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Evaluation of the effect of the Zielony Busz fertilizer on selected phytopathogenic fungi growth *in vitro*

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Abstract. The aim of the research was the laboratory evaluation of the fungistatic effect of the Zielony Busz (ZB) organic-mineral fertiliser on the linear growth of polyphagic phytopathogenic fungi *Alternaria alternata*, *Colletotrichum coccodes, Fusarium avenaceum, Fusarium equiseti* and *Fusarium oxysporum*. The preparation was applied to the medium (PDA potato-glucose agar) in concentrations of 2% and 5%, compared to 0.05% azoxystrobin (relative control) and pure PDA medium (absolute control). The strongest antifungal effect of the Zielony Busz fertilizer was found at the 5% concentration in reference to *A. alternata* (inhibition of colony growth at the level of 50–52% compared to the absolute control; 20–41.7% compared to azoxystrobin) and *C. coccodes* (18.2–53.3% growth inhibition compared to absolute control). Weaker inhibitory effect of 5% concentration of the fertilizer was noted against *F. avenaceum* (10.5% inhibition of colony growth compared to absolute control) and *F. oxysporum* (20% inhibition compared to absolute control), but only at the beginning of the experiment. The Zielony Busz fertilizer at a concentration of 2% caused the surface growth of all tested fungi, especially *F. equiseti*. The best effects of the fungistatic effect of the fertilizer were obtained in the first days of the experiment (4th day) with a 5% concentration of the product in the substrate. With time, the antifungal activity of fertilizer decreased significantly.

Keywords: plant extracts, *Alternaria alternata, Colletotrichum coccodes, Fusarium* spp., azoxystrobin

INTRODUCTION

Integrated pest management (IPM) is a system of protection that involves the use of various protection methods, primarily non-chemical methods (Kopacki et al., 2019; Sosnowska et al., 2016). The use of different forms of protection influences the condition of plants, including their growth and resistance to infectious agents. In plant production, mineral fertilisers are used in addition to plant protection products (pesticides). Irrational management of these products may favour the increase of diseases and pests in plantations (Mickiewicz, Mickiewicz, 2014). Commonly available chemicals are easy to use and are characterised by high efficacy, but at the same time they can adversely affect the environment (Lisiecki et al., 2014; Mickiewicz, Mickiewicz, 2014). Many synthetic chemicals are characterised by a broad spectrum of action, thus negatively affecting beneficial organisms and accumulating in plant tissues over a long period (Kordowska-Wiater, 2011). Thus, they pose a threat not only to the natural environment, but also to human life and health (Lisiecki et al., 2014). Moreover, the fact that resistance of pest organisms to chemicals can occur rapidly, even during one or more growing seasons, is of great importance (Perez-Garcia et al., 2011). Therefore, it is important to apply the system of integrated pest management, which aims to keep pests below the threshold of economic harmfulness of pests, using primarily non-chemical methods among others preparations of natural origin (Ciemniak, 2018; Mickiewicz, Mickiewicz, 2014).

The current concept, i.e. the promotion of the development of so-called sustainable agriculture, leads to a reduction in the use of chemical plant protection products and mineral fertilisers, in favour of products of natural origin such as biopreparations or plant-based liquid fertilisers (Mołoń, Durak, 2018; Sosnowska, 2018). Over the years, legal changes have led to the withdrawal of a large number of active substances and, consequently, plant protection products (Matyjaszczyk, 2012). In such a situation, a great opportunity to improve the quality and healthiness of plants is the use of preparations of natural origin. These include products that support the cultivation of plants (usually soil formulations), but also those registered as plant protection products for foliar application. Some of them can match the effectiveness of chemical agents (Kempka, 2014). One preparation of natural origin is an organic-mineral fertilizer with the trade name Zielony Busz (manufac-

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turer Himal Sp. z o.o., Łódź). It contains in its composition extracts from herbal plants (wormwood, garlic, nettle, algae), macro- (NPK) and microelements (Cu, Co, B, Zn, Mo) and potassium soap. According to the manufacturer's information, it is a foliar fertilizer intended for use in the cultivation of vegetables, fruit and ornamental plants. It is available also in the form of a spray for plants showing nutritional deficiencies and symptoms of infectious diseases.

