ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 16, No. 7 2009

Katarzyna GLEŃ[1] and Elżbieta BOLIGŁOWA[1]

EFFECT OF SELECTED FOLIAR FERTILIZERS ON PHYTOPATHOGENIC FUNGI UNDER CONDITIONS *IN VITRO*

WPŁYW WYBRANYCH NAWOZÓW DOLISTNYCH NA GRZYBY FITOPATOGENNE W WARUNKACH *IN VITRO*

Abstract: The research aimed at an assessment of the effect of foliar fertilizers, ie Mikrovit Fe – Iron chelate, Wapnovit and Fostar recommended for agronomic and vegetable crops and in orchards on linear growth, biomass and sporulation of the following fungi: *Sclerotinia sclerotiorum* (Lib.) de Bary, *Rhizoctonia solani* Kühn and *Phoma exiqua* Desm. var. *exiqua* under conditions *in vitro*.

The results obtained show a not unanimous response of the tested fungi species to applied foliar fertilizers and their various concentrations. Among the analyzed foliar fertilizers, Mikrovit Fe most strongly inhibited linear growth, biomass increment and mitigated spore production in all tested fungi. Also Fostar revealed a strong fungistatic effect on *Phoma exiqua* and *Rhizoctonia solani*. At the highest applied concentration (1.0 mm^3/cm^3) growth inhibition coefficients for these species were respectively 67.54 % and 46.45 %. On the other hand, Wapnovit revealed very weak fungistatic properties because it inhibited the linear growth of the analyzed fungal organisms only between 0.02 and 8.24 %. At the same time this fertilizer stimulated growth of the aerial mycelium and sporulation process in the tested fungi. Moreover, *Sclerotinia sclerotiorum* was the species which most weakly responded to fertilizer presence in the medium.

Keywords: foliar fertilizers, phytopathogenic fungi, growth, biomass, sporulation

The growing assortment of foliar fertilizers available on the market answers plant producers' requirements and provides a challenge to undertake new research on their effect on the amount of yield and the environment. The main objective of the introduction of foliar fertilizer to the agrocenosis is increasing the quantity of obtained yields [1-7]. While improving plant nutrition through foliar fertilization, one may also strengthen their resistance to pathogen infestation [8–14]. Moreover, the protective effect of foliar fertilizers is connected with their direct effect upon pathogens. Several studies in this area show that foliar fertilizers may inhibit development of plant pathogen under conditions *in vitro* [15–21].

[1] Departament of Agricultural Environment Protection, University of Agriculture in Krakow, al. A. Mickiewicza 21, 31-120 Kraków, Poland, email: rrglen@cyf-kr.edu.pl

The paper shows the influence of various concentrations of foliar fertilizers, ie Mikrovit Fe, Fostar and Wapnovit on linear growth, biomass increment and sporulation of phytopathogenic fungi: *Sclerotinia sclerotiorum* (Lib.) de Bary, *Rhizoctonia solani* Kühn and *Phoma exiqua* Desm. var. *exiqua* under conditions *in vitro*.

Material and methods

Foliar fertilizers: Mikrovit Fe, Fostar and Wapnovit manufactured by Intermag Enterprise in Olkusz were selected for a laboratory experiment. Mikrovit Fe – Iron chelate contained 3.0 % Fe, 32 g Fe/dm^3 of the fertilizer, 4.5 % N, pH – 32; Wapnovit had 12.16 % CaO, (256 g CaO/dm^3 of fertilizer), 10 % of nitrogen (N-NO_3), 0.48 % – MgO, 0.05 % – B, 0.02 % – Cu, 0.02 % – Zn, pH – 2.7,whereas Fostar; contained: 14.8 % – P, 34.1 % – P_2O_5 (500.3 g P_2O_5/dm^3 of fertilizer), pH – 1.4. Fungi species: *Sclerotinia sclerotiorum* (Lib.) de Bary, *Rhizoctonia solani* Kühn and *Phoma exiqua* Desm. var. *exiqua* originated from the cultures of the Agricultural Environment Protection Department. The fungi were cultured under conditions *in vitro,* in five replications, on the PDA medium (glucose-potato) and at the temperature of 23 °C. The PDA medium with added Mikrovit Fe, Wapnovit and Fostar were prepared to obtain their concentrations of : 0.01, 0.1 and 1 mm^3 per 1 cm^3 of the medium. Prior to the experiment, the outset pH was measured in the media. The media were inoculated with an agar ring, 5 mm in diameter, overgrown with two-week old mycelium. The control was provided by Petri dishes with the medium without the fertilizer supplement. The effect of individual foliar fertilizers and their concentrations on linear growth of the analyzed fungal organisms was presented as a difference between the mean fungus colony diameter on the control dishes and the diameter of the mycelium colony on dishes with individual concentrations of foliar fertilizers. Coefficients of linear growth rate and inhibition/stimulation coefficients were computed following the formula presented by Gleń [13]. After three weeks of the fungi culture growing on the PDA media with the foliar fertilizers and control the numbers of spores were assessed in Thom haemocytometer.

