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FUNGI SETTLING HORSERADISH ROOTS DEPENDING ON THE APPLIED PROTECTION

Summary

Investigations concerned the horseradish roots originating from a strict field experiment conducted in 2010-2011, in which chemical protection using: Topsin M 500, Dithane 455 SC, Penncozeb 80WP and Amistar 250 SC and non-chemical protection by Polyversum WP biopreparation and biotechnological preparations: Biosept 33SL, Bioczos BR and Biochikol 020 PC were applied. Irrespectively of the applied protection, a total of 755 fungi colonies of 14 genera were separated from the horseradish roots. The fungi classified to Fusarium genus were isolated most frequently, making up 35.7% of the total isolate number, subsequently Verticillium dahliae – 15.85%, Alternaria alternata – 6.09% and Pythium irregulare - 5.16%. The fungi community isolated from the horseradish roots from the object protected by synthetic fungicides was characterised by a greater number of fungi settling them, but at the same time it was less diversified regarding the species in comparison with the roots under biological protection. Chemical protection generally favoured the roots settling by pathogenic fungi (83.9%), whereas biological preparations favoured antagonistic species, such as Trichoderma harzianum and Trichoderma viride, which were not present in the objects under chemical protection.
***Key words**: horseradish roots, pathogenic fungi, biological and chemical protection*

GRZYBY ZASIEDLAJĄCE KORZENIE CHRZANU W ZALEŻNOŚCI OD ZASTOSOWANEJ OCHRONY

Streszczenie

Przedmiotem badań były korzenie chrzanu pochodzące ze ścisłego doświadczenia polowego przeprowadzonego w latach 2010-2011, w którym zastosowano ochronę chemiczną: Topsin M 500, Dithane 455 SC, Penncozeb 80 WP, Amistar 250 SC oraz niechemiczną z wykorzystaniem biopreparatu Polyversum WP i preparatów biotechnicznych: Biosept 33 SL, Bioczos BR, Biochikol 020 PC. Niezależnie od zastosowanej ochrony z korzeni chrzanu łącznie wyosobniono 755 kolonii grzybów, które należały do 14 rodzajów. Z największą częstotliwością izolowano grzyby należące do rodzaju Fusarium, które stanowiły 35,75% ogółu izolatów, kolejno Verticillium dahliae – 15,85%, Alternaria alternata – 6,09% oraz Pythium irregulare - 5,16%. Zbiorowisko grzybów wyizolowanych z korzeni chrzanu pochodzących z obiektu chronionego fungicydami syntetycznymi, charakteryzowało się większą liczebnością zasiedlających je grzybów, ale równocześnie było mniej zróżnicowane pod względem gatunkowym w porównaniu z tym pochodzącym z korzeni podlegających ochronie biologicznej. Chemiczna ochrona na ogół sprzyjała zasiedlaniu korzeni przez grzyby patogeniczne 83,9%, z kolei biologiczna - gatunkom antagonistycznym takim jak: Trichoderma harzianum oraz Trichoderma viride, których nie stwierdzono w ochronie chemicznej.
***Słowa kluczowe**: korzenie chrzanu, grzyby patogeniczne, biologiczna i chemiczna ochrona*

1. Introduction

Horseradish roots are a valuable raw material, which has been used in food and cosmetic industry for ages. At present the importance of this plant is growing worldwide as the compounds contained in its roots are widely analysed, including horseradish peroxidase, which finds numerous applications in medicine [1-4]. Therefore, the root quality, affected by a number of environmental and agrotechnical factors, is very important. Unfortunately, a long vegetation period is conducive to the colonisation of horseradish roots by microorganisms present in soil. *Verticillium* fungi are especially dangerous for horseradish, because they cause roots darkening, therefore disqualifying their industrial applications [5-8]. Other organisms, such as: *Albugo candida* or *Phoma lingam and Fusarium* fungi may also cause considerable losses and worsen the root storage [7, 9-11].

Control of infectious diseases intensification during the vegetation period ensures maintaining high quality and yielding of horseradish. Various methods of plant diseases prevention, alleviation or control are used for this purpose. Horseradish growers base mostly on the application of artificial fungicides, which undoubtedly contributes to a spectacular improvement in productivity and yield quality. However, the environmental pollution caused by an excessive use or overuse of agrochemicals leads to considerable changes in human attitudes towards pesticides application. Therefore, using biological protection methods may be a solution.

