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ORIGINAL PAPER

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EFFECT OF SELECTED PREPARATIONS ON GROWTH AND DEVELOPMENT *Boeremia strasseri*, THE CAUSAL AGENT OF BLACK STEM AND RHIZOMES ROT OF PEPPERMINT (*Mentha piperita*)

Beata Zimowska[✉], Ewa Dorota Król

Department of Plant Pathology and Mycology, University of Life Sciences in Lublin, Leszczyńskiego 7, 20-069 Lublin, Poland

ABSTRACT

The objective of undertaken studies was evaluation *in vitro* conditions efficiency of eight fungicides from different chemical groups and three preparations of natural origin *i.e.* Biosept Active, Beta-chikol and Bioczos towards isolate M 365 *Boeremia strasseri*, obtained from peppermint rhizomes showing symptoms of black rot. Test were performed by poisoning the culture media. The percentage of inhibition of the growth of four- and eight-day-old colonies on the medium with preparations in the control colonies was a measure of activity of the preparations. Moreover, the microscopic observations of morphological structures of fungus were conducted. Within chemical compounds trifloxystrobin, tiophanate methyl and mancozeb pointed to be the most effective. The effectiveness of Beta-chikol and Biosept Active in limiting *B. strasseri* colony growth was higher than the efficiency of Bioczos.

Key words: preparations of natural origins, fungicides, peppermint, *Boeremia strasseri*

INTRODUCTION

Diseases and pests are relevant yield-reducing factors of all crops, as well as medicinal and aromatic plants (MAPs). MAPs are considered minor crops and for this reason there are not many preparations registered for use in pests control [Carruba and Catalano 2009]. The cultivation area of MAPs in Poland reached 20,000 ha last year, which puts us at the second place with regard to the crop area in Europe. At present, more than 70 species of medicinal and aromatic plants are cultivated in Poland. During the last two decades, in southeastern Poland different species belonging to *Lamiaceae*, including peppermint (*Mentha piperita*), have been cultivated. The interest in the cultivation of this species is primarily determined by the content of essential oils or other secondary metabolites which are used for pharmaceutical, culinary, cosmetology and food industry usage. The concentration of the MAPs crops in this region led to an increase of pathogen occurrence, which results in a decrease of the yields, but also the quality of raw materials. Black stem and rhizomes rot, also called phomosis of peppermint caused by *Boeremia* (*Phoma*) *strasseri* is one of the most severe disease of peppermint plants [Boerema et al. 2004, Zimowska 2012, Rodeva et al. 2016]. The disease symptoms are visible on the stems, first in the form of necrotic, slightly hollow spots enfolding the stem around up to 10 cm from the base. With time, the

✉ beata.zimowska@up.lublin.pl

tissue in the place of the spots gets rotten. The rot proceeds very fast on the rhizomes. Young rhizomes rot away wholly, while the bark layer often comes off on older ones. The yield losses resulting from plant infection can reach 90% [Zimowska 2012].

The objective of the present study was to investigate the effect of preparations of natural origin and fungicides on *B. strasseri* in *in vitro* conditions.

MATERIAL AND METHODS

Three preparations of natural origin and 8 fungicides including: asoxystrobin, captan, mancozeb, chlorothalonil, tiophanate methyl, trifloxystrobin, cyprodynil, cymoksanil (tab. 1) were used. Those active ingredients according to Annex I of the Directive 91/414 are allow for use by the European Union

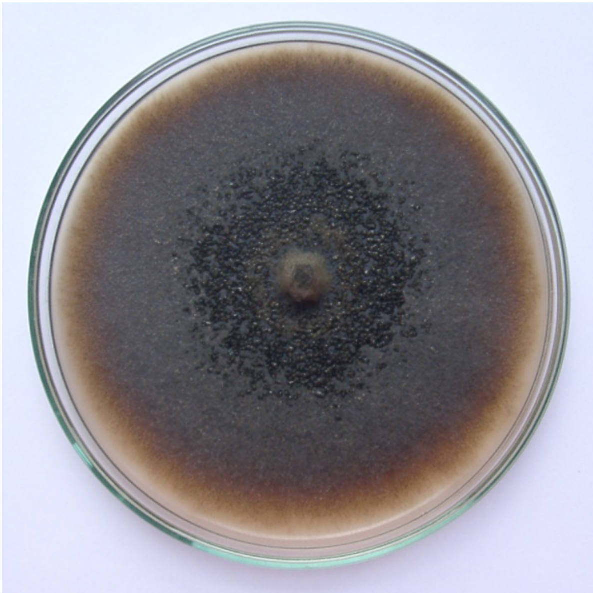

Fig. 1. 8-day-old colony of *B. strasseri* isolate M 365 on malt agar medium (phot. E. Zalewska)

