

## FUNGI ISOLATED FROM *Verbena hybrida* SEEDS, THEIR PATHOGENICITY TO SEEDLINGS AND ATTEMPTS AT THEIR CONTROL

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**Abstract.** As a result of the mycological analysis of vervein seeds, 17 fungi species were identified. They included typical saprobiotic ones, namely *Aspergillus*, *Penicillium*, *Rhizopus*, *Trichothecium* and pathogens such as *Alternaria*, *Botrytis*, *Fusarium*, *Rhizoctonia*. Fungi from the genera *Alternaria*, *Fusarium* and *Botrytis cinerea* constituted the highest proportion. In *in vitro* study the following fungi were the most pathogenic towards vervein seedlings: *A. alternata*, *F. oxysporum*, *F. culmorum*, *B. cinerea* and *R. solani*. The results showed that *in vitro* conditions the growth and development of *A. alternata* and *F. oxysporum* were most effectively inhibited by Zaprava Funaben T and Captan suspension, whereas Topsin M was much less effective and in the case of fungus *A. alternata* it even stimulated the development of mycelium.

**Key words:** *Verbena hybrida*, seeds, fungi, pathogenicity, control

### INTRODUCTION

The seeds of cultivated plants usually transfer numerous microorganisms, including saprobiotic ones and pathogens [Neergaard 1979, Czyżewska 1983]. Saprobionts can considerably lower the seeding quality of the seeds, even disqualifying them, while parasites threaten the germinating seeds, seedlings as well as plants at a later stage of development. Seeds can introduce harmful microorganisms into the soil. The knowledge about the fungi transferred with seeds can also help in the choice of proper seed dressings. The choice of vervein seeds is justified by great popularity of this plant and by a signal of the seedlings dying out.

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## MATERIAL AND METHODS

The experimental material comprised the seeds of garden vervein obtained from “Polan” company. It is a mixture of different cultivars belonging to Compacta group. Seedlings for the studies on pathogenicity were also obtained from the same group of seeds. The following were used in the studies on the effect of fungicides in limiting the growth of mycelium of *Alternaria alternata* and *Fusarium oxysporum*: Zaprava Funaben T (20% carbendazim, benzimidazole + 45% tiuram, ditiocarbaminiam), Captan suspension 50 WP (50% captan, ftalamide) and Topsin M (500 g methyl tiophane in 1 l, benzimidazole).

The mycological analysis was conducted on 200 seeds, including 100 that were disinfected on the surface (70% solution of ethanol, 1 min) and 100 that were not disinfected. The percentage proportion of particular fungi species was established and identification was performed according to the generally accepted methods [Kućmierz 1990].

In *in vitro* conditions studies were carried out on the pathogenicity of 8 fungi species isolated from seeds (*A. alternata*, *A. consortiale*, *Botrytis cinerea*, *Cladosporium herbarum*, *Epicoccum purpurascens*, *F. culmorum*, *F. oxysporum*, *Rhizoctonia solani*) towards vervein seedlings. The seedlings were obtained in flasks with a paper tube moistened with distilled water. The seeds without any disease symptoms were treated with a suspension of spores or the mycelium (1 ml) after they got the first leaves. 40 seedlings were treated with each of the studied fungi. 14 days after inoculation estimation of their healthiness was performed (dead plants, plants with disease symptoms and the ones without any).

The effect of the studied fungicides on the linear growth of the tested fungi, namely *A. alternata* and *F. oxysporum* in *in vitro* conditions was established by means of a generally accepted laboratory method of poisoned medium [Kowalik and Krechniak 1961]. The fungicide dilutions in the medium were 10, 1, 0.1 mg/l. The experiment was conducted in four replications. A replication was made of 2 Petri dishes with an agar ring overgrown with mycelium. Dishes with clear agar constituted the control. The effect of particular preparations was determined by means of Abbot's formula [Borecki 1984]:

$$S = \frac{K - A}{K} \cdot 100,$$

where S – inhibition of the mycelium growth in %, A – diameter of a colony in the combination with the tested fungicide, K – diameter of a colony in control combination.

## RESULTS AND DISCUSSION

As a result of the analysis of garden vervein seeds 167 fungi colonies and 67 bacteria colonies (about 40%) were isolated. The isolated fungi included both typical parasites such as *Alternaria* spp., *Botrytis cinerea* and *Rhizoctonia solani* and typical saprobes from the genera *Aspergillus*, *Penicillium*, *Rhizopus* and *Trichothecium*. The highest percentage proportion was found for *A. alternata*, *A. consortiale*, *F. oxysporum*, *F. culmorum*, *B. cinerea* and *Cladosporium herbarum* (tab. 1).

