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THE EFFECT OF PESTICIDES USED IN APPLE ORCHARDS ON ENTOMOPATHOGENIC FUNGI *IN VITRO*

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ABSTRACT

The isolates of fungi *Beauveria bassiana* (*Bb*), *Metarhizium anisopliae* (*Ma*) *and Paecilomyces fumosoroseus* (*Pf*) from the arable land (*Bb I, Ma I, Pf I*) as well as from the herbicide fallow (*Bb II, Ma II, Pf II*) of an apple orchard were inoculated on Sabouraud's medium with an addition of 2 herbicides (Chwastox Extra 300 SL and Roundup) and 3 insecticides (Zolone 35 EC, Basudin 205 EC and Sanmite 20 WG) in the dose 10 times higher than the recommended one (A), the recommended dose (B) and 10 times lower than the recommended one (C). It was shown that the isolates of fungi that in the natural conditions had a contact with herbicides were mostly more resistant to the toxic properties of pesticides as compared with the isolates from the arable land. Herbicides inhibited the growth of fungi more strongly than insecticides. Generally, the fungus *P. fumosoroseus* was more sensitive to herbicides than *B. bassiana.* Among the herbicides, Chwastox Ex. 300 SL proved more toxic to entomopathogenic fungi than Roundup. Chwastox Ex. 300 SL in the lowest dose (A) inhibited the growth of all the isolates till the end of the experiment. Roundup inhibited the growth of the studied fungi. At the lowest concentration (C), it even stimulated the growth of mycelium *Ma II* and *Bb II.* The studied insecticides were the most toxic towards *B. bassiana.* Zolone 36 EC was the insecticide that inhibited the growth in the strongest manner. At the highest dose it made the growth of all fungi isolates impossible till the end of the experiment. Sanmite 20 WG showed the least toxicity towards the studied fungi. At all the concentrations the colonies grew very intensively, frequently reaching the size approaching the control. Under the effect of herbicides and insecticides the majority if the fungi showed changes in the structure, colouring and germination of the colonies. The studies found no negative consecutive effect of pesticides on the growth of the mycelium of the examined isolates, which at the end of the experiment reached the size at the level of the control. It was also found out that changes in the germination of fungi colonies under the effect of pesticides disappeared with the lack of the factor that caused those changes. Pesticides had no negative effect on the sprouting of the spores.

Key words: pesticides, entomopathogenic fungi, growth, sprouting of spores.

INTRODUCTION

Most apple orchards in Poland are within the traditional scheme of plant protection, where several spraying treatments are performed in one vegetative season. It is estimated that in 1998 the level of the active substance used in pesticides in the orchards was 18.5 kg ha^{-1}, including 3.6 kg ha^{-1} of herbicides, 1.8 kg ha^{-1} of insecticides and as much as 13 kg ha^{-1} of fungicides [9]. Although fungicides dominate in orchard protection, the use of insecticides and herbicides themselves per 1 ha of an orchard is higher than the mean use of pesticides per 1 ha of the cultivated area in Poland. In 1998 it was estimated at the level of about 0.6-0.8 kg of the active substance [21].

In commercial orchards, the herbicide fallow is the most contaminated environment, where in one vegetative year several treatments of weed control are performed, and these are additionally supplemented by the chemicals used against pathogens and pests.

The advantages resulting from the application of synthetic means of plant protection are obvious; unfortunately, one of the consequences of their use is often contamination of the surface and subsoil waters with the remains; ecological threat of the agrocenoses or whole biotopes; selection of resistant breeds of pests; “generation” of new pests; dangers to the health and life of man and other homoeothermic organisms [20].

Entomopathogenic fungi belong to a group of useful microorganisms that in natural conditions play a very important role in limiting the populations of the agrophagous. They remarkably reduce the populations of such important orchard pests as codling moth, moths of the family *Lasiocampidae* or brown-tail moth [6, 7, 8, 10, 12]. The main habitat of entomopathogenic fungi is the soil. The populations of soil pests living there such as cockchafer gubs as well as the typical pests of apple trees such as codling moth, which have only a temporary contact with the soil can be subjected to fungous infections to a large extent [4, 5, 18].

Therefore, it seems purposeful to establish the effect of insecticides and herbicides used in apple orchards on entomopathogenic fungi coming from the environments of different degrees of pesticide contamination.

MATERIALS AND METHODS

The experiment tested the following species of entomopathogenic fungi: *Beauveria bassiana* (Bals.) Vuill., *Metarhizium anisopliae* (Metsch.) Sorok and *Paeciliomyces fumosoroseus* (Wize) Brown et Smith. The fungi were isolated from the soil using the method of bait insects [26, 27] against the larvae of the last-but-one larval stage of *Galleria mellonella* L. Two fungi isolates from each species were tested. The first isolate (*Bb I, Ma I, Pf I*) came from the soil from an arable field adjoining the orchard, where the use of pesticides was small, while the second isolate (*Bb II, Ma II, Pf II*) was isolated from the soil of the herbicide fallow, where the use of plant protection preparations was high.

The studies dealt with synthetic insecticides and herbicides (tab. 1) that were most frequently used in the protection of apple orchards, where the entomopathogenic fungi came from. Each of the preparations was applied in the following doses:

A – 10 times as high as the recommended one,
B – the one recommended in practice,
C – 10 times as low as the recommended one.

