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LABORATORY EFFECT OF AZOXYSTROBIN (Amistar 250 SC) AND GRAPEFRUIT EXTRACT (Biosept 33 SL) ON GROWTH OF FUNGI COLONIZING ZUCCHINI PLANTS

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Abstract. Zucchini (*Cucurbita pepo* L. var. *giromontina*) is a dependable vegetable in cultivation which is characterized by high fertility. Grown in the field and under cover, it is infected by a number of pathogens specific of the family *Curbitaceae* as well as those that are characteristic of other plantations. Actually, natural products such as plant extracts are more and more frequently used in plant protection from pathogens. The azoxystrobin (Amistar 250 SC, Syngenta) and extract of grapefruit (Biosept 33 SL, Cintamani Poland) were tested *in vitro* for their effectiveness to inhibit the linear growth of *Alternaria alternata, Botrytis cinerea, Fusarium avenaceum, F. culmorum, F. equiseti, F. oxysporum, Rhizoctonia solani* and *Trichoderma hamatum.* The azoxystrobin (0.05%, 0.1%) and extract of grapefruit (0.1%, 0.2%) were applied in different concentrations. The studies made use of a Petri dishes method recommended for testing fungicides in laboratory conditions. Extract of grapefruit was more effective than azoxystrobin. The extract of grapefruit inhibited the growth of *A. alternata, B. cinerea, R. solani* and *T. hamatum* already after 4 days and was the most effective after 12 days of experiment. Azoksystrobin inhibited the growth of *B. cinerea* and *R. solani* but did not inhibit the growth of *F. equiseti*. Microscopic analysis showed the morphological changes in fungal hyphae under the influence of tested substances.

Key words: synthetic fungicide, biological preparation, biocontrol, growth colonies *in vitro*

INTRODUCTION

Zucchini (*Cucurbita pepo* L. var. *giromontina*) grown under cover and in the field is usually infected by pathogens specific for *Cucurbitaceae* as well as organisms characteristic for other plants like *Pseudoperonospora cubensis* (Berk. et Curt.), *Erysiphe cichoracearum* D.C. et Merat, *Alternaria alternata* Keiss., *Botrytis cinerea* Pers., *Fusa-*

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rium spp., *Rhizoctonia solani* Kühn, *Sclerotinia sclerotiorum* (Lib.) de Bary [Gisi 2002]. Most of the diseases caused by these pathogens are rots that affect subterranean tissues (damping-off, root and crown rots) and vascular wilts initiated through root infection. The disease is controlled in commercial cucurbit crops by means of frequent applications of fungicides (azoxystrobin, Amistar 250 SC, Syngenta) [Reuveni and Sheglov 2002]. Chemical control causes not only fungal resistance and environmental pollution, but first of all, it directly influences the human health [Lingk 1991]. Recently, the tendency is to reduce the use of chemical preparations and to replace them with biocontrol products based on antagonistic microorganisms, organic compounds or plant extracts [Patkowska 2005, 2006, Orlikowski and Skrzypczak 2003, Rose et al. 2003]. Concerned about the protection of the natural environment, a lot of European countries take part in programs of reducing pesticides used in plant production (Europhyto 2018). Actually, biological preparations are more often used by producers to protect plants in ecological and integrated production. One of natural substances is the extract of grapefruit. The extract of grapefruit pulp and stones is the main content (33%) of the biopreparation Biosept 33 SL. The preparation contains endogenous flavonoids with strong antibiotic properties but without negative side-effects. Many researchers proved the inhibitory effect of grapefruit extract on the growth of pathogenic bacteria and fungi [Woedtke et al. 1999, Negi and Jayaprakasha 2001, Dłużniewska 2006, Lenc 2007, Jamiołkowska 2009]. Biosept 33 SL not only inhibits the development of harmful micro-organisms but also stimulates the resistance system of plants [Saniewska 2002, Dłużniewska 2006].

The aim of the study was the evaluation of the effect of azoxystrobin (Amistar 250 SC, Syngenta) and grapefruit extract (Biosept 33 SL, Cintamani Poland) on the growth of some fungi occurring on zucchini plants.

