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FUNGICIDAL ACTIVITY OF JUNIPER ESSENTIAL OIL (*Juniperus communis* L.) AGAINST THE FUNGI INFECTING HORSERADISH SEEDLINGS

Summary

The aim of the study was to evaluate in mycological aspect stored horseradish seedlings derived from the strict field experiment, with an application of chemical and non-chemical protection. Regardless of protection manner, the population of fungi present in horseradish seedlings was dominated by the following species: V. dahliae (14%), C. destructans (8%), E. purpurascens (7.7%), R. solani (7.3%), T. hamatum (6%). Protection improves the health status of the seedlings, and better protective effect is obtained by the application of synthetic fungicides which, compared to the control site (541), reduces the amount of fungal isolates to 266. The second stage of the study involved an evaluation of antifungal activity in vitro of 1.0; 0.5; 0.1 $mm^3 \cdot cm^{-3}$ juniper oil with respect to above mentioned fungi. In laboratory conditions, regardless of the concentration juniper oil strongly inhibits the linear growth and sporulation of fungi the most intensively colonizing horseradish seedlings. Mean values of colony surface growth limiting are in the range from 50.66% for V. dahliae to 90.1% for R. solani. Also, an increase in juniper oil concentration in the culture medium results in an increased inhibition of fungi sporulation. The presence of 1.0 $mm^3 \cdot cm^{-3}$ oil in the medium oil causes 90.4% reduction of C. destructans, and 82.1% of V. dahliae spores. A very strong fungistatic effect of juniper oil in vitro may be the basis for further research on its application in plants protection against root rots.
***Key words**: antifungal activity, juniper essential oil, pathogenic fungi, horseradish seedlings*

FUNGICYDALNA AKTYWNOŚĆ OLEJKU JAŁOWCOWEGO (*Juniperus communis* L.) WOBEC GRZYBÓW PORAŻAJĄCYCH SADZONKI CHRZANU

Streszczenie

Celem badań była mykologiczna ocena przechowywanych sadzonek chrzanu pochodzących ze ścisłego doświadczenia polowego, w którym zastosowano ochronę chemiczną oraz niechemiczną. Niezależnie od sposobu ochrony populacja grzybów zasiedlających sadzonki chrzanu była zdominowana przez gatunki: Verticillium dahliae (14%), Cylindrocarpon destructans (8%), Epicoccum purpurascens (7,7%), Rhizoctonia solani (7,3%), Trichoderma hamatum (6%). Ochrona polepsza zdrowotność sadzonek, przy czym lepszy efekt ochronny uzyskano po zastosowaniu syntetycznych fungicydów, zastosowanie których prowadziło do zmniejszenia ilości izolatów grzybów z 541 w obiekcie kontrolnym do 266. Celem drugiego etapu badań była ocena in vitro aktywności przeciwgrzybicznej 1.0; 0.5; 0.1 $mm^3 \cdot cm^{-3}$ stężenia olejku jałowcowego wobec ww. gatunków grzybów. W warunkach laboratoryjnych olejek jałowcowy niezależnie od stężenia bardzo silnie hamował rozrost liniowy oraz zarodnikowanie grzybów najintensywniej zasiedlających sadzonki chrzanu. Średnie wartości ograniczenia rozrostu powierzchniowego kolonii mieściły się w przedziale od 50,66% - V. dahliae do 90,1% dla Rhizoctonia solani. Poza tym zwiększenie stężenia w podłożu hodowlanym olejku jałowcowego skutkowało wzmożoną inhibicją sporulacji grzybów. W obecności 1,0 $mm^3 \cdot cm^{-3}$ olejku w pożywce ilość spor C. destructans zmniejszała się o 90,4%, a V. dahliae o 81,1%. Bardzo silne fungistatyczne oddziaływanie olejku jałowcowego w testach in vitro, może być podstawą do dalszych badań w kierunku wykorzystania go do ochrony roślin przed zgniliznami korzeniowymi, których sprawcami są testowane gatunki grzybów.
***Słowa kluczowe**: aktywność fungistatyczna, olejek jałowcowy, grzyby patogeniczne, sadzonki chrzanu*

