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THE INFLUENCE OF BIOPREPARATIONS AND SYNTHETIC FUNGICIDES ON GROWTH ON MICORRHIZAL FUNGUS *Oidiodendron* sp. *IN VITRO*

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Abstarct. The influence of biopreparations Bioczos SL, Biosept 33 SL, Biochikol 020 PC, Propolis and synthetic fungicides Euparen Multi 50 WG, Horizon 250 EW and Teldor 500 SC was examined on the growth of micorrhizal fungus *Oidiodendron* sp. Fungicides were added to PDA medium at the following concentrations: recommended dose, 5-times lower than the recommended and 5-times higher than the recommended. The synthetic fungicides were more toxic to *Oidiodendron* sp. than biopreparations. On the medium containing Horizon 250 EW fungus did not growth. The greather part of biopreparations inhibited growth of *Oidiodendron* sp. but only Bioczos SL and Propolis in dose 5-times higher than the recommended, toataly inhibited growth of fungus colonies.

Key words: synthetic fungicides, biopreparations, concentration, growth colonies, micorrhizal fungus, *Oidiodendron* sp.

INTRODUCTION

Symbiosis between higher plants and fungi is a common phenomenon in natural ecosystems [Paul and Clark 2000]. This close mycorrhizal relationship benefits both organisms is unquestionably, which has been repeatedly proven by relevant research [Davies 1987, Hall and Williams 2000, Kowalski 2000, Valleneuve et al. 1991, Krupa 2003, Sowik and Borkowska 2004, Borkowska 2004]. One of the many advantages of mycorrhiza is the induction of plant tolerance to biotic stresses (pathogenic fungi, pest) [Barea 1996, Dehne 1982, Kowalski 2004] and abiotic stresses (contamination, drought) [Schreiner et al. 2001, Borkowska 2002, Shi et al. 2002, Stahl et al. 1998, Aleksandrowicz-Trzcińska 2004b].

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Research into mycorrhiza is primarily concerned with heather plants and forest trees. It is multifarious, yet its final aim is to preserve the natural environment. In order for coexistence of plant roots with fungi to be established, favourable conditions must occur in the environment. In agro ecosystems natural conditions can deteriorate as a result of agronomical practices (irrigation, fertilization, soil cultivation) as well as the widespread use of chemicals to protect the crops. It is particularly evident in permanent-crop fruit culture, which is the one branch of arable farming where pesticides are most excessively used. Agricultural chemicals can obviously contaminate soil, which manifests itself in general impoverishment of edaphon [Ryszkowski 1981]. This can lead to the suppression of fungal mycorrhiza and a decrease in fungal biodiversity in soil [Kowalski 2000]. Integrated ecological fruit culture requires that the use of chemicals be limited or even abandoned altogether in favour of biologicals. Therefore, research seems advisable into the influence of biologicals and synthetic fungicides on the growth *in vitro* of the fungus *Oidiodendron* sp. forming mycorrhiza with heather plants, including the blueberry.

MATERIAL AND METHODS

The influence of 4 biologicals Bioczos SL, Biosept 33 SL, Biochikol 020 PC, Propolis and 3 synthetic fungicides Euparen Multi 50 WG, Horizon 250 EW, Teldor 500 SC, on the growth and ontogenesis of the mycorrhizal fungus *Oidiodendron* sp. was studied.

The above-mentioned preparations were added to a sterile PDA nutrient medium cooled to 55ºC in the following doses:

– 5 times higher than recommended dose – (X_1),
– recommended dose in agricultural practice – (X_2),
– 5 times lower than recommended dose – (X_3).

Successive concentrations were obtained by using a dilution method. The growth medium thus prepared was distributed to Petri dishes and then inoculated with the fungi *Oidiodendron* sp., which had been supplied by Mykoflor company from Puławy.

The reference colonies were fungi growing on a PDA medium with no biologicals or fungicides added. The experiment was set up in 5 repetitions at the temperature 22°C. The diameters of the *Oidiodendron* sp. colonies were measured at one-day intervals, on four consecutive days until the reference colonies covered the dishes thoroughly.