The aim of the presented investigations was to identify fungi communities settling horseradish roots originating from plantations diversified regarding their protection where synthetic fungicides (Topsin M 500, Dithane NeoTec 75, Amistar 250 SC, Penncozeb 80WP and Tebu 250 EW) were applied as well as non-chemical protection by Polyversum WP biopreparation and biotechnological means: Biochikol 020PC, Biosept 33SL and Bioczos BR.

2. Material and methods

Horseradish roots originating from a strict field experiment conducted in 2010-2011 on a private farm in Łukomierz in the Łódzkie voivodship onstituted the experimental materials. The experimental factor in the kind of applied protection. Biological protection during the vegetation period comprised: horseradish seedlings dressing with Polyversum

WP (*Pythium oligandrum*) biopreparation and six-time foliar application of biotechnological means: 2x Biochicol 020PC (chitosan), 2 x Biosept 33SL (extract from the grapefruit seeds and flesh) and 2 x Bioczos (garlic pulp in a paraffin shell). The chemical protection included seedlings dressing with Topsin M 500 (tiophanate-methyl) and four-time single applications of fungicides: Dithane NeoTec 75 WG (mancozeb), Amistar 250 SC (azoxystrobin), Penncozeb 80WP (mancozeb) and Tebu 250 EW (tebuconazole) – 1.25 l/ha. No protection was used on the control object. The horseradish roots were dug out in the third decade of October. A hundred main root pieces from each combination were selected for the analyses and cleaned superficially under running water. Samples – pieces 5mm thick were cut out of the boundary of necrotic changes on the root skin, disinfected by immersing for 30 seconds in 50% ethanol solution, rinsed under sterile distilled water and dried on a filter paper. In the inoculation chamber the material was placed – 10 pieces on PDA (Potato-Dextrose Agar) culturing medium in Petri dishes with 200 mm diameter. The culturing was maintained in a climatic chamber for 10 days at the temperature of 23^{o}C. Appearing fungi colonies were successively split off diagonally and then macro- and microscope observations were conducted, which provided the basis for the fungi classification to species using fungal identification keys and monographs [12-18]. The frequency of occurrence of individual species and genera was established on the basis of the number of obtained individual fungi isolates. Its value was expressed in percentage with reference to the total number of the isolates (100%) obtained for individual portions of roots.

3. Results

A total of 755 fungal colonies were obtained from the horseradish roots in result of mycological analysis (tab. 1). Classification included 19 fungi species, which were ascribed to 14 genera, and three other genera: *Aspergillus, Mucor and Penicillium* and yeast like fungi. The applied protection did not diversify the fungal community settling horseradish roots regarding the quality. *Fusarium* fungi dominated – a total of 270 colonies were isolated, which made up 35.76% of the total isolate number, represented by four species with a dominating *F. oxysporum*. A considerable proportion of the total fungal population existing on the roots was also composed of *Verticillium dahliae* 15.85%, *Alternaria alternata* – 6.09% and *Pythium irregulare* – 5.16%.

On the basis of conducted mycological analysis a diversification in the number of isolated fungal colonies was found depending on the applied plant protection. Each year, a lower number of fungi isolates was obtained from the portion of roots of the plants protected by the biotechnological and biological preparations in comparison with the chemical protection or the control object (tab.1). Moreover, this kind of protection favoured root colonisation by antagonistic fungi*: Trichoderma harzianum* and *T. viride*, whose share in 2010 constituted 5.41% (fig. 1a). On the other hand, the year 2011, in comparison with 2010 was characterised by an excessive amount of rainfall, from the third decade of May to the second decade of July, revealed a more favourable thermal and moisture conditions for the development of horseradish. At a uniform rainfall distribution during the vegetation season, the number of *Trichoderma* fungi increased to 11.76% (fig. 1b).

