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COMPARISON OF FUNGICIDAL PROPERTIES OF GERANIUM AND TEA TREE OILS

Summary

The aim of laboratory analyses was to compare the effect of geranium and tea tree oils on linear growth, biomass increment and sporulation of phytopathogenic fungi: Fusarium culmorum (W.G. Smith) Sacc., F. solani var. coeruleum (Sacc.) Booth and Sclerotinia sclerotiorum (Lib.) de Bary, Botrytis cinerea Pers. Ex Nocca and Balb. The fungi were cultured on PDA medium with oils added in various concentrations. Irrespective of the applied concentration, geranium oil revealed very strong fungistatic effect toward the tested fungi species. It was seen as the total inhibition of their surface growth, inhibition of biomass increment between 70% and 85.29% and total blocking of sporulation process in S. sclerotiorum and B. cinerea. The response of individual fungi species to tea tree oil presence in the medium depended on the oil concentration. The highest concentrations (1.00 and 0.80 mm^3) caused the 100% inhibition of linear growth of all species colonies, spore production by S. sclerotiorum and reduced (64.7-98.4%) their biomass increment.
***Key words**: plant oils, pathogenic fungi*

PORÓWNANIE FUNGICYDALNYCH WŁAŚCIWOŚCI OLEJKU GERANIOWEGO I OLEJKU Z DRZEWA HERBACIANEGO

Streszczenie

Celem badań laboratoryjnych było porównanie oddziaływania olejków geraniowego i drzewa herbacianego na rozrost liniowy, przyrost biomasy oraz zarodnikowanie grzybów fitopatogennych: Fusarium culmorum (W.G. Smith) Sacc., F. solani var. coeruleum (Sacc.) Booth oraz Sclerotinia sclerotiorum (Lib.) de Bary, Botrytis cinerea Pers. Ex Nocca i Balb. Grzyby hodowano na podłożu PDA z udziałem olejków w różnych stężeniach. Olejek geraniowy niezależnie od zastosowanego stężenia wykazywał bardzo silne oddziaływanie fungistatyczne w odniesieniu do testowanych gatunków grzybów. Wyrażało się to całkowitym zahamowaniem ich rozrostu powierzchniowego, hamowaniem przyrostu biomasy w zakresie od 70 do 85,29% oraz zupełnym blokowaniem procesu sporulacji S. sclerotiorum i B. cinerea. Reakcja poszczególnych gatunków grzybów na udział w podłożu hodowlanym olejku drzewa herbacianego zależała od zastosowanego stężenia. W najwyższych stężeniach (1.00 i 0.80 mm^3) olejek ten w 100% hamował wzrost liniowy kolonii wszystkich gatunków, wytwarzanie zarodników przez S. sclerotiorum oraz ograniczył (64,7-98,4%) przyrost ich biomasy.
***Słowa kluczowe**: olejki roślinne, grzyby chorobotwórcze*

1. Introduction

In search of new substances useful for plant protection against harmfull organisms, considerably more attention is paid to biological compounds, produced both by microorganisms and plants. The preparations based on natural compounds may decrease the level of chemicals used for plant protection. Moreover, they contribute to the natural environment protection owing to easier biodegradation [14, 24].

Compounds synthetized from plants differ from one another by their chemical structure, activity towards pathogenic microorganisms and pest insects [2, 26, 30]. These compounds comprise phenol derivatives, alkaloids, glycosides, saponins, essential oils and others. According to Łakota et al. [26] the quantity and quality of compounds accumulated in plants depend on many factors (plant species and development phase, the weather and soil conditions). Moreover, some of them are characterized by a broad spectrum of action, other reveal a higher activity against facultative than specialized microorganisms. In literature, the issue of inhibitory effect of some plant water extracts towards phytopathogenic fungi has been addressed by many authors [3, 4, 6, 7, 27, 32]. There are fewer papers discussing potential applications of anti-bacterial and antifungal properties of essential oils from aromatic plants [1, 5, 8, 18]. In previous investigations geranium oil and tea tree oil were used for combating microorganisms, such as: *Staphylococcus aureus*, *Escherichia coli*, *Lactobacillus* spp. and *Candida albicans* [15]. Moreover, since 2010 tea tree oil (as the active substance) has been registered for application on organic farms for plant protection against some pathogenic fungi. On the other hand, we lack studies on geranium oil effect on plant pathogenic fungi.

