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THE EFFECT OF ENTOMOPATHOGENIC FUNGI ON THE GROWTH OF *FUSARIUM* FUNGI IN BIOTIC TESTS

Summary

Insecticidal soil fungi are used for the biological control of pests of cultivated plants. Fungi of the Fusarium genus are economically important phytopathogens that produce mycotoxins. The aim of the study was to identify the effect of selected insecticidal fungi on the growth and development of Fusarium fungi. Study material were the strains: F. culmorum, F. avenaceum, F. poae, Beauveria bassiana, Isaria fumosorosea and Metarhizium anisopliae. Identification of interactions between strains was conducted in laboratory conditions using three media: Potato Dextrose Agar, Sabouraud Agar, Czapek-Dox. The cultures were incubated and surface growth of colonies was observed. After culturing, interactions between species were assessed using the individual biotic effect score by Mańka. Fusarium fungi prevailed over entomopathogenic fungi in plate cultures. In most cases, Fusarium fungi obtained a positive and relatively high score when cultured in the presence of entomopathogenic fungi. It was demonstrated that entomopathogenic fungi have a poor antagonistic potential against Fusarium fungi. The entomopathogenic species I. fumosorosea, in some cases limiting the growth of phytopathogens, constituted the exception.
***Key words**: Fusarium, insecticidal fungi, interaction*

WPŁYW GRZYBÓW ENTOMOPATOGENNYCH NA WZROST GRZYBÓW Z RODZAJU *FUSARIUM* W TESTACH BIOTYCZNYCH

Streszczenie

Glebowe grzyby owadobójcze wykorzystuje się do biologicznego zwalczania szkodników roślin uprawnych. Grzyby z rodzaju Fusarium to ważne gospodarczo fitopatogeny wytwarzające mykotoksyny. Celem badań było rozpoznanie wpływu wybranych grzybów owadobójczych na wzrost i rozwój grzybów z rodzaju Fusarium. Materiał badawczy stanowiły szczepy F. culmorum, F. avenaceum, F. poe, Beauveria bassiana, Isaria fumosorosea i Metarhizium anisopliae. Badanie wzajemnych oddziaływań pomiędzy szczepami przeprowadzono w warunkach laboratoryjnych z wykorzystaniem trzech podłoży: Potato Dextrose Agar, Sabouraud Agar, Czapek-Dox. Kultury inkubowano i obserwowano wzrost powierzchniowy koloni. Po hodowli oceniono interakcje między gatunkami według skali indywidualnego efektu biotycznego Mańki. Grzyby Fusarium dominowały w hodowli szalkowej nad grzybami entomopatogennymi. W większości przypadków uzyskano dodatni i stosunkowo wysoki wskaźnik skali dla grzybów Fusarium hodowanych w obecności grzybów entomopatogennych. Wykazano, że grzyby entomopatogenne posiadają słaby potencjał w zakresie działania antagonistycznego względem grzybów Fusarium. Wyjątkiem był entomopatogenny gatunek I. fumosorosea, który w pewnych przypadkach ograniczał wzrost fitopatogenów.
***Słowa kluczowe**: Fusarium, grzyby owadobójcze, interakcja*

1. Introduction

In the contemporary agriculture, biological methods to eliminate harmful organisms are commonly used. Observation of interactions occurring in nature is also gaining popularity. The main mechanisms in the interactions between fungi are: competition for living space and nutrients, mycoparasitism and antibiosis. Various species of fungi react to each other in different way, both in terms of growth and later pathogenicity [2].

The fungi of the Fusarium genus are filamentous fungi considered as highly toxic and common throughout the world [3]. In Europe, the prevailing species are *F. culmorum*, *F. avenaceum*, *F. poae*, *F. solani* and *F. graminearum* [8, 14]. *F. graminearum* mainly occurs in warmer regions where cereal crops are cultivated (USA, Australia, South America and Central Europe), while *F. culmorum* prevails in colder regions (Northwestern Europe) [9]. *Fusarium* fungi are cosmopolitan pathogens of cereals and many other plants. They constitute a serious problem in agriculture, as they infect plants of high economic importance for man [3]. The loss of cereal crops due to their presence may be from 7% up to 70%. By infecting plants at different developmental stages, they lead to many diseases, i.e. fusarioses and stem-base diseases [9]. They are ubiquitous organisms, demonstrating a high tolerance to changing weather and soil conditions, and quickly adapting to the medium on which they live [3]. *Fusarium* fungi also produce hazardous mycotoxins. These compounds are toxic not only for humans and animals, but also for plants and microorganisms [4]. Among soil microorganisms there are few fungal species that limit or completely inhibit the development of *Fusarium* fungi.