1. Introduction

Compared to the phyllosphere, the underground parts of plants are much more colonized by microorganisms. Special risk is caused by phytopathogens developing, intracellularly, as well as in tissues and vascular bundles of the plants [43]. Over 80% of diseases are caused by the fungi, that in the global world production result in about 12% crop yields reduction [39]. Additional fungal infections of post-harvest period adversely affect the market value of agricultural products, reduce their storage stability and contaminate with mycotoxins [10, 11, 37, 46]. Qualitative and quantitative composition of fungi colonizing plant roots depend on plant species as well as climate and soil conditions. Due to the long vegetation period, just horseradish is the most predestined to microbial contamination. Many authors believe that horseradish roots are the most abundantly inhabited by fungi of Verticillium genus [1, 9, 13, 29]. Typical pathogens for this species are *Albugo candida* and *Phoma lingam* [1, 12, 15, 20]. Quality deterioration can be the result of roots colonization by soil polyphagous pathogens such as *Cdestructans*, *Rsolani*, *Alternaria* spp., *Fusarium* spp., [14, 25, 33, 38]. Further destruction of already infected root tissues occurs during the storage. Elongation of that period may additionally favor the occurrence of saprotrophic fungi. Undoubtedly, protective treatments carried out during vegetation season improve the health status and form fungi population colonizing the roots. Chemical protection is still the main strategy limiting diseases occurrence in horseradish cultivation. The use of fungicides rises

some public debate concerning contamination with the remains, and on the other hand, the spread of resistance in pathogen populations [40, 42]. In this case, there is an increasing interest in the use of natural products such as essential oils and plant extracts for plants protection against pathogens during vegetation, as well as plant raw material protection against storage rots. Numerous reports confirm fungicidal activity of various essential oils and plant extracts [3-7, 26, 28, 39, 42]. Essential oils and plant extracts are a rich source of bioactive substances, which could lead to the development of a new generation of safe antifungal agents. For example, common juniper (*Juniperus communis* L.) is a well known and respected medicinal plant, essential oil produced of juniper berry demonstrates diuretic, anti-inflammatory, choleretic, antioxidant, and a range of other pharmacological properties [2]. Also its antibacterial and antifungal activity was demonstrated [27, 34, 36, 41].

The study was aimed at quantitative and qualitative evaluation of fungi colonizing stored horseradish seedlings derived from crops protected with fungicides and synthetic biopreparations, as well as determination of organisms responsible for necrotic lesions within inner bark and roots pulp. The market still lacks effective protection measures for the treatment of seedlings and their protection against storage rots. This study is an attempt to look for natural products, which would give a chance to reduce fungal diseases of horseradish. The second aim consisted thus in an evaluation of fungistatic effect of juniper oil on dominant species of fungi isolated from horseradish seedlings.

2. Materials and methods

Isolation of fungi from horseradish seedlings was performed in order to evaluate fungi communities associated with diseases presence inside the seedlings during storage. The seedlings originated from a strict field experiment conducted in the years 2010–2012 in the individual farm in Łukomierz/Łódź Voivodship. The research factor included the kind of applied protection. Non-chemical protection included horseradish seedlings treatment with biological preparation Polyversum WP (*Pythium oligandrum*) – 10g/kg of seedlings, and during the vegetation season six times foliar application of biotechnical preparations: 2 × Biochikol 020 PC (chitosan) - 2.5%, 2 × Biosept 33Sl (grapefruit seeds and pulp extract) – 0.2%, 2 × Bioczos BR (garlic pulp in paraffin coating) – 4 pieces/L of water. Chemical protection of the plants included seedlings treatment with Topsin M 500 SC (methyl thiophanate) and four individual applications of fungicides: Dithane NeoTec 75 WG (mancozeb), Amistar 250 SC (azoxystrobin), Penncozeb 80 WP (mancozeb) and Tebu 250 EW (tebuconazole). No protection was applied in the control site. Horseradish roots were dug in the third decade of October, the seedlings were separated from the maternal root and stored in prisms covered with sand for five months. In the spring, 100 pieces of seedlings from each combination were randomly collected for laboratory analysis, and their surface was purified under running water. The samples of a thickness of 5 mm were collected in the border region of necrotic lesions (bark and pulp inner necrosis) visible in seedlings cross-section, they were disinfected for 30 seconds plunging in 50% ethanol solution, rinsed twice in sterile distilled water and dried on sterile filter paper. In the inoculation chamber, each 10 pieces of the material was transferred on PDA culture medium (potato dextrose agar) in Petri dishes of a diameter of 200 mm. The culturing was carried out in a climatic chamber for 10 days at 23°C. Emerging fungal colonies were gradually cleaved on the slants. The fungi were identified using mycological keys and monographs [8, 21, 23, 24, 30, 31, 35]. The frequency of particular species or genera occurrence was determined based on the obtained number of fungus isolates. Its value was expressed in percentage, referring to the total number of isolates (100%) obtained for a batch of seedlings.