Table 1. Pesticides used in the experiment

Commercial name of preparation	Name and content of active substance g or %	Recommended dose g or ml l^{-1}	Toxicity class	Producer
Insecticides				
Basudin 25 EC	diazynon 25%	1.5 ml	III	Novartis Crop Protection AG – Szwajcaria
Sanmite 20 WP	pirydaben 20%	1 g	IV	NM Agro – Polska Sp. z. o. o.
Zolone 35 EC	fozalon 35%	2.9 ml	II	Rhone-Poulenc Agrochimie
Herbicides				
Chwastox Extra 300 SL	natrium salt MCPA 300 g	13 ml	III	Zakłady Chemiczne Organika-Sarzyna w Nowej Sarzynie
Roundup	isopropyloamine gliphosate salt 41%	24 ml	IV	Monsanto Europe S. A – Belgia Monsanto Polska Sp. zo.o.

Pesticides were added to the sterile medium of Sabouraud (SDA) cooled to the temperature of about 55°C, while the subsequent concentrations were obtained by the method of dilutions. The medium prepared in such a way was poured into the Petri dishes. The control consisted of the fungi growing on SDA medium without an addition of pesticide. The experiment was set in 5 repetitions. The culture was kept at the temperature of 22°C for the period of 2 days. Every five days the diameter of the fungi colonies was measured and observations on the morphological properties of the colonies were carried out.

When significant changes were found in the appearance of the colonies, after the experiment they were inoculated to the SDA medium without an addition of pesticide in order to determine the persistence of those changes.

When significant deviations were found in the germination of fungi growing on the media with pesticide addition, the consecutive effect of pesticides on the sprouting of the germs was established. To this aim, the suspension of spores coming from the selected combinations was placed on the basic slides with a layer of 2% sterile agar. The experiment was set in 3 repetitions and it was continued at the temperature of 22-23°C. The sprouting spores, in the number of 100 in each repetition, were counted under a microscope for the 3 successive periods of 24 hours.

RESULTS AND DISCUSSION

The effect of pesticides on the growth and morphology of entomopathogenic fungi colonies *in vitro*

Out of the group of the tested pesticides, herbicides were stronger in inhibiting the growth of entomopathogenic fungi than insecticides (tab. 2 and 3). The negative effect of herbicides on different entomopathogenic fungi is also reported by Gardner and Storey [3] as well as Miętkiewski et al. [17].

The preparation that was the most toxic towards the studies fungi was Chwastox Ex. 300 SL (active substance 9s.a.) MCPA), which in the dose 10 times higher than the recommended one (A) inhibited their growth throughout the experiment, and in the recommended dose (B) limited this growth strongly, especially in the case of both isolates of *P. fumosoroseus* (fig. 1). The toxic effect of high doses of sodium salt MCPA towards different species of *Hyphomycetales* and *Mycophyta* is also described by Miętkiewski et al. [16, 17].

Table 2. The size of colonies of isolates *B. Bassiana, M. anisopliae* and *P. fumosoroseus* on the medium with herbicides (% in relation to the control)

No. of isolate	Day after inoculation	Preparation					
		Chwastox Ex 300 SL			Roundup		
		A	B	C	A	B	C
Bb I	5	bw	48.7±0.57	89.9±0.61	pw	79.2±0.61	79.2±0.35
	20	bw	51.3±1.92	74.5±0.71	47.0±1.52	78.1±1.15	86.3±2.46
Bb II	5	bw	60.3±3.40	93.5±2.14	pw	65.9±0.87	82.9±0.57
	20	bw	40.0±2.45	89.5±2.22	76.0±0.55	94.8±0.79	105.9±0.75
Ma I	5	bw	42.7±1.82	80.2±1.44	bw	65.7±0.45	99.3±0.22
	20	bw	47.7±1.71	93.3±1.66	6.7±0.76	56.0±0.55	80.5±1.08
Ma II	5	bw	46.2±0.96	100.0±0.96	pw	62.0±0.71	94.9±0.71
	20	bw	44.8±1.52	72.1±3.73	21.1±1.64	81.3±1.68	121.9±1.35
Pf I	5	bw	52.0±1.35	92.1±1.49	pw	41.5±0.55	73.1±0.61
	20	bw	33.2±2.67	71.5±3.80	42.0±2.48	60.0±2.04	57.7±1.14
Pf II	5	bw	47.7±0.84	108.7±0.84	pw	57.2±0.74	67.4±0.76
	20	bw	28.1±3.35	65.3±3.84	47.0±2.04	59.3±1.88	67.7±1.25