The obtained results were presented in the form of tables and figures. Statistical calculations were done using the Tukey test, the assumed significance level being $\alpha = 0.01$.

RESULTS

The fungus *Oidiodendron* sp. was found out to generally be more susceptible to synthetic fungicides than to biologicals (fig. 1), although the latter did inhibit the fungal growth by 53%.

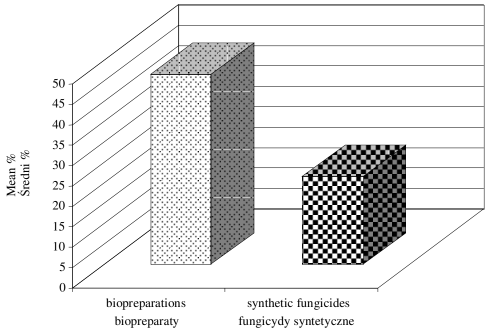


Fig. 1. The influence of biopreparations and synthetic fungicides on the growth *Oidiodendron* sp. (growth in % of the control)
Rys. 1. Wpływ biopreparatów i syntetycznych fungicydów na wzrost grzyba *Oidiodendron* sp. (wzrost w % w stosunku do kontroli)

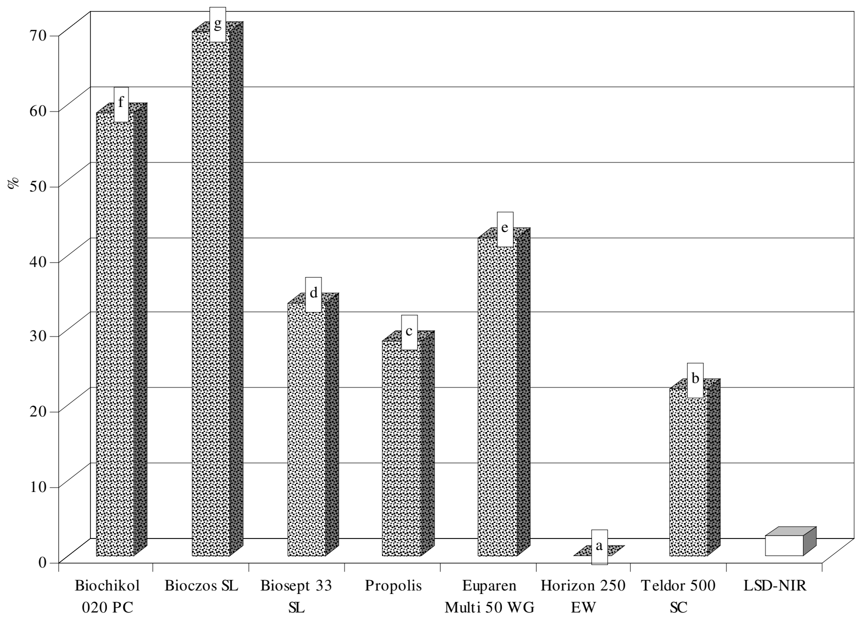


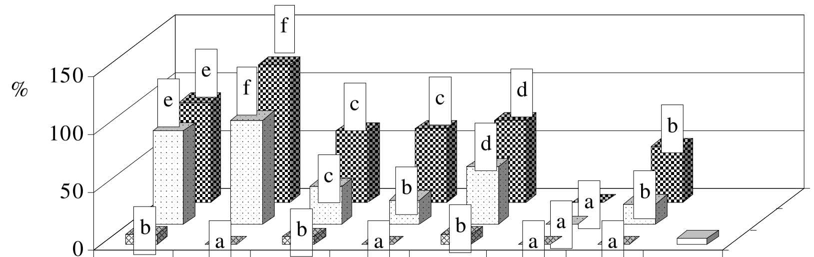
Fig. 2. The growth of colonies *Oidiodendron* sp. on media with biological and synthetic fungicides (in % of the control); a, b, c, d, e, f, g – means followed by the some letters do not significantly different
Rys. 2. Wzrost kolonii grzyba *Oidiodendron* sp. na podłożach z biopreparatami i fungicydami syntetycznymi (w % w stosunku do kontroli); a, b, c, d, e, f, g – średnie wielkości kolonii w obrębie preparatów oznaczone tymi samymi literami nie różnią się istotnie

