhizoctonia [2, 28]. In Poland the disease is known as vascular blackening, whereas in the United States, which has the highest horseradish production, it is simply referred to as root discoloration. In order to reduce the root destruction by fungi, protection must be commenced already at the stage of horseradish seedling planting. There are no preparations for seedling treatment in Polish market. This doubtlessly constitutes a serious problem for horseradish producers. On the other hand, plant protection requires preparations safe for the public health, non-toxic for the environment and biodegradable. In such a situation, the use of chitosan may pose an alternative, as it fulfills these criteria. Chitosan is a chitin derivative, a polysaccharide obtained from crustacean exoskeleton, such as lobsters and crabs and from cellular walls of fungi [16]. The bioactivity of this natural compound consists in inducing plant immunity to microorganisms: viruses, bacteria and fungi [9]. Numerous publications indicate high efficiency of preparations based on chitosan in the protection of different plant species against infections [3, 10, 23, 26, 30, 35, 36, 38, 39]. Furthermore, chitosan increases the growth and development of plants [15, 17].

The study aimed at the evaluation of horseradish seedling treatment with chitosan (Biochikol 020 PC) on yield and root fractions and the protective efficiency against Verticillium wilt. On the other hand, in the *in vitro* conditions we tested the effect of different chitosan concentrations on

surface growth of colonies of the following fungi: *Verticillium dahliae* Kleb., *Fusarium culmorum* (W.G.Smith) Sacc., *Fusarium solani* (Mart.) Sacc., *Fusarium oxysporum* Schlecht, *Phoma exiqua* Desm., *Sclerotinia sclerotiorum* (Lib.) de Bary, *Rhizoctonia solani* Kühn., contributing to the occurrence of horseradish Verticillium wilt.

2. Materials and methods

2.1. Soil conditions

The field experiment was conducted in the period 2010–2012 in an agricultural holding in Łukomierz (Pajęczno County, Łódź Voivodeship). Following the soil-agricultural maps, the experiment was established on a podzolic soil formed out of light loam with high sand content with granulometric content of fine sand, included in the IVb soil quality class, and good rye agricultural usability. The topsoil contained 0.126% N. Moreover, it was characterized by a high phosphorus content, average potassium content and low magnesium content. The humus content in the topsoil was 1.03% and pH 5.43. The tested soil was characterized by an average zinc content and low copper and manganese content.

2.2. Field work range

The one-factor field experiment was established in random blocks in four repetitions. The tested factor was horseradish seedling treatment with biotechnological preparation Biochikol 020 PC containing 20 g/dm^3 of chitosan (ß-1,4-D-glucosamine). In the first/second third of April, directly prior planting, the horseradish seedlings of the Alpo cultivar were soaked for half an hour in a 2.5% solution of the Biochikol 020 PC preparation. Control consisted of non-treated seedlings. Horseradish was planted in rows with spacing of 62.5 cm × 35 cm, the field size was 20 m^2, and the total working surface area was 160 m^2. Triticale was used as the forecrop in every year. With the winter plowing a full dose of manure, approx. 35 t·ha^{-1}was used along with mineral fertilizers: Polidap NP-100kg $P_2O_5 \cdot ha^{-1}$, potassium salt – 100 kg $K_2O \cdot ha^{-1}$. In the spring, potassium sulfate – 80 kg $K_2O \cdot ha^{-1}$, magnesium superhposphate – 60kg $P_2O_5 \cdot ha^{-1}$ and boronated ammonium nitrate –100 kg N· ha^{-1} were introduced.

After the harvest conducted in the third part of October, the roots were washed and then the following were determined: total yield, percentage contribution of root fractions in the yield, commercial yield and the percentage of the main roots with the symptoms of Verticillium wilt. The evaluation of root infection was conducted on a sample of 50 main roots (nurseries) from each field. To this end, crosswise cuts were performed at a distance of approx. 4 cm from the rosette.

The obtained results were subjected to variance analysis, and the difference significance was tested using the Student's t-Test at the significance level of α=0.05.

3. Laboratory research

3.1. Tested fungi: the study used the following fungi: *Verticillium dahliae* Kleb., *Fusarium culmorum* (W.G.Smith) Sacc., *Fusarium solani* (Mart.) Sacc., *Fusarium oxysporum* Schlecht, *Phoma exiqua* Desm., *Sclerotinia sclerotiorum* (Lib.) de Bary, *Rhizoctonia solani* Kühn, which were isolated from the symptoms of main Verticillium wilt of horseradish from the field experiment.