The research was conducted to learn the effect of various concentrations of geranium and tea tree oils on linear growth, biomass and sporulation of phytopathogenic fungi: *Fusarium culmorum* (W. G. Smith) Sacc., *F. solani* var. *coeruleum* (Sacc.) Booth and *Sclerotinia Sclerotiorum* (Lib.) de Bary, *Botrytis cinerea* Pers. Ex Nocca and Balb. in *in vitro* conditions.

2. Material and methods

Laboratory tests were conducted on essential oils, Dr Beta series - made by Pollena Aroma. Geranium oil was obtained from *Pelargonium graveolens* and tea tree oil from *Melaleuca alternifolia*. According to Procyk [28], the main components of geranium oil are: geraniol, citronellol, linallol, terpineol and alcohols. In the opinion of Różański [29], geraniol constitutes between 25 and 26% of this oil, cit-

ronellol 27-49%, whereas the other components have a smaller share. According to Carson and Riley [16], tea tree oil is a mixture of about a hundred hydrocarbon compounds and terpens. The components of this oil have very strong antibacterial and antiseptic properties. Kędzia et al. [19] think that the main component of fresh tea tree oil is terpinen-4-ol, in proportion of between 29 and 45%. Similar compounds are present in slightly lower proportions [19, 29].

The plant oils were tested on pathogenic fungi from the own collection of the Department of Agricultural Environment Protection: *Fusarium culmorum* (W. G. Smith) Sacc., *F. solani* var. *coeruleum* (Sacc.) Booth and *Sclerotinia Sclerotiorum* (Lib.) de Bary, *Botrytis cinerea* Pers. Ex Nocca and Balb. In the laboratory, culturing media were prepared with an addition of geranium and tea tree oils. The plant oils (dissolved in1ml of 5% ethyl alcohol) were added to a standard Potato Dextose Agar (PDA) medium with the temperature of 45^o so that their concentrations in the culturing medium were 0.10; 0.25; 0.50; 0.80 and 1.00 mm^3/cm^3. The media prepared in this way were then inoculated with 5mm agar disc overgrown with mycelium of the tested fungi species. Petri dishes with medium without the oils were the control. Culturing was conducted in a thermostat (23^{o}C) in five replications. From the moment of mycelium increments appearance, the colony diameter was measured daily until the whole surface of any petri dish was overgrown with mycelium. The effect of individual plant oils on linear growth of tested phytopathogenic fungi was presented as the difference between the diameter of the fungus colony on control petri dishes and the diameter of the fungus colony on petri dishes with oils in respective concentrations. The result was converted into the inhibition-stimulation coefficient expressed in percentage, according to Abbot formula [22]:

$$I = \frac{K - A}{K} * 100\% ,$$

where:
I - coefficient of mycelium linear growth inhibition,
K – mean of fungus colony on control petri dish,
A – diameter of fungus colony on petri dish with determined plant oil concentration.

The biomass of tested fungi was inserted in 300 ml Erlenmayer flasks on 100ml of modified PDA medium (without agar) with added plant oils in the above mentioned concentrations. Inoculum of the pathogenic fungi was introduced to the prepared medium and the flasks were protected with aluminium foil. The culturing was conducted for 21 days at the room temperature of about 23^{o}C. After that time the culturing liquid with mycelium was filtered through filter paper discs with 150mm diameter. Subsequently, the mycelium was dried on sterile glass at the temperature of 85^{o}C and weighed until constant weight. The obtained results of research on the increment of the tested fungi biomass were subjected to the analysis of variance. The significance of differences between mean values were verified by t-Student test on the significance level α=0.5.