MATERIALS AND METHODS

Fungicide azoxystrobin (Amistar 250 SC, Syngenta) in the concentrations of 0.05%, 0.1% and extract of grapefruit (Biosept 33 SL, Cintamani Poland) in the concentrations 0.1%, 0.2% was added to potato-dextrose agar (PDA Difco) according to the method described by Thanassoulopoulos et al. [1971]. It consists in adding the examined substance to PDA medium cooled to 50^{o}C and inoculating into the set in medium of the studied fungi. The medium, together with the studied substance, was poured into sterile Petri dishes with the diameter of 9 cm. Control dishes contained PDA without azoxystrobin or grapefruit extract. The mycelium of eight fungi (*Alternaria alternata, Botrytis cinerea, Fusarium avenaceum, F. culmorum, F. equiseti, F. oxysporum, Rhizoctonia solani, Trichoderma hamatum*) in the form of 3 mm disks was placed on the medium in Petri dishes. Five dishes were used for each combination. The dishes were stored at 25°C for 24 days. The diameter of fungal colonies was measured after 4, 8, 12 and 24 days. Inhibition of mycelium growth on the medium enriched with azoxystrobin and grapefruit extract to that on control medium was the measure of antifungal activity.

The antifungal efficiency of azoxystrobin and extract of grapefruit was calculated from Abbot's formula:

$$I = \frac{C - T}{C} \cdot 100\%$$

where:

I – fungus linear growth inhibition index (percentage)

C – fungus colony diameter in the control combination

T – fungus colony diameter in combination containing a tested substance concentration in the agar [Kurzawińska and Duda-Surman 2008].

Data were analyzed with Tukey's test using the SAS statistical system, one-way ANOVA [SAS Version 9.1, SAS Inst., Cary, N. C., USA].

Changes in the morphology of the fungi were observed in an optical microscope after 12 days of the experiment. Changes in the mycelium colour and the presence or absence of sclerotia, spores and chlamidospores were observed.

RESULTS AND DISCUSSION

The results presented in the work indicated the effect of Amistar 250 SC and Biosept 33 SL toward fungal organisms depends on the kind of tested substances, their concentrations and fungus species used in experiment.

Fig. 1a. Effect of Amistar 250 SC and Biosept 33 SL on the growth of fungi on potato-dextrose agar after 12 days (photo by A. Jamiołkowska): A1 – Amistar concentration 0.05%; A2 – Amistar concentration 0.1%; B1 – Biosept concentration 0.1%, B2 – Biosept concentration 0.2%

Ryc. 1a. Wpływ preparatów Amistar 250 SC i Biosept 33 SL na wzrost grzybów na pożywce ziemniaczano-glukozowej (PDA) po 12 dniach (fot. A. Jamiołkowska): A1 – stężenie preparatu Amistar 0.05%; A2 – stężenie preparatu Amistar 0.1%; B1 – stężenie preparatu Biosept 0.1%; B2 – stężenie preparatu Biosept 0.2%;

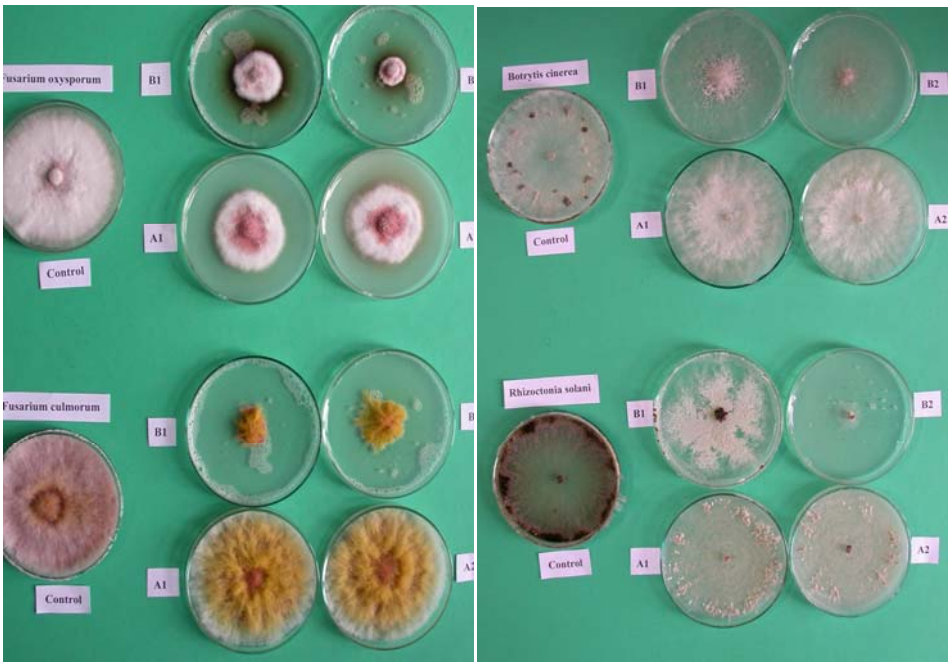

Fig. 1b. Effect of Amistar 250 SC and Biosept 33 SL on the growth of fungi on potato-dextrose agar after 12 days (photo by A. Jamiołkowska): A1 – Amistar concentration 0.05%; A2 – Amistar concentration 0.1%; B1 – Biosept concentration 0.1%, B2 – Biosept concentration 0.2%