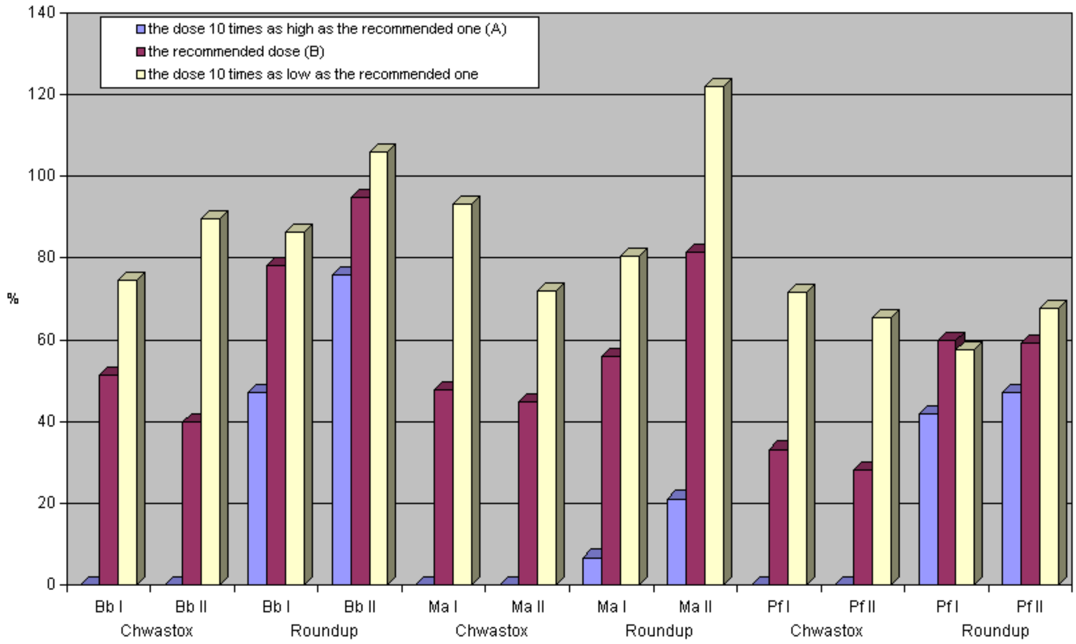
Abbreviations:
BbI, II – isolates of fungus *Beauveria bassiana*
MaI, II – isolates of fungus *Metarhizium anisopliae*
PfI, II – isolates of fungus *Paecilomyces fumosoroseus*
A – concentration of the preparation in the medium 10 times higher than the recommended one
B – concentration of the preparation corresponding to the field dose
C – concentration of the preparation in the medium 10 times lower than the recommended one
bw – no growth
pw – beginning of growth
± – standard deviation

Table 3. The size of colonies of isolates *B. Bassiana, M. anisopliae* and *P. fumosoroseus* on the medium with insecticides (% in relation to the control)

No. of isolate	Day after inoculation	Preparation								
		Basudin 25 EC			Sanmite 20 WG			Zolone 35 EC		
		A	B	C	A	B	C	A	B	C
Bb I	5	bw	18.5±1.50	51.7±1.10	60.4±0.74	68.3±0.74	68.3±0.65	bw	pw	69.3±0.94
	20	17.1±2.25	30.9±1.55	73.5±0.58	71.0±2.38	74.0±2.37	69.5±0.82	bw	26.9±1.44	71.7±2.08
Bb II	5	bw	pw	60.9±1.71	54.3±0.50	75.2±0.48	76.0±0.57	bw	pw	36.4±0.67
	20	15.5±2.96	46.9±1.06	81.5±3.18	80.0±1.68	84.7±1.78	82.4±1.64	bw	30.5±1.87	54.2±1.64
Ma I	5	bw	15.4±1.00	54.2±1.89	35.7±0.71	65.7±0.57	77.9±1.82	bw	pw	45.0±0.76
	20	15.6±1.84	30.4±1.19	79.3±4.24	53.3±1.27	76.8±0.42	90.6±1.92	bw	28.4±3.70	38.4±1.15
Ma II	5	bw	13.9±0.65	75.5±0.58	57.7±0.74	73.0±0.79	92.0±0.82	bw	pw	45.3±0.91
	20	20.1±1.85	34.7±2.47	69.5±0.00	92.5±0.61	95.9±3.34	100.2±1.88	bw	46.0±1.60	71.0±1.62
Pf I	5	bw	39.2±2.01	77.1±0.00	63.1±0.91	83.1±0.97	80.0±0.89	bw	pw	78.5±1.44
	20	19.7±2.50	54.1±3.55	95.3±3.82	64.4±1.08	69.1±0.89	74.2±1.44	bw	53.0±1.85	87.7±1.29
Pf II	5	pw	52.3±1.04	81.0±1.19	59.4±0.84	71.7±1.47	84.1±0.65	bw	pw	74.6±1.68
	20	12.7±0.96	48.9±2.08	73.8±4.44	72.1±1.52	74.2±0.76	77.0±0.55	bw	54.2±2.45	89.5±1.46

Abbreviations:
BbI, II – isolates of fungus *Beauveria bassiana*
MaI, II – isolates of fungus *Metarhizium anisopliae*
PfI, II – isolates of fungus *Paecilomyces fumosoroseus*
A – concentration of the preparation in the medium 10 times higher than the recommended one
B – concentration of the preparation corresponding to the field dose
C – concentration of the preparation in the medium 10 times lower than the recommended one
bw – no growth
pw – beginning of growth
± – standard deviation

Fig. 1. The size of isolate colonies *B. bassiana* (*BbI, BbII*), *M. anisopliae* (*Ma I, Ma II*) and *P. fumosoroseus* (*Pf I, Pf II*) on the beddings with herbicides on the 20th day of the culture (% in relation to the control)



Chwastox Ex. 300 SL in the recommended dose (B) affected the change of the morphological properties of the studied fungi (photo 1). The colonies of isolate *Bb I* formed a flat mycelium, which was slightly protuberant in the central part, covered with delicate hyphae of the air mycelium, where no sporulation was observed (photo 2). Colonies *Bb II* were slightly folded, with clusters of high air mycelium on the edges, where no sporulation was observed either (photo 2). Colonies *Ma II* were grey, with visible light hyphae of air mycelium on the sides, coming off from the bedding (photo 3). The colonies of isolate *Pf II* did not sporulate and formed a white, pinnate mycelium creeping on the surface (photo 4).