Spore number was assessed on each dish. Five discs of medium overgrown with mycelium were cut out using 1mm cork borer of petri dish with the phytopathogenic fungi species. The discs were then transferred to test tubes and submerged in 10ml of sterile distilled water. Following a thorough crushing and shaking, the whole content was filtered through a three-layered gauze. Subsequently, a droplet of spore suspension was placed in Thom haemocytometer and spore number was counted under a light microscope.

3. Results and discussion

3.1. The effect of plant oils on linear growth of phytopathogenic fungi

The conducted research demonstrated that in the first place the kind of applied plant oil was the factor modifying surface growth of the tested fungi. Irrespectively of the applied concentration, very strong fungistatic effect of geranium oil was revealed towards the tested fungi species *(F. culmorum, F. solani* var. *coeruleum, S. sclerotiorum i B. cinerea).* Mean growth inhibition coefficients for all concentrations of this oil was 100% (fig. 1-4). Obtained values of the coefficients evidence a total inhibition of the tested mycelium hyphae in all concentrations. Górski et al. [13] obtained similar results for another fungus species. Geranium oil, which they tested, also inhibited growth of *Trichoderma harzianum*, irrespectively of its concentration in the medium. Kędzia [20] revealed great sensitivity of yeast-like fungi *Candida albicans, C. krusi, Geotrichum candidum)* and mould fungi (*Aspergillus niger, Alternaria solani, Penicillium digitatum)* to geranium oil. It was emphasized in the paper that geraniol was primarily responsible for strong anti-fungal properties of this oil.

On the other hand the Authors' own investigations demonstrated a variable sensitivity of the tested fungi species to the presence of tea tree oil in the culturing medium. Among the tested species, *F. culmorum* and *S. sclerotiorum* were characterised by a very strong sensitivity to applied tea tree oil. Mean values of the coefficient of these fungi surface growth were on the same very high, 90% level (Fig. 1, 2). On the other hand, the oil slightly less affected the surface development of the *B. cinerea* and F.solani var. *coeruleum* colonies. Coefficients of linear growth inhibition for these fungi were, respectively 70% and 75% (Fig. 3, 4). In the opinion of many authors [9-12, 21] the same researched factor under *in vitro* conditions may differently affect even phytopathogenic fungi which belong to the same species. Usually, also changes in phytopathogenic fungi response are observed within the same species (strains, special pathogens).

Unlike in case of geranium oil, the strength of tea tree oil effect on the coefficient of surface growth inhibition in the tested fungi depended on its concentration. Boligłowa et al. [5] were of a similar opinion. In the Authors' own investigations, the 1.00, 0.80 and 0.50 mm^3 tea tree oil supplement in the culturing medium totally inhibited surface growth of *Fusarium culmorum* (Fig. 1). Slightly lower inhibition coefficients (88.4% and 62.9%) were registered on the media with the 0.25 and 0.10 mm^3 addition of this oil.

A similar sensitivity to the share of individual concentrations of tea tree oil in the culturing medium characterised *S. sclerotiorum* fungus (Fig. 2). In this case, similarly the lowest concentrations of this oil caused a reduction of the colony growth between 57.6% and 94.5%, whereas *B. cinerea* revealed a bigger resistance to tea tree oil. Total inhibition (100%) of its mycelium growth was registered only on the media with the highest oil concentrations (1.00 and 0.80 mm^3) (Fig. 3). This oil concentration of 0.50 mm^3 inhibited the development of mycelium hyphae almost twice stronger (86.2%) than the 0.25mm^3 concentration. On the other hand, the lowest concentration of the tea tree only to a

slight degree (18.4%) reduced *B. cinerea* colony growth. This oil modified the surface growth inhibition coefficient also in *F. solani* var. *coeruleum* (Fig. 4). Lower concentrations (0.25 mm^3 and 0.10 mm^3) of tea tree oil caused the inhibition of *F.solani* var. *coeruleum* colony on the levels, respectively 51.1 and 22.8%. The same oil applied in higher concentrations (1.00, 0.80 and 0.50 mm^3) contributed to the total inhibition of this fungus colony surface growth.