Ryc. 1b. Wpływ preparatów Amistar 250 SC i Biosept 33 SL na wzrost grzybów na pożywce ziemniaczano-glukozowej (PDA) po 12 dniach (fot. A. Jamiołkowska): A1 – stężenie preparatu Amistar 0.05%; A2 – stężenie preparatu Amistar 0.1%; B1 – stężenie preparatu Biosept 0.1%; B2 – stężenie preparatu Biosept 0.2%

The results showed Biosept 33 SL inhibited strongly the growth and formation of morphological elements of all tested fungi. The concentration of 0.2% was more effective than that of 0.1% (tab. 1, 2). Already after 4 days of experiment the grapefruit extract showed the strong inhibitory effect on *A. alternata, B. cinerea, R. solani* and *T. hamatum* (59.3–86.7%). The diameters of colonies were significantly smaller than those grown on PDA without amendment (tab. 1, 2). The inhibitory effect of Biosept 33 SL on *F. avenaceum, F. culmorum, F. equiseti* and *F. oxysporum* was more prolonged than on other species and was the strongest after 12 days (34.6–79.4%) (tab. 1, 2, fig. 1a, b). Presented results confirm many authors [Woedtke et al. 1999, Pięta et al. 2004, Patkowska 2005, 2006]. Kurzawińska and Duda-Surman [2008] confirmed a high inhibitory effect of grapefruit extract on mycelium growth of *Stewartia pseudocamellia, Alternaria alternata, Cylindrocarpon radicicola, Fusarium oxysporum, F. avenaceum* and *Phomopsis thea*. The results of investigations conducted by Orlikowski et al. [2002] showed that grapefruit extract as a biologically active substance in

Table 1. Colonies diameter (cm) of fungi growing on potato-dextrose agar (PDA) with different concentrations of Amistar 250 SC and Biosept 33 SL

Tabela 1. Średnica kolonii (cm) grzybów rosnących na pożywce ziemniaczano-glukozowej (PDA) z różnym stężeniem preparatów Amistar 250 S.C. i Biosept 33 SL

Fungus species Gatunek grzyba	Experimental combination Kombinacja doświadczalna	Average of diameter of colony (cm) – Średnica kolonii (cm)			
		4 days – dni	8 days – dni	12 days – dni	24 days – dni
Alternaria alternata	control – kontrola	3.64 A	5.08 A	6.38 A	8.36 A
	A1	2.12 B	3.18 B	4.36 B	7.94 A
	A2	1.98 B	3.26 B	4.44 B	8.30 A
	B1	1.16 C	1.52 C	3.02 C	6.42 B
	B2	1.06 C	1.26 C	1.92 D	3.66 C
LSD (0.05) – NIR (0.05)		0.265	0.3561	0.5595	0.8297
Botrytis cinerea	control – kontrola	9.00 A	9.00 A	9.00 A	9.00 A
	A1	3.18 C	6.22* B	8.90* A	9.00 A
	A2	2.56 D	5.92* B	9.00* A	9.00 A
	B1	4.38 B	6.52* B	9.00* A	9.00 A
	B2	3.50 C	5.68* B	7.56* B	8.20 B
LSD (0.05) – NIR (0.05)		0.5366	0.8664	0.7692	0.6418
Fusarium avenaceum	control – kontrola	2.14 A	3.70 A	5.74 A	9.00 A
	A1	1.68 B	2.70 B	3.94 C	6.90 C
	A2	1.74 B	2.98 B	4.72 B	7.62 B
	B1	1.88 AB	2.34 C	3.16 D	4.54 D
	B2	1.72 B	1.76 D	2.20 E	3.40 E
LSD (0.05) – NIR (0.05)		0.2703	0.352	0.5014	0.3970
Fusarium culmorum	control – kontrola	3.90 A	7.66 A	9.00 A	9.00 A
	A1	2.70 BC	5.38 B	8.76 A	9.00 A
	A2	2.84 B	5.64 B	8.86 A	9.00 A
	B1	2.06 C	2.48 C	2.22 C	3.62 B
	B2	3.24 B	3.30 C	4.06 B	3.92 B
LSD (0.05) – NIR (0.05)		0.6670	0.8631	0.8510	0.5703
Fusarium equiseti	control – kontrola	2.14 A	3.34 B	5.20 C	9.00 A
	A1	2.26 A	3.68 AB	6.12 A	9.00* A
	A2	2.36 A	3.98 A	6.56 B	9.00* A
	B1	1.60 B	2.26 C	3.40 D	6.12 B
	B2	1.26 C	1.40 D	2.02 E	4.20 C
LSD (0.05) – NIR (0.05)		0.330	0.4935	0.3851	0.3757
Fusarium oxysporum	control – kontrola	3.44 A	5.84 A	8.84 A	9.00 A
	A1	2.04 C	3.18 C	5.10 C	8.52 B
	A2	2.32 B	3.64 B	5.46 B	8.74 AB
	B1	1.40 D	2.10 D	3.04 D	5.40 C
	B2	1.16 D	1.48 E	1.82 E	2.56 D
LSD (0.05) – NIR (0.05)		0.2553	0.2676	0.1815	0.4085
Rhizoctonia solani	control – kontrola	9.00 A	9.00 A	9.00 A	9.00 A
	A1	3.84** B	5.62 B	9.00 A	9.00 A
	A2	3.24** B	5.76 B	9.00 A	9.00 A
	B1	3.62** B	5.38 B	7.34 B	7.88 B
	B2	1.65** C	1.72 C	1.92 C	1.96 C
LSD (0.05) – NIR (0.05)		0.7207	1.6146	0.2158	0.2743
Trichoderma hamatum	control – kontrola	9.00 A	9.00 A	9.00 A	9.00 A
	A1	4.34 B	7.04* B	8.96 AB	9.00 A
	A2	4.34 B	7.04* B	9.00 A	9.00 A
	B1	3.24 C	5.52* C	8.74* B	9.00 A
	B2	1.20D	1.90* D	3.22* C	5.84 B
LSD (0.05) – NIR (0.05)		0.3631	0.6121	0.2438	0.481