Regardless of the concentration of a preparation in medium and the time of observation, all the experimental preparations were shown to strongly inhibit the growth of fungal colonies (fig. 2).

Table 1. Size of colonies of the fungus *Oidiodendron* sp. on media with biological and synthetic fungicides (in % of the control)
Tabela 1. Wielkość kolonii grzyba *Oidiodendron* sp. na podłożu z biopreparatami i fungicydami syntetycznymi (w % w stosunku do kontroli)

Preparation Preparat	Concentration Koncentracja	Day – Doba			
		1	2	3	4
Biochikol 020 PC	X_1	A 0.0 a	A 0.0 a	A 12.1 b	A 20.5 c
	X_2	A 0.0 a	B 100.4 b	B 106.3 b	B 116.6 c
	X_3	A 0.0 a	C 117.3 bc	B 111.8 b	B 120.3 c
Bioczos SL	X_1	A 0.0 a	A 0.0 a	A 0.0 a	A 0.0 a
	X_2	B 94.6 c	B 117.4 d	B 80.7 b	B 65.7 a
	X_3	C 117.6 a	C 126.6 b	C 115.1 a	C 118.0 a
Biosept 33 SL	X_1	A 0.0 a	A 0.0 a	A 8.3 ba	A 15.7 b
	X_2	A 0.0 a	B 41.7 b	B 41.1 b	B 47.5 b
	X_3	A 0.0 a	C 84.9 b	C 77.8 b	C 85.8 b
Propolis	X_1	A 0.0 a	A 0.0 a	A 0.0 a	A 0.0 a
	X_2	A 0.0 a	B 24.3 b	B 29.8 b	B 29.5 b
	X_3	A 0.0 a	C 89.2 b	C 82.8 b	C 86.3 b
Horizon 250 EW	X_1	A 0.0 a	A 0.0 a	A 0.0 a	A 0.0 a
	X_2	A 0.0 a	A 0.0 a	A 0.0 a	A 0.0 a
	X_3	A 0.0 a	A 0.0 a	A 0.0 a	A 0.0 a
Euparen Multi 50 WG	X_1	A 0.0 a	A 0.0 a	A 13.8 b	A 17.2 b
	X_2	A 0.0 a	B 58.3 b	B 65.9 bc	B 70.3 c
	X_3	A 0.0 a	C 88.3 b	C 92.0 b	C 101.2 c
Teldor 500 SC	X_1	A 0.0 a	A 0.0 a	A 0.0 a	A 0.0 a
	X_2	A 0.0 a	A 0.0 a	B 33.4 b	B 35.8 b
	X_3	A 0.0 a	B 56.2 b	C 63.2 b	C 77.6 c

X_1 – the dose 5 times higher than the recommended – koncentracja preparatu w pożywce 5-krotnie większa od zalecanej
X_2 – the recommended dose – koncentracja zalecana
X_3 – the dose 5 times lower than the recommended – koncentracja preparatu w pożywce 5-krotnie mniejsza od zalecanej
$LSD_{0.01}$ interaction C/AB (day/preparation × concentration) = 8.17 – $NIR_{0,01}$ interakcja C/AB (doba/preparat × koncentracja) = 8,17
$LSD_{0.01}$ interaction B/AC (concentration/preparation × day) = 7.64 $NIR_{0,01}$ interakcja B/AC (koncentracja/preparat × doba) = 7,64
a, b, c, d – means for days fallowed by the some letters do not significantly different – średnie wielkości kolonii w kolejnych dobach oznaczone tymi samymi literami nie różnią się istotnie (wiersze)
A, B, C – means for ccentrations fallowed by the some letters do not significantly different – średnie wielkości kolonii dla koncentracji w obrębie preparatu oznaczone tymi samymi literami nie różnią się istotnie (kolumny)