3.2. The influence of plant oils on biomass of the tested fungi

The tested plant oils to a various degree affected biomass increment of the tested fungi. The phenomenon of diversified effect of oils on plant phythopathogenic microorganisms is connected with the specific character of chemical compounds forming a part of these oils, their complex effect and sensitivity of the phytopathogen. Daferera [8] and Lo Cantore et al. [25] also reported the fact. In the Authors' own investigations, generally the oils inhibited the increment of the tested fungi biomass in comparison with the control (Fig. 1). However, the geranium oil which in the analysed concentrations totally inhibited surface growth of the fungi colonies, also significantly stronger reduced the biomass increment (from 70 to 85%) of the tested phytopathogens than the tea tree oil.

The divergences between linear growth and biomass increment often happen in the *in vitro* research [9-11, 17, 31]. In the Authors' own investigations, the fungi culturing in the presence of oils lasted for three weeks. In the first days the growth inhibition was observed, but later they may adapt themselves to the environment conditions. Such response of fungi has been noted in papers by other authors [23].

In the Authors' own studies only *F. culmorum* and *F. solani* var. *coeruleum* revealed significantly higher biomass in the presence of tea tree oil in comparison with geranium oil. *S. sclerotiorum* fungus responded differently, whereas *B. cinerea* mass did not markedly depend on the kind of the oil used for the experiment.

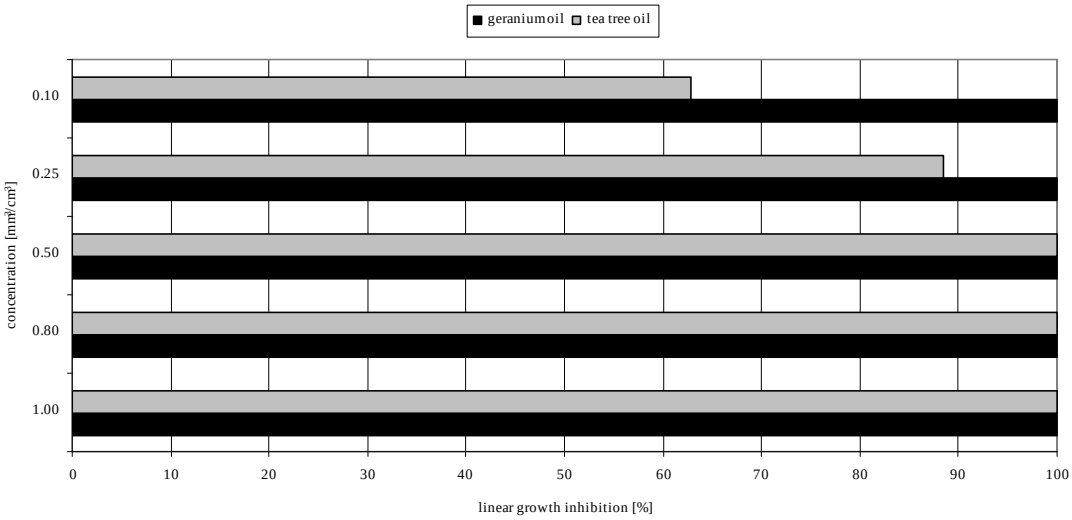

Fig. 1. Influence of plant oils concentration on surface growth of *F. culmorum*
Rys. 1. Wpływ stężenia olejków roślinnych na rozrost powierzchniowy F. culmorum