Entomopathogenic fungi are an important and widespread component of most ecosystems throughout the world. To date, more than 1000 species have been identified, with approx. 90 genera of insecticidal fungi. Among the most common species are: *Metarhizium anisopliae*, *Isaria fumosorosea* and *Beauveria bassiana*. Their presence has been noted in tropical forests, Canada, Norway, Finland, Greenland and Antarctica [7]. Colonization of the environment by fungi is dependent on many factors, i.e. soil type, insolation, season, temperature and soil use [15]. Entomopathogenic fungi are an important component of inte-

grated plant protection as a limiting factor for arthropod population in crops and a group of pathogens of harmful insects. Studies conducted in some production greenhouses in Poland have demonstrated a high efficiency of biopreparations based on insecticidal fungi in combating the greenhouse whitefly, that reached 75-85% [13].

The aim of the study was to evaluate *in vitro* the individual biotic effect between selected phytopathogenic and entomopathogenic fungi on media with different composition.

2. Methodology

The study material included the following strains of fungi: *F. culmorum* (M40), *F. avenaceum* (M48.1), *F. poae* (M82), *B. bassiana*, *I. fumosorosea* and *M. anisopliae*. The molecularly characterized *Fusarium* strains originated from the Institute of Genetics of the Polish Academy of Sciences and had been isolated from durum wheat. The insecticidal fungi originated from the collection of the Institute of Plant Protection of the National Research Institute in Poznan. Interaction between the entomo- and phytopathogens was tested on commercially available solid media: Potato Dextrose Agar (PDA), Sabouraud Agar (SB) and Czapek-Dox (CD) containing [$g \cdot L^{-1}$]:

– PDA (potato extract 4; glucose 20; agar 15);
– SB (combination of peptones 10; glucose 40; chloramphenicol 0.5; agar 15);
– CD (sodium nitrate 2; magnesium glycerophosphate 0.5; potassium sulphate 0.35; potassium chloride 0.5; iron sulphate 0.01; agar 12).

The tested fungi were inoculated in pairs (entomopathogenic fungus / phytopathogenic fungus) onto pre-poured sterile media in Petri dishes: two discs (∅10 mm) with the inocula of the appropriate fungi in the centre of the plate, at a distance of 20 mm from each other. The plates were incubated at 25°C. Individual cultures of each species were used as controls. Cultures for every combination were conducted in quintuplicate. The diameters of the fungal colonies were measured daily from the inoculation. The growth measurements were used to calculate growth index taking into account the rate of growth of each colony in mm per 1h. Interactions between the fungal species were determined based on the method of assessment of the individual biotic effect, as proposed by Mańka [5], using photographic documentation, conducted after the cultures had ended, using a 4-grade score (4 grades for the assessment of the line of contact of the colonies and 4 grades for the assessment of the inhibition zone, with the maximum antagonistic interaction score of 8).

