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THE EFFECT OF MANGANESE TREATMENT ON PATHOGENIC FUNGI ISOLATED FROM BARLEY KERNELS

ODDZIAŁYWANIE NAWOZU MANGANOWEGO NA GRZYBY PATOGENICZNE IZOLOWANE Z ZIARNIAKÓW JĘCZMIENIA

Abstract: Teprosyn Mn manganese seed treatment, manufactured by a British firm Phosyn Chemicals Ltd., which was tested in the experiment, has been available on the Polish market for several years. Applied as a seed treatment it results in more intensive development of root system and improves general plant condition. However, in the available literature lacks information on Teprosyn Mn effect on plant healthiness or pathogenic organisms.

The aim of the paper was to compare the effect of Teprosyn Mn fertilizer and Raxil Gel 206 chemical seed treatment on pathogenic fungi species: *Fusarium culmorum* (W.G. Smith) Sacc., *Fusarium poae* (Peck) Wollen., *Fusarium avenaceum* (Fr.) Sacc., *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem. and *Botrytis cinerea* Pers.

In laboratory conditions the dynamics of growth and sporulation of the above mentioned fungi were assessed on PDA medium with a supplement of 0.1; 0.5 and 1.0 $mm^3 \cdot cm^{-3}$ of Teprosyn Mn and 0.005; 0.05 and 0.5 $mm^3 \cdot cm^{-3}$ of Raxil Gel 206. *In vitro* Teprosyn Mn manganese fertilizer reveals weak and diversified effect on linear growth of the studied phytopathogens. In the highest concentration (1.0 $mm^3 \cdot cm^{-3}$) it reduces the growth of *Fusarium avenaceum*, *Botrytis cinerea* and *Fusarium poae* colonies on the level of 7.3–10.1 %, whereas all its concentrations inhibit the sporulation process in *B. cinerea* i *F. avenaceum* from 35.3 % to 66 %. Along with increasing concentration in the medium its stimulating effect on linear growth of *Bipolaris sorokiniana* raises (5.7–18.3 %) and the spore number increases from 40 to 271.5 %.

Keywords: manganese treatment, pathogenic fungi, linear growth, sporulation

Introduction

Increasing the area under cereal crops greatly influences the species composition and harmfulness of numerous pathogens of these plants. On the other hand, among cereals,

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spring barley is the most infected by pathogenic fungi [1]. Obligatory pests, such as *Blumeria graminis* f. sp. *hordei* causing powdery mildew or *Puccina hordei* causing barley leaf rust are particularly oppressive on barley plantations [2–4] Also fungi transferred with the seeding material, such as *Bipolaris sorokiniana* or numerous *Fusarium* spp. species, which contribute to seedling blight or barley leaf spot, are also dangerous [5, 6]. In soil these organisms find convenient conditions for development, while a polyphagous character of *Fusarium* ssp. causes that they provide the main source of infection, also for numerous plant species. It has been estimated, that the losses of barley yield due to fungal diseases reach from 10 to 30 % [7, 8]. Chemical treatment of seed material is the most environment friendly, the most efficient and also the cheapest method of protection. Certified and carefully treated grain to a considerable extend guarantees reduction of diseases transferred with seed material and therefore obtaining better plant emergences leading to bigger yields [4, 9–12]. It is known from the literature reports that foliar macro and microelement fertilizers may also reduce the development of plant infectious diseases to a great extent [13–16]. On the other hand, *in vitro* they inhibit surface growth of phytopathogenic fungi [17, 18]. Teprosyn Mn manganese fertilizer, available on the market in Poland for several years, has been used as a seed treatment stimulating the development of cereal root system. The identification of the direct effect of this treatment on development of the pathogens colonizing barley kernels seems a necessary stage in the research on its influence on plant healthiness.

The paper aims to compare the effect of Teprosyn Mn seed treatment and Raxil Gel 206 chemical treatment on *Fusarium culmorum* (W.G. Smith) Sacc., *Fusarium poae* (Peck) Wollen., *Fusarium avenaceum* (Fr.) Sacc., *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem. and *Botrytis cinerea* Pers pathogenic fungi colonizing seeds of spring barley, 'Poldek' c.v.