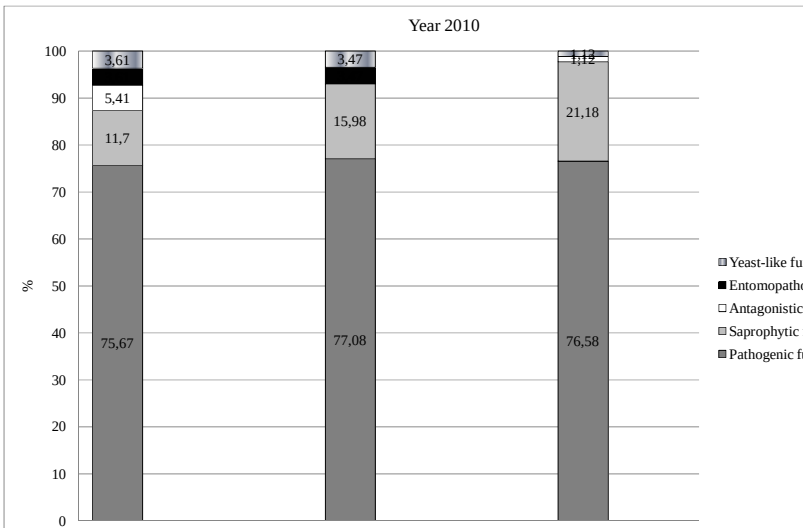
The pathogens share was the highest in fungal community from the roots under combined chemical protection, respectively 77.08% and 90.7% (fig. 1a and b). It should be emphasized, that in 2010 pathogenic fungi species were isolated from the combinations of protection methods with the same frequency (fig. 1a).

Table 1. Fungi isolated from horseradish roots depending on the applied protection (number of isolates)
Tab. 1. Grzyby wyizolowane z korzeni chrzanu w zależności od zastosowanej ochrony (liczba kolonii)

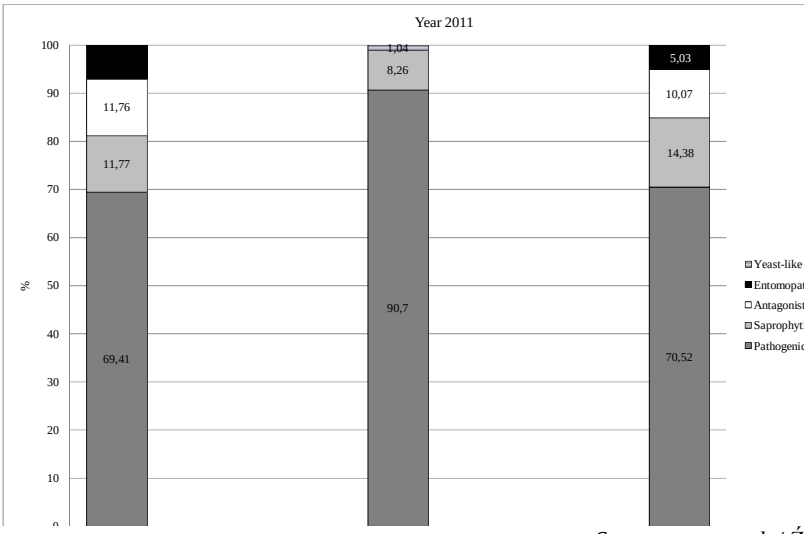
Fungus species	Biological protection			Chemical protection			Control			Total	%
	Lata– Years			Lata– Years			Lata– Years				
	2010	2011	□	2010	2011	□	2010	2011	□		
Acremonium roseum (Oud.) W. Gams	2	1	3		1	1		2	2	6	0,79
Alternaria alternata (Fr.) Keissler	4		4	11	6	17	11	14	25	46	6,09
Aspergillus spp.	1		2				3	1	4	6	0,79
Botrytis cinerea Pers.	2	1	3	8	1	9	4	1	5	9	1,19
Cladosporium cladosporioides (Fres) de Vries				12	3	15	11	7	18	33	4,37
Epicoccum purpurascens Ehrenb. ex Schlecht	4	2	6	5	12	17		5	5	28	3,70
Fusarium culmorum (W.G.Smith) Sacc.	3	6	9	13	7	20	11	6	17	46	6,09
Fusarium equiseti (Corda) Sacc.	5	10	15	20	8	28	19	12	31	74	9,80
Fusarium oxysporum Schlecht	18	12	30	24	14	38	27	15	42	110	14,57
Fusarium solani (Mart.) Sacc.	5	3	8	9	5	14	10	8	18	40	5,29
Mucor spp.	2	3	5	11	1	12	13	2	15	32	4,23
Paecilomyces variotti Bainier	4	6	10	5		5		7	7	35	4,63
Penicillium spp.	6	1	7				10	2	12	19	2,51
Phoma eupyrena Sacc.		3	3	1	3	4	3	5	8	15	1,98
Phoma exiqua Desm.				2	3	5		2	2	7	0,93
Pythium irregulare Buisman	11	4	15				16	8	24	39	5,16
Rhizoctonia solani Kühn	2	3	5	3	6	9	3		3	17	2,25
Rhizopus nigricans Ehrenberg	2	5	7		3	3	2	6	8	18	2,38
Sclerotinia sclerotiorum (Lib.) de Bary	2		2	3	5	8	5	2	7	12	1,59
Trichoderma harzianum Rifai	2	7	9					5	5	14	1,85
Trichoderma viride Pers. Ex. Gray	4	3	7				2	9	11	18	2,37
Verticillium dahliae Kleb.	28	15	43	12	18	30	27	20	47	120	15,85
Grzyby drożdżopodobne Yeast-like fungi	4		4	5	1	6	2		2	12	1,59
Razem - Total	111	85	19	144	97	24	179	139	318	755	100