3.2. Antifungal chitosan activity: In the laboratory experiment, the fungicidal activity of chitosan contained in the Biochikol 020 PC recorded as immunity stimulator was compared to the biotechnological protection chemical Biosept 33 SL (33% grapefruit extract). In aseptic conditions, appropriate amounts of preparations were added to standard potato-dextrose agar (PDA) in temperature approx. 45°C in order to obtain concentrations: 2.5%; 1.0%; 0.5%. Pure PDA medium on Petri dishes constituted control. Inoculum of test fungi in the form of a 5 mm agar disk overgrown with a 10-day mycelium was placed in the middle of the established substrate. The experience was conducted in five repetitions. Daily measurements of fungi colony width were used for the calculation of the growth rate coefficient [T] of the test fungi following the formula by Kowalik and Krechniak [18].

$$T = \frac{A}{D} + \frac{b_1}{d_1} + \cdots + \frac{b_x}{d_x}$$

T – linear growth rate,
A – diameter from diameter measurements,
D – number of days from the experiment outset,
b_1, b_2 – increment of colony diameter since the last measurement [mm],
d_1, d_2 – number of days since the last measurement.

The influence of individual preparation concentrations on the linear fungi growth was determined on the basis of the difference between the fungi colony on Petri dishes with specific concentrations, and colony width on the control dishes. The results were presented in the form of percentage coefficient of growth inhibition/stimulation following the formula presented in the work of Gleń and Boligłowa [11].

$$H = \frac{K-A}{K} \cdot 100\%$$

H – index of fungi linear growth,
K – mean diameter of fungi colony on a plate of control,
A – mean diameter of fungi colony in individual test object.

The study results were subjected to a two-factor variance analysis, where the first factor was the preparation type, and the second its concentration. Significance was assessed on the basis of Duncan test with significance level α = 0.05.

4. Results and discussion

In the individual cultivation years, the total yield and the occurrence of Verticillium wilt was significantly variable (Tables 1–3). Horseradish is a plant that has a relatively good temperature drop tolerance, but it is less tolerant of water shortages. The experimental area, due to the neighboring Bełchatów and Szczerców mines has low water resources, which is particularly severe in the years with low precipitation. In the studied years, seasonal and unequal changes of rainfall were significantly pronounced, and to a lesser extent also temperature changes. The vegetative season of 2010 in comparison to 2011 and 2012 was characterized by the highest amount of rainfall and lower temperatures. However, only the beginning of vegetation (3rd part of April up to 2nd part of June) 2010 was characterized by the excessive precipitation, and in the subsequent months these amounts did not differ from the decade averages. The most stable humidity conditions were observed in the vegetative season of 2012. In particular, the rainfall in August

and September favored the accumulation of root mass. Independently of the seedling treatment with Biochikol 020 PC, the total horseradish yield in 2012 was significantly higher by 2.6 and 1.0 t·ha^{-1} in comparison to 2010 and 2011 (Table 1). In each year of cultivation, the horseradish seedling treatment contributed to the obtainment of significantly higher root yields. However, the treatment effect was best pronounced in the year with the most favorable distribution of rainfall (2012), when the reported yield was over 2 t·ha^{-1} higher than in the control. On the other hand, the lower efficiency of horseradish seedling treatment in 2010 and 2011 can be explained by the fact of excessive soil moisture directly after planting. Following Kowalski et al. [19], chitosan is characterized by good water solubility, which could weaken its effect. On the other hand, due to decreased transpiration, it protects plants against water loss, which is of paramount importance during drought periods [4, 9]. The favorable influence of chitosan on the biomass accumulation, and thus increased plant yielding is corroborated by other authors [5, 6, 20, 22, 24, 32, 34, 37].

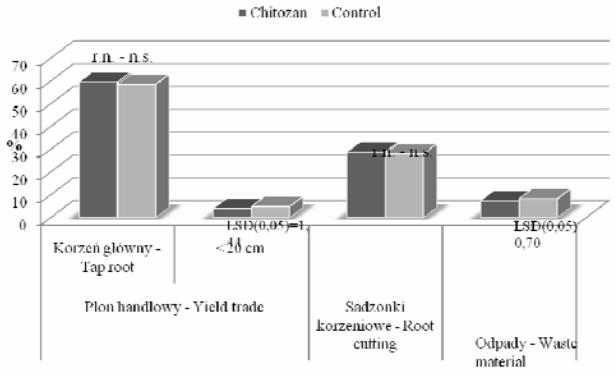
Table 1. Total root yield of horseradish [t·ha^{-1}]
Tab. 1. Całkowity plon korzeni chrzanu [t·ha^{-1}]