Materials and methods

Pathogenic fungi*: Fusarium culmorum* (W.G. Smith) Sacc., *Fusarium poae* (Peck) Wollenw., *Fusarium avenaceum* (Fr.) Sacc., *Bipolaris sorokiniana* (Sacc.) Shoemaker and *Botrytis cinerea* Pers, most frequently isolated from malting barley, 'Poldek' c.v., were selected for the laboratory experiment which was conducted in 2012. Teprosyn Mn seed treatment with guaranteed 27.4 % content of total manganese and chemical treatment Raxil Gel 206 with active substances: tiuram – 200 g and tebukonazol – 6 g per 1 dm^3 of the substance, was used in the experiment.

Seed treatments were added to the PDA medium in the amounts allowing to obtain their concentrations, respectively for Teprosyn Mn 0.1; 0.5 and 1.0 $mm^3 \cdot cm^{-3}$ and for Raxil Gel 206: 0.005; 0.05 and 0.5 $mm^3 \cdot cm^{-3}$. Prepared media were inoculated with an agar disc, 5 mm in diameter, overgrown with three-week old mycelium of the analysed organism. The control was provided by Petri dishes with clean PDA medium. The test fungi were cultured *in vitro* in five replications for each combination. 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]:

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

where: I – 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 [14]:

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

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 t-Student test on the significance level $\alpha = 0.05$.

Results and discussion

The experiments confirmed a high fungistatic activity of Raxil Gel 206 chemical seed treatment, which was the point of reference for an assessment of Teprosyn Mn seed treatment effect on fungal organisms. In the first place the treatment guarantees a better development and linear growth of cereal crops. The phytopathogenic fungi analyzed *in vitro* responded to Teprosyn Mn treatment supplement to the medium quite variably (Table 1).

Table 1

Effect of seed treatments on the tested fungi linear growth rate

Type of preparation	Tested fungi linear growth rate, T				
	Fusarium avenaceum	*Fusarium culmorum*	*Fusarium poae*	*Botrytis cinerea*	*Bipolaris sorokiniana*
Raxil Gel 206	8.41	3.09	4.68	10.10	9.36
Teprosyn Mn	73.92	81.39	83.01	70.82	65.12
Control	72.34	77.12	88. 49	73.65	62.79
$LSD_{0.05}$	1.86	5.44	2.81	4.08	4.88

Irrespective of the applied concentration, the treatment stimulated surface growth of *Fusarium avanaceum*, *Fusarium poae* and *Bipolaris sorokiniana* mycelium, however, no significant differences in the values of linear growth coefficients were registered in comparison with the control. Moreover, despite small reduction observed in *Botrytis cinerea* fungus growth rate on the medium with added Teprosyn Mn, statistical analysis did not reveal any significant differences in comparison with the control. Only in case of *Fusarium poae,* the preparation markedly limited the rate of its colony surface growth. On the other hand, Raxil Gel 206 chemical treatment very strongly inhibited growth of all tested fungi, as evidenced by very low values of the linear growth rate coefficients.

In the presented experiments the kind of seed treatment modified not only the surface growth of the tested phytopathogen colonies, but also their concentration in the medium (Table 2). Moreover, individual fungi revealed different sensitivity to the applied preparations and their concentrations. Obtained results prove a lack of fungistatic effect of the analysed concentrations of Teprosyn Mn on *B. sorokiniana*.

Table 2

Linear growth rate coefficient, *T*, of tested fungi depending on the kind and concentration of seed treatments

Type of preparation	Concentration [$mm^3 \cdot cm^{-3}$]	*Fusarium avenaceum*	*Fusarium culmorum*	*Fusarium poae*	*Botrytis cinerea*	*Bipolaris sorokiniana*
Teprosyn Mn	1.0	66.44 c*	80.24 b	80.12 c	66.63 c	68.42 c
	0.5	76.16 e	79.24 b	82.39 cd	73.00 c	65.99 c
	0.1	79.15 e	84.69 b	86.54 de	72.83 c	62.95 c
Raxil Gel 206	0.5	2.76 a	1.00 a	0.60 a	5.52 a	5.72 a
	0.05	5.57 a	3.73 a	1.79 a	11.40 ab	8.12 ab
	0.005	16.89 b	4.55 a	11.65 b	13.38 b	14.25 b
Control		72.34 d	77.12 b	88.49 e	73.65 c	62.79 c

* Values in columns marked by the same letter are not significantly different.