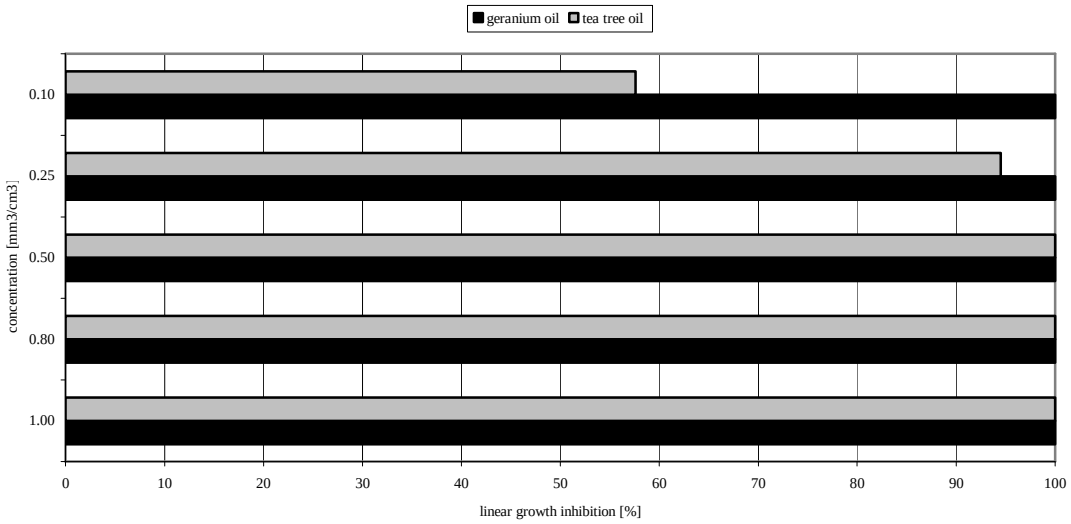

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Fig. 2. Influence of plant oils concentration on surface growth of *S. sclerotiorum*
Rys. 2. Wpływ stężenia olejków roślinnych na rozrost powierzchniowy S. sclerotiorum

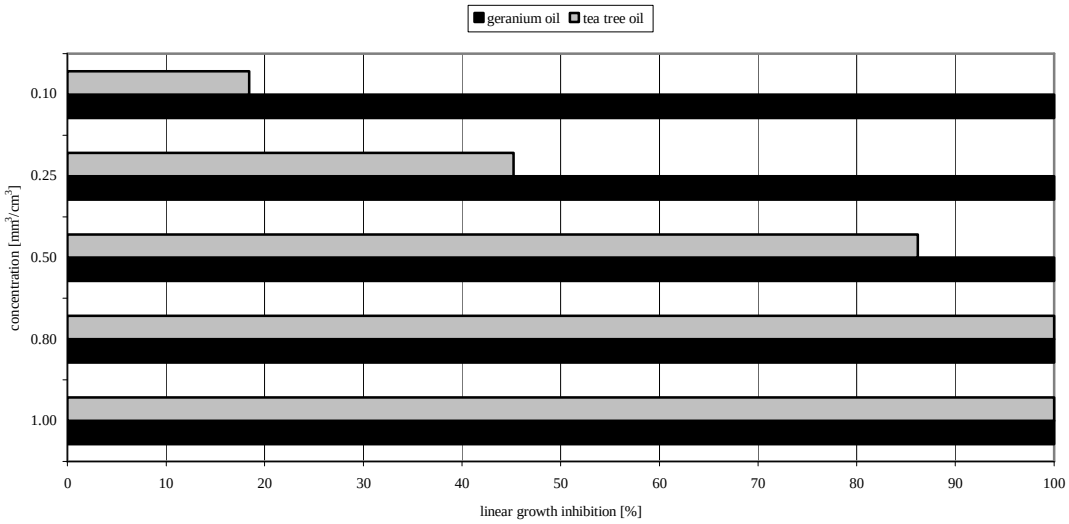

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Fig. 3. Influence of plant oils concentration on surface growth of *B. cinerea*
Rys. 3. Wpływ stężenia olejków roślinnych na rozrost powierzchniowy B. cinerea