Photo 1. 20-day-old colonies of *Beauveria bassiana, Metarhizium anisopliae* and *Paecilomyces fumorosoreus* on the medium with herbicide Chwastox Ex 300 SL:
K – control, A – concentration 10 times as high as the recommended one, B – concentration corresponding to the recommended one, C – concentration 10 times as low as the recommended one, *Bb I, II* – isolates of fungus *Beauveria bassiana, Ma I, II* – isolates of fungus *Metarhizium anisopliae, Pf I, II* – isolates of fungus *Paecilomyces fumosoroseus*

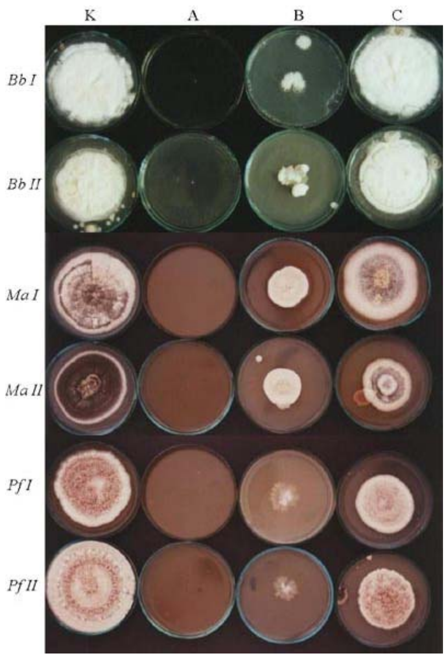


Photo 2. Morphology of 20-day-old colonies *Beauveria bassiana*:
K – control, B – Chwastox Ex 300 SL in the concentration corresponding to the field dose, *Bb I, II* – isolates of fungus *B. bassiana*

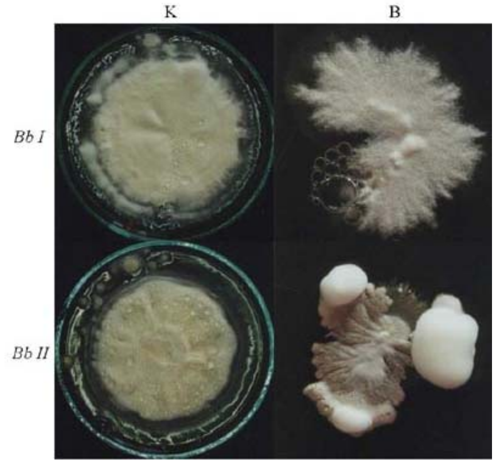


Photo 3. Morphology of 20-day-old colonies *Materhizium anisopliae*:
K – control, B – Chwastox Ex 300 SL in the concentration corresponding to the field dose, *Ma I, II* – isolates of fungus *M. anisopliae*

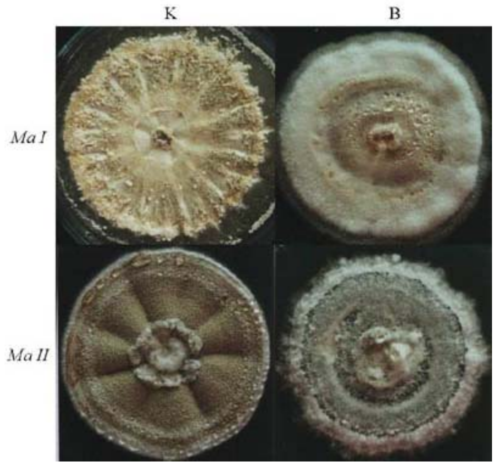
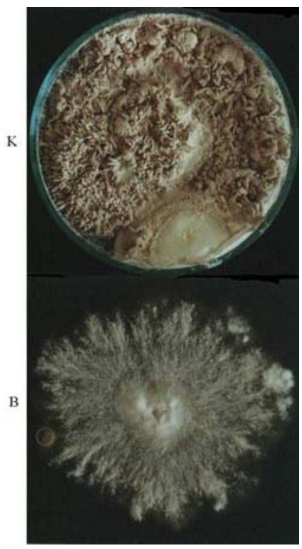


Photo 4. Morphology of 20-day-old colonies of isolate *Pf II* (*Paecilomyces fumosoroseus*):
K – control, B – Chwastox Ex 300 SL in the concentration corresponding to the field dose

