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INFLUENCE OF PREPARATIONS CONTAINING NATURAL SUBSTANCES ON INHIBITION OF *MONILIA LAXA* AND *M. FRUCTIGENA* MYCELIUM GROWTH

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Abstract

Fungi of the genus *Monilia* cause some economically important diseases that result in huge crop losses, especially in orchards where no intensive chemical protection is conducted. The selected preparations containing natural substances (Biosept 33 SL and Biochikol 020 PC) were checked for its effect on the growth of mycelium of the pathogens *Monilia laxa* and *M. fructigena*.

The biotech agent Biosept 33 SL effectively inhibited the mycelium growth of both pathogens of the genus *Monilia* under *in vitro* conditions, comparably to that of the chemical preparation Topsin M 500 SC. Biochikol 020 PC displayed the lowest effectiveness in mycelium linear growth inhibition of *Monilia laxa* and *M. fructigena*.

Key words: preparations containing natural substances, growth of mycelium, *Monilia laxa*, *Monilia fructigena*

Introduction

Fungi of the genus *Monilia* are responsible for such economically significant diseases as brown rot of pome fruit trees and brown rot of stone fruit trees. The pathogens occur on all main fruit trees: apple trees, pear trees and stone fruit-bearing trees. They often lead to significant crop losses, cause damage to fruits and the trees themselves (Gril et al. 2007). They infect not only fruits but also flowers, short shoots and shoots.

In Poland the two fungi species, namely *Monilia laxa* and *M. fructigena* are responsible for brown rot. The third species causing the disease, namely *M. fructicola*, is not discussed here. In the EU this pathogen is listed among quarantine species

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(Holb 2006, Ondejková et al. 2010). However, it was already identified in many European countries (Bosshard et al. 2006, Petróczy and Palkovics 2006, Ondejková et al. 2010, Grabke et al. 2011).

Orchard protection against pathogens causing brown rot of pome fruit trees and brown rot of stone fruit trees consists primarily of chemical agents use that may have an adverse effect on human health. In addition, the diseases caused by fungi of the genus *Monilia* are a great threat to trees in orchards where no intensive chemical protection is provided. This is why it is important to look for pro-ecological methods of controlling the pathogens. The seeking of non-chemical control methods against pathogens belonging to the genus *Monilia* was justified by many authors (Bryk 2006, Holb 2006, Arrebola et al. 2010). The first step to assess the suitability of natural substances for protection against these pathogens is checking its activity against the pathogen growth under *in vitro* conditions. In experiments an effect of natural substances contained in the preparations Biochikol 020 PC and Biosept 33 SL on the mycelium growth of fungi of the genus *Monilia* under laboratory conditions was tested.

Materials and methods

During the seasons of 2009–2011 experiments were carried out to find an effect of chitosan and grapefruit extract contained in available preparations on inhibition of the mycelium growth of the causal agents of brown rot of pome fruit trees and brown rot of stone fruit trees. The test material used were isolates of pathogenic fungi *Monilia laxa* and *M. fructigena*. The pathogens were isolated during both seasons from apples and plums in summer (vegetation period) and in spring (after overwintering – from fruit mummies), on Potato Dextrose Agar (PDA) by using standard phytopathological methods. The following preparations were used for testing: Biochikol 020 PC, containing chitosan as the active substance, and Biosept 33 SL, containing grapefruit extract 33%. For comparison the chemical agent Topsin M 500 SC was applied.

The test of inhibiting effect on the mycelium linear growth for pathogens of the genus *Monilia* was performed by using the intoxinated substrate method developed by Kowalik and Krechniak (1961). Each of the examined preparations was used in the three different concentrations: 1.0%, 2.0%, 4.0% Biochikol 020 PC, 0.025%, 0.05%, 0.1% Biosept 33 SL, 0.05%, 0.1%, 0.2% Topsin M 500 SC. The second value was equal to the recommended dose, while the first and the third ones corresponded to the concentration lowered and increased by half as compared to that of the recommended value, respectively. Into Potato Dextrose Agar cooled to 40°C an appropriate amount of the preparation was added with an automatic measuring pipette and poured into Petri dishes. Then, 5-millimetre disks of 14-day-old cultures of *M. laxa* and *M. fructigena* were applied. Five repetitions were made for each fungi species and preparation concentration. The control combination contained no preparations in the medium. The colony diameter was measured for next days until complete over-