2.1. Antifungal activity of juniper essential oil

Juniper oil used in the experiment was manufactured by Dr. Beta company. According to the label, the oil was obtained from common juniper berries. Juniper berries contain about 1.5% of essential oil on average, which includes monoterpenes: 80% α- and β-pinene, thujene, sabinene, 5% terpinene-4-ol, α-terpineol, borneol and geraniol. In turn, sesquiterpenes (α- and β-cadinene, caryophyllene) are present in trace amounts.

Dominant pathogenic species to horseradish plants were used in order to examine antifungal activity of juniper essential oil: *V. dahliae*, *C. destructans*, *E. purpurascens*, *R. solani*, *T. harzianum*.

Juniper oil dissolved in 1 ml of 5% ethanol was added to PDA medium (potato-glucose) in amounts such that its concentration in the medium was 1.0; 0.5; 0.1 $mm^3 \cdot cm^{-3}$. Prepared media were inoculated with agar disk of a diameter of 5mm overgrown with three-week mycelium of the examined organism. The controls consisted of Petri dishes with clean PDA medium and PDA + 0.01% ethanol. The experiment was performed in 5 replications. The culture was carried out in a controlled conditions with 12 hour light cycle and at a temperature of 23°C. The assessment of sporulation was conducted on 20-day old fungi cultures. A drop of spore suspension was placed in Thom hemocytometer under the light microscope and the spore number was counted.

The effect of individual seed treatment and its concentration on linear growth and sporulation of the analysed fungi was expressed as linear growth/sporulation inhibition index according to Abbot formula [19]:

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

where:
H – index of fungi linear growth/sporulation inhibition,
K – mean diameter of fungi colony on a plate/number of control spores,
A – mean diameter of colony/number of fungi spores in individual test object.
Moreover, the coefficient of linear growth rate was computed on the basis of daily measurements of fungi surface growth in each combination:

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

where:
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 results were subjected to the analysis of variance and the significance of differences was verified by Duncan test on the significance level α = 0.05 (STATISTICA 10).

3. Results

Mycological analysis demonstrated differentiation in the number of fungal isolates obtained from horseradish seedlings derived from three vegetation seasons during which chemical and non-chemical protection was used.

In the period covered by the study, 1175 fungal colonies were isolated in total from the seedlings, and almost half (526) of isolates were obtained in the first year of the study (Table 1). In turn, number of isolates in the next two storage seasons was on a similar level of 340 and 309, respectively. Compared to the control, protection methods used in each year of vegetation affected reduction in the number of fungal isolates obtained from the stored horseradish seedlings. However, chemical protection to a greater extent contributed to the reduction in fungi population colonizing the seedlings, 88 isolates on average were recorded in this combination, and three fold more in the control site (180).

Isolated fungi were classified into 20 genera, 12 species were determined among them. Regardless of the protection and storage season, *V. dahliae* was isolated from horseradish seedlings with the highest frequency of 13.96%. The next positions were occupied by the species such as *C. destructans* (8.0%), *E. purpurascens* (7.66), *R. solani* (7.32) and antagonistic *T. hamatum* (6.04). In addition, a large share in the total population of fungi colonizing the seedlings was noted for Fusarium genus (20.77%). It was represented by 5 species, and *F. solani* was the most abundant (4.68%). Applied protection did not differentiate fungi communities present in horseradish seedlings in terms of quality. However, it affected proportions in the share of various species and genera in general population of fungi. In total 68 isolates of Trichoderma genus were observed in fungi community isolated from seedlings obtained from the site protected with biological and biotechnological preparations, while only 14 in the site of chemical protection (Table 1). In contrast, the use of synthetic fungicides limited horseradish seedlings colonization by dominant pathogens such as *C. destructans* and *R. solani*. The number of these species isolates was 16 and 14, respectively, and was twofold lower than in the case of biological protection. Besides, a significantly greater share of species belonging to Fusarium genus was noted in fungi community isolated from chemically protected seedlings.