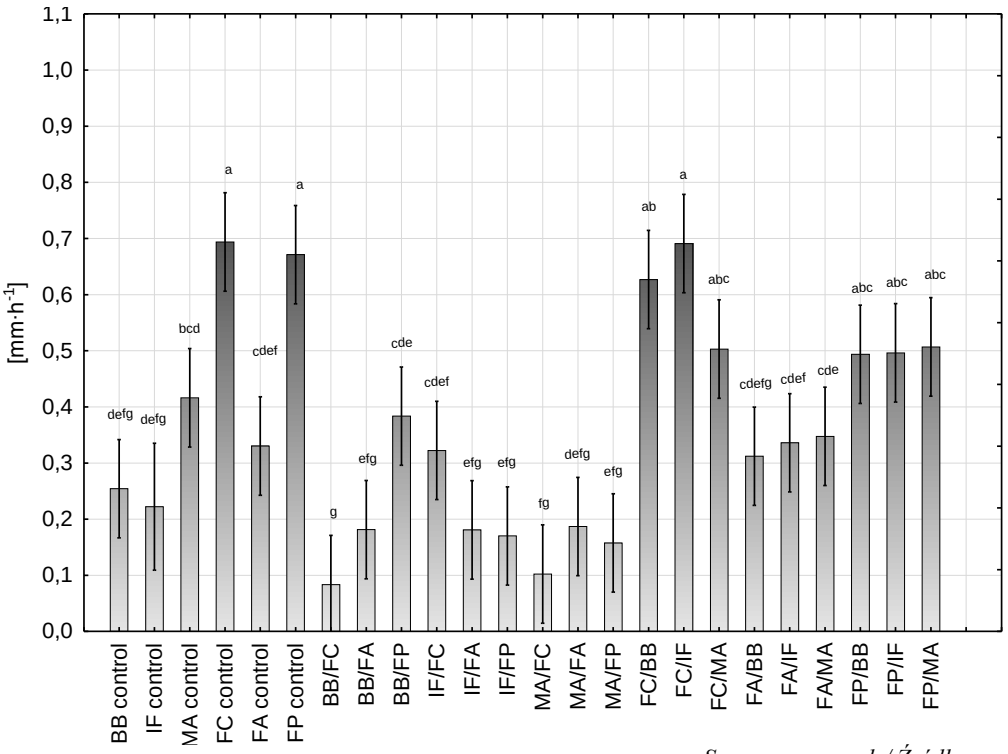
The results were developed statistically in the Statistica 10 software using analysis of variance (ANOVA) and Tuckey's test, with a significance threshold at α=0.05.

3. Results and discussion

The tested species demonstrated various growth rates that depended on the type of medium used. On the PDA medium (Fig. 1), the fastest growth rate was shown by *F. culmorum* and *F. poae*, and the presence of entomopathogenic fungi did not significantly modify their growth rate. It was found that *F. culmorum* had a somewhat lower growth rate in the presence of *B. bassiana* and *M. anisopliae*, and *F. poae* showed a slightly inhibited growth when cultured with all tested insecticidal fungi. On the SB medium (Fig. 2), *Fusarium* fungi demonstrated a slower growth, similar to that of the entomopathogens, with the exception of *B. bassiana*. The tendency observed in the latter case was a poorer growth of *B. bassiana* in cultures with phytopathogenic fungi. The growth of the tested fungi on the CD medium (Fig. 3) was similar to that observed on the PDA medium, but a significant stimulation of *F. culmorum* growth was found in the culture with *B. bassiana*.