growing Petri dishes with control (*M. laxa* – 10 days, *M. fructigena* – 12 days). Based on these measurements the coefficient of inhibition of fungal growth for fungi under examination (Kowalik and Krechniak 1961) was calculated. The obtained results were subjected to statistical analysis by employing analysis of variance and t-Duncan test, at the significance level of $\alpha = 0.05$.

Results

The chemical preparation Topsin M 500 SC was most effective in inhibiting the mycelium growth of both pathogens of the genus *Monilia* isolated from the fruits during the vegetation season (Table 1). The fungistatic activity of this agent was statistically almost unchanged regardless of the concentration used. The tests indicated high effectiveness of the preparations containing natural substances. The Biosept 33 SL at any concentration had a high coefficient of the mycelium linear growth inhibition for both pathogens isolated during the vegetation season, as compared to that of the preparation Topsin M 500 SC. For the concentration of 0.1% this coefficient was 94.86% to *M. laxa* and 97.29% to *M. fructigena*. Biochikol 020 PC was less effective and its fungistatic activity depended on the concentration used. The coefficient of the mycelium linear growth inhibition for the concentration of 4% was equal to 70.07% to *M. laxa* and 92.63% to *M. fructigena*.

Table 1

Linear growth inhibition of fungi isolates mycelium of the genus *Monilia* collected from infected fruits during the vegetation period (%)

Preparation	Concentration (%)	*Monilia laxa*	*Monilia fructigena*
Control		0.00 f	0.00 e
Biochikol 020 PC	1.00	44.49 e	72.05 d
	2.00	65.42 d	84.17 c
	4.00	70.07 c	92.63 b
Biosept 33 SL	0.025	84.53 b	95.11 ab
	0.05	86.30 b	96.32 ab
	0.10	94.86 a	97.29 a
Topsin M 500 SC	0.05	96.94 a	95.15 ab
	0.10	97.43 a	97.76 a
	0.20	98.44 a	98.19 a

Values in columns marked with the same letter do not differ significantly at $\alpha = 0.05\%$ (t-Duncan test).

Comparable results were obtained for fungi of the genus *Monilia* isolated from fruit mummies (after overwintering). Biosept 33 SL was most effective among the preparations containing natural substances (Table 2) and its fungistatic activity depended on the concentration used. It should be noted that the highest concentration of this preparation (0.1%) showed higher coefficient of the mycelium linear

Table 2

Linear growth inhibition of fungi isolates mycelium of the genus *Monilia* collected from fruit mummies (%)

Preparation	Concentration (%)	*Monilia laxa*	*Monilia fructigena*
Control		0.00 f	0.00 e
Biochikol 020 PC	1.00	38.76 e	79.80 d
	2.00	57.26 d	82.86 cd
	4.00	73.15 c	89.99 ab
Biosept 33 SL	0.025	82.92 b	85.78 bc
	0.05	86.54 b	86.51 bc
	0.10	92.96 a	94.46 a
Topsin M 500 SC	0.05	96.39 a	89.84 ab
	0.10	97.24 a	89.13 ab
	0.20	97.97 a	93.58 a

Values in columns marked with the same letter do not differ significantly at $\alpha = 0.05\%$ (t-Duncan test).

growth inhibition than that of the chemical agent for *M. fructigena* and was equal to 94.46%. The poorest fungistatic activity was recorded for Biochikol 020 PC. The coefficient of the mycelium linear growth inhibition at concentration of 4% was 73.15% to *M. laxa* and 89.99% to *M. fructigena*.