Table 1. Characteristic of examined preparations

No.	Preparations	Name of active ingredient and its content in the preparations	Producer
1.	Beta-chikol	20 g·dm^{-3} chitosan	Gumitex Poli-Farm
2.	Biosept Active	33% extract from grapefruit	Cintamani
3.	Bioczos	garlic extract	Himal
4.	Amistar 250 SC	250 g·dm^{-3} azoxystrobin	Syngenta Ltd.
5.	Captan 50 WP	50% captan	Arysta LS
6.	Curzate M 72,5 WP	4.5% cymoksanil; 68% mancozeb	DuPont
7.	Dithane Neo Tec 75 WG	75% mancozeb	Dow AgroSciences
8.	Gwarant 500 SC	500 g·dm^{-3} chlorothalonil	Arysta
9.	Switch 62,5 WG	cyprodynil 37.5%, fludioksonil 25%	Syngenta
10.	Topsin M 500 SC	500 g·dm^{-3} tiophanate methyl	Nippon Soda
11.	Zato 50 WG	50% trifloksystrobin	Bayer AG

Fig. 2. Fragments of peppermint rhizomes: A) with symptoms of black rot; B) symptomless (phot. E. Zalewska)

Fig. 3. Black stem and rhizomes rot of peppermint plants (phot. B. Zimowska)

Member States. The isolate of *B. strasseri* M 365 (fig. 1) obtained from the peppermint rhizome showing symptoms of black rot (figs 2 and 3) coming from our own professional collection was chosen for study [Zimowska 2012].

The tests were performed on malt agar medium (MA) in two series, using the method of poisoning the culture medium and inoculating the fungal inoculum on them [Zalewska 2016]. The effect of Biosept Active on *B. strasseri* was tested at the concentrations 0.05, 0.1, 0.2 and 0.3%, Beta-chikol at 0.01, 0.025, 0.05 and 0.1% while Bioczos at 0.5, 1, 2 and 5%. The fungicides were tested at the concentrations 1, 10 and 100 g·cm^{-3} in order to determine an approximate ED_{50} dose and classify the tested fungicides within one of four groups of fungicidal activity [Zalewska 2016]. The mycelial disk (3 mm diameter) taken from two-week-old, one-spore colonies growing on MA medium, at the temperature 24°C constituted the infection material. For each preparation as well as for each studied concentration of the preparation four replications were made. The colonies of *B. strasseri* growing on the medium without the preparations constituted the control. The measure of the activity of the test preparations was the percentage of growth inhibition after 4 and 8 days of cultivation of fungus colonies in the presence of the preparations in relation to the control colony [Machowicz-Stefaniak i Zalewska 2011].

Microscopic observations of 4- and 8-day-old colonies of *B. strasseri* were also carried out, in order to detect changes in the appearance of morphological structures of the fungus under the influence of preparations.

RESULTS

As the result of studies the tested fungicides were included within three groups of fungicidal activity towards *B. strasseri* (tabs 2 and 3). The highest percentage of inhibition of growth of 4 and 8-day-old colonies of pathogens was caused by in Zato 500 WG,

Table 2. Effect of fungicides tested on growth of 4-days-old colonies of *B. strasseri*