A1 – Amistar concentration 0.05%; A2 – Amistar concentration 0.1%; B1 – Biosept concentration 0.1%; B2 – Biosept concentration 0.2%; * no sporulation as compared to the control; ** no formation of sclerotia as compared to the control; values in column designated with the same letters (A, B, C…..) do not significantly differ at 5% error (Tukey's test)

A1 – stężenie preparatu Amistar 0.05%; A2 – stężenie preparatu Amistar 0.1%; B1 – stężenie preparatu Biosept 0.1%; B2 – stężenie preparatu Biosept 0.2%; * brak zarodnikowania względem kontroli; ** brak tworzenia sklerocjów względem kontroli; wartości w kolumnach oznaczone tą samą literą (A, B, C….) nie różnią się istotnie na poziomie istotności 5% (test Tukeya)

Table 2. Linear growth inhibition (%) of fungus colony on potato-dextrose agar (PDA) with different concentrations of Amistar 250 SC and Biosept 33 SL

Tabela 2. Ograniczenie wzrostu liniowego (%) kolonii grzybów na pożywce ziemniaczano-glukozowej (PDA) z różnym stężeniem preparatów Amistar 250 SC i Biosept 33 SL

Fungus species Gatunek grzyba	Growth inhibition (%) as compared to the control Ograniczenie wzrostu (%) względem kontroli															
	A1				A2				B1				B2			
	4 days dni	8 days dni	12 days dni	24 days dni	4 days dni	8 days dni	12 days dni	24 days dni	4 days dni	8 days dni	12 days dni	24 days dni	4 days dni	8 days dni	12 days dni	24 days dni
Alternaria alternata	41.8	37.4	31.7	5.0	45.6	35.8	30.4	0.7	68.1	70.1	52.7	23.2	70.9	75.2	69.9	56.2
Botrytis cinerea	64.7	30.9*	1.1*	0.0	71.6	34.2*	0.0*	0.0	51.3	27.6*	0.0*	0.0	61.1	36.9*	16.0*	8.9
Fusarium avenaceum	21.5	27.0	31.4	23.3	18.7	19.5	17.8	15.3	12.1	36.8	44.9	49.6	19.6	52.4	61.7	62.2
Fusarium culmorum	30.8	29.8	2.7	0.0	27.2	26.4	1.6	0.0	47.2	67.6	75.3	59.8	16.9	56.9	54.9	56.4
Fusarium equiseti	-5.6[1]	-10.2[1]	-17.7[1]	0.0*	-22.0[1]	-19.2[1]	-26.2[1]	0.0*	25.2	32.3	34.6	32.0	41.1	58.1	61.2	53.3*
Fusarium oxysporum	40.7	45.5	42.3	5.3	32.6	37.7	38.2	2.9	59.3	64.0	65.6	40.0	66.3	74.7	79.4	71.6
Rhizoctonia solani	61.3**	37.6	0.0	0.0	64.0**	36.0	0.0	0.0	59.8**	40.2	18.4	12.4	81.7**	80.9	78.7	78.2
Trichoderma hamatum	51.8	21.8*	0.4	0.0	51.8	21.8*	0.0	0.0	64.0	38.7*	2.9*	0.0	86.7	78.9*	64.2*	35.1