The preparation Biosept 33 SL, containing grapefruit extract 33% as an active substance, inhibited effectively the mycelium growth of pathogens belonging to the genus *Monilia* under *in vitro* conditions in a way comparable of that of the chemical preparation Topsin M 500 SC. Chitosan contained in the biotechnical preparation Biochikol 020 PC showed the lowest effectiveness in the mycelium linear growth inhibition of *M. laxa* and *M. fructigena*. The results obtained under *in vitro* conditions clearly indicate that it is necessary to conduct further studies to investigate an effect of natural substances contained in the tested preparations under field conditions so that they can be used for protecting fruit trees against pathogens of the genus *Monilia*.

Discussion

A number of authors seek natural methods for control of pathogens responsible for brown rot of pome fruit trees and brown rot of stone fruit trees. Witting et al. (1997) demonstrated that the application of *Aureobasidium pullulans* and *Epicoccum purpurascens* during sweet cherry flowering significantly reduced the number of latent infections of fruit primordia with *M. laxa*. Holb (2006) shows also that it is possible to use living organisms, e.g. *Trichoderma viride, Aspergillus flavus, Epicoccum nigrum* and *Bacillus subtilis* B-3 strain to control diseases caused by fungi of the genus *Monilia*. Hou et al. (2010) have proved that berberine present in the following plant species:

Coptis chinensis, *Tripterygium wilfkrdii*, *Artemisia apiacea* and *Melisa toosendan*, as a natural agent significantly reduces the incidence of brown rot on stone fruit trees.

In Poland there are no extensive studies on the use of "environmentally friendly" agents for orchard protection, as emphasised, for example, by Bryk (2006). The author indicates a possibility of using of the preparation Biochikol 020 PC in orchards. Zydlik (2008) claims that this preparation can limit apple infestation with scab up to 70–90%, and leaves by 50–70% as well as limit the infestation of apple powdery mildew even by 98%. In addition, Orlikowski and Skrzypczak (1997) as well as Pięta and Pastucha (2002) showed that chitosan (an active substance in the preparation Biochikol 020 PC) has an advantageous effect on plant health and induces a wide spectrum of immunological reactions.

The tests have indicated an inhibitory effect of the Biochikol 020 PC to fungi of the genus *Monilia* under *in vitro* conditions, with the coefficient of the mycelium linear growth inhibition higher for *M. fructigena* than that for *M. laxa*. The results indicate that the preparation Biosept 33 SL can be also used for control of pathogens belonging to the genus of *Monilia*, as its active substance demonstrated a higher fungistatic effectiveness than that of Biochikol 020 PC, comparable even with Topsin M 500 SC. According to Wielgusz and Andruszewska (2008) grapefruit extract being an active substance of the preparation Biosept 33 SL has antifungal, antiviral and antibacterial properties known for many years. Advantageous properties of this substance were confirmed also, for example by Andrzejak (2008) and Bisek (2008).

Under *in vitro* conditions an effect of preparations on a specified factor only is examined. It is impossible to take into account their influence on other organisms that can inhibit or stimulate such factor. This is why it would be justified to verify fungistatic activity of the preparations Biochikol 020 PC and Biosept 33 SL to pathogens of the genus *Monilia* in field experiments.

Streszczenie

WPŁYW PREPARATÓW ZAWIERAJĄCYCH NATURALNE SUBSTANCJE NA HAMOWANIE WZROSTU GRZYBNI *MONILIA LAXA* I *M. FRUCTIGENA*

Grzyby rodzaju *Monilia* są sprawcami ważnych gospodarczo chorób, powodujących ogromne straty owoców, zwłaszcza w sadach, gdzie nie jest prowadzona intensywna ochrona chemiczna. Sprawdzano działanie wybranych preparatów zawierających naturalne substancje (Biosept 33 SL i Biochikol 020 PC) na wzrost grzybni patogenów *Monilia laxa* i *M. fructigena*.

Środek biotechniczny Biosept 33 SL skutecznie ograniczał wzrost grzybni patogenów rodzaju *Monilia* w warunkach *in vitro*, w sposób porównywalny z preparatem chemicznym Topsin M 500 SC. Najmniejszą skutecznością w hamowaniu wzrostu liniowego grzybni *M. laxa* i *M. fructigena* odznaczał się Biochikol 020 PC.

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