The aim of this study was to laboratory evaluate the fungistatic effect of Zielony Busz (ZB) fertilizer on the linear growth of polyphagous phytopathogenic fungi: *Alternaria alternata* (Fr.) Keissl., *Colletotrichum coccodes* (Wallr.) S. Hughes, *Fusarium avenaceum* (Fr.) Sacc., *Fusarium equiseti* (Corda) Sacc. and *Fusarium oxysporum* Schltdl.

MATERIALS AND METHODS

Mycological material

The test material consisted of plant pathogenic fungi: *Alternaria alternata* (Fr.) Keissl., *Colletotrichum coccodes* (Wallr.) S. Hughes, *Fusarium avenaceum* (Fr.) Sacc., *Fusarium equiseti* (Corda) Sacc. and *Fusarium oxysporum* Schltdl., isolated from sweet pepper (*Capsicum annuum* L.) plants in 2018–2019 and from the fungal collection of the Department of Plant Protection of the University of Life Sciences in Lublin.

Evaluation of the fungistatic effect of the preparation

In the study, the organic-mineral fertiliser Zielony Busz (ZB), based on extracts from herbal plants (wormwood – *Artemisia absinthium* L., garlic – *Allium sativum* L., common nettle – *Urtica dioica* L., algae – *Algae*), enriched with macro- and microelements, was used. ZB fertiliser, at concentrations of 2% (ZB2) and 5% (ZB5) recommended for application by the manufacturer, was added directly to the culture medium, which was potato-glucose agar (PDA Difco). The control sample consisted of fungal colonies growing on the medium without the addition of fertiliser (absolute control C) and colonies growing on the medium with the addition of 0.05% azoxystrobin (Amistar 250 SC, Syngenta Polska Sp. z o.o.) (relative control A). The poisoned media method was used in the study (Jamiołkowska, Kowalski, 2012). Fungal inoculum was derived from 10-day-old mono-spore colonies of *A. alternata*, *C. coccodes*, *F. avenaceum*, *F. equiseti*, *F. oxysporum,* cultured on PDA medium. Three replicates were prepared for each experimental combination. The experimental combinations were kept in a thermostat for 12 days at 25 °C. After 4, 8, 12 days, the diameter of the fungal colonies was measured (mm). The measure of antifungal activity was the inhibition of mycelial growth on the culture medium with ZB fertiliser compared to growth on the control medium. The antifungal efficacy of the tested extracts was calculated based on the Abbott formula:

$$I = [(K - T)/K] \times 100\%;$$

where:

I – inhibition/stimulation index of linear growth of the fungus,

K – diameter of the fungus colony in the control sample (C – absolute control, A – relative control),

T – diameter of the fungus colony in the test sample – containing the concentration of the test substance in the agar (ZB2, ZB5).

Statistical analysis

The data collected were presented as experimental means and analyzed using standard statistical procedures ANOVA (one-way analysis of variance). Assessment of the significance of differences between the means was based on Tuckey's post-hoc test).

RESULTS

Zielony Busz (ZB) fertiliser based on plant extracts showed differentiated antifungal activity depending on the fungus species and the concentration used (Table 1, Fig. 1). ZB at a concentration of 2% did not inhibit the growth of the tested fungi, but strongly stimulated their surface growth (Table 1, Figs. 1, 2). The best anti-fungal effect of ZB fertiliser was obtained at a concentration of 5%, where the preparation had a fungistatic effect significantly inhibiting the linear growth of *A. alternata* (inhibition index 50–52%), *C. coccodes (*18.2–53.3%), *F. oxysporum* (20–24%) compared to the absolute control (C) (Fig. 1). In contrast, for 0.05% azoxystrobin, a reduction in surface proliferation was recorded only for *A. alternata* colonies (20–41.7%) (Fig. 2, 3). Of the fungi of the genus *Fusarium* tested, the species *F. oxysporum* showed the greatest sensitivity to a 5% concentration of fertiliser in the culture medium. The highest growth inhibition rate for this species was 24% relative to the absolute control (day 12). However, the 5% concentration of fertiliser was less effective in inhibiting the growth of the fungus than the 0.05% concentration of azoxystrobin (Fig. 1, 2). For both ZB concentrations tested, surface colony growth of *F. equiseti* was strongly stimulated compared to the control treatments (Table 1, Fig. 3).