Fungicides*	Percent of inhibition in relation to a.i. concentration (g·cm^{-3})		
	1	10	100
	I		
Zato 500 WG	100 h	100 h	100 h
Topsin M 500 SC	75.81 f	65.12 e	100 h
Dithane Neo Tec 75 WG	12.09 b	23.72 c	100 h
	III		
Captan 50 WP	19.99 bc	23.56 c	86.97 g
Switch 62,5 WG	76.73 f	73.02 ef	85.11 g
	IV		
Amistar 250 SC	33.48 d	39.07 d	36.27 d
Gwarant 500 SC	27.44 c	38.16 d	39.07 d
Curzate M 72,5 WP	9.30 a	12.55 b	20.47 c
Control	0.0 a	0.0 a	0.0 a
LSD		9.551	

Values marked with the same letter do not differ significantly, $p \leq 0.05$; * fungicides compared according to fungicidal activity group

Table 3. Effect of fungicides tested on growth of 8-days-old colonies of *B. strasseri*

Fungicides*	Percent of inhibition in relation to a.i. concentration ($g \cdot cm^{-3}$)		
	1	10	100
	I		
Zato 500 WG	100 f	100 f	100 f
Topsin M 500 SC	77.22 e	76.94 e	100 f
Dithane Neo Tec 75 WG	11.66 b	17.49 b	100 f
	II		
Captan 50 WP	17.50 b	13.61 b	76.38 e
Switch 62,5 WG	55.28 d	68. 61 e	74.72 e
	III		
Amistar 250 SC	16.94 b	34.99 c	30.55 c
Gwarant 500 SC	13.05 b	16.38 ab	22,22 b
Curzate M 72,5 WP	1.21 a	9.72 a	15.83 b
Control	0.0 a	0.0 a	0.0 a
LSD		10.228	

Explanations like in Table 2

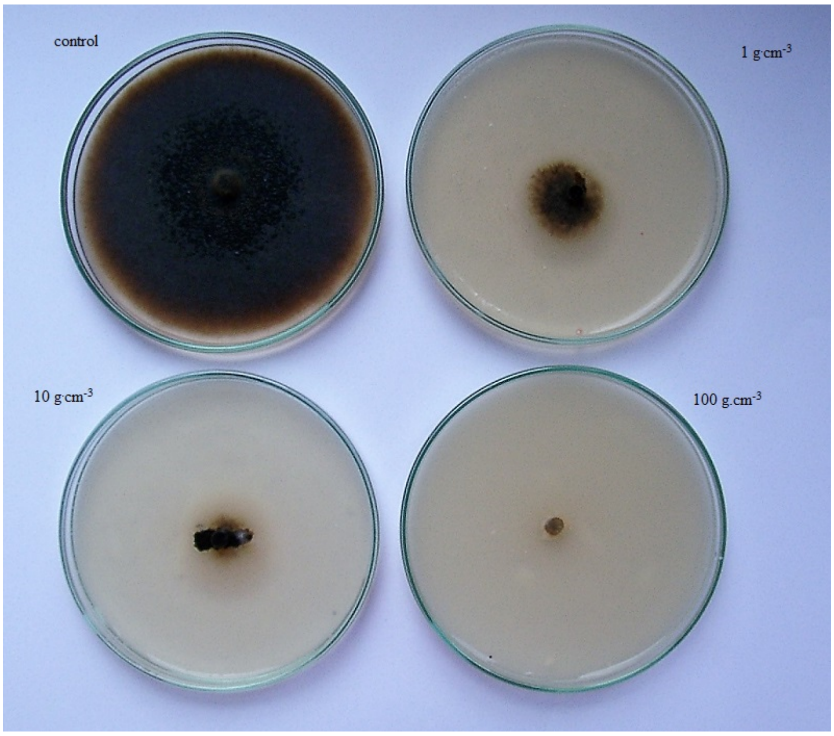

Fig. 4. 8-day-colonies of *B. strasseri* M 365 on malt agar medium with Topsin M 500 SC (phot. E. Zalewska)

Table 4. Toxic activity of fungicides on *B. strasseri*

Fungicides	Toxic activity (g·cm^{-3})		
	1	10	100
Amistar 250 SC	+	+	+
Captan 50 WP	+	+	+
Curzate M 72,5 WP	+	+	+
Dithane Neo Tec 75 WG	+	+	+
Gwarant 500 SC	+	+	–
Switch 62,5 WG	+	+	+
Topsin M 500 SC	+	+	–
Zato 50 WG	–	–	–