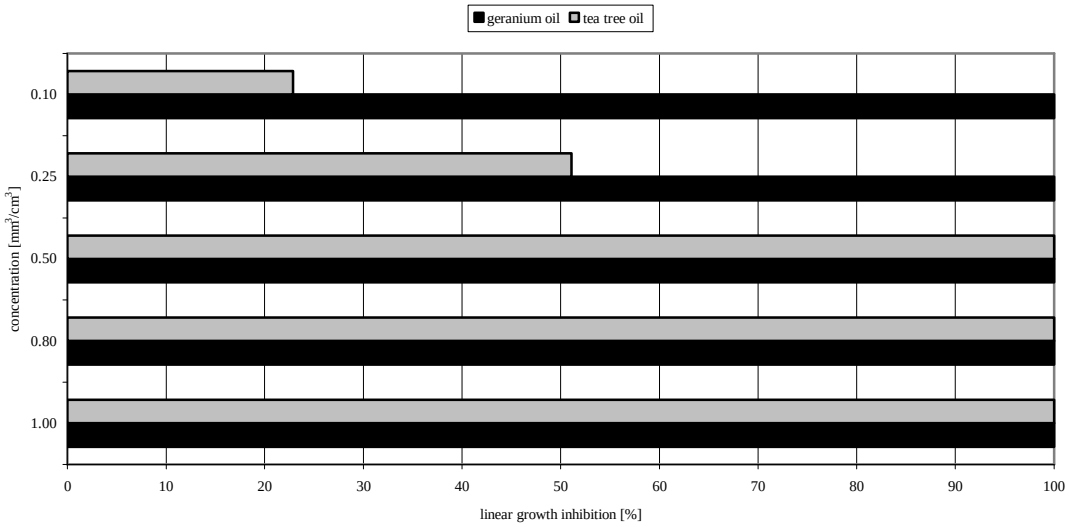

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Fig. 4. Influence of plant oils concentration on surface growth of *F. solani* var. *coeruleum*
Rys. 4. Wpływ stężenia olejków roślinnych na rozrost powierzchniowy F. solani var. coeruleum

Table 1. Effect of plant oils on the tested fungi biomass
Tab. 1. Wpływ olejków roślinnych na biomasę grzybów testowych

Plant oil	Biomass [g]			
	F. culmorum	*F. solani var. coeruleum*	*S. sclerotiorum*	*B. cinerea*
Control	0.31 c*	0.30 c	0.63 c	0.34 b
Geranium	0.07 a	0.08 a	0.16 b	0.05 a
Tea tree	0.11 b	0.11 b	0.10 a	0.05 a

*Values in columns marked with the same letters do not differ significantly
**Wartości w kolumnach oznaczone tymi samymi literami nie różnią się istotnie*

Source: own work / Źródło: opracowanie własne

In the Authors' own investigations the fungi biomass, except *B. cinerea* significantly depended on the applied oil concentration (Tab. 2). Geranium oil concentrations from 1.00 to 0.25 mm^3 in the culturing medium, markedly stronger inhibited the increment of *F. culmorum F. solani var. coeruleum, S. sclerotiorum* biomass in comparison with the lowest, 0.10 mm^3 concentration. On he the other hand, tea tree oil concentrations significantly stronger diversified biomass of these fungi. In case of *F. culmorum*, only the highest tea tree oil concentration in the culturing medium (1.00 mm^3) considerably reduced its biomass. On the other hand, *F. solani* var. *coeruleum* biomass was markedly inhibited by this oil concentrations from 1.00 to 0.50 mm^3, whereas *S. sclerotiorum* by the concentrations from 1.00 to 25 mm^3.

3.3. The effect of plant oils on fungi sporulation

Microscopic fungi produce spore of various kinds, which guarantee the continuation of the species. It is a common opinion, that in plant phytopathogenic fungi, a limited growth of hyphae occurs under unfavourable environmental conditions, whereas spore formation processes intensify [17]. The tested plant oils, beside a strong influence on mycelium hyphae development, also modified their sporulation process. Results of Authors' own studies presented in Table 3 evidence that geranium oil, irrespectively of the applied concentration totally blocked the spore formation process in *B. cinera* and *S. sclerotiorum*. A similar reaction was observed also in *F. culmorum* i *F. solani var. coeruleum* fungi on culturing media with 1.00, 0.80 and 0.50mm^3 of this oil. Moreover, a lack of conidia was also observed in *S. sclerotiorum* on the culture media containing 1.00, 0.80 and 0.50mm^3 of tea tree oil. Moreover, lower concentrations stimulated sporulation in this fungus species. On the other hand, all concentrations of tea tree oil reduced the number of *F. culmorum* macroconidia - a greater decrease in their number was observed with growing oil concentration. However, the number of microconidia was increasing. The effect of individual concentrations of tea tree oil on spore formation process in *F. solani* var. *coeruleum* proved complex. At the highest concentration a lack of both both macro- and microconidia was noted. On the other hand, on the medium with 0.80, 0.50 and 01.0mm^3 oil concentrations, the number of macroconidia was lower than in the control, whereas microconidia formation by *F. solani* var. *coeruleum* was stimulated at the two first oil concentrations. Moreover, culturing of this pathogen on the medium containing 0.25 mm^3/cm^3 of tea tree oil favoured production of macro- and microconidia by the fungus.