A comparison of coefficients of the rate and inhibition of linear growth shows that *B. sorokoniana* species was the most resistant both to Raxil Gel 206 and Teprosyn Mn (Tables 2 and 3).

Table 3

Coefficients of inhibition/stimulation, *I,* of tested fungi linear growth

Type of preparation	Concentration [$mm^3 \cdot cm^{-3}$]	*Fusarium avenaceum*	*Fusarium culmorum*	*Fusarium poae*	*Botrytis cinerea*	*Bipolaris sorokiniana*
Teprosyn Mn	1.0	7.28	+5.66	10.09	8.16	+18.29
	0.5	+4.46	+3.43	7.76	2.92	+16.77
	0.1	+8.45	3.88	3.33	+6.12	+5.70
Raxil Gel 206	0.5	90.38	95.86	95.73	86.91	83.51
	0.05	86.62	90.42	93.20	79.36	79.78
	0.005	73.44	89.11	82.33	76.19	69.52

On media with Raxil Gel 206 chemical treatment supplement linear growth was inhibited starting from the lowest concentration in the range from 69.52 % to 83.51 % (Table 3). On the other hand, the manganese fertilizer concentrations of 1.0 and 0.5 $mm^3 \cdot cm^{-3}$ caused stimulation of *B.sorokiniana* colony growth by 18.29 % and 16.77 %, respectively.

Fusarium species more strongly responded to the presence of tested preparations in the medium. However, among them *F. poae* proved the most sensitive, as its strong inhibition was noted on the media containing Raxil Gel 206. All concentrations (0.5, 0.05 and 0.005 $mm^3 \cdot cm^{-3}$) inhibited its colony linear growth within the range of 95.73–82.33 % (Tables 2 and 3). Similarly, with increasing concentration of Teprosyn in the medium a stronger inhibition of *F. poae* hyphae growth was registered. It is worth emphasizing that for the same pathogen Teprosyn Mn revealed an opposite effect than on *B. sorokiniana*. Higher concentrations 1.0 and 0.5 $mm^3 \cdot cm^{-3}$ of the manganese treatment stimulated the growth of *F. culmorum* mycelium only to a small extend (5.66–3.43 %) (Table 3). The preparation concentrations of 0.5 and 0.1 $mm^3 \cdot cm^{-3}$ contributed to a faster growth of *F. avenaceum* colony, whereas the lowest concentration favoured also *B. cinerea* fungi growth (Table 3). On the other hand, weak limiting effect on surface growth of *B. cinerea* colony was observed on media containing higher concentrations (1.0 and 0.5 $mm^3 \cdot cm^{-3}$) of Teprosyn Mn treatment. Only 7.0 % inhibition of *F. avenaceum* colony linear growth was registered in the combination with 1.0 $mm^3 \cdot cm^{-3}$ (Teprosyn Mn dose recommended for seed treatment) (Table 3).

Authors' own investigations revealed that the tested preparations and their concentrations more strongly affected sporulation in the tested fungi than the surface growth of hyphae. Spores produced by fungi existing on seeds play a crucial role in the pathogenesis process, since they are responsible for plant infection which is the first stage of disease process. Moreover its endospores allow to survive under unfavourable environmental conditions [20–22]. Therefore, pathogenic fungi which have no conditions for developing hyphae start to product a greater number of spores and these in turn ensure viability and the continuity of the species [23].

The strongest stimulating effect of manganese treatment on linear growth of *B. sorokiniana* was reflected in its sporulation process. In combination with the highest concentration of Teprosyn Mn, the registered number of spores was 271 % bigger than in the control and was diminishing successively to 129 % and 46 % along with decreasing concentration of the preparation in the medium. On the other hand, on media containing 1.0 $mm^3 \cdot cm^{-3}$ of Teprosyn Mn treatment an increase in macroconidia number was observed in *F. poae* by 75.75 % and in *F. culmorum* by 53.11 % (Fig. 1), whereas a relatively strong inhibition of sporulation, between 61 and 66 % was noted in *F. avenaceum* and slightly lower (35–61 %) for *B. cinerea*.

With few exceptions, Raxil Gel 206 seed treatment strongly inhibited sporulation process in the tested fungal organisms (Fig. 2). The preparation in concentrations of 0.5 and 0.05 $mm^3 \cdot cm^{-3}$ almost totally blocked spore production by *B. sorokiniana* fungus.