Fungi biomass growth was maintained in 300 cm^3 Erlenmayer flasks on 100 cm^3 of the modified PDA medium (without agar) with the supplements of foliar fertilizers in the same concentrations as in the above mentioned experiment. The culture was maintained for 21 days at the temperature of ca 23 °C. After this period the post-culture liquid with mycelium was filtered through filter paper. Then the mycelium was dried on a sterile glass at 80 °C to constant weight and weighed. The results were verified statistically using ANOVA and the significance of differences was assessed on the basis of the t-Student test.

Results and discussion

The laboratory experiments allowed to assess the direct effect of foliar fertilizers, ie Mikrovit Fe, Fostar and Wapnovit on linear growth, biomass and sporulation of *Sclerotinia sclerotiorum* (Lib.) de Bary, *Rhizoctonia solani* Kühn and *Phoma exiqua* Desm. var. *exiqua* fungi. However, the obtained results do not provide grounds for unanimous de-

termination of the fertilizer effect on the fungal organisms, since each investigated fungus species responded differently to individual fertilizer preparations and their concentrations in the medium. This fact was confirmed by other authors [15–22].

Among the analyzed foliar fertilizers Mikrovit Fe, irrespective of the concentration applied, revealed strong fungistatic properties towards *Phoma exiqua* and *Rhizoctonia solani.* It has been visible as a significantly slower rate of the linear growth of these species in comparison with the growth on Petri dishes, inhibition of surface growth of these species colonies by respectively 49.28 % and 44.76 %, biomass increments on average by 41.36 % and 64. 36% and sporulation (Fig. 1, Table 1–3).

Table 1

Coefficients of rate [T] and inhibition of the linear growth of the tested fungi [%]

Foliar fertilizers	Concentration [mm^3/cm^3]	*Rhizoctonia solani*		*Sclerotinia sclerotiorum*		*Phoma exiqua*	
		[T]	[%]	[T]	[%]	[T]	[%]
Mikrovit Fe – Iron chelate	1	33.38 a*	59.97	76.94 b	4.82	20.83 a	74.99
	0.1	40.75 b	51.14	68.14 a	15.71	37.45 c	55.05
	0.01	64.12 d	23.18	80.56 c	0.35	68.48 f	17.8
Fostar	1	44.66 c	46.45	80.44 c	0.49	27.04 b	67.54
	0.1	64.03 d	23.22	79.92 c	1.14	55.17 d	33.77
	0.01	65.30 e	21.70	83.55 d	+3.35	56.09 e	32.67
Wapnovit	1	76.53 f	8.24	80.42 c	0.52	79.00 g	5.17
	0.1	81.94 g	1.75	79.66 bc	1.46	80.23 e	3.70
	0.01	83.96 i	+0.67	79.66 bc	1.46	83.29 i	0.02
Control		83.40 h		80.84 cd		83.31 i	

* Values in columns marked by the same letter are not significantly different,
\+ means stimulation of biomass increment.

At the same time it was found that the inhibitory effect of Mikrovit Fe on *P. exiqua* and *R. solani* was diminishing with their decreasing concentrations in the media (Table 1–4). A similar relationship was found in the research on the influence on Mikrovit Fe on *Botrytis cinerea* [21]. Moreover, Mikrovit Fe used in 0.1 mm^3/cm^3 concentration very strongly (91.46 %) inhibited *S. sclerotiorum* biomass growth, but it limited its linear growth only in 15.40 % (Table 1–2). However, in the same object a stimulation of *S. sclerotiorum* sporulation was detected (Table 3). In the opinion of Hodges [23] such a result may be considered as a defensive response of the fungus to unfavourable conditions and protection of the colony durability.