A1 – Amistar concentration 0.05%; A2 – Amistar concentration 0.1%; B1 – Biosept concentration 0.1% ; B2 – Biosept concentration 0.2%; * no sporulation as compared to the control; ** no formation of sclerotia as compared to the control, [1] – stimulation of mycelia growth

A1 – stężenie preparatu Amistar 0.05%; A2 – stężenie preparatu Amistar 0.1%;; B1 – stężenie preparatu Biosept 0.1%; B2 – stężenie preparatu Biosept 0.2%; * brak zarodnikowania względem kontroli; ** brak tworzenia sklerocjów względem kontroli, [1] – stymulowanie wzrostu grzybni

Table 3. Effect of Amistar 250 SC and Biosept 33 SL on the macro- and microscopic changes of mycelium fungi growing on potato-dextrose agar (PDA) after 12 days

Tabela 3. Wpływ preparatów Amistar 250 SC i Biosept 33 SL na zmiany makro- i mikroskopowe grzybni na pożywce ziemniaczano-glukozowej (PDA) po 12 dniach

Fungus species Gatunek grzyba	Experimental combination Kombinacja doświadczalna	Morfologiczne zmiany w obrębie grzybni Morphological changes of mycelium fungi
Alternaria alternata	control kontrola A1, A2, B1, B2	Black-grey air mycelium, black reverse, present spores Grzybnia powietrzna czarno-szara, rewers czarny, obecność zarodników
Botrytis cinerea	control kontrola	White-grey air mycelium, scarce dark brown sclerotia over the whole dish, colourless reverse, spores present Grzybnia powietrzna biało-szara, obecne nieliczne ciemnobrązowe sklerocja na całej szalce, rewers bezbarwny, obecność zarodników
	A1, A2	White, fluffy air mycelium, colourless reverse, no sclerotia, numerous spores present Grzybnia powietrzna puszysta, biała, rewers bezbarwny, brak sklerocjów, obecność licznych zarodników
	B1, B2	White air mycelium of a very delicate structure, colourless reverse, white sclerotia in the central part of the dish, spores present Grzybnia powietrzna biała o bardzo delikatnej strukturze, rewers bezbarwny, białe sklerocja w centralnej części szalki, obecność zarodników
Fusarium avenaceum	control kontrola	Pink-red air mycelium, carmine reverse, no spores Grzybnia powietrzna różowo-czerwona, rewers karminowy, brak zarodników
	A1, A2	Pink-red air mycelium, carmine reverse, no spores Grzybnia powietrzna różowo-czerwona, rewers karminowy, brak zarodników
	B1, B2	Pink-red air mycelium, carmine reverse, no spores Grzybnia powietrzna żółto-czerwona, rewers karminowy, brak zarodników
Fusarium culmorum	control kontrola	Red air mycelium, yellow-red in the central part, carmine reverse, no spores Grzybnia powietrzna czerwona, w centralnej części żółto-czerwona, rewers karminowy, brak zarodników
	A1, A2	Yellow-red air mycelium, carmine reverse, no spores Grzybnia powietrzna żółto-czerwona, rewers karminowy, brak zarodników
	B1, B2	Yellow-red air mycelium of irregular growth, carmine reverse, no spores Grzybnia powietrzna żółto-czerwona, nieregularna we wzroście, rewers karminowy, brak zarodników

Fusarium equiseti	control kontrola	White-cream abundant air mycelium, beige reverse, spores and chlamidospores present Grzybnia powietrzna obfita biało-kremowa, rewers beżowy, obecność zarodników i chlamydospor
	A1, A2	White-cream abundant air mycelium, beige reverse, no spores and chlamidospores Grzybnia powietrzna obfita biało-kremowa, rewers beżowy, brak zarodników i chlamydospor
	B1, B2	White-cream abundant air mycelium, beige reverse, spores and chlamidospores present Grzybnia powietrzna biało-kremowa, rewers beżowy, brak zarodników i chlamydospor
Fusarium oxysporum	control kontrola	White-purple abundant air mycelium , pink reverse, no spores Grzybnia powietrzna obfita biało-fioletowa, rewers różowy, brak zarodników
	A1, A2	White-pink abundant air mycelium, intensively pink in the central part, pink reverse, no spores Grzybnia powietrzna biało-różowa, w centralnej części intensywnie różowa, rewers różowy, brak zarodników
	B1, B2	White-pink air mycelium , dark red reverse, no spores Grzybnia powietrzna biało-różowa, rewers ciemnoczerwony, brak zarodników
Rhizoctonia solani	control kontrola	Poor brown-grey air mycelium, colourless reverse, brown sclerotia on the edges of the dish Grzybnia powietrzna uboga brązowo-szara, rewers bezbarwny, brązowe sklerocja na brzegach szalki
	A1, A2	Poor white-cream air mycelium, colourless reverse, cream colour sclerotia on the edges of the dish Grzybnia powietrzna uboga, biało-kremowa, rewers bezbarwny, kremowe sklerocja na brzegach szalki
	B1, B2	Poor white-cream air mycelium, colourless reverse, no sclerotis Grzybnia powietrzna uboga, biało-kremowa, rewers bezbarwny, brak sklerocjów
Trichoderma hamatum	control kontrola	White air mycelium, colourless reverse, green spores Grzybnia powietrzna biała, rewers bezbarwny, zielone zarodnikowanie
	A1, A2	White air mycelium, colourless reverse, green spores Grzybnia powietrzna biała, rewers bezbarwny, zielone zarodnikowanie
	B1, B2	White air mycelium, colourless reverse, no spores Grzybnia powietrzna biała, rewers bezbarwny, brak zarodników