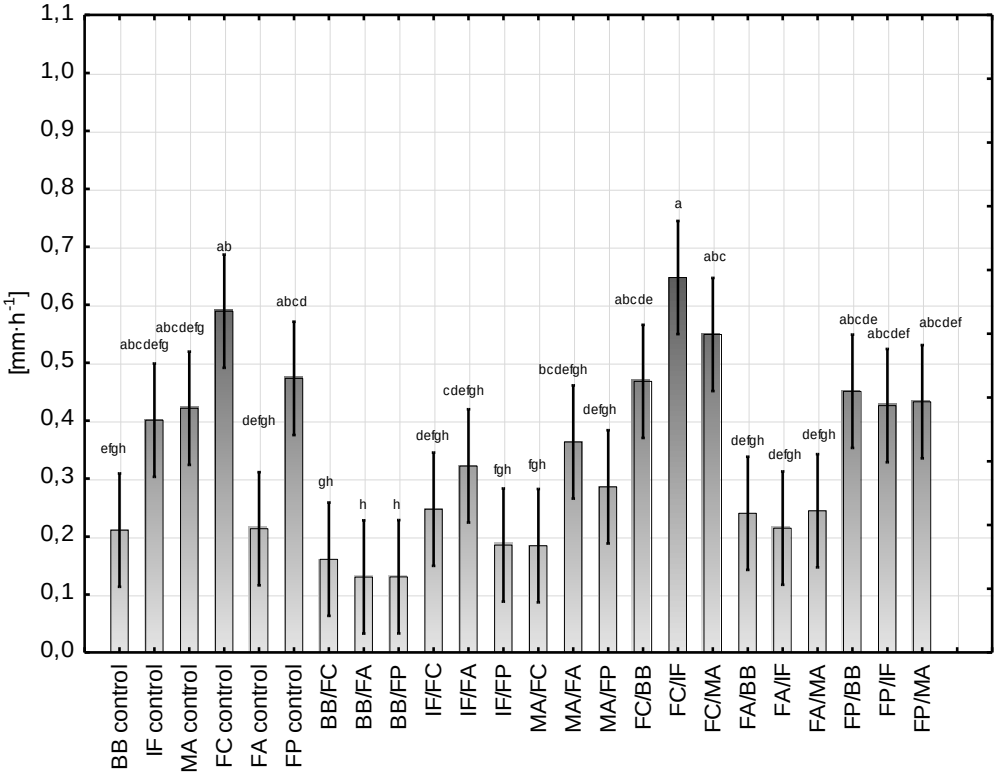
Assessment of individual biotic relations of the tested fungal species after the cultures had ended indicates that *Fusarium* fungi prevail over entomopathogenic fungi in dish cultures (Tab. 1). In most cases, *Fusarium* fungi obtained a positive and relatively high score when cultured in the presence of entomopathogenic fungi, which means that the entomopathogenic fungal colonies were surrounded by *Fusarium* fungi and smaller in size. The exception was the interaction between *F. poae* and *I. fumosorosea* on the CD medium, where the entomopathogenic species relatively strongly prevailed over the phytopathogen. A weaker inhibitory effect on the growth of phytopathogens was noted for: *F. culmorum* co-cultured with *I. fumosorosea* on the PDA medium, and *F. avenaceum* co-cultured with *I. fumosorosea* on the SB medium. Generally, the prevalence of phytopathogens was the most pronounced on the PDA medium dedicated to the phytopathogenic species (mean score of +4), and on the CD (poor mineral medium) and SB (dedicated to entomopathogenic fungi) media, with the mean scores of +3 and +2, respectively. In most cases, the tested entomopathogenic fungi demonstrated negligible antagonistic properties against the *Fusarium* species.

The biotic tests conducted for the two different trophic groups of filamentous fungi indicated that entomopathogenic fungi have a low antagonistic potential against *Fusarium* fungi. The exception was the entomopathogenic species *I. fumosorosea* that, in some cases, limited the growth of phytopathogens. Piegza et al. [10], testing *Trichoderma* (a genus widely recognized as an antagonist of phytopathogenic fungi), observed a similar correlation – the individual biotic effect was dependent on the medium on which the co-culture was conducted and not all strains or species of the *Trichoderma* genus showed antagonistic potential against *Fusarium*. Popiel et al. [11] tested 92 strains of 29 species potentially antagonistic against *Fusarium* and only a few of those strains were deemed as promising, while the study assessed not only the inhibition of growth, but also inhibition of the synthesis of mycotoxins. The authors emphasized that the antagonistic effect can be specified not only for a species, but also for a strain of a given species. It was determined that the fungi strongly antagonistic against toxin-producing *Fusarium* species limited the production of moniliformin (MON) in the co-culture by over 90%, until the total cease of production of this mycotoxin. Cooney et al. [1] demonstrated that 6-pentyl-alpha-pyran (6PAP), a metabolite of *T. harzianum*, can reduce the production of deoxynivalenol (DON) by *F. graminearum* on an agar medium by 66–81%. A very strong reduction of the synthesis of mycotoxins was also found by Matarese et al. [6], with the antagonist often limiting the synthesis of DON by over 90%. In turn, Schöneberg et al. [12] in their assessment of *Trichoderma*, *Clonostachys* and *Cladosporium*, observed that the absence of strong growth inhibition can lead to a serious inhibition of formation of the ascocarps and ascospores of *Fusarium*, which also significantly reduces their pathogenesis in plants.