4. Conclusions

1. Under *in vitro* conditions plant oils strongly influenced the colony surface growth, biomass and intensity of sporulation in phytopathogenic fungi: *Fusarium culmorum*, *F. solani* var. *coeruleum, Sclerotinia sclerotiorum* oraz *Botrytis cinerea.*
2. Irrespective of the applied concentration, geranium oil, in comparison with the tea tree oil, caused a total inhibition of the tested fungi surface growth, inhibited biomass increment in between 70 and 85.29% and totally blocked sporulation process in *S. sclerotiorum* i *B. cinerea*.
3. Response of individual fungi species to the tea tree oil supplement in the culturing medium depended significantly on the applied concentration. In the highest concentrations (1.00 and 0.80 mm^3) the oil inhibited 100% of all fungi species linear growth, while it reduced their biomass increment in 64.7-98.4% and *S. sclerotiorum* sporulation.
4. Among the tested plant phytopathogenic fungi, *S. sclerotiorum and B. cinerea* revealed the greatest sensitivity to the analysed oils.

Table 2. The influence of plant oil concentrations on fungi biomass [g]
Tab. 2. Wpływ stężeń olejków roślinnych na biomasę grzybów [g]

Tested fungi	Control	Oil concentration (mm^3/cm^3 culture medium)						$LSD_{0.05}$
		Oil	**0.10**	**0.25**	**0.50**	**0.80**	**1.00**	
F. culmorum	0.31	A	0.13	0.09	0.08	0.07	0.07	0.03
		B	0.17	0.13	0.11	0.11	0.08	0.04
F. solani var. coeruleum	0.30	A	0.27	0.04	0.06	0.04	0.02	0.04
		B	0.26	0.22	0.10	0.05	0.08	0.09
S. sclerotiorum	0.63	A	0.61	0.02	0.07	0.04	0.07	0.05
		B	0.40	0.01	0.03	0.03	0.02	0.08
B. cinerea	0.34	A	0.03	0.08	0.07	0.06	0.02	n.s.
		B	0.09	0.04	0.04	0.04	0.02	n.s.

A – geranium oil; B – tea tree oil; n.s. - non significant
A – olejek geraniowy; B – olejek drzewa herbacianego; różnica nieistotna

Source: own work / Źródło: opracowanie własne

Table 3. Influence of plant oils on test fungi sporulation
Tab. 3. Wpływ olejków roślinnych na zarodnikowanie grzybów testowych

Plant oil	Oil concentration [mm^3/cm^3 medium]	Spore numer in 1cm^{3}x [10^8]					
		F. culmorum		*F. solani var. coeruleum*		*S. sclerotiorum*	*B. cinerea*
		Macroconidia	Microconidia	Macroconidia	Microconidia		
Geranium	1.00	-*	-	-	-	-	-
	0.80	-	-	-	-	-	-
	0.50	-	-	-	-	-	-
	0.25	-	-	6.71	56.38	-	-
	0.10	2.06	14.05	25.21	18.6	-	-
Tea tree	1.00	0.03	23.15	-	-	-	2.09
	0.80	0.05	12.28	7.25	66.18	-	4.38
	0.50	1.18	10.25	8.09	32.65	-	10.00
	0.25	5.28	6.10	24.76	29.9	4.93	2.61
	0.10	7.63	3.93	10.53	13.13	5.48	3.5
Control	-	8.5	12.25	18.41	22.28	3.68	7.3

* (-) denotes lack of sporulation/ ** (-) oznacza brak zarodnikowania*

Source: own work / Źródło: opracowanie własne

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