Table 1. Occurrence of fungal genera and species isolated from diseased horseradish seedlings
Tab. 1. Występowanie rodzajów i gatunków grzybów izolowanych z chorych sadzonek chrzanu

Genus, Fungus species	Number of isolates									Total	%
	Biological protection			Chemical protection			Control				
	Years			Years			Years				
	2010	2011	2012	2010	2011	2012	2010	2011	2012		
Cylindrocarpon destructans (Zins.) Scholten	21	6	3	13	3	-	26	20	2	94	8,00
Epicoccum purpurascens Ehrenb. ex Schlecht.	14	8	2	21	4	-	30	11	-	90	7,66
Rhizoctonia solani Kühn	19	3	8	5	-	9	22	9	11	86	7,32
Trichoderma hamatum Persoon.	11	7	15	4	-	7	17	5	5	71	6,04
Verticillium dahliae Kleb.	27	16	12	12	11	19	32	25	10	164	13,96
Inne grzyby – other fungi											
Acremonium sp.	1	1	-	-	1	-	2	1	-	6	0,51
Alternaria spp.	4	2	4	6	2	10	9	15	21	73	6,21
Aspergillus spp.	3	1	5	1	-	3	5	3	1	22	1,87
Botryosphaeria spp.	-	3	2	-	1	2	1	5	7	21	1,79
Botrytis cinerea Pers.	1	-	-	2	-	1	2	2	-	8	0,68
Chaetomium sp.	-	1	4	-	-	1	-	3	1	10	0,85
Cladosporium sp.	-	2	-	3	-	1	2	-	1	9	0,77
Fusarium avenaceum	-	-	1	-	-	-	1	1	-	3	0,26
Fusarium culmorum (W.G.Smith) Sacc.	2	2	1	5	3	3	7	2	3	28	2,38
Fusarium equiseti (Corda) Sacc.	3	7	2	9	3	-	7	3	4	38	3,23
Fusarium oxysporum Schlecht	2	-	4	5	3	1	6	2	2	25	2,13
Fusarium solani (Mart.) Sacc.	6	7	10	8	3	-	11	6	4	55	4,68
Fusarium spp.	14	8	11	9	9	11	7	18	8	95	8,09
Mucor spp.	5	3	1	5	-	9	7	8	4	42	3,57
Penicillium spp.	10	2	5	3	-	7	17	4	6	54	4,60
Phoma sp.	-	4	1	3	8	1	6	7	2	32	2,72
Pythium spp.	5	-	-	1	-	-	10	2	-	18	1,53
Rhizopus spp.	3	5	1	-	6	2	1	7	10	35	2,98
Sclerotinia sclerotiorum (Lib.) de Bary	1	-	-	3	1	-	2	6	3	16	1,36
Trichoderma sp.	10	8	17	-	2	1	14	19	5	76	6,47
Trichothecium sp.	-	-	1	-	-	-	2	-	1	4	0,34
Total	162	96	110	118	60	88	246	184	111		
			368			266			541	1175	100
Together in the years: 2010 – 526; 2011 – 340; 2012 – 309 isolates											