Source: own work / Źródło: opracowanie własne

Fig. 1. Growth index of entomopathogenic fungi and *Fusarium* fungi on the PDA medium. *Designation of species: F. culmorum (FC), F. avenaceum (FA), F. poae (FP), B. bassiana (BB), I. fumosorosea (IF), M. anisopliae (MA). Index data for a culture of two species regard the species listed first in the description. Values marked with the same letters do not differ significantly at α=0.05*
Rys. 1. Indeks wzrostu grzybów entomopatogennych i grzybów z rodzaju Fusarium na podłożu PDA. Oznaczenie gatunków: F. culmorum (FC), F. avenaceum (FA), F. poae (FP), B. bassiana (BB), I. fumosorosea (IF), M. anisopliae (MA). Dane indeksu dla hodowli dwóch gatunków dotyczą gatunku wymienionego jako pierwszy w opisie. Wartości oznaczone tymi samymi literami nie różnią się istotnie statystycznie na poziomie α=0,05



Source: own work / Źródło: opracowanie własne

Fig. 2. Growth index of entomopathogenic fungi and *Fusarium* fungi on the SB medium. Values marked with the same letters do not differ significantly at α=0.05
Rys. 2. Indeks wzrostu grzybów entomopatogennych i grzybów z rodzaju Fusarium na podłożu SB. Wartości oznaczone tymi samymi literami nie różnią się istotnie statystycznie na poziomie α=0,05

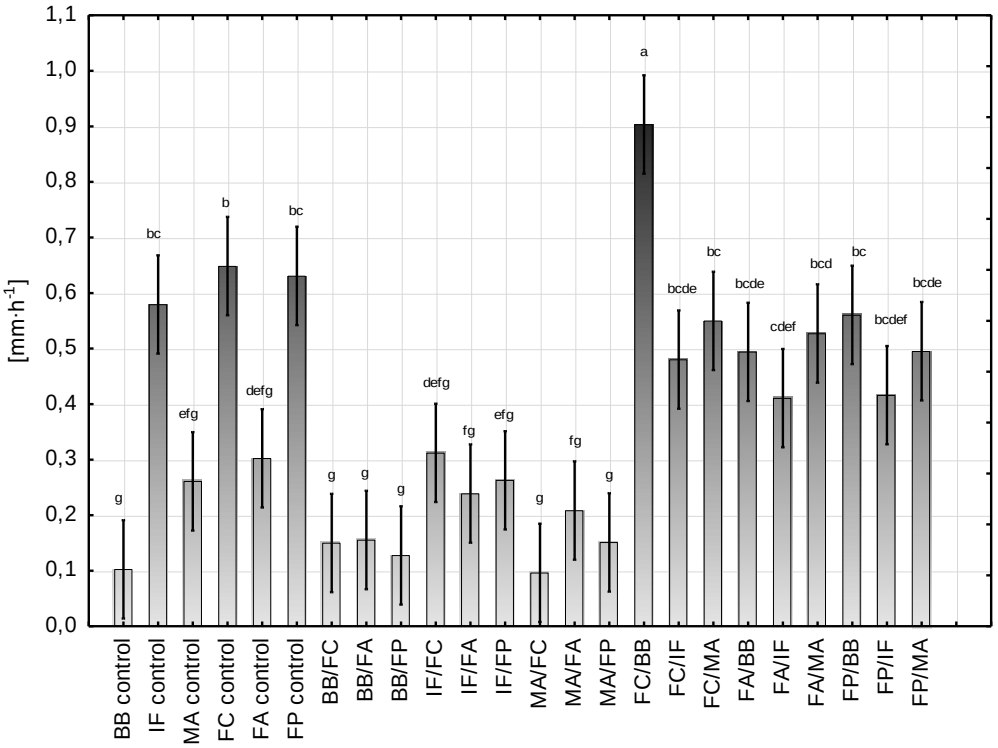


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Fig. 3. Growth index of entomopathogenic fungi and *Fusarium* fungi on the CD medium. Values marked with the same letters do not differ significantly at α=0.05