The activity of foliar fertilizers on fungal organisms under *in vitro* conditions greatly depends not only on the kind and dose of the fertilizer preparation but, as reported by Weber and Wyrwa [16], also on the medium reaction. On the other hand, in common opinion, microscopic fungi may develop within a wide range of environment reaction values, but for the majority the optimum pH ranges from 4 to 7 [24–26]. In the Authors' studies the share of individual concentrations of Mikrovit Fe changed the medium pH to a slight extent (4.34–5.20). Moreover, a characteristic feature of Mikrovit Fe used in

the experiment was the presence of chelated Fe cations. Gumiński [27] reports that Fe^{3+} is the most strongly bound at pH of 3 and the bond strength decreases with growing pH. Therefore, the role of the medium reaction is connected rather with the availability of individual nutrients to fungi. In their own studies the Authors observed a significant and at the same time very strong inhibition of biomass increment, particularly in *S. sclerotiorum* and *Rhizoctonia solani* at 1.0 and 0.1 mm^3/cm^3 of Mikrovit Fe supplement (Table 4). On the other hand, it may be supposed that at a lower concentration of this preparation in the medium and pH 5.20, Fe ions might have been better utilized by the analyzed fungal organisms to build fungal structures. On the other hand, irrespectively on the applied concentration, Mikrovit Fe strongly reduced sporulation process in the tested fungi. This fact was also corroborated by the studies of Glen and Boliglowa [19].

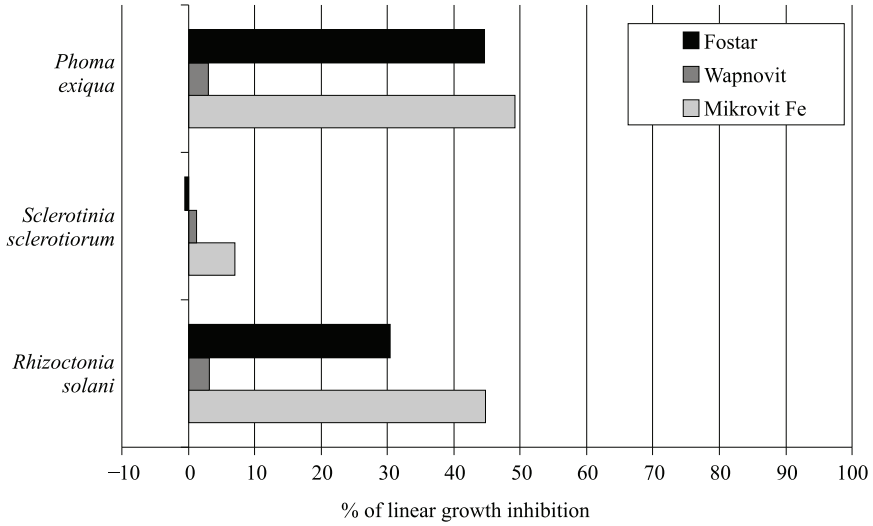

Fig. 1. Effect of foliar fertilizers on tested fungi growth inhibition

The response of the tested fungal organisms to Fostar was rather diversified. Generally the fertilizer revealed weaker fungistatic properties. In its highest concentrations (1.0 and 0.1 mm^3/cm^3) it notably reduced the development of all tested fungi colonies, but as in the case of Mikrovit Fe, *P. exiqua* and *R. solani* proved to be the most sensitive species (Table 1). An apparent inhibition of their linear growth (ca 34 and 23 %) was observed also on media containing 0.1 mm^3/cm^3 of this fertilizer preparation (Table 1). However, on liquid media Fostar, especially in the lowest concentration it stimulated biomass increments in the tested organisms (Table 2). *R. solani* biomass increment reached even 68.34 %. In the Authors' former studies [22] a strong inhibition of linear growth reaching ca 80 % was noted as well as very intensive biomass increments (54.62–106.17 %) of *Fusarium* fungi in the presence of 0.01 mm^3/cm^3 of Fostar. ANOVA conducted in the Authors' own research did not reveal any notable effect of individual Fostar concentrations on *S. sclerotiorum* growth rate (Table 1). However, in each of the