	Biochikol	Bioczos SL	Biosept 33 SL	Propolis	Euparen Multi	Horizon 250	Teldor 500 SC	LSD-NIR
the dose 5 times higher than the recommended-dawka 5-krotnie większa niż zalecana	8,2	0	6	0	7,8	0	0	4,53
the recommended dose-dawka zalecana	80,8	89,6	32,6	20,9	48,6	0	17,3	
the dose 5 times lower than the recommended-dawka 5-krotnie	87,4	119,3	62,1	64,6	70,4	0	49,3	

Fig. 3. The influence of concentration on the growth of colonies *Oidiodendron* sp. (in % of the control); a, b, c, d, e, f – means for preparations fallowed by the some letters do not significantly different

Rys. 3. Wpływ koncentracji preparatu w pożywce na wzrost kolonii grzyba *Oidiodendron* sp. (w % w stosunku do kontroli); a, b, c, d, e, f – średnie wielkości kolonii w obrębie preparatów przy określonej dawce oznaczone tymi samymi literami nie różnią się istotnie

Of the synthetic fungicides Horizon proved to have the most toxic effect on *Oidiodendron* sp. (no growth), while Euparen Multi was least effective. As far as the biologicals are concerned, Propolis inhibited the fungal growth most strongly (fig. 2). The use of Bioczos caused a reverse response-the fungus produced colonies of the significantly biggest growth compared with the use of both the remaining biologicals and synthetic fungicides. The fungal growth reached 70% of the reference colony (fig. 2).

Regardless of the time of observation and the type of preparation the dose 5 times higher than recommended – (X_1) proved to be most harmful to the fungus (tab. 1). The lower the concentration of preparations became in the medium, the less harmful their toxic effects were. The only exception was Horizon, which produced total effect throughout the experiment regardless of its concentration (fig. 3). At highest concentrations, also Teldor as well as Propolis and Bioczos showed their strong toxic effect (fig. 3). The largest doses of Euparen Multi, Biosept and Biochikol caused the fungus *Oidiodendron* sp. to produce significantly bigger colonies than the remaining ones, yet their growth was severely inhibited and did not exceed 8.2% of the colonies at control (fig. 3). As for the biologicals, Propolis was found to bring about the most inhibitory effect. *Oidiodendron* sp. was shown to be most tolerant of Bioczos, producing the biggest colonies compared with the remaining ones (fig. 3). A similar response was also observed at the lowest concentration of Bioczos – X_3.

During the first day of the experiment all the preparations at all their concentrations were found to affect the fungus *Oidiodendron* sp. in a similar way: inhibiting the colony growth thoroughly (tab. 1). The only exception was Bioczos, which, in a recommended dose, did not interfere with the fungal growth on the first day. At the lowest concentration – X_3 the preparation not only failed to inhibit the growth, but it simply stimulated it – it exceeded the control colony growth by 18%. As the experiment proceeded, the toxic effect of the preparations at all concentrations generally were off considerably (only Biochikol in a recommended dose even stimulated the mycelium growth on the last day) or kept at a steady level, as in the case of Horizon, which inhibited the mycelium growth throughout the experiment (tab. 1). A similar effect was demonstrated by Teldor, Propolis and Biczos, but only at the highest concentrations (tab. 1).