Treatments	Years			Mean	LSD (0,05)
	2010	2011	2012		
Chitozan	10,70	12,15	13,52	12,12	0,56
Control	9,10	10,76	11,43	10,43	
Mean	9,90	11,45	12,47		
LSD (0,05)	0,69				

Source: own work / Źródło: opracowanie własne

The main roots and I, II and III level suckers not exceeding 20 cm constitute the commercial yield in horseradish root structure. Chitosan used for seedling treatment did not have a significant effect on the main root mass and the total seedling fraction, yet it significantly limited the number of suckers <2 cm and waste material (too fine roots) (Fig. 1). The study demonstrated, that the contribution of horseradish seedlings with the length from 25–30 cm is stable in the experimental years and it is not modified by treating seedlings with chitosan (Table 2). Furthermore, chitosan did not have an influence on the fractions of the main roots, the longest seedlings (>35 cm) and with the length of 25–30 cm. However, it was determined, that the better water availability in the beginning of vegetation causes a significant increase of the contribution of the main roots in the total yield. Thus the over 60% contribution of this fraction in 2010, whereas in the years with a more equal rainfall distribution and their dominance shift toward the end of vegetation, it amounts to 56.5%. Also the previous study of Gleń [12, 13] demonstrated, that main root has the highest percentage contribution in the yield structure of horseradish roots.

In each vegetative season, a significantly lower percentage of roots with the symptoms of Verticillium wilt were reported from the fields, on which chitosan treatment was used (Table 3). The causes of this disease are primarily the fungi of the Verticillium genus, with a clear dominance of *V. dahliae* and *V. longisporum* [2, 8, 12, 14, 25]. These pathogens are often accompanied by different Fusarium species: *F. culmorum, F. oxysporum*, F. *equiseti*, *F. solani* and other soil fungi such as: *Sclerotinia sclerotiorum, Rhizoctonia solani* and *Phoma* spp. [14]. Previous studies corroborate the high efficiency of chitosan in the plant root protection against soil pathogens [6, 20, 26, 29]. The use of chitosan for the treatment of numerous plant species results in the increase of their immunity towards *F. oxysporum* [33]. It is also known, that chitosan is efficient in inhibiting the development of *F. oxysporum* f. sp. radicis-lycopersici on tomato roots [21]. Furthermore, carrot seed treatment protects the plant against the development of *Sclerotinia* spp. [7]. On the other hand, Mariańska-Cichoń et al. [23] determined, that soaking strawberry seedlings in chitosan does not provide enough protection against verticiliosis.



Source: own work / Źródło: opracowanie własne

Fig. 1. The structure of the total yield of horseradish (average years) [%]
Rys. 1. Struktura plonu ogólnego chrzanu (średnie z lat) [%]

Table 2. Fraction of root horseradish [%]
Tab. 2. Frakcje korzeni chrzanu [%]

Treatments	Years	Tap root	Lateral roots					Waste material
			> 35 cm	30-35 cm	25-30 cm	20-25 cm	< 20 cm	
Chitozan	2010	60,51	10,99	9,10	4,77	5,65	2,73	6,23
	2011	60,37	7,56	7,15	5,64	6,85	3,54	8,64
	2012	57,73	8,52	10,99	4,63	4,39	5,67	7,79
Mean		59,54	8,97	9,02	5,00	5,59	3,89	7,52
Control	2010	60,83	9,52	7,20	5,27	6,86	3,48	6,62
	2011	59,30	6,72	5,74	5,98	8,26	4,85	9,08
	2012	55,45	7,76	9,57	3,87	7,26	6,53	9,36
Mean		58,54	7,96	7,43	5,00	7,45	4,88	8,31
$LSD_{0,05}$ for treatments		r.n.*	r.n.	0,88	r.n.	1,09	1,28	0,70
$LSD_{0,05}$ for years		1,12	1,28	1,07	r.n.	1,33	1,57	1,40

r.n.*- różnica nieistotna / nonsignificant difference

Source: own work / Źródło: opracowanie własne

Table 3. Verticillium wilt of horseradish roots [%]
Tab. 3. Czernienie pierścieniowe korzeni chrzanu [%]

Treatments	Years			Mean	LSD (0,05)
	2010	2011	2012		
Chitozan	23,84	16,95	14,82	18,39	1,23
Control	25,21	20,34	17,51	21,02	
Mean	24,52	18,62	16,14		
LSD (0,05)	1,51				