The best fungistatic effects of the ZB 5% fertiliser were obtained during the first days of the experiment (day 4), while with the passage of time its anti-fungal activity significantly decreased The results of the study, however, indicate the possibility of prophylactic use of the Zielony Busz preparation at a concentration of 5% to protect plants against polyphagous phytopathogens, particularly against A. *alternata* and *C. coccodes,* and the need for repeated treatments to maintain proper plant protection.

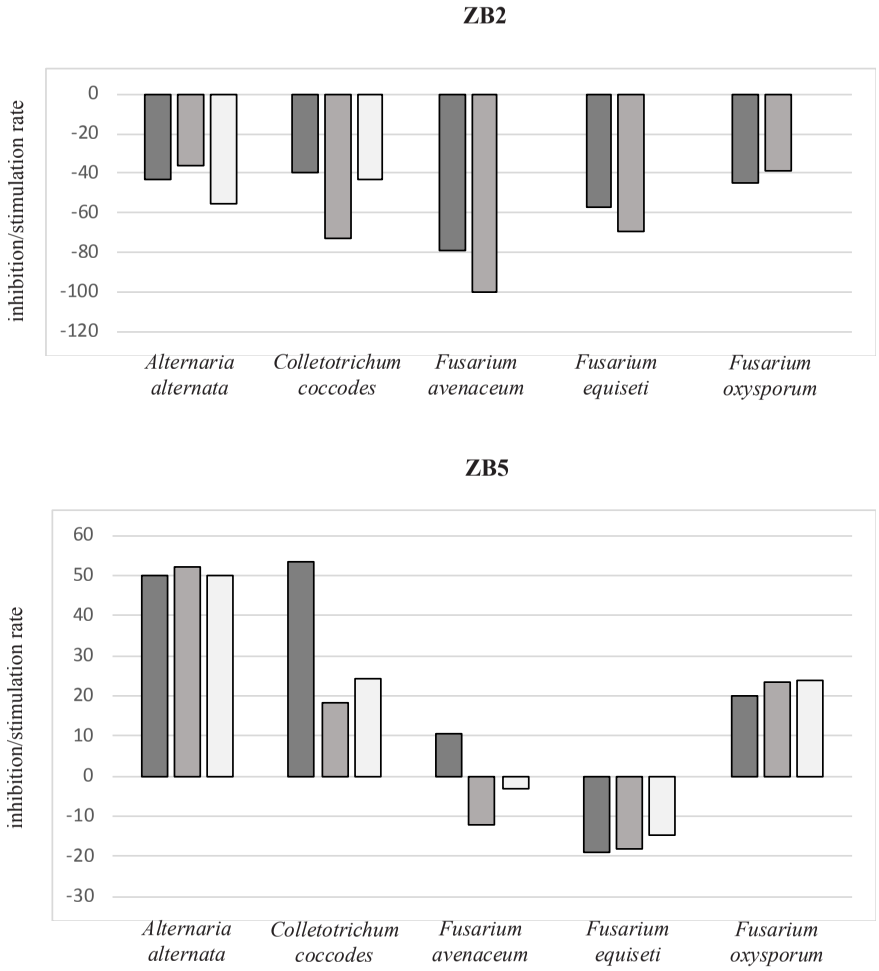

Figure 1. Fungi growth inhibition index (%) compared to the absolute control (C) after application of Zielony Busz (ZB) fertilizer
No measurements were done for ZB2% on the 12th day of the experiment as the fungi *Fusarium* spp. due to their rapid growth have overgrown the entire plates, hence the result would be incorrect
ZB2 – 2% concentration of the preparation, ZB5 – 5% concentration of the preparation