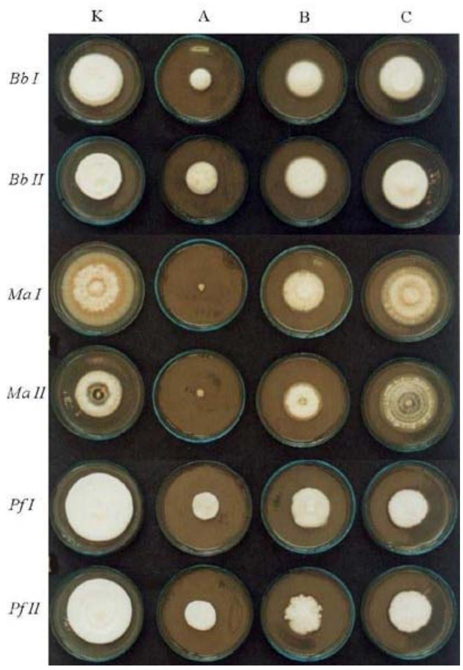


Chwastox Ex. 300 SL in the concentration 10 times of lower than the recommended one had only a slight effect on the studied fungi. Both isolates of *P. fumosoroseus* which are the most susceptible to this preparation developed like in the control in the first stage of the experiment. However, on the 20the day of the control the rate of their growth clearly decreased (tab. 2). Similar results were obtained by Miętkiewski and Sapieha [14].

Chwastox Ex. 300 SL in the dose 10 times lower than the recommended one did not affect the change of the appearance of the colonies of the tested fungi.

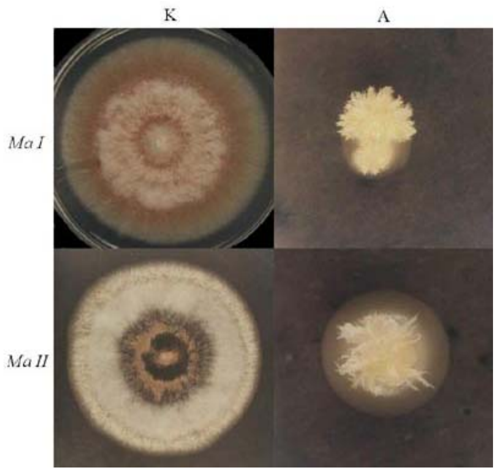
Preparation Roundup (s. a. gliphosat) at the concentration 10 times higher than the recommended one (A) initially had a similar effect to Chwastox Ex. 300 SL, completely inhibiting the growth of fungi (tab. 2). However, on the 20^{th} day of the culture the growth of all isolates was fairly intensive (photo 5). The isolates of fungi from the herbicide fallow were more resistant to the dose of A Roundup. Isolate *Bb II*, growing most intensively, formed colonies of the size of about 76% as compared to the control, while *Ma I,* the most susceptible one, formed colonies of the size of only about 6.7% in comparison to the control colonies (fig. 1). Similar reactions of the insecticide hyphae to high doses of gliphosat were also recorded by Miętkiewski et al. [15, 16].

Photo 5. 20-day-old colonies of *B. Bassiana, M. anisopliae* and *P. fumosoroseus* on the medium with herbicide Roundup:
K – control, A – concentration 10 times as high as the recommended one,
B – concentration corresponding to the recommended one,
C – concentration 10 times as low as the recommended one, *Bb I, II* – isolates of fungus *Beauveria bassiana, Ma I, II* – isolates of fungus *Metarhizium anisopliae, Pf I, II* – isolates of fungus *Paecilomyces fumosoroseus*



Isolate *Bb II*, growing on the bedding with Roundup in the dose 10 times as high as the recommended one (A) formed flat, cream-yellow colonies with no signs of sporulation. Fungus *M. anisopliae* formed yellow-beige colonies, mucilaginous with thick spiked hyphae (photo 6). Fungus *P. fumosoroseus* had the mycelium of atypical white colouring with no signs of the colonies' sporulation.

Photo 6. Morphology of 20-day-old colonies *Metarhizium anisopliae*:
K – control, A – Roundup in the concentration 10 times as high as the recommended one, *Ma I, II* – isolates of fungus *M. anisopliae*



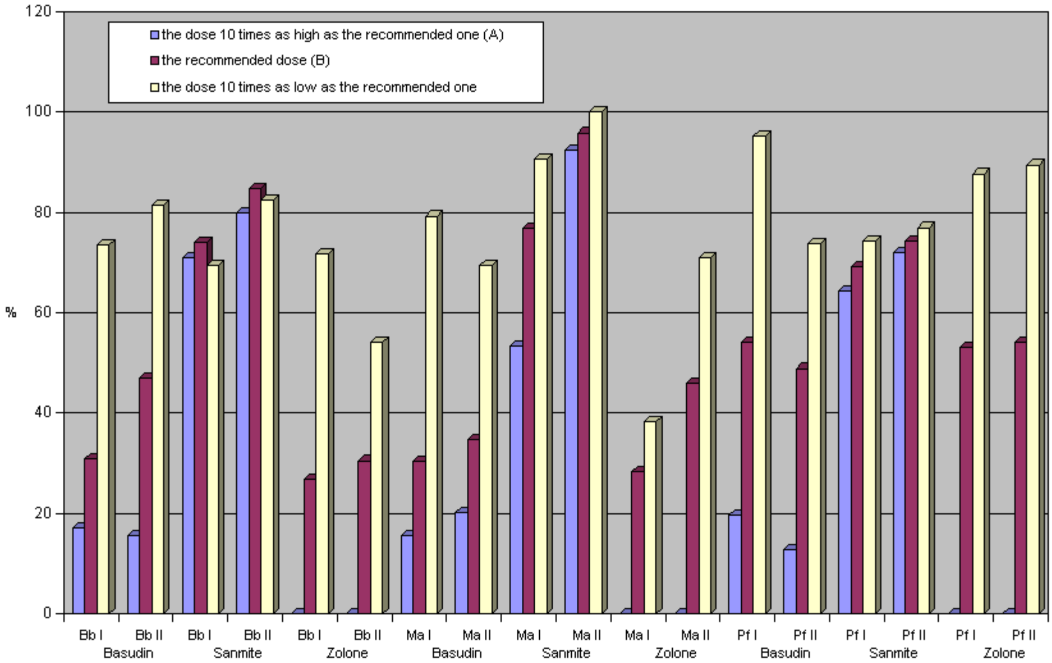
The recommended dose (B) of preparation Roundup and the dose 10 times lower (C) had a smaller effect on the studied entomopathogenic fungi (tab. 2). A specially intensive growth was observed in the case of both isolates *B. bassiana* and isolate *Ma II* from the herbicide fallow (fig. 1). Colonies *Bb II* and *Ma II* on the beddings with the lowest dose (C) of gliphosat were bigger than the control colonies on the 20th day after inoculation. The stimulating effect of low doses of certain herbicides is confirmed in the studies by Wojciechowska et al. [25].