It should be noticed that the linear growth tests revealed the weakest fungistatic effect of Raxil Gel 206 on this pathogen (Table 3).

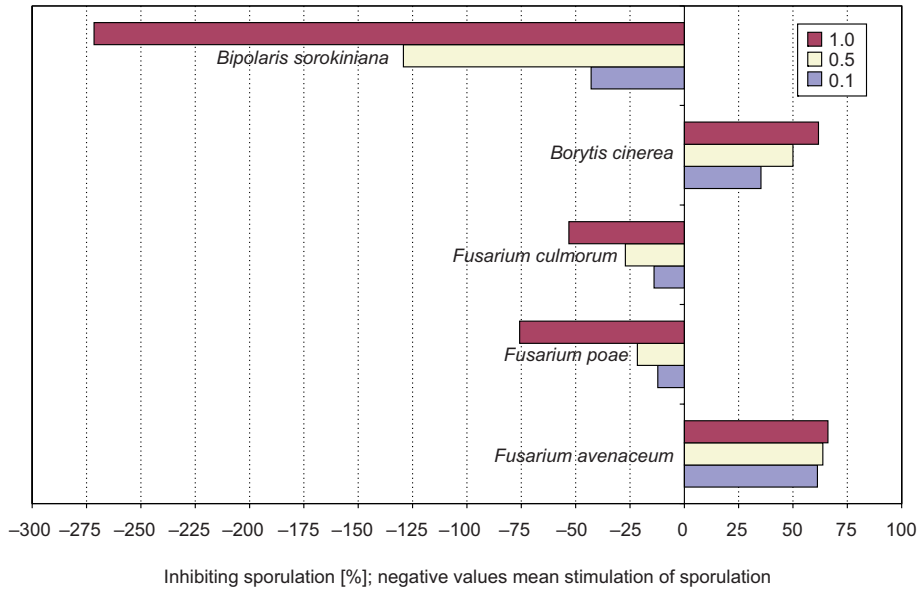

Fig. 1. Effect of Teprosyn Mn treatment on test fungi sporulation

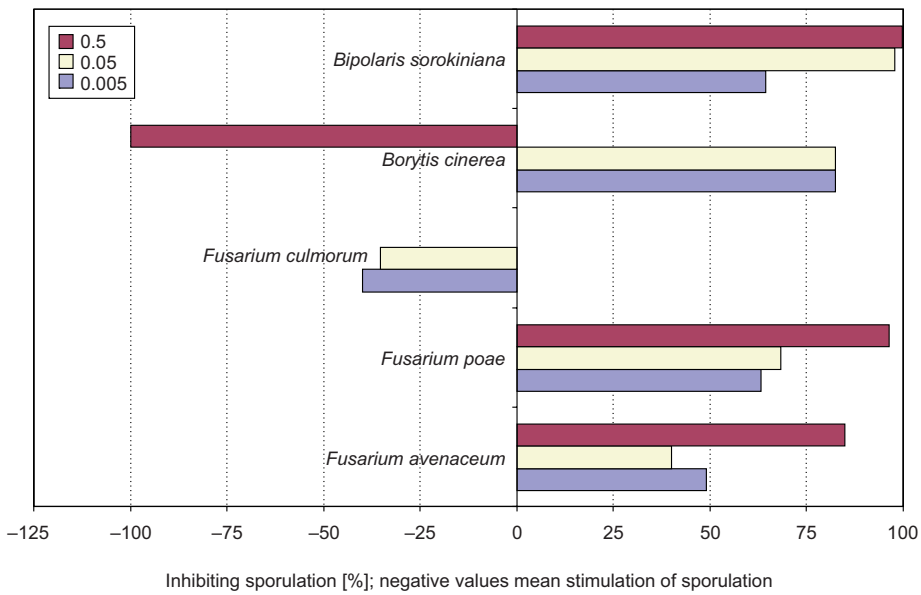

Fig. 2. Effect of Raxil Gel 206 seed treatment on the test fungi sporulation

Very strong inhibition of *F. poae* sporulation, reaching 96.30 %, was also registered on the medium with 0.5 $mm^3 \cdot cm^{-3}$ of this seed treatment (Fig. 2). In the conducted experiments Raxil Gel 206 revealed quite changeable effect on sporulation process in