Source: own work / Źródło: opracowanie własne

a)

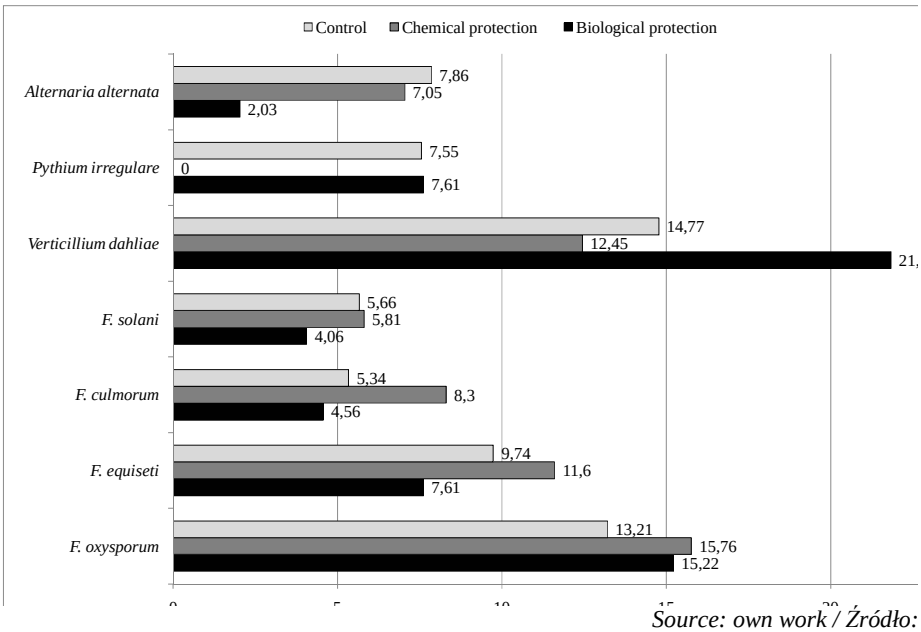


b)



Source: own work / Źródło: opracowanie własne

Fig. 1. The influence of biological and chemical protection on the percentage of fungi isolated from horseradish roots
Rys. 1. Wpływ biologicznej i chemicznej ochrony na procentowy udział grzybów wyizolowanych z korzeni chrzanu a) 2010, b) 2011



Source: own work / Źródło: opracowanie własne

Fig. 2. Dominating fungi species colonising horseradish roots depending on the applied protection
Rys. 2. Dominujące gatunki grzybów kolonizujace korzenie chrzanu w zależności od zastosowanej ochrony

The kind of protection applied during the vegetation period considerably modified the frequency with which the dominating fungi species were isolated (fig. 2). *Verticillium dahliae* definitely prevailed (21.83%) among the fungal community settling the roots of plants which received biotechnological and biological preparations. On the other hand, the fungi community isolated from the horseradish roots protected by chemicals was dominated by *Fusarium* species, whose share was 41.47%.