Generally, Roundup in doses B and C did not have any significant effect on the appearance of fungi colonies. The majority of isolates showed poorer sporulation. Only isolate *Ma II* formed a flat colony, with strong fructification.

Out of the insecticides used in the experiment, Zolone 35 EC (s. a. phosalon) was characterized by high toxicity towards the studied fungi (tab. 3). This preparation applied in the dose 10 times as high as the recommended one (A) inhibited the growth of all fungi (tab. 3).

Phosalon in the field concentration (B) strongly limited the growth of fungi colonies at the fist stage of the experiment – signs of growth were observed. On the 20th day after inoculation, colonies *B. bassiana* and isolate *Ma I* reached the size that did not exceed 30% of the control (fig. 2).

Fig. 2. The size of isolate colonies *B. bassiana* (*BbI, BbII*), *M. anisopliae* (*Ma I, Ma II*) and *P. fumosoroseus* (*Pf I, Pf II*) on the beddings with insecticides on the 20th day of the culture (% in relation to the control)



Zolone 35 EC on the beddings in the dose 10 times as low as the recommended one (C) caused a more intensive growth of all fungi, and fungus *P. fumosoroseus* formed colonies comparable to the control as for the size. The toxic effect of Zolone 35 EC, belonging to photoorganic insecticides towards entomopathogenic fungi, is also reported by Olmert and Kenneth [19], Bajan et al. [2] and Vänninen and Hokkanen [23].

The presence of preparation Zolone 35 EC in the bedding affected the morphology of growing fungi (photo 7). Isolate *Bb I*, under the effect of the recommended dose, formed flat colonies, with visible cracks in the central part, with a fluffy roll of air mycelium on the edges (photo 8). Isolate *Ma I* formed a beige-pink mycelium, slightly protuberant (photo 9). The colonies of isolate *Ma II* were light, slightly folded, with hyphae fragments coming off the bedding (photo 9). Fungi colonies were characterized by poor sporulation. Phosalon in the dose C – 10 times as low as the recommended one caused changes in the appearance of cultures *B. bassiana* and *M. anisopliae*. Isolate *Bb I* formed flat colonies, radially cracked, with a belt of air mycelium on the edges (photo 8). Colonies *Bb II* were more compact and they sporulated very intensively. Fungus *M. anisopliae* formed a protuberant, domed mycelium of white colouring, which in *Ma II* in its central part was lightly cream-coloured, fluffy and strongly sporulating.

Photo 7. 20-day-old colonies of *B. bassiana, M. anisopliae* and *P. fumosoroseus* on the medium with insecticide Zolone 35 EC:
K – control, A – concentration 10 times as high as the recommended one, B – concentration corresponding to the recommended one,
C – concentration 10 times as low as the recommended one, *Bb I, II* – isolates of fungus *Beauveria bassiana, Ma I, II* – isolates of fungus *Metarhizium anisopliae, Pf I, II* – isolates of fungus *Paecilomyces fumosoroseus*

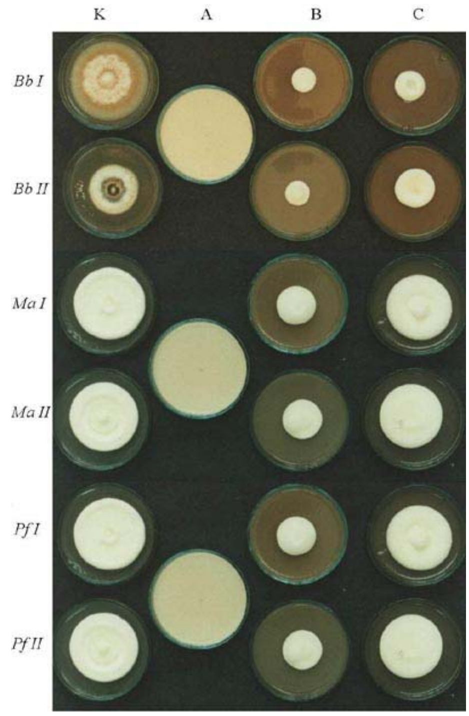


Photo 8. Morphology of 20-day-old colonies of isolate *Bb I (Beauveria bassiana)*:
K – control, B – Zolone 35 EC in the concentration corresponding to the field dose, C – Zolone 35 EC in the concentration 10 times as low as the recommended one