analyzed concentrations of this fertilizer a four time smaller number of *S. sclerotiorum* spores was registered (Table 3). On the other hand at 1.0 mm^3/cm^3 of Fostar content in the medium the number of *P. exiqua* spores declined even ten times. Also Gleń [22] reported that irrespective of the applied concentration, this fertilizer totally blocked the macroconidia forming the process in *F. coeruleum*, *F. culmorum* and *F. graminearum*. In the Authors' own research, pH of media containing 1.0, 0.1 and 0.01 mm^3/cm^3 of Fostar was respectively: 3.61, 3.95 and 4.32. A strongly acid medium pH assuredly did not favour the growth of the tested phytopathogenic fungi, particularly during the short experiment on Petri dishes. It was confirmed by the research by Gleń and Boligłowa [19]. Foliar fertilizers characterized by a low pH applied in 1.0 mm^3/cm^3 concentration led to a complete inhibition of *F. avenaceum, F. coeruleum, F. graminearum.*

The Wapnovit foliar fertilizer revealed a lack of fungistatic activity. Slight inhibition of *R. solani*, *P. exiqua* and *S. sclerotiorum* colony growth was registered only when the highest concentrations were used (Table 1). Irrespective of its concentration Wapnovit had a significant influence on stimulation *R. solani* biomass increments (Table 3). The 0.01 mm^3/cm^3 concentration also greatly affected intensive growth of *P. exiqua* biomass. On the other hand, although higher concentrations of Wapnovit revealed a tendency to stimulate this species, the biomass increments did not differ notably from the control. Moreover, a considerable (nine-fold) smaller *P. exiqua* spore number was observed (Table 3). The analyzed foliar fertilizer did not have any marked influence either on linear growth or biomass of *S. sclerotiorum* (Table 1, 2). Very weak fungistatic properties of Wapnovit applied in 1.0 mm^3/cm^3 concentration (field dose) towards *Fusarium* fungi were also observed by Gleń [22]. Unlike Fostar, Wapnovit is a mutlicomponent fertilizer which apart from calcium contains also nitrogen, magnesium, boron, copper and zinc. Moreover the pH of the media with added Wapnovit ranged from 4.51 to 5.75, remaining within the range optimal for the fungi tested in the experiment. The tested fungi species revealed a great tolerance to Wapnovit presence in the medium.

Table 2

Effect of foliar fertilizers on biomass increment of tested fungi

Foliar fertilizers	Concentration [mm^3/cm^3]	*Rhizoctonia solani*		*Sclerotinia sclerotiorum*		*Phoma exiqua*	
		Biomas [g]	T [%]	Biomas [g]	T [%]	Biomas [g]	T [%]
Mikrovit Fe – Iron chelate	1	0.046 a*	92.29	0.181 a	80.43	0.259 a	53.75
	0.1	0.164 a	72.53	0.079 de	91.46	0.311 ab	44.46
	0.01	0.424 b	28.98	0.442 b	52.21	0.415 c	25.89
Fostar	1	0.525 bc	12.06	0.755 c	18.38	0.394 bc	29.64
	0.1	0.533 bc	10.72	0.893 cde	3.46	0.569 de	+1.61
	0.01	1.005 d	+68.34	0.994 e	+7.46	0.664 ef	+18.57
Wapnovit	1	1.009 d	+69.01	0.953 de	+3.03	0.563 d	+0.53
	0.1	0.977 d	+63.65	0.814 cd	12.00	0.608 de	+8.57
	0.01	0.970 d	+62.48	0.863 cde	6.70	0.714 f	+27.50
Control		0.597 c		0.925 de		0.560 d	

* values in columns marked with the same letter do not differ significantly,
\+ means stimulation of biomass increment.

Table 3

Sporulation of tested fungi

Fungus species	Spore number per 1 cm^3 x [10^8]									
Fungus species	Control	Mikrovit Fe			Fostar			Wapnovit		
		concentration [mm^3/cm^3]								
		1.0	0.1	0.01	1.0	0.1	0.01	1.0	0.1	0.01
Sclerotinia sclerotiorum	12.48	8.36	7.40	6.79	4.06	4.21	4.93	13.61	8.59	8.39
Phoma exiqua	72.24	0.23	5.47	7.38	7.18	8.10	11.69	7.23	8.02	8.14

Conclusions

1. Among the tested fungal organisms, *Phoma exiqua* and *Rhizoctonia solani* revealed high sensitivity to the applied fertilizer preparations, particularly to Mikrovit Fe and Fostar.

2. Mikrovit Fe revealed the strongest fungistatic properties, which became apparent as strong inhibition of biomass growth of all tested fungi species, a considerable limitation of *P. exiqua* and *R. solani* linear growth, and inhibition of sporulation of *P. exigua* and *S. sclerotiorum*.