Source: own work / Źródło: opracowanie własne

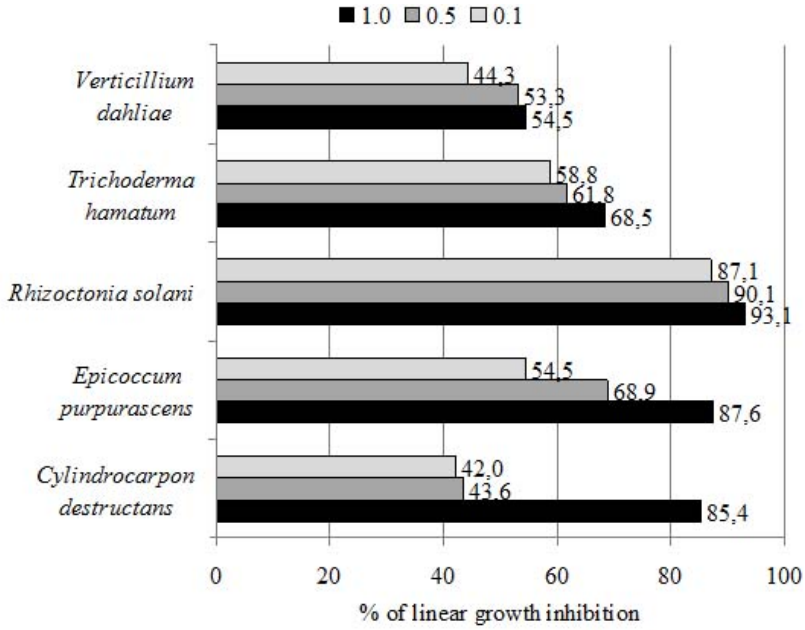
The study demonstrated high fungistatic activity of juniper oil. The applied concentrations significantly affected the reduction in examined fungi growth rate (Table 2). It was found that the decreasing concentration of juniper oil in a culture medium causes a decrease in fungistatic effect. However, significant differences in the growth rate under the influence of used concentrations were found with respect to *C. destructans* and *T. hamatum* (Table 2). *R. solani* to the highest degree reacted to juniper essential oil presence in the medium. This was reflected in very high values of the coefficients of its colony surface growth inhibition amounting to 87.1% for the concentration of 0.1 $mm^3 \cdot cm^{-3}$ (the lowest) even up to 93.1% in the presence of the highest concentration (Fig. 2). In turn, almost half lower (44.2–54.5%) indices of hyphae linear growth inhibition were recorded for *V. dahliae* and *C. destructans* (42.01–43.62%) on the media containing 0.1 and 0.5 $mm^3 \cdot cm^{-3}$ of juniper oil, respectively.

Fungistatic effect of juniper essential oil was also reflected in the process of examined fungi sporulation. Clear trend of increasing inhibition of sporulation with increasing oil concentration in the medium was noted with respect to sporulating fungi species (Table 3). Moreover, a significant differentiation in the number of spores between the applied concentrations was recorded within the species. Analyzing the values of coefficients of sporulation and linear growth inhibition, it was observed that juniper oil, especially at lower concentrations, more strongly inhibits *C. destructans* sporulation than mycelium growth (Fig. 1-2).

Table 2. The impact of juniper essential oil on linear growth rate of tested fungi
Tab. 2. Wpływ olejku jałowcowego na tempo wzrostu liniowego grzybów testowych

Concentration of essential oil [$mm^3 \cdot cm^{-3}$]	Growth rate index [T]				
	Cylindrocarpon destructans	*Epicoccum purpurascens*	*Rhizoctonia solani*	*Trichoderma hamatum*	*Verticillium dahliae*
1,0	1,27a*	11,89a	3,75a	13,33a	24,31a
0,5	17,83b	10,32a	7,82a	16,60ab	23,55a
0,1	19,40b	17,45a	14,98a	21,92b	28,87a
Control PDA+ethanol	27,01c	35,25b	90,57b	48,97c	47,61b
Control PDA	27,42c	34,78b	92,47b	45,04c	50,04b

* Values in columns marked by the same letter are not significantly different *Source: own work / Źródło: opracowanie własne*