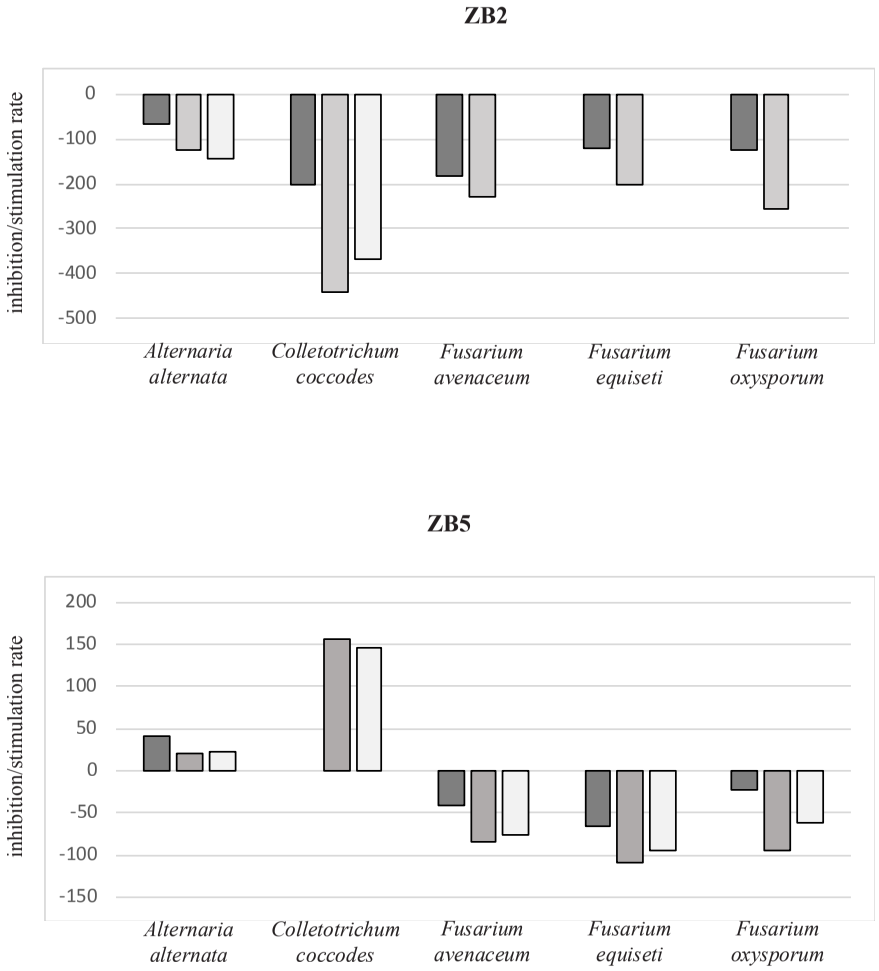

Figure 2. Fungi growth inhibition index (%) compared to the relative control (A) after application of Zielony Busz (ZB) fertilizer
No measurements were done for ZB2% on the 12th day of the experiment as the fungi *Fusarium* spp. due to their rapid growth have overgrown the entire plates, hence the result would be incorrect
ZB2 – 2% concentration of the preparation, ZB5 – 5% concentration of the preparation