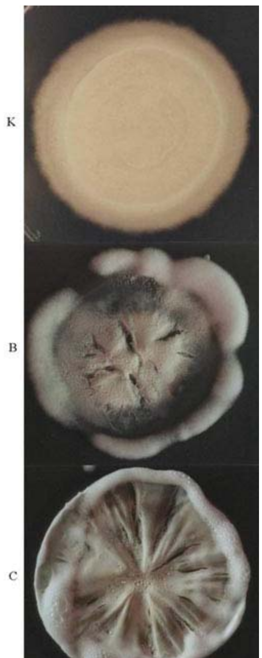
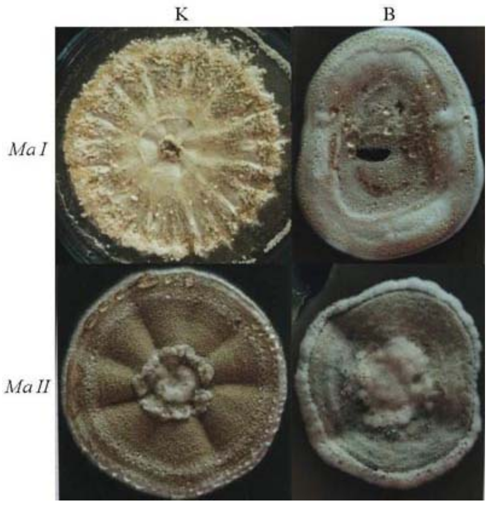


Photo 9. Morphology of 20-day-old colonies of *Metarhizium anisopliae*:
K – control, B – Zolone 35 EC in the concentration corresponding to the field dose, *Ma I, II* – isolates of fungus *M. anisopliae*



Basudin 25 EC (s. a. diasinone), at the concentration 10 times higher than the field one (A) completely inhibited the growth of the studied fungi for the first 5 days of the experiment (tab. 3). In the later period, the growth of the colonies was slow and on the 20th day of the culture the colonies were smaller than the control by more than 80%.

Colonies *P. fumosoroseus* on the bedding with diasinone in dose A were white and fluffy, while the mycelium of the other species did not differ from the control with an exception of isolate *Ma II*, which did not form sporules.

Basudin 25 EC in the recommended dose (B) was the most effective in inhibiting the growth of isolate *Bb II* from the herbicide fallow, where on the fifth day of the culture only the initial growth of the colonies was observed and it was strongly limited till the end of the experiment (fig. 2). Concentration B also had a negative effect on the other isolates and *P. fumosoroseus*, which was the most resistant, formed colonies as much as by about 50% smaller than the control (fig. 2).

On the beddings with the dose of Basudin 25 EC – C (10 times as low as the recommended one), the growth of all fungi was intensive and only isolate *Pf I* reached the value comparable to the control (tab. 3). The studies by Väninnen and Hokkanen [23] confirm that fungus *P. fumosoroseus* was relatively resistant to the effect of Basudin 25 EC. The authors report that the species *P. farinosus* and *B. bassiana* were the most susceptible to the aforementioned preparation. The present studies do not confirm this phenomenon – isolates *M. anisopliae* and *B. bassiana* showed a similar reaction to the applied diasinone. A strong reaction of *B. bassiana* to Basudin 25 EC was also observed by Miętkiewski [11].

Diasinone in the field and lower doses did not cause any changes in the appearance of fungi colonies. However, it was observed that both isolates *B. bassiana* and isolate *Ma I* did not sporulate on the bedding with the field dose of this insecticide.

Preparation Sanmite 20 WG (s. a. pirydaben) was characterized by the least toxicity towards the studied fungi (photo 10). It is interesting that the preparation dose differentiated the size of the colonies only in a small degree (tab. 3), and isolates *Bb II* and *Ma II* reached the size comparable to the control even in the presence of the dose 10 times as high as the recommended one (A) (fig. 2, photo 10). Only isolate *Ma I* on the bedding with the highest concentration of pirydaben initially formed colonies 35.7% of the size of the control, and at the end of the experiment the growth rate was the highest – colonies *Ma I* reached the size of about 50% of the control (tab. 3).