Rys. 3. Indeks wzrostu grzybów entomopatogennych i grzybów z rodzaju Fusarium na podłożu CD. Wartości oznaczone tymi samymi literami nie różnią się istotnie statystycznie na poziomie α=0,05

Tab. 1. Response of phytopathogenic fungi to entomopathogenic fungi following the biotic relations score by Mańka (1974)

Tab. 1. Reakcja grzybów fitopatogennych na grzyby entomopatogenne według skali stosunków biotycznych Mańki (1974)

Culture medium	Enthomopathogenic species	Phytopathogenic species		
		F. culmorum	*F. avenaceum*	*F. poae*
PDA	*I. fumosorosea*	-2*	0	+7
	B. bassiana	+7	+3	+5
	M. anisopliae	+5	+2	+6
Mean for culture medium		+4**		
SB	*I. fumosorosea*	0	-2	+2
	B. bassiana	+5	nt / nb	+5
	M. anisopliae	+6	nt / nb	+1
Mean for culture medium		+2		
CD	*I. fumosorosea*	0	+5	-5
	B. bassiana	+6	+6	+7
	M. anisopliae	+7	+1	+3
Mean for culture medium		+3		
Mean for phytopathogen species		+4***	+2	+3
Mean for the tested species of phytopathogens		+3****		

nt – not tested / nb – nie badano

**Arithmetic mean grade of each interaction on the repeated plate cultures concerning the degree of surrounding one colony by another, the diameter of the inhibition zone and limited growth in the scale of 0 to 4 °, with negative results indicating an inhibitory effect on the growth of the phytopathogen in the presence of the entomopathogen, and positive results indicating an opposite response.*

***Arithmetic mean grade of each interaction on the medium used.*

****Arithmetic mean grade of each interaction for a given phytopathogenic species.*

*****Arithmetic mean grade of all identified interactions.*

**Średnia arytmetyczna ocena poszczególnych interakcji na powtórzeniach płytkowych dotycząca stopnia otoczenia jednej kolonii przez drugą, szerokości strefy inhibicyjnej, ograniczonego wzrostu w skali 0-4 °, wynik ujemny wskazuje na wpływ ograniczający wzrost fitopatogena w obecności entomopatogena, wynik dodatni na reakcję odwrotną.*

***Średnia arytmetyczna ocena poszczególnych interakcji na zastosowanym podłożu.*

****Średnia arytmetyczna ocena poszczególnych interakcji dla danego gatunku fitopatogena.*

*****Średnia arytmetyczna ocena wszystkich stwierdzonych interakcji.*

Source: own work / Źródło: opracowanie własne

In the case of the species *I. fumosorosea* used in this study, its practical use is aimed at combating pests of plants, but the inhibiting potential of the strain against *Fusarium* fungi, particularly in relation to the possible inhibition of mycotoxin synthesis, can be a significant additional value of using this entomopathogen in biological plant protection. On the other hand, the potential of *Fusarium* fungi to inhibit the growth and development of fungi useful in agrocenoses, as demonstrated in our experiment, is very unfavourable. When insecticidal fungi are used in practice to combat insects, the possibility of less effective action in agrocenoses infested with the pathogenic *Fusarium* fungi will have to be taken into account.

4. Conclusions

1. The tested entomopathogenic fungal strains of the *B. bassiana*, *I. fumosorosea* and *M. anisopliae* species demonstrate a poor potential antagonistic effect against the *F. culmorum*, *F. avenaceum* and *F. poae* fungal strains isolated from durum wheat.
2. Only the *I. fumosorosea* species shows a relatively significant inhibition of *F. poae* growth on the Czapek-Dox medium, and a poor inhibition of *F. culmorum* growth on Potato Dextrose Agar and *F. avenaceum* growth on Sabouraud Agar.
3. The tendency of the *I. fumosorosea* strain to inhibit *Fusarium* fungi can be considered as favourable, but the relatively significant inhibition of the growth and development of insecticidal fungi, useful in agrocenoses, by *Fusarium* fungi observed *in vitro* is highly unfavourable.

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