4. Discussion

Irrespectively of protection applied during the vegetation period, 755 fungal colonies were obtained from the horseradish roots, among which pathogens prevailed greatly, making up 76.67%. They were represented by 13 pathogenic species including 4 from *Fusarium* genus with a total share of 35.76%. *F. oxysporum*, *F. equiseti* and *F. culmorum* were the most frequently isolated in this group of pathogenic fungi, followed by less frequently isolated *F. solani*, whereas the other dominant pathogenic species comprised *Verticillium dahliae* (15.85%), *A. alternata* (6.09%) and *Pythium irregulare* (5.16%). Moreover, less numerous species, such as *Epicoccum purpurascens*, *Rhizoctonia solani*, *Phoma eupyrena*, *S. sclerotiorum* were isolated and single *B.cinerea* and *Phoma exiqua* fungi. The available literature lacks information about horseradish roots colonization by pathogenic fungi, however the obtained results partially overlap with those obtained by Yu [19]. During the mycological analysis of discoloured horseradish roots the author found that the changes were caused by 18 fungi species, among which *Verticillium* genus was most numerously represented (38%) by two species: *V. dahliae i V. longisporum.* Moreover, similar as in the presented work, *Fusarium* fungi were most frequently isolated (31.8%), comprising *F. oxysporum*, *F. solani* and *F. equiseti.* Convergent results were obtained also for other species, such as: *A. alternata*, *R. solani*, *Phoma* spp. and saprophytic fungi *Penicillium* and *Rhizopus*. Majchrzak et al. [20] isolated the four *Fusarium* sp., presented in this paper, also from the roots of other *Brassicaceae* plants, such as spring rapeseed, white mustard, brown mustard, oil radish, camelina or crambe, as well as seven others, which were not isolated from horseradish roots. It has been commonly known that *F.* oxysporum, *F. solani* and *F. culmorum* are considered the main agents causing root and stem rot in any plant species [21, 20]. The use of seedlings settled by these pathogenic species may prove exceptionally risky, because it may result in developing rot diseases or later plant dieback owing to *Fusarium* wilting.

Presented research demonstrated quantitative differences in fungal populations settling horseradish roots depending on the applied protection. In comparison with the biological control, chemical protection contributed to an increase in the total number of isolated fungi and in the first place influenced increased share of pathogenic fungi. *Fusarium* fungi found especially favourable conditions for development in the portion of roots originating from the combination protected by chemical means and made up even 42% in comparison with 32% on the roots protected with biopreparations. Moreover, this kind of protection more favoured roots settling by *R. solani* and *S. sclerotiorum* and potential pathogens *A. alternate* and *B. cinerea*. According to Patkowska [22] Polyversum WP preparation reveals a better efficiency in limiting these fungi species occurrence in comparison with synthetic fungicides. Other investigations have shown that the use of *Pythium oligandrum* for tomato seeds dressing contributed to strong reduction of the pathogen number settling the roots [23]. On the other hand, in the Author's own research, application of Topsin M 500 SC preparation for seedlings dressing and subsequent foliar application of fungicides completely protected horseradish roots against their colonisation by *Pythium irregulare* and saprophytes Aspergillus spp. and Penicillium spp.. Koutb and Ali [24] reported that *Epicoccum purpurascens* fungus reveals antagonism through the antibiosis or competitiveness towards *Pythium irregulare* causing root rots. The Authors' own investigations demonstrated that the horseradish roots from the chemically protected object were to a greater degree settled by *E. purpurascens*, which to some extend may explain eliminating *P. irregulare.*

5. Conclusions

1. The applied biological and chemical protection reduced the number of fungi, including pathogens, settling horseradish roots. However, fungal population colonizing horseradish roots from the objects protected by biological means were on average 19% less numerous than the population from objects receiving chemical control.
2. *Fusarium* fungi were the most frequently isolated from the horseradish roots – 35.75% of the total isolate number, *Verticillium dahliae* – 15.85%, *Alternaria alternata* – *6.09%* and *Pythium irregulare* - 5.16%.
3. Chemical protection generally favoured roots settling by pathogenic fungi – 83.9%, whereas biological control encouraged antagonistic species, such as *Trichoderma harzianum* and *Trichoderma viride*, which were not registered under chemical control.

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