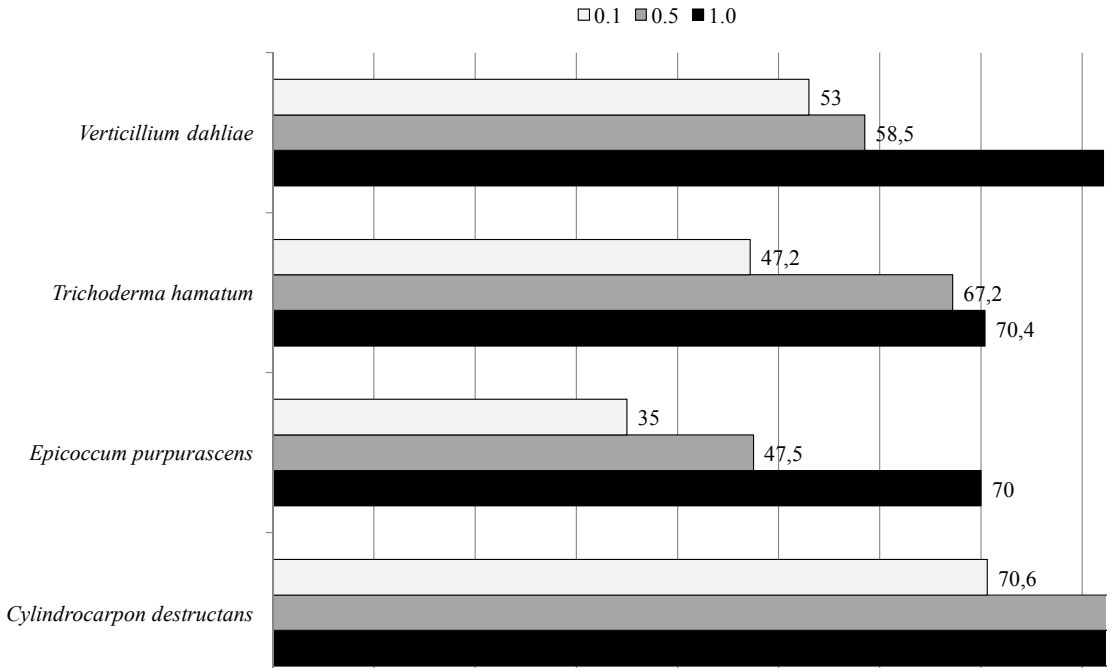
Source: own work / Źródło: opracowanie własne

Fig. 1. Effect of juniper essential oil on linear growth of tested fungi
Rys. 1. Wpływ olejku jałowcowego na wzrost liniowy testowanych grzybów

Table 3. The impact of juniper essential oil on sporulation of tested fungi
Tab. 3. Wpływ olejku jałowcowego na zarodnikowanie grzybów testowych

Concentration of essential oil [$mm^3 \cdot cm^{-3}$]	Number of spor in $cm^3 \cdot 10^7$			
	Cylindrocarpon destructans	*Epicoccum purpurascens*	*Trichoderma hamatum*	*Verticillium dahliae*
1,0	16,20a	1,80a	5,55a	8,40a
0,5	22,31b*	3,15b	6,15b	19,33b
0,1	49,83c	3,90c	9,90c	22,03c
Control PDA+ethanol	60,23c	6,50d	10,80c	51,65d
Control PDA	169,2d	6,00d	18,75d	46,31d

* Values in columns marked by the same letter are not significantly different *Source: own work / Źródło: opracowanie własne*



Source: own work / Źródło: opracowanie własne

Fig. 2. Effect of juniper essential oil on inhibition sporulation coefficient of the tested fungi
Rys. 2. Wpływ olejku jałowcowego na współczynnik zahamowania zarodnikowania grzybów testowych

4. Discussion

Five-month storage period favored the occurrence of disease lesions in the inner part of horseradish seedlings. *V. dahliae* was isolated with the highest frequency from necrotic tissue in every storage season. In turn, the species classified as dominants such as *C. destructans* and *E. purpurascens* were three-fold, while *R. solani* and *T. hamatum* two-fold more frequently noted in the population of fungi isolated from the seedlings collected in 2010. Intense colonization of the roots by these species was certainly encouraged by hydro-thermal conditions during the vegetation, the year 2010, compared to 2011 and 2012, was characterized by more precipitation and lower temperatures. In the available literature there is no information on horseradish roots colonization by fungi during the storage. Yu [45] reported that internal discoloration of horseradish roots in post-harvest evaluation is the result of complex infestation by 18 species of fungi, among which Verticillium genus with 38% share is dominating. Consistent results were also obtained for other fungi like *Alternaria spp*., *Rhizoctonia solani*, *Phoma* spp., and saprophytes *Penicillium* spp. and *Rhizopus* spp. In turn, Fusarium genus in the study of Yu [45] accounted for 31.8%, while in our study it was 20.8% It is known from the study conducted by Gleń-Karolczyk [14], that in the autumn post-harvest evaluation *E. purpurascens* had 3.7% and *P. irregulare* 5.2% share in the community of fungi colonizing the roots of horseradish. However, after the storage first of them was isolated with a two-fold higher frequency (7.7%) and *Phytium* spp. with four times lower (1.5%) (Tab. 1). Koutb and Ali [18] indicate that *E. purpurascens* exhibits an antagonism to *P. irregulare* which may confirm the obtained results. In this study, *C. destructans* (8%) was observed as a dominant. That species was not noted on dry-rotting outer skin of horseradish mother plantations [14]. So there is a presumption that *C. destructans* mainly infects the youngest shoots of the roots (first and second row) and colonizes the tissue inside the roots. Marek [25] claims that the decrease in roots firmness during the storage promotes greater invasiveness of this fungus. The used applied protection methods affected an improved mycological purity of horseradish seedlings. A total of 1175 isolates were obtained, including at least 266 from the combination protected chemically, 368 from non-chemical protection, and 541 from the control (Table 1). *E. purpurascens* had better conditions for development in the batch of seedlings derived from chemically protected combination, while *C. destructans*, *R. solani* and antagonist *T. hamatum* in the combination with non-chemical protection. The use of seedlings colonized by pathogenic fungi can be extremely risky, because the consequence may be developing rot diseases or subsequent plants dying as a result of wilting (*Verticillium* spp.).