– fungicidal activity; + fungistatic activity

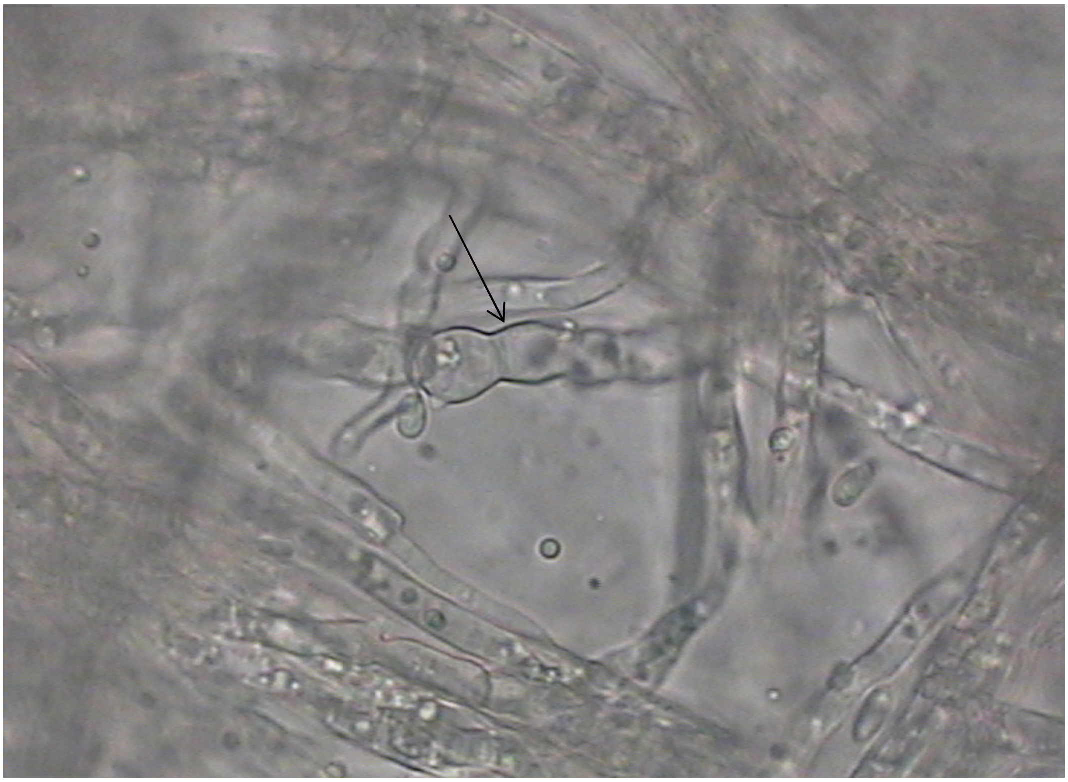

Fig. 5. Swollen cells of hyphae observed on malt agar medium with Topsin 500 SC, magnification ×500 (phot. E. Zalewska)