DISCUSSION

One of the basic factors taken into consideration while estimating mycorrhizal properties of fungi is their susceptibility to agricultural chemicals [Kowalski 2004]. Although long-standing research has been conducted into the influence of fungicides on the occurrence of mycorrhiza, the results are still inconclusive. Mycorrhizal fungi are known to respond to pesticides in a number of ways. Some chemicals can interfere with or even inhibit symbiosis between a fungus and a plant, others, on the contrary, stimulate mycorrhiza [Aleksandrowicz-Trzcińska and Kieliszewska-Rokicik 1999; Aleksandrowicz-Trzcińska 1999, 2002]. Fungicides, widely used in plant protection against phytopathogens, can alter the colonization of roots by mycorrhizal fungi [Owson et al. 1986]. It is hard to give the explanation of a diversified effect of fungicides in identical conditions on the onset and development of mycorrhiza. If a mycorrhizal relationship between the host plant and the fungus is not established, tolerance to biotic and abiotic stresses, which are integral part of agroecosystems, decreases [Borkowska 2004, Orlikowski 2004, Dehne 1982]. The effect of fungicides on mycorrhizal fungi depends on the type of agent, its usage and concentration. It is confirmed by the results of our experiment. All the experimental preparations considerably curbed the growth of fungal colonies. The synthetic fungicides tended to inhibit the growth of *Oidiodendron* sp. more strongly than the biologicals did.

The toxic effect of the preparations on *Oidiodendron* sp. depended primarily on their concentration in the medium. Horizon proved to be most toxic at all concentrations. It inhibited the colony growth throughout the experiment. Similar results were obtained by Aleksandrowicz-Trzcińska [2004a] for the fungus *Hebeloma crustuliniforme*. She suggests that the cause of a strong toxic effect of the fungicides Bayleton and Tilt of IBE group, which also includes Horizon, lies in these preparations inhibiting the biosynthesis of ergosterol known to be produced by pathogenic fungi as well. Triazole compounds are presumed to affect mycorrhizal fungi in a similar way. Teldor curbed the colony growth to a considerably smaller extent, still, at a recommended concentration of the preparation, the fungus did yield colonies smaller than the reference colony by as much as 65%. Of the examined synthetic fungicides Euparen Multi showed the least toxic effect on *Oidiodendron* sp. Admittedly, in the initial phase of the experiment at the highest concentration – (X_1) no sign of the colony growth was observed, but finally the colonies reached only 20% of the reference culture growth. At a recommended concentration the fungus produced colonies smaller than the reference colony by only 30%. At the lowest concentration X_3 - the colonies were even bigger. As Aleksandrowicz-Trzcińska and Kieliszewska-Rokicik [1999] reported, dichlofluanide, an active agent in Euparen 50 WP, had no harmful influence on the degree of controllable mycorrhizal association between the fungus *H. crustuliniforme* and the Scots pine roots. A recommended concentration of Euparen did not curb the growth of the fungus *Laccaria bicolor* in the Scots pine roots, either [Aleksandrowicz-Trzcińska 2002]. The author also found out that Euparen did not affect spontaneous mycorrhiza.

The majority of biologicals, on the other hand, curbed the growth of *Oidiodendron* sp. Generally, of the experimental preparations Propolis proved the most effective and Bioczos the least effective, the mycelium being smaller than in the reference culture by as little as 30%. Only the highest concentrations of Bioczos and Propolis guaranteed total inhibition of the fungal growth throughout the experiment. Recommended doses of Bioczos and Propolis curbed the colony growth to a smaller or larger extent respectively. In the case of Biochikol both recommended and the smallest doses stimulated the growth of *Oidiodendron* sp. colony. The research carried out by Guerin-Laguette et al. [2003] showed that natural preparations with an oil base and olive oil applied to the medium strongly stimulated the growth of the edible ectomycorrhizal mushroom *Tricholoma matsutake*. Moreover, olive oil contributed to a considerable gain in the mushroom biomass. This influence was correlated with the concentration of the preparation.

CONCLUSIONS

1. The synthetic fungicides inhibited the growth of *Oidiodendron* sp. more than the biologicals did.

2. Of the synthetic fungicides, Horizon 250 EW proved to have the most toxic effect on the fungus *Oidiodendron* sp., while Euparen Multi 50 WG inhibited the mycelium growth to the slightest extent.