Source: own work / Źródło: opracowanie własne

In the laboratory test, the used chitosan (Biochikol 020 PC) concentrations exhibited fungistatic activity toward such fungi species as: *F. culmorum F. oxysporum*, *F. solani*, *P. exiqua*, *R. solani* and *V. dahliae.* This is demonstrated by significantly lower values of linear growth rate coefficients of colonies of these fungi on media with chitosan in comparison to the control (Tab. 4). However, in comparison to the biotechnological preparation Biosept 33 SL, the Biochikol 020 PC limited growth of the tested fungi to a lesser extent. Only in the case of *F. culomrum* on the media containing 0.5% and 1.0% of the tested preparations a significantly higher chitosan activity than grapefruit extract was observed, although the growth inhibition coefficients were only 6.9% and 2.2% higher (Fig. 2). The analyzed fungi were characterized by a different sensitivity to the presence of the tested chitosan concentrations in medium. The strongest and similar reaction was observed for the species: *F. culmorum* and *Phoma exiqua* (Figs. 2 and 5). *V. dalhiae* was another fungi, for which surface growth was inhibited independently of the chitosan concentration and remained within the range from 42.4% to 45.7% (Fig. 8). Furthermore, a still lower and similar sensitivity characterized *Rhizoctonia solani*, *F. oxysporum* and *F. solani* (Figs. 3, 4, 6). However, with the increase of chitosan concentration, the inhibitory effect on the linear growth of these fungi also increased (Table 4). In reference to *S. sclerotiorum*, chitosan did not exhibit fungistatic activity (Fig. 7). It is worth pointing out, that all Biosept 33 SL concentrations limited the growth of its colonies in over 90%. (Table 4, Fig. 7).

El Hadrami [9] reports, that the use of natural chitosan in 1 mg/ml concentration in *in vitro* conditions enables the decrease of growth of numerous fungi and Oomycetes. On the other hand, the study of Saharan et al. [31] demonstrates, that chitosan nanoparticles in 0.1% concentration limited the colony growth of *R. solani* in 34.4%. In the own study, at the lowest tested concentration of natural chitosan (0.5%), the growth of this fungi mycelium was limited in 29.3%, and the most intensive inhibition of 43.4% was reported from the medium containing 2.5% chitosan.

Table 4. The coefficient of linear growth of the tested fungi [T]
Tab. 4. Współczynnik tempa wzrostu liniowego testowanych grzybów [T]

Treatments	Concentration [%]	*Fusarium culmorum*	*Fusarium oxysporum*	*Fusarium solani*	*Phoma exiqua*	*Rhizoctonia solani*	*Sclerotinia sclerotiorum*	*Verticillium dahliae*
Chitozan (Biochikol 020 PC)	2,5	15,07 b*	31,25 b	17,76 d	7,62 c	21,57 c	50,88 b	13,76 c
	1,0	19,99 c	34,58 c	20,13 e	9,66 d	25,49 d	51,08 b	16,75 d
	0,5	24,82 d	38,20 d	21,83 f	10,88 e	33,84 e	53,67 b	17,52 e
Biosept 33 SL	2,5	4,87 a	10,95 a	2,95 a	2,75 a	2,01 a	1,65 a	2,77 a
	1,0	27,69 b	30,19 b	6,63 b	2,84 a	16,08 b	1,87 a	10,66 b
	0,5	33,19 f	44,76 e	8,54 c	4,78 b	42,51 f	2,21 a	16,33 d
Control		75,49 g	65,85 f	28,59 g	34,79 f	49,83 g	52,95 f	33,87 f

*values in columns marked with the same letter do not differ significantly

Source: own work / Źródło: opracowanie własne

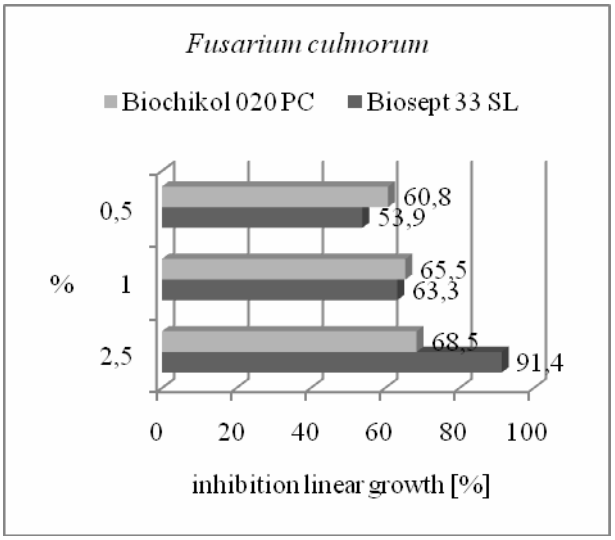


Fig. 2. Growth inhibition factor *F. culmorum*
Rys. 2. Współczynnik zahamowania wzrostu F. culmorum