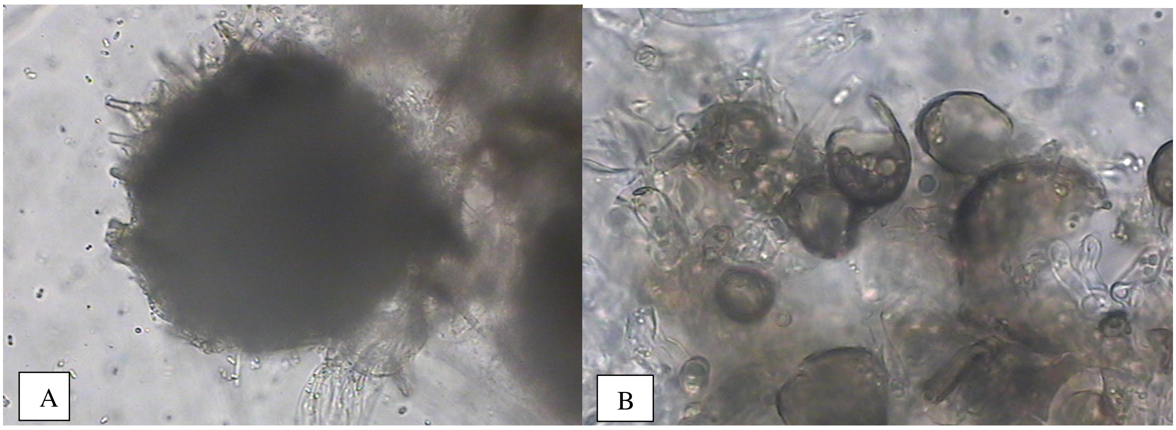

Fig. 6. Pycnidia of *B. strasseri* A) control; B) on malt agar medium with Topsin 500 SC, magnification ×160 (photo E. Zalewska)

Table 5. Impact of the preparations of natural origin on the growth of *B. strasseri* colony

Preparations	Concentrations (%) 0.01	0.025	0.05	0.1	Control	LSD
	Percent of inhibition of the colony growth					
Beta-chikol						
effect after 4 days	91.56 a	93.16 ab	93.66 ab	100 b	0.0 c	7.6864
effect after 8 days	98.00 ab	98.00 ab	93.66 ab	100 b	0.0 c	7.6865
Biosept Active	0.05	0.1	0.2	0.3		
effect after 4 days	85.13 b	71.48 a	87.14 b	89.55 b	0.0 c	9.9232
effect after 8 days	86.38 b	73.60 a	88.88 b	92.21 b	0.0 c	5.6962
Bioczos	0.5	1	2	5		
effect after 4 days	32.12 c	14.05 b	10.03 a	35.33 c	0.0 d	3.8225
effect after 8 days	25.83 c	11.94 a	19.71 b	41.10 d	0.0 e	5.2543

Values marked with the same letter do not differ significantly, $p \leq 0.05$

regardless of the concentration. The maximum percentage of growth inhibition at 100 $g \cdot cm^{-3}$ also was shown by Topsin M 500 SC (fig. 4) and Dithane Neo Tec 75 WG. The above-mentioned preparations were qualified for the first group of toxicity, *i.e.* substances of the heaviest fungicidal activity towards *B. strasseri* (tabs 2 and 3). Captan 50 WP and Switch 62.5 WG were qualified within the formulations with moderate fungicidal activity, III toxicity group. Fungicides with poor fungicidal activity were found to be Amistar 250 SC, Gwarant 500 SC and Curzate M 72,5 WP, and on this basis they were classified into group IV toxicity (tabs 2 and 3). Among the tested fungicides Zato 500 WG, Topsin M 500 SC and Dithane Neo

Tec 75 WG showed fungicidal activity towards *B. strasseri* (tab. 4). Microscopic studies carried out under MBL-N 120 light microscope shown that in the concentrations 1 g·cm^{-3} and 10 g·cm^{-3} Topsin 500 SC and Switch 62,5 WG, Amistar 250 SC and Captan 50 WP fungicides limited sporulation of the pathogen. Moreover, hyphae of *B. strasseri* at the presence of the above mentioned preparations were deformed and produced swollen cells resembling chlamydospores (fig. 5). Pycnidia were formed very rarely, they were smaller than in control and after dissection no conidia were observed (fig. 6).

Studies on the possibility of reducing the growth of *B. strasseri* by preparations of natural origin showed that the percentage of inhibition of growth of 4 and 8-day-old colonies of the pathogens was significantly higher than that in control in all tested formulations (tab. 5). The highest percentage of growth inhibition was observed in case of the Beta-chikol. This preparation at the concentration of 0.1% inhibited the growth of the pathogen completely, but these values did not differ significantly with the percentage of inhibition for the remaining tested concentrations except for 0.01% concentration (tab. 5). Biosept Active, independently of the concentration, inhibited the growth of 8-day-old colonies of *B. strasseri* more strongly than 4 day-old ones. The most effective concentration was found to be the highest concentration of the preparation, the least effective proved to be the concentration of 0.1% while the percentage of inhibition of growth of the fungal colonies at the concentration of 0.1% was significantly lower that for the other concentrations (tab. 5).