3. Of the biologicals, Propolis inhibited the fungal growth most strongly. *Oidiodendron* sp. was shown to tolerate Bioczos SL, which had the least toxic effect among both the biologicals and the synthetic fungicides.

4. Biochikol 020 PC was the only preparation which, in a recommended dose, stimulated the mycelium growth on the last day of the experiment.

REFERENCES

Aleksandrowicz-Trzcińska M., 1999. Impact of fungicides used at protection of forest nurseries on the develop of pine seedling micorrhizae. Part II. Shares of mycorrhizal and autotrophic roots. Sylwan. 11, 37–46.

Aleksandrowicz-Trzcińska M., 2002. Wpływ fungicydów na wzrost i kolonizację mikoryzową sadzonek sosny zwyczajnej (*Pinus silvestris* L.) hodowanych w kontenerach. Rozpr. habil. Wyd. SGGW. Warszawa.

Aleksandrowicz-Trzcińska M., 2004a. Wpływ fungicydów na mikoryzy sadzonek drzew leśnych. [w:] Dlaczego mikoryza jest szansą sukcesu dla roślin ogrodniczych i leśnych? Wyd. Wieś Jutra. Warszawa, 61–69.

Aleksandrowicz-Trzcińska M., 2004b. Kolonizacja mikoryzowa i wzrost sosny zwyczajnej (*Pinus silvestris* L.) w uprawie założonej z sadzonek w różnym stopniu zmikoryzowanych. Acta Sci. Pol. Silv. Colendar. Rat. Ind. Lignar 3(1), 5–15.

Aleksandrowicz-Trzcińska M., Kieliszewska-Rokicik B., 1999. The impact of fungicides used at forest nursery protection on the development of pine seedling mycorrhiza. Part. 1. Research on the content of ergosterol in roots. Sylwan. 6, 73–81.

Barea J. M., Calvet C., Estaun V., Camprubi A., 1996. Biological control as key component in sustainable agriculture. Plant and Soil. 185, 171–172.

Borkowska B., 2002. Growth and photosynthetic activity of mikropropagated strawberry plants inoculated with endomicorrhizal fungi (AMF) and growing under drought stress. Acta Physiol. Plantarum. 24(4), 365–370.

Borkowska B., 2004. Dlaczego mikoryza. [w:] Dlaczego mikoryza jest szansą sukcesu dla roślin ogrodniczych i leśnych? Wyd. Wieś Jutra. Warszawa, 14–18.

Davies F. T. Jr., 1987. Effect of VA-mycorrhizal fungi on growth and nutrien uptake of cuttings of *Rosa multiflora* in two container media with three levels fertilizer application. Plant and Soil. 104, 31–35.

Dehne H. W., 1982. Interaction between vesicular-arbuscular mycorrhizal fungi and plant pathogens. Phytopathology 72, 1115–1119.

Guerin-Laguette A., Vaario L-M., Matsushita N., Shindo K., Suzuki K., Lapeyrie F., 2003. Growth stimulation of a Shiro-like, mycorrhiza forming, mycelium of *Tricholoma matsutake* on solid substrates by non-ionic surfactants or vegetable oil. Myc. Prog. 2(1), 37–44.

Hall J. L., Williams L. E., 2000. Assimilate transport and partitioning in fungal biotrophic interaktions. Austr. J. Plant Physiol. 27(6), 549–560.

Kowalski S., 2000. Mikoryzy i ich znaczenie dla optymalnego wzrostu i rozwoju drzew leśnych oraz potrzeby i możliwości sztucznej mikoryzacji materiału sadzeniowego. Leśny Bank Genów Kostrzyna, Miłków. 19, 18–30.

Kowalski S., 2004. Stosowanie biopreparatu z grzybami mikoryzowymi w pojemnikowej hodowli siewek drzew leśnych. [w:] Dlaczego mikoryza jest szansą sukcesu dla roślin ogrodniczych i leśnych? Wyd. Wieś Jutra. Warszawa, 23–32.