Table 1. Fungi isolated from *Verbena hybrida* seeds  
 Tabela 1. Grzyby izolowane z nasion werbeny ogrodowej (*Verbena hybrida*)

Species of fungus Gatunek grzyba	Nondisinfected seeds Nasiona niedezynfekowane		Disinfected seeds Nasiona dezynfekowane	
	Number of isolates	%	Number of isolates	%
	Liczba izolatów		Liczba izolatów	
<i>Alternaria alternata</i>	10	9.62	14	22.23
<i>Alternaria chartarum</i>	2	1.92	0	0
<i>Alternaria consortiale</i>	14	13.46	10	15.88
<i>Aspergillus niger</i>	3	2.88	1	1.58
<i>Botrytis cinerea</i>	6	5.76	4	6.35
<i>Cladosporium cladosporioides</i>	7	6.74	3	4.76
<i>Cladosporium herbarum</i>	8	7.70	4	6.35
<i>Epicoccum purpurascens</i>	10	9.62	0	0
<i>Fusarium culmorum</i>	7	6.74	6	9.53
<i>Fusarium oxysporum</i>	10	9.62	9	14.29
<i>Humicola fuscoatra</i>	1	0.96	1	1.58
<i>Penicillium expansum</i>	4	3.89	2	3.17
<i>Penicillium waksmani</i>	3	2.88	0	0
<i>Rhizoctonia solani</i>	4	3.85	4	6.35
<i>Rhizopus nigricans</i>	6	5.76	2	3.17
<i>Trichoderma koningii</i>	3	2.88	3	4.76
<i>Trichothecium roseum</i>	6	5.76	0	0
Total – Suma	104	100.00	63	100.00

Table 2. Pathogenicity of tested fungi (isolated from seeds) to *Verbena hybrida* seedlings  
 Tabela 2. Patogeniczność wybranych gatunków grzybów (izolowanych z nasion) dla siewek *Verbena hybrida*

Fungus Grzyb	Percentage of dead seedlings	Percentage of seedlings with disease symptoms	Percentage of seedlings without disease symptoms
	Procent siewek zmarłych	Procent siewek z objawami chorobowymi	Procent siewek bez objawów chorobowych
<i>Alternaria alternata</i>	6.2	43.2	50.6
<i>Alternaria consortiale</i>	2.1	29.6	68.3
<i>Botrytis cinerea</i>	5.0	40.1	53.9
<i>Cladosporium herbarum</i>	0	17.8	82.2
<i>Fusarium culmorum</i>	3.8	44.5	51.7
<i>Fusarium oxysporum</i>	6.1	37.1	56.8
<i>Epicoccum purpurascens</i>	0	18.8	81.2
<i>Rhizoctonia solani</i>	5.6	38.1	56.3
Control – Kontrola	0	0	100.0

Surface disinfection of the seeds only insignificantly limited the number of the isolated fungi species, mainly saprobic ones (*A. chartarum*, *E. purpurascens*, *P. waksmani*, *T. roseum*), while clearly inhibiting the number of isolated fungi colonies. The number of the colonies of the pathogen increased only in the case of *A. alternata* after surface disinfection, which can suggest internal seed colonization.

As a result of *in vitro* experiments on the pathogenicity of 8 fungi species, all showed pathogenic properties towards the seedlings of vervein (tab. 2). The highest pathogenicity was observed in the case of seed inoculation with the fungi *A. alternata*, *F. culmorum*, *B. cinerea*, *F. oxysporum* and *R. solani*. It is interesting to find out patho-

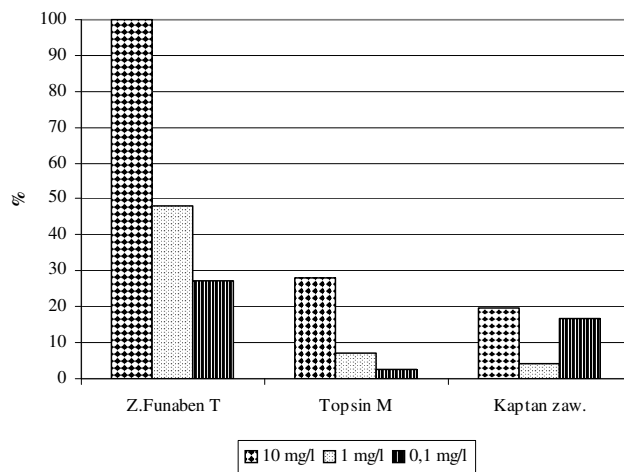


Fig. 1. The extent of linear growth decrease (in %) of *Fusarium oxysporum* according to fungicide concentration (*in vitro*)