The result of Bioczos activity on the growth of 4-day-old colonies as well as 8-day-old colonies of *B. strasseri* at the highest concentration of the preparation was significantly higher than at the concentrations 0.5%, 1% and 2% with an exception of the percentage of growth inhibition of 4-days-colonies at the concentration of 0.5% (tab. 5).

It was found that the colonies of *B. strasseri* growing in the presence of Beta-chikol and Biosept Active produced poor and compact aerial mycelium with short, dark hyphae with numerous swollen cells. There were no pycnidia and conidia. In the case of Bioczos, swollen cells were observed only at the highest concentrations, but pycnospores and pycnidia were formed.

DISCUSSION

Studies on the diseases of peppermint conducted since 2004 in central and south-east Poland indicate serve harmfulness of *B. strasseri* towards plants which results in an even 90% decrease of the yield [Zimowska 2012]. Information on pests and diseases affecting MAPs in their typical cultivation areas represents an important finding of the experiment. Many methods may be suggested for their management, involving agronomic, biological and chemical strategies [Isman 2000].

Studies *in vitro* point to the differences in the development of *B. strasseri* colonies on media containing various concentrations of the active substance of fungicides tested. Out of eight compounds belonging to distinct chemical groups trifloxystrobin, tiophanate methyl and mancozeb were noteworthy due to the highest activity. Trifloxystrobin belonging to the group of strobilurine fungicides was characterized by the ability to limit the growth of a wide spectrum of fungal pathogens, including pathogens affecting MAPs [Król 2005, Machowicz-Stefaniak and Zalewska 2011, Zalewska 2016]. A high fungicidal activity of thiophanate methyl and mankozeb was shown for other fungi infecting herbs, i.e. *Alternaria alternata*, *Phoma anethi* and *Fusarium* spp. or pathogens of orchard plants i.e. *Phomopsis viticola* [Król 2005, Mačkinaitė 2012]. An additional advantage of the above mentioned preparations is their strong antisporulation effect, which is accompanied by limitation of hyphae development, observed in the present studies. It could be relevant in the case of reduction of primary and secondary sources of infection on peppermint plantations [Zimowska 2011].

However, it is widely acknowledged that in the cultivation of MAPs, chemical products for diseases control should be kept to a minimum, and applied only when no alternative measures are available [WHO 2003]. Taking into consideration the above mentioned suggestion the present studies included preparations of natural origin. Beta-chikol proved to be a very effective preparation. In the highest concentration it completely inhibited the pathogen's growth, while in the other concentrations in the course of time its effectiveness remained at a high level. Chitosan contained in the preparation showed high effectiveness in inhibiting the growth of a number of other pathogens, i.e. *Phoma narcisii* [Saniewska 2002], *Seimatosporium hypericinum* [Zimowska 2006] and *Septoria carvi* [Zalewska 2016].

The efficiency of Biosept Active increased slightly with an increase of the active ingredient in the medium. The substantial role in action mechanism in grapefruit extract was played mainly by endogenous flavonoides and glycosides of grapefruit extract. These compounds inhibit the germination of spores and growth of the hyphae by damaging the membrane systems, and inhibiting the activity of respiratory enzymes [Dakora 1995], and probably causing inhibition of the growth of *B. strasseri* colonies, as well as changes in the pathogen's morphological structures.

Within the preparations of natural origin, Bioczos turned out to be the least effective. The literature provides information on the possibility of using garlic extract in the plant's protection against plant pathogens. Garlic extract has antibacterial and antifungal properties. The main ingredient playing a relevant role in the mechanism of action is allicin. This natural biological substance, which is volatile phytoanticipin produced in garlic upon wounding, is active against a broad range of phytopathogenic organisms [Miron et al. 2000]. In the case of *B. strasseri* colonies, a low inhibitory effect of Bioczos was observed. Moreover, the preparation did not limit the pathogen's sporulation.

Despite being able to produce a wide range of chemical substances that are toxic to pathogens, medicinal and aromatic plants are not able to protect themselves from these organisms. Therefore, many methods should be suggested for plant protection management, involving agronomic, biological and chemical strategies. Hence, further and more detailed studies concerning the effectiveness of the tested preparations in *in vivo* conditions will be undertaken and the outcomes will be published later on.

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