Krupa P., 2003. Mikoryzy drzew rosnących na terenach zdegradowanych przez przemysł. XXXVIII Międzynar. Symp. Mikrobiol. „Efektywne mikroorganizmy w rolnictwie zrównoważonym i ochronie środowiska". SGGW. Rogów k. Łodzi.

Orlikowski L., 2004. Mikoryzowanie roślin a rozwój fytoftorozy (*Phytophthora* spp.). [w:] Dlaczego mikoryza jest szansą sukcesu dla roślin ogrodniczych i leśnych? Wyd. Wieś Jutra. Warszawa, 93–96.

Owson P. W., Thies W. G., Fender W., 1986. Field performance of Douglas-fir seedlings after treatment with fungicides. Can. J. For. Res. 16, 1369–1371.

Paul E. A., Clark F. E., 2000. Mikrobiologia i biochemia gleb. Przekł. Kurek E., Kobus J., Wyd. UMCS. Lublin, 295–314.

Ryszkowski L., 1981. Wpływ intensyfikacji rolnictwa na faunę. Zesz. Probl. Post. Nauk Roln. 233, 3–38.

Schreiner P. R., Ivors K. L., Pinkerton J. N., 2001. Soil solarization reduces arbuscular mycorrhizal fungi as a consequence of weed suppression. Mycorrhiza. 1(6), 273–277.

Shi L., Guttenberg M., Kottke I., Hampp R., 2002. The effect drought on mycorrhizas of beech (*Fagus sylvatica* L.): changes in community structure and the content of carbohydrates and nitrogen storage bodies of the fungi. Mycorrhiza. 12(6), 303–311.

Sowik I., Borkowska B., 2004. Wpływ mikoryzy na stosunki wodne w truskawkach rosnących w warunkach suszy i porażonych *Verticillium dahliae* Kleb. 2004. Ogólnopol. Konf. Sad. Skierniewice, 263–265.

Stahl P., Schuman G. E., Frost S. M., Williams S. E., 1998. Arbuscular mycorrhizae and water stress tolerance of wyoming big sagebrush seedlings. Soil Sci. Am. J. 62, 1309–1313.

Villeneuve N., Le Tacon F., Bouchard D., 1991. Survival of inoculated *Laccaria bicolor* in competition with native ectomycorrhizal fungi and effects on the growth of outplanted Douglas – fir seedlings. Plant Soil. 135, 95–107.

WPŁYW BIOPREPARATÓW I SYNTETYCZNYCH FUNGICYDÓW NA WZROST *IN VITRO* GRZYBA MIKORYZOWEGO *Oidiodendron* sp.

Streszczenie. Badano wpływ biopreparatów Bioczos SL, Biosept 33 SL, Biochikol 020 PC, Propolis oraz fungicydów syntetycznych Euparen Multi 50 WG, Horizon 250 EW oraz Teldor 500 SC, na wzrost i rozwój grzyba mikoryzowego *Oidiodendron* sp. Do pożywki PDA dodawano badane preparaty w dawkach: zalecanej w praktyce rolniczej, 5-krotnie mniejszej i 5-krotnie większej od zalecanej, a następnie inokulowano grzybem *Oidiodendron* sp. Wykazano, że fungicydy syntetyczne okazały się bardziej toksyczne w stosunku do badanego grzyba niż biopreparaty. Preparat Horizon 250 EC przy koncentracji zalecanej uniemożliwił wzrost grzyba do końca trwania eksperymentu. Większość biopreparatów ograniczało wzrost grzyba *Oidiodendron* sp., lecz tylko przy stężeniu najwyższym, w przypadku Bioczosu SL oraz Propolisu, obserwowano całkowite zahamowanie wzrostu kolonii grzyba.

Słowa kluczowe: fungicydy syntetyczne, biopreparaty, koncentracja, wzrost kolonii, grzyb mikoryzowy, *Oidiodendron* sp.

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