A1 – Amistar concentration 0.05%; A2 – Amistar concentration 0.1%; B1 – Biosept concentration 0.1%, B2 – Biosept concentration 0.2%
A1 – stężenie preparatu Amistar 0.05%; A2 – stężenie preparatu Amistar 0.1%; B1 – stężenie preparatu Biosept 0.1%; B2 – stężenie preparatu Biosept 0.2%

Biosept 33 SL can be successfully applied in ornamental plants protection against *F.oxysporum* special forms. Similar results are provided by Dłużniewska [2004], who presented the inhibiting effect of the preparation on the growth of *Coniothyrium fuckeli, Botrytis cinerea* and *Fusarium sp*. on rose stems.

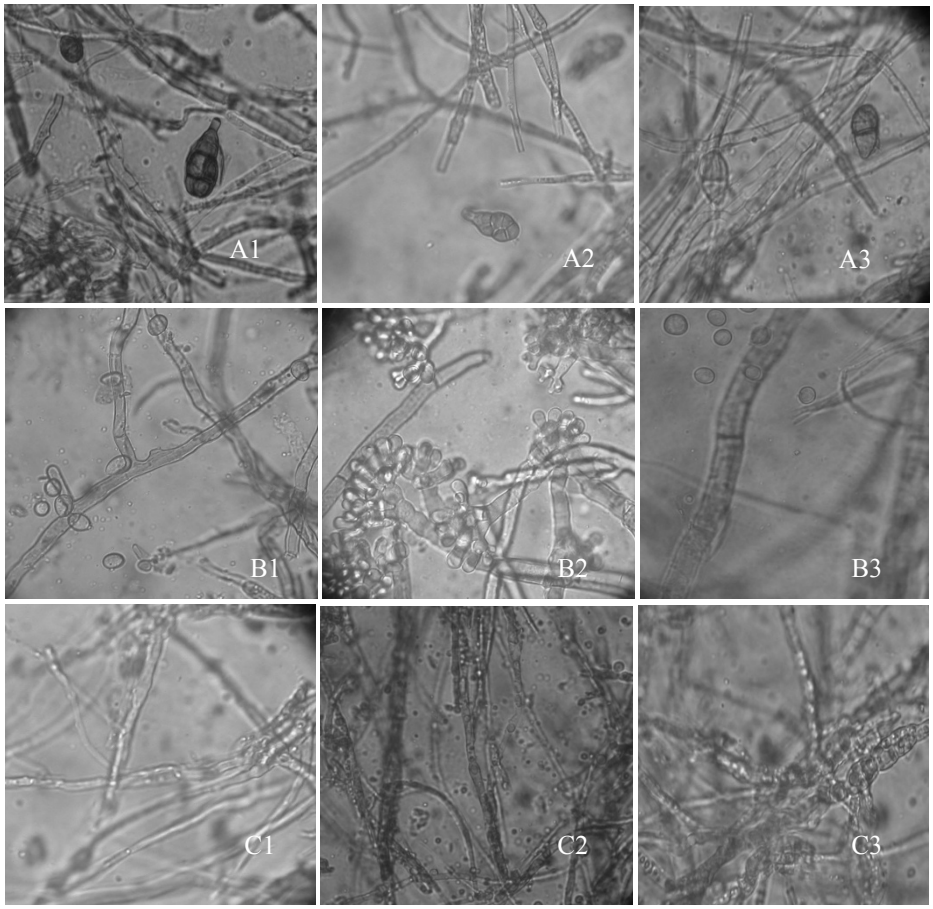

Fig. 2a. Influence of azoxystrobin (Amistar 250 SC) and grapefruit extract (Biosept 33 SL) on mycelium growth of tested fungi after 12 days (photo by A. Jamiołkowska): *A. alternata*: A1 – control, A2 – concentration of Amistar 0.1%, A3 – concentration of Biosept 0.2%; *B. cinerea*: B1 – control, B2 – concentration of Amistar 0.1%, B3 – concentration of Biosept 0.2%; *F. avenaceum*: C1 – control, C2 – concentration of Amistar 0.1%, C3 – concentration of Biosept 0.2%