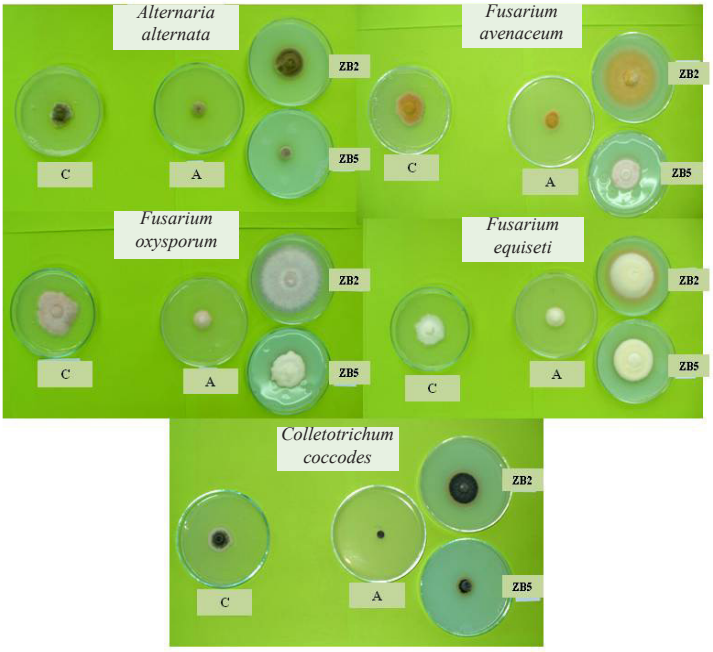

Figure 3. Eight-day-old test fungi colonies.
ZB2 – 2% concentration of the preparation, ZB5 – 5% concentration of the preparation, C – absolute control, A – relative control (azoxystrobin 0.05%)

Table 1. Growth of test fungi on media containing Zielony Busz (ZB) fertiliser.

Experimental combination		Colony diameter [mm]		
		4th day	8th day	12th day
Alternaria alternata	ZB2	20.0a	34.0a	56.0a
	ZB5	7.0c	12.0d	18.0d
	C	14.0b	25.0b	36.0b
	A	12.0b	15.0c	23.0c
	$LSD_{0.05}$	2.1	2.3	3.3
Colletotrichum coccodes	ZB2	21.0a	38.0a	70.0a
	ZB5	7.0c	18.0c	37.0c
	C	15.0b	22.0b	49.0b
	A	7.0c	7.0d	15.0d
	$LSD_{0.05}$	2.2	2.8	6.3
Fusarium avenaceum	ZB2	34.0a	66.0a	90.0a
	ZB5	17.0b	37.0b	60.0b
	C	19.0b	33.0c	62.0b
	A	12.0c	20.0d	34.0c
	$LSD_{0.05}$	2.2	2.8	6.3
Fusarium equiseti	ZB2	33.0a	66.0a	90.0a
	ZB5	25.0b	46.0b	78.0b
	C	21.0c	39.0c	68.0c
	A	15.0d	22.0d	40.0d
	$LSD_{0.05}$	2.6	3.0	1.5
Fusarium oxysporum	ZB2	29.0a	71.0a	90.0a
	ZB5	16.0c	39.0c	60.0c
	C	20.0b	51.0b	79.0b
	A	13.0d	20.0d	37.0d
	$LSD_{0.05}$	1.7	2.7	3.7

ZB2 – 2% concentration of ZB, ZB5 – 5% concentration of ZB, C – absolute control, A – relative control (azoxystrobin)

a, b, c...– values in the columns, for a given fungus species, marked with the same letter are not significantly different at the significance level $p \leq 0.05$

DISCUSSION

Environmental protection is not only one of the greatest challenges for the European Union, but also a priority for action. Increasing climatic and environmental problems are forcing more and more effective solutions, also in agriculture (Wrzaszcz, Prandecki, 2020). These actions result, among other things, in the withdrawal of chemical plant protection products (Góral, Rembisz, 2017), and research into biological preparations is gaining increasing importance (Krzepiłko et al., 2020).

In this study, laboratory tests made it possible to determine the direct effect of the Zielony Busz fertiliser on the growth of phytopathogenic fungi. The preparation, which contains, among others, plant extracts (from wormwood, nettle, garlic, algae) and copper, shows a varied antifungal effect, and the results of the research do not provide a basis for unambiguous determination of the fungistatic effect of the preparation. This is because each species of fungus tested reacted differently to the addition of the fertiliser to the culture medium and its concentration. It should be assumed that plant extracts contained in the ZB preparation with a higher concentration (5%), had a biocidal effect. As reported by many authors, plant extracts are a source of many valuable biologically active substances (Jamiołkowska, 2013; Nabrdalik, Grata, 2015; Sultana et al., 2007). The antimicrobial activity of garlic is attributed to a major active constituent called allicin (Ejaz et al., 2003). This natural biological substance has strong antimicrobial activity both *in vitro* and *in vivo* (Curtis et al., 2004; Hadian, 2012; Jamiołkowska, 2013; Jamiołkowska, Wagner, 2011). The effectiveness of the Zielony Busz fertiliser can also be attributed to the algae in its formulation.