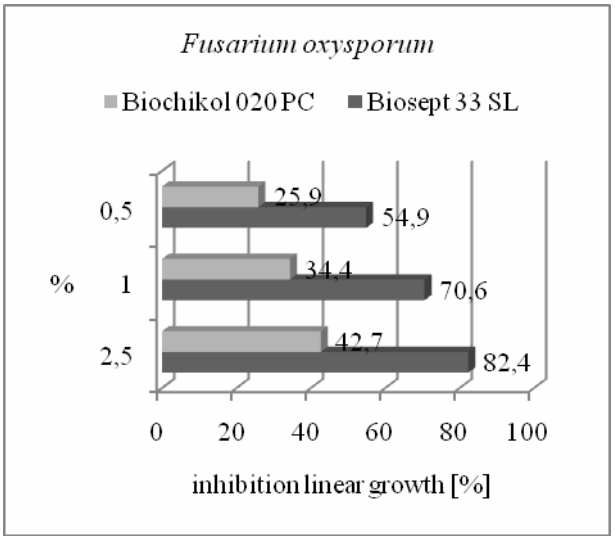


Fig. 3. Growth inhibition factor *F. oxysporum*
Rys. 3. Współczynnik zahamowania wzrostu F. oxysporum

Source: own work / Źródło: opracowanie własne

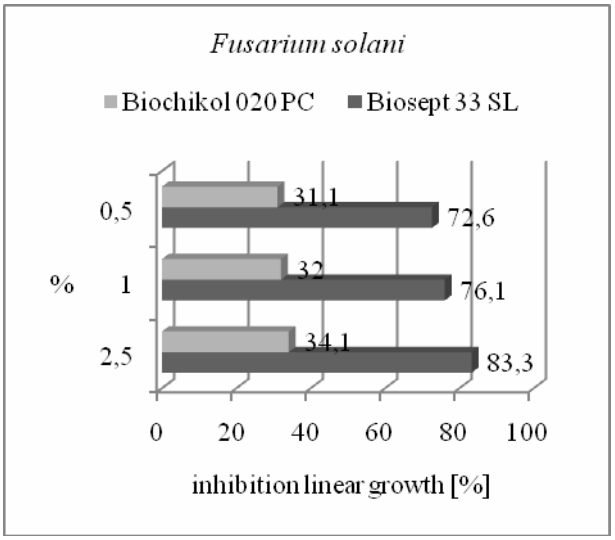


Fig. 4. Growth inhibition factor *F. solani*
Rys. 4. Współczynnik zahamowania wzrostu F. solani

Source: own work / Źródło: opracowanie własne

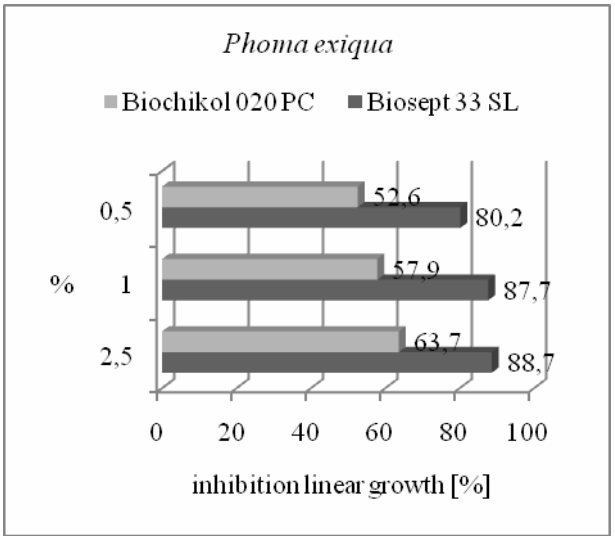


Fig. 5. Growth inhibition factor *P. exiqua*
Rys. 5. Współczynnik zahamowania wzrostu P. exiqua

Source: own work / Źródło: opracowanie własne

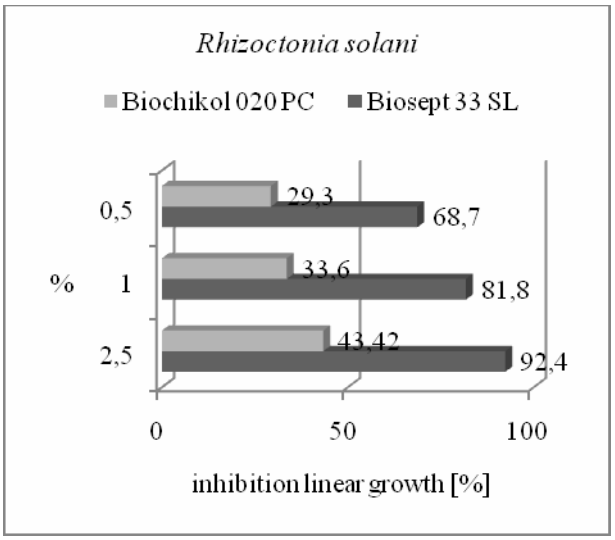


Fig. 6. Growth inhibition factor *R. solani*
Rys. 6. Współczynnik zahamowania wzrostu R. solani