Ryc. 2a. Wpływ azoksystrobiny (Amistar 250 SC) i ekstarktu z grejpfruta na wzrost badanych grzybów po 12 dniach (fot. A. Jamiołkowska): *A. alternata*: A1 – kontrola, A2 – stężenie preparatu Amistar 0.1%, A3 – stężenie preparatu Biosept 0.2%; *B. cinerea*: B1 – kontrola, B2 – stężenie preparatu Amistar 0.1%, B3 – stężenie preparatu Biosept 0.2%; *F. avenaceum*: C1 – kontrola, C2 – stężenie preparatu Amistar 0.1%, C3 – stężenie preparatu Biosept 0.2

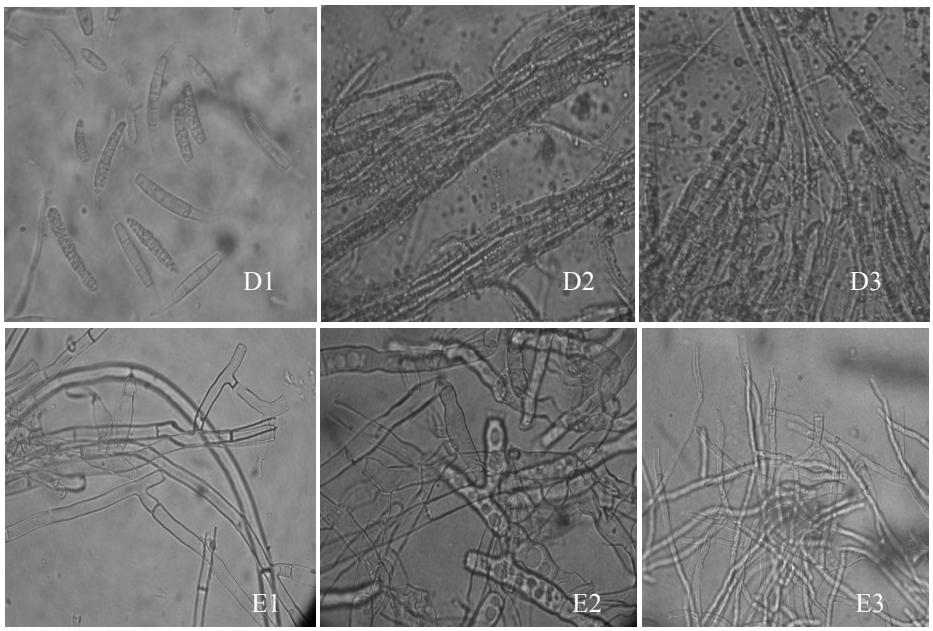

Fig. 2b. Influence of azoxystrobin (Amistar 250 SC) and grapefruit extract (Biosept 33 SL) on mycelium growth of tested fungi after 12 days (photo by A. Jamiołkowska): *F. equiseti:* D1 – control, D2 – concentration of Amistar 0.1%, D3 – concentration of Biosept 0.2%; *R. solani*: E1 – control, E2 – concentration of Amistar 0.1%, E3 – concentration of Biosept 0.2%

Ryc. 2b. Wpływ azoksystrobiny (Amistar 250 SC) i ekstarktu z grejpfruta na wzrost badanych grzybów po 12 dniach (fot. A. Jamiołkowska): *F. equiseti:* D1 – kontrola, D2 – stężenie preparatu Amistar 0.1%, D3 – stężenie preparatu Biosept 0.2%; *R. solani*: E1 – kontrola, E2 – stężenie preparatu Amistar 0.1%, E3 – stężenie preparatu Biosept 0.2%

Grapefruit extract in tested concentrations was more effective than azoxystrobin. The influence of azoxystrobin was the strongest at the beginning of experiment. After 4 days the diameters of colonies were significantly smaller than those in control combination (18.7–71.6%). Azoxystrobin showed the strongest inhibitory effect in the concentration of 0.1% on the growth of *B. cinerea* and *R. solani* (71.6% and 64.0%, respectively) but after 12 days the fungicide did not inhibit the growth of these fungi as well as that of *T. hamatum*. The growth of *F. equiseti* was not affected at all by azoxystrobin amendment (tab. 1, 2, fig. 1a, b).