Rys. 1. Procent zahamowania wzrostu liniowego grzybnii *Fusarium oxysporum* w zależności od badanego fungicydu i jego stężenia (*in vitro*)

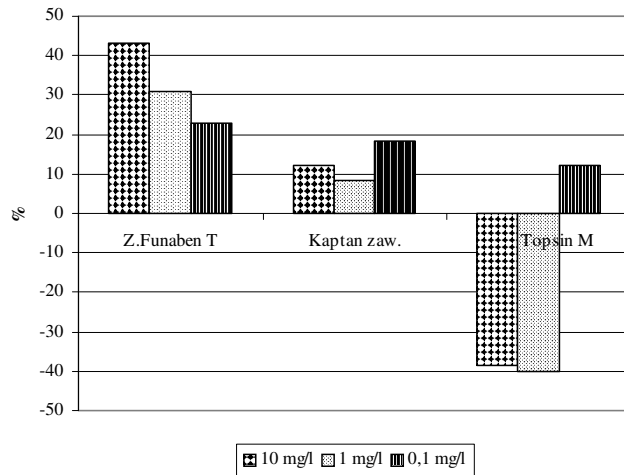


Fig. 2. The extent of linear growth decrease (in %) of *Alternaria alternata* according to fungicide concentration (*in vitro*)

Rys. 2. Procent zahamowania wzrostu liniowego grzybnii *Alternaria alternata* w zależności od badanego fungicydu i jego stężenia (*in vitro*)

genic properties of *C. herbarum* and *Epicoccum purpurascens*, which both in older and new literature [Filipowicz and Sońta 2000] are often considered to be saprobiotic. Already in 1988 it was stated that these fungi show pathogenic properties towards carrot roots [Kućmierz 1988]. Then a few papers appeared which treated about pathogenic properties of *C. herbarum* and *E. purpurascens* towards the seedlings of garden pansy [Kućmierz and Cerek 1990], spinach [Kućmierz et al. 2004], sweet basil, common bahu and peppermint [Szczeponek 2004]. It follows from the studies by Bartyńska and Kochmańska [1998] that *E. purpurascens* belonged to the strongest pathogens of the seedlings of *Gypsophila paniculata* L. at the initial stages of their development. Hence, it should be admitted that both *C. herbarum* and *E. purpurascens* have pathogenic properties and their intensity is related to the environmental conditions for the growth and development of the plant (weakening or injuring).

As follows from laboratory studies, the highest effectiveness in inhibiting the growth of *F. oxysporum* mycelium was characteristic of preparation Zaprawa Funaben T, while Topsin M and Captan suspension had a much poorer effect. Certain differentiation in the effect depended on the concentration of the preparation in the medium (fig. 1). Funaben T also proved to be the most effective in limiting the mycelium growth in the case of fungus *A. alternata*, although this effect was much weaker than for *F. oxysporum*. Topsin M turned out to be the weakest and in higher concentrations it even stimulated the mycelium growth (fig. 2). A similar phenomenon of stimulation of the fungi from genus *Alternaria* under the effect of benzimidazoles was observed by other authors [Borecki 1984].

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### **GRZYBY WYIZOLOWANE Z NASION WERBENY OGRODOWEJ (*Verbena hybrida*) ORAZ PATOGENICZNOŚĆ WYBRANYCH GRZYBÓW DLA SIEWEK I PRÓBY ICH ZWALCZANIA**

**Streszczenie.** W wyniku analizy mikologicznej nasion werbeny ogrodowej zidentyfikowano 17 gatunków grzybów. Są wśród nich zarówno typowe saprobionty: *Aspergillus*, *Penicillium*, *Rhizopus*, *Trichothecium*, jak i patogeny: *Alternaria* spp., *Botrytis cinerea*, *Fusarium* spp., *Rhizoctonia solani*. Największy udział procentowy miały grzyby z rodzajów: *Alternaria*, *Fusarium*, *B. cinerea*, *R. solani* oraz *C. herbarum*. W doświadczeniach *in vitro* najbardziej chorobotwórczymi dla siewek werbeny okazały się: *A. alternata*, *F. oxysporum*, *F. culmorum*, *B. cinerea* i *R. solani*, zaś znacznie słabszymi: *Epicoccum purpurascens* i *Cladosporium herbarum*. W warunkach laboratoryjnych najskuteczniej hamowały wzrost grzybnii *A. alternata* i *F. oxysporum* Zaprawa Funaben T i Captan zawieszinowy, znacznie słabiej Topsin M, który w przypadku grzyba *A. alternata*, w wyższych stężeniach nawet stymulował wzrost grzybnii.

**Słowa kluczowe:** *Verbena hybrida*, nasiona, grzyby, patogeniczność, zwalczanie

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