Source: own work / Źródło: opracowanie własne

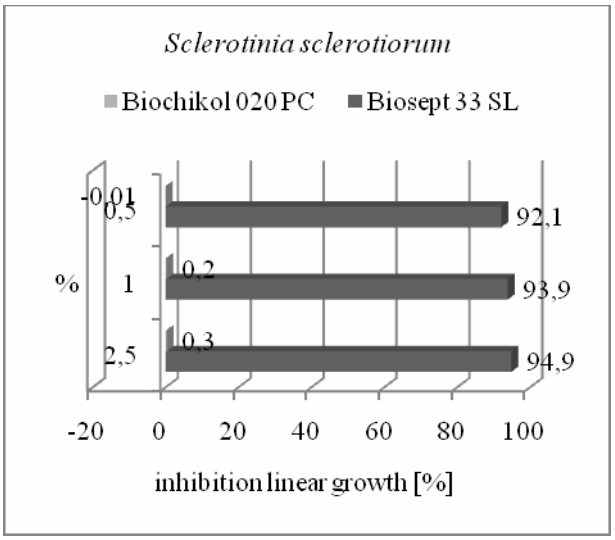
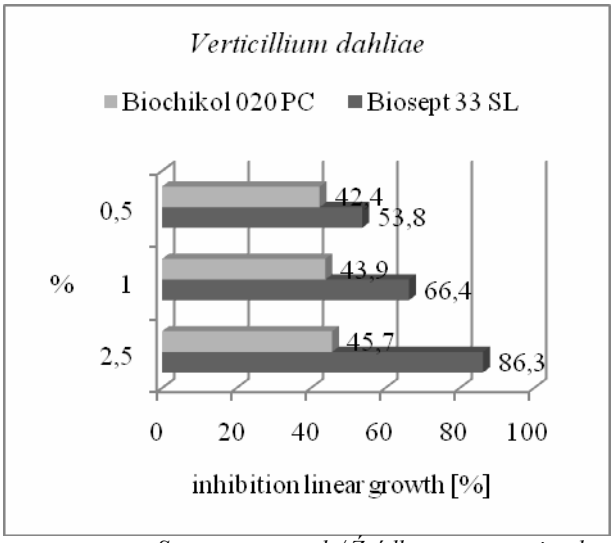


Fig. 7. Growth inhibition factor *S. sclerotiorum*
Rys. 7. Współczynnik zahamowania wzrostu S. sclerotiorum

Source: own work / Źródło: opracowanie własne



Source: own work / Źródło: opracowanie własne

Fig. 8. Growth inhibition factor *V. dahliae*
Rys. 8. Współczynnik zahamowania wzrostu V. dahliae

In conclusion, the obtained results of the field experiment, indicating the limitation of horseradish infection with the Verticillium wilt causing fungi, could be the result of the direct inhibitory effect of chitosan (Biochikol 020 PC) on these pathogens.

5. Conclusions

1. Chitosan (Biochikol 020 PC) used for the horseradish seedling treatment contributes to a significant increase of total yield, contribution of seedling fractions with the length of 30–35 cm and a decrease in the amount of 20–25 cm seedlings, roots to 20 cm constituting the commercial yield and the amount of waste material.
2. Independently of the year of cultivation, the intensity of horseradish Verticillium wilt decreases under the influence of the used chitosan.
3. *In vitro* fungistatic effect of chitosan on the Verticillium wilt causing fungi depends on its concentration (it increases with the increase of the dose) and fungi species.
4. The tested fungi were ranked according to their decreasing sensitivity to chitosan: *F. culmorum* 64.9%, *P. exiqua*

58.1%, *V. dahliae* 44%, *R. solani* 35.4%, *F. oxysporum* 34.3%, *F. solani* 32.4%. At the same time, *S. sclerotiorum* exhibited immunity, the colony surface growth inhibition was only 0.2%.

6. References

[1] Azevedo A.M., Martins V., Prazeres D.M.F., Vojinovic V., Cabral J.M.S., Fonseca L.P.: Horseradish peroxidase: a valuable tool in biotechnology. Biotechnology Annual Review, 2003, Vol. 9, 199-247.