3. Fostar foliar fertilizer applied in 1.0 mm^3/cm^3 concentration strongly inhibited linear growth of *R.solani* and *P. exiqua*, reduced their biomass increment by 12.06–29.64 %. Irrespective of its concentration, it inhibited sporulation in *P. exiqua* and *S. sclerotiorum.*

4. The share of 1.0 mm^3/cm^3 of Wapnovit in the medium stimulates biomass increment in all tested fungi species and favours intensification of the sporulation process in *S. sclerotiorum.*

References

[1] Czuba R.: *Efekty dolistnego dokarmiania roślin uprawnych. Cz. II. Reakcja roślin na dolistne stosowanie mikroelementów i azotu łącznie z mikroelementami*. Rocz. Glebozn. 1993, **XLIV**(3/**4**), 79–87.

[2] Boligłowa E.: *Wpływ dolistnego dokarmiania na plonowanie i jakość bulw ziemniaka*. Rozp. Habilit., **4**1. Wyd. WSR-P, Siedlce 1995.

[3] Jabłoński K. and Bernat E.: *Wpływ dolistnego nawożenia Mikrosolem Zn na kształtowanie się plonu ziemniaka i jego jakość oraz możliwość ograniczenia stosowania fungicydów do zwalczania zarazy ziemniaka*. Progr. Plant Protect., 2001, **41**(1), 299–305.

[4] Boligłowa E.: *Wpływ dolistnego dokarmiania ziemniaka na plon jego strukturę, zdrowotność i trwałość przechowalniczą bulw*. Acta Agrophys., 2003, **85**, 99–106.

[5] Tobiasz-Salach R. and Borecka-Jamro D.: *Wpływ wieloskładnikowych nawozów dolistnych na plonowanie i skład chemiczny owsa*. Acta Agrophys., 2003, **85**, 89–98.

[6] Chwil S. and Szewczuk C.: *Wpływ dolistnego dokarmiania buraka cukrowego na jego plon i niektóre cechy jakościowe*. Acta Agrophys., 2003, **85**, 117–12**4**.

[7] Sady W.: Nawożenie warzyw polowych. Plantpress, Kraków 2000.

[8] Nowosielski O.: *FloroGama (FGO) – Pierwszy nawóz nalistny ochroniarski*. Ochr. Rośl., 1986, **7**, 1**4**–16.

[9] Solarska E.: *Ocena przydatności działania ochronnego nawozów dolistnych na chmielu*, Mat. 35 Sesji Naukowej IOR, cz. II Postery, Poznań 1995.

[10] Piszczek J.: *Wpływ dokarmiania dolistnego preparatami Bornit i Tytanit na zdrowotność i jakość buraka cukrowego*. Progr. Plant Protect., 2001, **41**(1), 306–311.

[11] Kapsa J.: *Możliwość ograniczania dawek fungicydów przez dodatek Insolu 7 w ochronie plantacji ziemniaka przed Phytophthora infestans (Mont.) de Bary*, Zesz. Nauk. AR Kraków, 2002, nr 387, 82, 75–79.