The results indicate that juniper oil may be the chance for limiting of horseradish infestation by previously mentioned pathogens. Its 1.0 $mm^3 \cdot cm^{-3}$ concentration in *in vitro* tests in the culture medium strongly inhibited the linear growth of *R. solani* (93.1%) *E. purpurascens* (87.63) *C. destructans* (85.4%) colonies, and to a lesser extent *T. hamatum* (68.5%) and *Vdahliae* (54.5%) (Fig. 1). Particularly high fungistatic activity of all analyzed concentrations of juniper essential oil with respect to *R. solani* can be of an utilitarian significance. This species is the reason of seedling rot of many species of plants [32]. Besides, it causes large losses in potato production, there are also cases of intensive occurrence of cereals black speck [22, 44]. In this study, oil juniper to the highest degree limited the sporulation process in *C. destructans*. 70.6% and 90.4% lower number of spores was noted for it on the media with the share of 0.1–1.0 $mm^3 \cdot cm^{-3}$, respectively (Fig. 2). *C. destructans* is also polyphagous pathogen which infect mainly the root system [17]. Its presence is increasingly often noted at the base of the stalk, where it produces "black-foot disease" [16]. In medicine, juniper essential oil is widely

used inter alia for the treatment of microbial infections and even malaria [34]. Sati and Joshi [36] reported a very high effectiveness of methanol extract from juniper leaves against bacteria pathogenic to plants. Unfortunately, still very little research concerns the fungi pathogenic to plant. Only Modnicki and Łabędzka [27] indicate that fungicidal activity of juniper oil to *R. solani* and *R. stolonifer* is an effect of monoterpenes activity.

5. Conclusions

1. The main causes of necrosis of inner bark and pulp of horseradish seedlings are the following fungi: *V. dahliae* (13.96%), *C. destructans* (8.0%), *E. purpurascens* (7.66%), *R. solani* (7.32%) and *T. hamatum* (6.04%).
2. In *in vitro* tests, juniper oil, regardless of used concentration very strongly inhibits linear growth of above mentioned fungi. Average values of colony surface growth limiting are in the range from 50.66% for *V. dahliae* to 90.1% for *R. solani.*
3. An inhibition of sporulation process of the examined fungi increases in a direct proportion to increasing concentrations of juniper oil. The share of 1.0 $mm^3 \cdot cm^{-3}$ oil in the medium results in 90.4% decrease in the amount of *C. destructans* spores, and 82.1% in case of *V. dahliae*.
4. An inhibition of sporulation process of the examined fungi increases in a direct proportion to increasing concentrations of juniper oil. The share of 1.0 $mm^3 \cdot cm^{-3}$ oil in the medium results in 90.4% decrease in the amount of *C. destructans* spores, and 82.1% in case of *V. dahliae*.
5. Horseradish protection during the vegetation season positively affects the quality of stored seedlings. This is proved by total number of obtained fungal isolates: 226 for chemical protection, 368 for non-chemical, and 541 for the control.

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