[2] Babadoost M., Chen W., Bratsch A.D., Eastman C.E.: *Verticillium longisporum* and *Fusarium solani*: two new species in the complex of internal discoloration of horseradish roots. Plant Pathology, 2004, 53, 669-676.

[3] Benhamonu N.: Ultrastructural and cytochemical aspects of chitosan of Fusarium oxysporum f. sp. Radicis-lycopersici, agent of tomato crown and root rot. Phytopathology, 1992, 82 (10): 1185-1193.

[4] Bittelli M., Flury M., Campbell G.S., Nichols E.J.: Reduction of transpiration through foliar application of chitosan. Agric. Forest Meteorol., 2001, 107: 167-175.

[5] Boonlertnirun S., Suvannasara R., Promsomboon P., Boonlertnirun K.: Chitosan in combination with chemical fertilizer on Agronomic traits and some physiological responses relating to yield potential of rice (Oryza sativa L.). Research Journal of Biological Sciences, 2012, 7(2): 64-68.

[6] Borkowski J., Dyki B., Felczyńska A., Kowalczyk W.: Effect of Biochikol 020 pc (chitosan) on the plant growth, fruit yield and healthiness of tomato plant roots and stems. Polish Chitin Society, Monograph XII, 2007, 217-223.

[7] Cheah L.H.; Page, B.B.C.: *Trichoderma* spp. for potential biocontrol of clubroot of vegetable brassicas. Crop Food Res., 1997, 150–153.

[8] Eastburn D.M., Chang R.J.: *Verticillium dahliae*: a causal agent of root discoloration of horseradish in Illinois. Plant Disease, 1994, 78, 496-498.

[9] El Hadrami A., Lorne R. Adam 1, Ismail El Hadrami 2 and Fouad Daayf.: Chitosan in Plant Protection. Mar. Drugs, 2010, 8, 968-987. doi:10.3390/md8040968.

[10] Ghaouth El., Arul J., Grenier J., Banhamou N., Asselin A., Belanger R.: Effect of chitosan on cucumber plants, suppression of *Pythium aphanidermatum* and induction of defence reaction. Phytopathology, 1994, 84: 313-320.

[11] Gleń K., Boligłowa E.: Ocena aktywności fungistatycznej wyciągów roślinnych w testach *in vitro*. Journal of Research and Applications in Agricultural Engineering, 2012, 57 (3): 104-109.

[12] Gleń K.: Wpływ nawożenia dolistnego na plonowanie oraz porażenie chrzanu (Armoracia rusticana Gaertn) przez *Verticillium dahliae* i *Phoma lingam*. Prog. Plant Prot./Post.Ochr. Roślin, 2009, 49 (4): 2047-2053.

[13] Gleń K.: Wpływ niechemicznej i chemicznej ochrony na zdrowotność i plonowanie chrzanu. Prog. Plant Prot./Post.Ochr. Roślin, 2012, 52 (4):1165-1169.

[14] Gleń-Karolczyk K.: Fungi settling horseradish roots depending on the applied protection. Journal of Research and Applications in Agricultural Engineering, 2015, 60 (3): 52-56.

[15] Gornik K., Grzesik M., Duda BR.: The effect of chitosan on rooting of gravevine cuttings and on subsequent plant growth under drought and temperature stress. J Fruit Ornamental Plant Res., 2008, 16: 333-343.

[16] Katiyar D., Hemantaranjan A., Bharti S., Nishant Bhanu: A future perspective in crop protection: Chitosan and its oligosaccharidea. Advences in Plants Agriculture Research, 2014, 1 (1): 00006. http://dx.doi.org/10.15406/apar.2014.01.00006.

[17] Khan W.M., Prithiviraj B., Smiyh D.L.: Effect of foliar application of chitin oligosaccharides on photosynthesis of maize and soybean. Photosynthetica, 2002, 40 (4): 621-624.

[18] Kowalik R., Krechniak E.: Szczegółowa metodyka biologicznych i laboratoryjnych badań środków grzybobójczych. Materiały do metodyki badań biologicznej oceny środków ochrony roślin. Poznań IOR, 1961.

[19] Kowalski B., Jimenez Terry F., Herrera L., Agramonte Peñalver D.: Application of soluble chitosan in vitro and in the greenhouse to increase yield and seed quality of potato minitubers. Potato Res., 2006, 49, 167-176.

[20] Kurzawińska H.: Potential use of chitosan in the control of lettuce pathogens. Polish Chitin Society, Monograph XII. 2007: 173-178.