[12] Sawicka B.: *Wpływ łącznego stosowania agrochemikaliów na tempo szerzenia się Phytophthora infestans na roślinach ziemniaka*. Acta Agrophys., 2003, **85**, 157–167.
[13] Kruczek A.: *Wpływ dolistnego dokarmiania kukurydzy azotem i nawozem wieloskładnikowym na porażenie roślin przez choroby i szkodniki oraz wyleganie.* Acta Agrophys., 2003, **85**, 77–87.
[14] Gleń K.: *Evaluation of foliar fertilizers for horse radish Armoracia rusticana Gaertn protection against fungal diseases*. Ecol. Chem. Engin., 2007, **14**, 527–532.
[15] Przeździecki Z., Wojciechowska-Kot H., Mikołajska I. and Murawa D.: *Wpływ mikroelementów na rozwój grzybni, zarodnikowanie i zawartość kwasów tłuszczowych Fusarium i Fusarium avenaceum.* Acta Acad. Agricullt. Tech., Olsztyn 1991, **53**, 229–239.
[16] Weber Z. and Wyrwa P.: *Wpływ nawozu dolistnego Bonga na wzrost czterech gatunków grzybów in vitro*. Rocz. Akad. Roln. Poznań, Roln., 1993, **42**(247), 133–137.
[17] Skrzypczak Cz., Orlikowski L.B. and Matysiak B.: *Effect of some fertilizer chelates on the growth on pathogenic fungi and development of Phytophtora foot-rot of gerbera and population dynamics of Phytophtora cryptogen.* Phytopathol. Polon., 1996, **11**, 41–50.
[18] Boligłowa E. and Gleń K.: *Wpływ nawozów dolistnych na wzrost i zarodnikowanie grzybów patogenicznych z rodzaju Fusarium w warunkach in vitro*. Acta Agrophys., 2003, **85**, 107–116.
[19] Gleń K. and Boligłowa E.: *Effect of Mikrovit Fe and Mikrovit Mn on development of Fusarium culmorum (W.G. Smith) Sacc.* Ecol. Chem. Eng. 2006, **8**(13), 743–750.
[20] Gleń K., Boligłowa E. and Trela S.: *Assessment of Tytanit in vitro effect on selected phytopathogenic fungi.* Ecol. Chem. Eng. 2006, **7**(13), 743–750.
[21] Gleń K. and Boligłowa E.: *Response of some polyphagous fungi on microelement foliar fertilizers under conditions in vitro.* Ecol. Chem. Eng. 2007, **14**, 537–543.
[22] Gleń K.: *Comparison of Fostar and Wapnovit foliar fertilizers effect on phytopathogenic Fusarium fungi of genus*. Ecol. Chem. Eng. 2007, **14**, 529–536.
[23] Hodges C.F: *Vegetative growth and sporulation of Biopolaris sorokiniana on sequentially older infected leaves of Poa pratensis exposed to postemergence herbicides*, Mycopathologia. 1994, **128**(2), 105–109.
[24] Kirlay Z., Klement Z. and Solymosy F.: *Fitopatologia*. *Wybór metod badawczych*. PWRiL. Warszawa 1977
[25] Kochman J. and Węgorek W.: Ochrona roślin. Plantpress. Kraków 1997.
[26] Borecki Z.: *Nauka* o chorobach roślin. PWRiL, Warszawa 2001.
[27] Gumiński S.: Znaczenie chelatów i związków próchniczych w odkażaniu środowiska, Bioindykacja skażeń przemysłowych i rolniczych. PAN, Wrocław 1983, 57–66.

WPŁYW WYBRANYCH NAWOZÓW DOLISTNYCH NA GRZYBY FITOPATOGENNE W WARUNKACH *IN VITRO*

Katedra Ochrony Środowiska Rolniczego, Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie

Abstrakt: Celem podjętych badań było określenie wpływu nawozów dolistnych, tj. Mikrovit Fe – Chelat żelaza, Wapnovit i Fostar zalecanych do stosowania w uprawach rolniczych, warzywniczych i sadach, na wzrost liniowy, biomasę i zarodnikowanie grzybów: *Sclerotinia sclerotiorum* (Lib.) de Bary*, Rhizoctonia solani* Kühn i *Phoma exiqua* Desm. v. *exiqua* w warunkach *in vitro.*

Uzyskane wyniki wskazują, że reakcja badanych gatunków grzybów na zastosowane nawozy dolistne i ich różne stężenia nie jest jednoznaczna. Spośród badanych nawozów dolistnych Mikrovit Fe niezależnie od stężenia w podłożu najsilniej ogranicza rozrost liniowy, przyrost biomasy oraz osłabia proces sporulacji wszystkich grzybów testowych. Silne właściwości fungistatyczne w odniesieniu do *Phoma exiqua* i *Rhizoctonia solani* wykazywał również Fostar. Szczególnie zaś w największym z zastosowanych stężeń (1.0 mm^3/cm^3) – współczynnik zahamowania wzrostu dla tych gatunków wynosił odpowiednio 67,54 % i 46,45 %. Natomiast Wapnovit wykazał bardzo słabe właściwości fungistatyczne, bowiem hamował on rozrost liniowy badanych organizmów grzybowych w zakresie 0,02–8,24 %. Jednocześnie nawóz ten stymulował wzrost grzybni powietrznej i proces sporulacji testowanych gatunków. Ponadto gatunkiem najsłabiej reagującym na obecność nawozów w podłożu okazał się *Sclerotinia sclerotiorum*.

Słowa kluczowe: nawozy dolistne, grzyby fitopatogenne, wzrost, biomasa, zarodnikowanie