The effect of azoxystrobin was of short duration comparing to that of grapefruit extract as after 24 days the fungicide inhibited only slightly the growth of some fungi and even the absence of inhibition was noticed. In the case of *F. equiseti*, the fungus growth was stimulated by asoxistrobin and after 24 days sporulation inhibition was only observed in the control. The research of Reuveni and Sheglov [2002] showed that azoxystrobin added to PDA significantly inhibited the growth and conidia production of *A. alternata* but not as strongly as difenoconazole or trifloxystrobin. Strobilurin ana-

logues (azoxystrobin) inhibit mitochondrial respiration by blocking electron transfer at the cytochrome b_{c1} complex [Anke 1995]. Circumvention of this cytochrome b_{c1} target site by induction of the alternative oxidase respiratory pathway has been proposed as the likely reason for the low mycelial sensitivity to strobilurins displayed by several pathogens [Olaya et al. 1998]. This alternative oxidase respiratory pathway is utilized by fungi growing on agar, especially nutrient-rich agar, and could account for the low sensitivity to azoxystrobin that observed Reuveni and Sheglov [2002] for *A. alternata* grown on PDA amended with this compound. It is possible that less effective activity of azoxystrobin *in vitro* is a result of mechanism described above. Microscopic observation showed morphological changes in fungal hyphae growing on the medium amended with azoxystrobin and grapefruit extract. These substances caused the delay of conidia production of *A. alternata* and *B. cinerea*, while in the combinations with *Fusarium* spp. the inhibition of conidia production, deformation, thickening and breaking of hyphae were noticed. The hyphae of *R. solani* were thinner in the combination with Biosept 33 SL, while thicker and deformed in the combination with azoxystrobin (fig. 2a, b). Changes in the colour and structure of the air mycelium were also observed (tab. 3). The inhibiting effect of biopreparations on the growth of phytopathogenic fungi *in vitro* conditions was studied by Pięta et al. [2004]. According to Orlikowski et al. [2001a, 2001b], compounds found in grapefruit extract, witch were active substances of Biosept 33 SL, inhibited not only the germination of *Fusarium oxysporum* and *Botrytis cinerea* spores but they also caused growth inhibition of germ hyphae through dehydration of the cytoplasm of the mycelium cells.

The high antifungal activity of grapefruit extract shows the possibility of its use in the protection of zucchini against pathogenic fungi what will be the subject of further studies.

CONCLUSIONS

1. Extract of grapefruit was more effective than azoxystrobin in inhibition the growth of tested fungi.
2. Azoksystrobin the most inhibited the growth of *B. cinerea* and *R. solani* but it was not effective against *F. equiseti*.
3. Microscopic observations showed the morphological changes in fungal hyphae under the influence of tested substances.

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LABORATORYJNA OCENA WPŁYWU AZOKSYSTROBINY I EKSTRAKTU Z GREJPFRUTA NA WZROST GRZYBÓW WYSTĘPUJACYCH NA CUKINII

Streszczenie. Cukinia (*Cucurbita pepo* L. var. *giromontina*) to warzywo niezawodne w uprawie charakteryzujące się wysoką plennością. Uprawiane w polu i pod osłonami porażane jest przez wiele patogenów specyficznych dla rodziny *Curbitaceae*, jak i charakterystycznych dla innych plantacji. Obecnie w ochronie roślin przed patogenami stosuje się coraz częściej produkty naturalne, takie jak wyciągi roślinne. Celem badań była laboratoryjna ocena wpływu azoksystrobiny (Amistar 250SC, Syngenta) i ekstraktu z grejpfruta (Biosept 33SL, Cintamani Polska) na niektóre grzyby występujące na cukinii. W doświadczeniu użyto izolaty *Alternaria alternata*, *Botrytis cinerea*, *Fusarium avenaceum, F. culmorum, F. equiseti, F. oxysporum, Rhizoctonia solani, Trichoderma hamatum* wyosobnione z roślin cukinii. Amistar stosowano w stężeniach: 0.05%, 0.1% a Biosept w stężeniach: 0.1%, 0.2%. W badaniach zastosowano metodę szalkową zalecaną do testowania fungicydów w warunkach laboratoryjnych. Ekstrakt z grejpfruta był skuteczniejszy niż azoksystrobina i wykazywał bardziej długotrwałe działanie względem badanych gatunków grzybów. Obserwacje mikroskopowe wykazały zmiany morfologiczne w strzępkach grzybów pod wpływem działania azoksystrobiny i ekstraktu z grejpfruta. Badane substancje opóźniały zarodnikowanie lub całkowicie je zahamowały, powodowały deformację, zgrubienie i załamywanie się strzępek.

Słowa kluczowe: syntetyczny fungicyd, preparat biologiczny, biologiczna ochrona, wzrost grzybów *in vitro*

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