[21] Lafontaine, J.P.; Benhamou, N.: Chitosan treatment: an emerging strategy for enhancing resistance of greenhouse tomato plants to infection by *Fusarium oxysporum* f. sp. *Radicis-lycopersici*. Biocontrol Sci. Technol., 1996, 6, 111-124.

[22] Lee Y.S., Kim Y.H., Kim S.B.: Changes in the respiration, growth, and vitamin C content of soybean sprouts in response to chitosan of different molecular weights. Hort. Science, 2005, 40, 1333-1335.

[23] Marjańska-Cichoń B., Sapieha-Waszkiewicz A., Miętkiewski R.: Zastosowanie biopreparatów Biosept 33 SL i Biochikol 020 PC w formie moczenia sadzonek do ochrony truskawki przed wertycyliozą. Zesz. Nauk. Instytutu Sadownictwa i Kwiaciarstwa, 2008, 16: 1-7.

[24] Mondal M.M.A., Malek M.A., Puteh A.B., Ismail M.R., Ashrafuzzaman M., Naher L.: Effect of foliar application of chitosan on growth and yield in okra. Aust. J. Crop, 2012, 6: 918-921.

[25] Mueller J.P., Percich J.A.: Root deterioration associated with *Verticillium* wilt of horseradish. Plant Disease, 1982, 66 (5), 410-414.

[26] Nawrocki J.: Wpływ wybranych preparatów stosowanych do zaprawiania korzeni wysadkowych na zdrowotność nasienników selera korzeniowego. Prog. Plant Prot./Post.Ochr. Roślin, 2010, 50(1): 240-243.

[27] Palanisamy S., Unnikrishnan B., Chen S.M.: An Amperometric Biosensor Based on Direct Immobilization of Horseradish Peroxidase on Electrochemically Reduced Graphene Oxide Modified Screen Printed Carbon Electrode. Int. J. Electrochem. Sci., 2012, 7, 7935-7947.

[28] Percich J.A., Johnson D.R.: A root rot complex of horseradish. Plant Disease, 1990, 74 (5): 391-393.

[29] Pięta D.: The use of Biosept 33 SL, Biochikol 020 PC and Polyversum to control soybean (Glycine max (L.) Merill diseases against pathogens. Part I. Healthiness and yielding of soybean after using biopreparations. Acta Sci. Pol., Hortorum Cultus, 2006, 5 (2): 35-41.

[30] Pięta D., Pastucha A.: The protective effect of chitosan in limiting fungi diseases of soybean. Acta Sci.Pol. Hortorum Cultus, 2002: 1 (1): 31-43.

[31] Saharan V., Akanksha Mehrotra, Rajesh Khatik, Pokhar Rawal, S.S.Sharma, Ajay Pal.: Synthesis of chitosan based nanoparticles and their in vitro evaluation against phytopathogenic fungi. International Journal of Biological Macromolecules, 2013, 62 :677-683.

[32] Salachna P., Zawadzińska A.: Effect of chitosan on plant growth, flowering and corms yield of potted freesia. Journal of Ecological Engineering, 2014, 15(3): 97-102. DOI: 10.12911/22998993.1110223.

[33] Savard T., Beaulieu C., Boucher I., Champagne C.P.: Antimicrobial action of hydrolyzed chitosan against spoilage yeasts and lactic acid bacteria of fermented vegetables. J. Food Prot., 2002, 65, 828-833.

[34] Sheikha S.A.A.K., AL-Malki F.M.: Growth and chlorophyll responses of bean plants to the chitosan applications. European Journal of Scientific Research, 2011, 50 (1): 124-134.

[35] Szczeponek A., Mazur S., Nawrocki J.: The usage of chitosan in protection of some peppermint and lemon balm pathogens. Polish Chitin Soc., Monograph XI, 2006, 193-200.

[36] Thakur M., Sohal B.S.: Role of elicitors in inducing resistance in plants against pathogen infection: A Review ISRN Biochemistry, 2013, Article ID 762412, 10 pages http://dx.doi.org/10.1155/2013/762412.

[37] Uthairatanakij A., Teixeira da Silva J.A., Obsuwan K.: Chitosan for improving orchid production and quality. Orchid Science and Biotechnology, 2007, 1(1), 1-5.

[38] Wojdyła A.T.: Chitosan in the control of rose diseases – 6 year – trials. Bull. Pol. Ac. Sci., Biol. Sci., 2001, 49 (3): 243-252.

[39] Xing K., Zhu X., Peng X., Qin S.: Chitosan antimicrobial and eliciting properties for pest control in agriculture: A review. Agronomy for Sustainable Development, 2015, 35, 569-588.

This Research was financed by the Ministry of Science and Higher Education of the Republic of Poland.