B. cinerea and *F. culmorum*. In case of the first species, on the media containing 0.05 and 0.005 $mm^3 \cdot cm^{-3}$ of this preparation the number of spores was by 82.35 % lower than on the control, whereas for *F. culmorum* it was bigger by 35 and 40 %, respectively. On the other hand, on the medium with the highest concentration of Raxil Gel 206 a strong stimulation (100 %) of *B. cinerea* fungus sporulation was observed (Fig. 2). Intensified spore production by fungi mentioned above confirms the Hodges' rule [25], *ie* at confirmed strong inhibition of *F. culmorum* and *B. cinerea* surface growth on the medium with 0.5 $mm^3 \cdot cm^{-3}$ of Raxil gel 206 (Fig. 2) scarce fungi hyphae were producing a greater number of spores. The phenomenon should be treated as a defence mechanism of these fungal organisms against the unfavourable environmental conditions. In the light of conducted research, Teprosyn Mn seed treatment may in agricultural practice reveal poor protective properties of cereal grain against such fungi as *F. avenaceum* and *B. cinerea*.

Conclusions

1. Raxil Gel 206 used for *in vitro* tests very strongly inhibited surface growth of the tested fungi, irrespective of the applied concentration, whereas in 0.5 $mm^3 \cdot cm^{-3}$ concentration almost totally blocked sporulation process in *B. sorokiniana* (99.7 %) and *F. poae* (96.3 %) and in 100 % stimulated spore production by *B. cinerea*.

2. Teprosyn Mn manganese treatment in laboratory conditions revealed weak and diverse effect on linear growth of tested phytopathogens. Its highest concentration (1.0 $mm^3 \cdot cm^{-3}$) limited *F. avenaceum*, *B. cinerea* and *F. poae* colony growth by between 7.3 and 11.0 %.

3. All concentrations of Teprosyn Mn inhibited sporulation process in *B. cinerea* and *F. avenaceum* by between 35.3 and 66 %. With growing concentration in the medium its stimulating effect on *B. sorokiniana* linear growth increased (5.7–18.3 %) and the spore number grew from 40 to 217.5 %.

References

[1] Walters D, Avrova A, Bingham IJ, Burnett FJ, Fountaine J, Havis ND, Hoad SP, et al. Eur J Plant Pathol. 2012;133(1):33-73. DOI 10.1007/s10658-012-9948x.
[2] Backes G, Madsen LH, Jaiser H, Stougaard J, Herz M, Mohler V, Jahoor A. Theor Appl Genet. 2003;106:353-362. DOI: 10.1007/s00122-002-11481.
[3] Bankina B, Gaile Z. Agron Res. 2009;7(Spec Issue I):198-203.
[4] Sawińska Z, Krzyzińska B, Kazikowski P, Głazek M. Fragm Agron. 2014;31(4):85-91. http://www.up.poznan.pl/pta/pdf/2014/FA%2031%284%29%202014%20Sawinska.pdf.
[5] Al-Sadi AM, Deadman ML. J Phytopathol. 2010;158:683-690. DOI: 10.1111/j.1439-0434.2010.01684.x.
[6] Zare L. Int J Agric Crop Sci. 2013;5(4):332-335. www.ijagcs.comIJACS/2013/5-4/332-335.
[7] Knudsen GR, Stack JP. Modeling growth and dispersal of fungi in natural environments. In: Handbook of applied mycology, DK Arora, B Rai, KG Mukerji, G Knudsen (eds.), New York: Marcel Dekker; 1991:625-645.
[8] Wiewióra B. Pam Puł. 2007;146:139-154.
[9] Dawson WAJM, Bateman GL. Plant Pathol. 2000;49:477-486. DOI: 10.1046/j.1365-3059.2000.00479.x.
[10] Wiewióra B. Biul Inst Hod Rośl. 2003;228: 89-94.
[11] Baturo A. Acta Agrobot. 2006;58(2):347-358.