Algae from the *Rhodomelaceae* family produce antibiotics and inhibit the growth of *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Streptococcus pyogenes* and *Proteus subtilis* (Arunkumar et al., 2010; Sultana et al., 2007, 2008, 2011). Also, nettle extract contains many valuable biologically active compounds (including lectins, sterols, terpenes, flavonoids, volatile compounds) with potent antifungal (against *Alternaria alternata, Fusarium oxysporum, F. solani, Rizoctonia solani*) and antibacterial activities (Asgarpanah, Mohajerani, 2012; Bisht et al., 2012; Hadizadeh et al., 2009; Modarresi-Chahardehi et al. , 2012). High fungistatic activity of nettle extract against *A. solani was* also demonstrated by Tapwal et al. (2011) and Nabrdalik, Grata (2015).

When applied at higher concentrations, Zielony Busz fertiliser proved particularly effective in limiting the growth of *A. alternata*, exerting a strong fungistatic effect even compared to 0.05% azoxystrobin (the fungicide's active ingredient). *In vitro* tests also showed an inhibitory effect of ZB in concentration of 5% on *C. coccodes*, the causal agent of plant anthracnose. The results show that the preparation could be used prophylactically to protect plants against anthracnose or in the early stages of the disease, as ZB inhibited the growth of the fungus by 50% in the first days. A major challenge in plant protection is Fusarium blight caused by fungi of the genus *Fusarium* (Arie, 2019; Jamiołkowska, 2013; Mielniczuk, Skwaryło-Bednarz, 2020). *In vitro* tests conducted showed the lack of effective action of the ZB preparation on this group of phytopathogens. A slight fungistatic effect of the preparation was observed at the beginning of the experiment, only with respect to *F. oxysporum*, while the growth of other species was even stimulated. Similar results were obtained by Jamiołkowska and Wagner (2011) indicating that there was no significant effect of a garlic-based preparation (Bioczos Płynny, Himal) on the linear growth of *F. equiseti*, *F. culmorum* and *F. avenaceum in vitro*. Different results are presented by Świerczyńska et al. (2011), who noted an inhibitory effect of Bioczos under laboratory conditions against fungi of the genus *Fusarium*. The antifungal effect of the preparation was enhanced by the content of algae and copper. Ethanolic solutions from marine algae limit the development of grey mould on strawberry fruit and inhibit the growth of *Verticillium* spp., *Rhizoctonia solani*, *Botrytis cinerea*, *Phytophthora cinnamomi under in vitro* and *in vivo* conditions (Arunkumar et al., 2010; Jimenez et al., 2011). Copper, even in the form of nanoparticles, has been shown to inhibit mycelial growth and spores of phytopathogens (Szaniawski et al., 2015). The fungistatic effect of 5% concentration of Zielony Busz fertiliser on species important in phytopathology, such as *A. alternata* or *C. coccodes,* demonstrated in *in vitro* studies, is an encouragement to more extensive research in this area, mainly field experiments determining the effect of the fertilizer on plant health and yield.

CONCLUSIONS

1. The effect of Zielony Busz fertiliser on phytopathogenic fungi, under laboratory conditions, depended on the species of fungus and the concentration of fertiliser in the culture medium.
2. The fertiliser showed the strongest antifungal effect at a concentration of 5% against *A. alternata* and *C. coccodes,* especially during the initial period of the experiment.
3. Zielony Busz applied to the culture medium at a concentration of 5% inhibited the growth of *F. oxysporum,* while at a concentration of 2% it promoted the surface proliferation of all *Fusarium* species tested.
4. A concentration of 5% of the tested formulation provides the most effective fungistatic effect of the fertiliser. In order to maintain the protective efficacy of the preparation, subsequent treatments should be carried out at an interval of a maximum of every 12 days.

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