[12] Kurowski P, Damszel M, Wysocka U, Sadowski T, Rychcik B. Prog Plant Prot./Post Ochr Roślin. 2013;53(2):351-355. http://www.uwm.edu.pl/fitent/publikacje.html.
[13] Reuveni R, Reuveni M. Crop Prot. 1998;17(2):111-118. DOI:10.1016/S0261-2194(97)00108-7.
[14] Gleń K. Ecol Chem Eng A. 2008;15(4-5):331-336.
[15] Gleń K. Prog Plant Protect/Post Ochr Roślin. 2009;49(4):2047-2051.
[16] Cwalina-Ambroziak B, Wierzbowska J, Damszel M, Bowszys T. Acta Sci Pol., Hortorum Cultus. 2012;11(4):157-168. http://www.aqua.ar.wroc.pl/acta/pl/full/7/2012/000.
[17] Gleń K, Boligłowa E. Ecol Chem Eng A. 2009;16(7):751-757. http://tchie.uni.opole.pl/ece_a/A_16_7/ECE_A16(7).pdf.
[18] Cwalina-Ambroziak B. Pol J Environ Stud. 2012;21(3):589-594. http://www.pjoes.com/pdf/21.3/Pol.J.Environ.Stud.Vol.21.No.3.589-594.pdf.
[19] Kowalik R, Krechniak E. Szczegółowa metodyka biologicznych i laboratoryjnych badań środków grzybobójczych. In: Materiały do metodyki badań biologicznej oceny środków ochrony roślin. Poznań: Instytut Ochrony Roślin; 1961.
[20] González-Fernández R, Prats E, Jorrín-Novo1 JV. J Biomed Biotechnol. 2010;1:1-36. http://dx.doi.org/10.1155/2010/932527.
[21] Ikeda K, Inoue K, Kitagawa H, Meguro H, Shimoi S, Park P. Plant Pathol. 2012;131-151. DOI: 10.5772/30801.
[22] Lejman A, Ogórek R, Sobkowicz P. Pol J Environ Stud. 2015;24(1):141-149. DOI: 10.15244/pjoes/24923.
[23] Hodges CF. Mycopathologia. 1994;128(2):105-109. DOI: 10.1007/BF01103017.

ODDZIAŁYWANIE NAWOZU MANGANOWEGO NA GRZYBY PATOGENICZNE IZOLOWANE Z ZIARNIAKÓW JĘCZMIENIA

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Abstrakt: W doświadczeniu testowano nawóz manganowy Teprosyn Mn, produkowany przez angielską firmę Phosyn Chemicals Ltd., który od kilku lat jest on dostępny na polskim rynku. Efektem jego stosowany jako zaprawy nasiennej (nawóz donasienny) jest intensywniejszy rozwój systemu korzeniowego oraz polepszenie ogólnej kondycji roślin. W dostępnej literaturze brakuje informacji dotyczących oddziaływania Teprosynu Mn na zdrowotność roślin oraz organizmy patogeniczne.

Celem pracy było porównanie oddziaływania nawozu donasiennego Teprosyn Mn i chemicznej zaprawy nasiennej Raxil Gel 206 na patogeniczne gatunki grzybów: *Fusarium culmorum* (W.G. Smith) Sacc., *Fusarium poae* (Peck) Wollen., *Fusarium avenaceum* (Fr.) Sacc., *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem. i *Botrytis cinerea* Pers.

W warunkach laboratoryjnych oceniono dynamikę wzrostu i sporulację wymienionych grzybów na podłożu (PDA) z udziałem 0.1; 0.5; 1.0 $mm^3 \cdot cm^{-3}$ Teprosynu Mn oraz 0.005; 0.05; 0.5 $mm^3 \cdot cm^{-3}$ Raxil Gel 206. Nawóz manganowy Teprosyn Mn w warunkach *in vitro* wykazuje słabe i zróżnicowane oddziaływanie na rozrost liniowy badanych fitopatogenów. W największym stężeniu (1.0 $mm^3 \cdot cm^{-3}$) ogranicza o 7,3 do 10,1 % rozrost kolonii: *Fusarium avenaceum*, *Botrytis cinerea* i *Fusarium poae*. Natomiast we wszystkich stężeniach hamuje proces wytwarzania zarodników przez *B. cinerea* i *F. avenaceum* w zakresie od 35,3 % do 66 %. Wraz ze zwiększaniem stężenia w podłożu hodowlanym rośnie jego stymulujące oddziaływanie na wzrost liniowy *Bipolaris sorokiniana* (od 5,7 do 18,3 %) oraz zwiększa się ilość zarodników od 40 do 271,5 %.

Słowa kluczowe: nawóz manganowy, grzyby fitopatogenne, wzrost liniowy, zarodnikowanie