Photo 10. 20-day-old colonies of *Beauveria bassiana, Metarhizium anisopliae* and *Paecilomyces fumosoroseus* on the medium with insecticide Sanmite 20 WG: K – control, A – concentration 10 times as high as the recommended one, B – concentration corresponding to the recommended one, C – concentration 10 times as low as the recommended one, *Bb I, II* – isolates of fungus *Beauveria bassiana, Ma I, II* – isolates of fungus *Metarhizium anisopliae, Pf I, II* – isolates of fungus *Paecilomyces fumosoroseus*

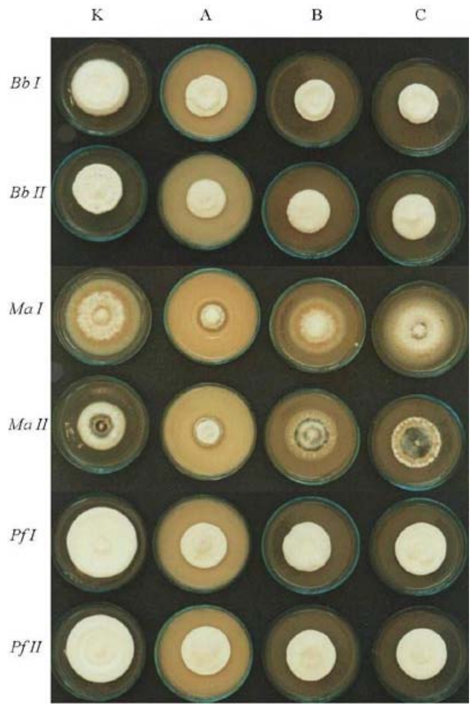
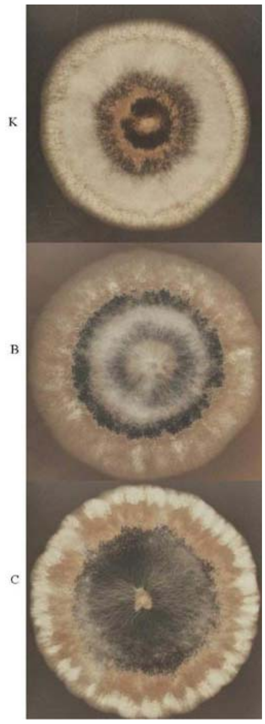


Photo 11. Morphology of 20-day-old colonies of isolate *Ma II* (*Metarhizium anisopliae*):
K – control, B – Sanmite 20 WG in the concentration corresponding to the field dose, C – Sanmite 20 WG in the concentration 10 times as low as the recommended one



Sanmite 20 WG at the highest concentration (A) did not affect the appearance of the colonies of *B. bassiana* or *P. fumosoroseus*. Under the effect of high doses of the preparation the colonies of those fungi sporulated more intensively than in the control. On the other hand, morphological changes of the colonies were observed in the case of *M. anisopliae* (photo 10). The colonies of *Ma I* were dark beige, slightly mucilaginous at the sides, with a visible concentric air mycelium. The mycelium of isolate *Ma II* was protuberant in the central part, with a fluffy structure, around which a clear ring of forming sporules was seen. The colonies of *Ma I* on the beddings in the recommended (B) and the lowest (C) concentrations of Sanmite 20 WG were covered with delicate hyphae of air mycelium. The colonies of *Ma II* on the bedding with the field dose of the insecticide were flat and mucilaginous, in the central part covered with delicate air hyphae and with strong sporulation on the edges of the cultures (photo 11). In the lowest dose – C – the colonies of isolate *Ma II* sporulated very strongly in the central part. The colonies were encircled by a belt of snow-white air mycelium on the edges (photo 11).

The consecutive effect of pesticides on the growth of fungi colonies and the germination of spores *in vitro*
Selected fungi, showing significant changes in the morphology of the colonies under the effect of the tested pesticides, were inoculated again to the bedding SDA without a pesticide with the aim of determining the persistence of those changes (tab. 4).

Table 4. The consecutive effect of selected pesticides on the growth of isolates *B. bassiana. M. anisopliae* and *P. fumosoroseus* on the bedding without an addition of pesticide (% in relation to the control)

No. of isolate	Day after inoculation	Preparation				
		Zolone 35 EC		Chwastox Ex 300 SL		Roundup
		B	C	B	C	A
Bb I	5	126.7±0.76	82.1±0.76	110.1±2.18	X	152.5±0.65
	20	94.4±1.49	104.0±0.00	111.2±4.64	X	100.4±0.42
Bb II	5	121.2±1.06	129.5±0.76	132.7±0.35	X	X
	20	107.7±7.07	103.8±0.00	100.0±0.00	X	X
Ma I	5	95.9±0.86	102.5±0.70	105.8±1.00	X	68.6±1.64
	20	107.5±0.00	97.7±0.00	107.5±0.00	X	102.6±2.32
Ma II	5	33.1±2.02	X	106.5±0.00	X	91.2±1.62
	20	93.8±0.00	X	101.9±0.00	X	111.4±3.33
Pf I	5	116.4±0.86	X	116.4±1.80	113.3±1.50	X
	20	94.1±7.07	X	88.2±0.00	100.0±0.00	X
Pf II	5	99.2±1.00	X	91.4±0.29	X	X
	20	104.6±0.00	X	92.3±0.00	X	X

Abbreviations:
BbI, II – isolates of fungus *Beauveria bassiana*
MaI, II – isolates of fungus *Metarhizium anisopliae*
PfI, II – isolates of fungus *Paecilomyces fumosoroseus*
A – concentration 10 times higher than the recommended one
B – concentration corresponding to the field dose
C – concentration 10 times lower than the recommended one
± – standard deviation
X – combination not studied in respect of the consecutive effect of pesticides on the growth of entomopathogenic fungi

It was found out that the changes in the growth intensity and the morphology of fungi colonies observed during their contact with pesticides were not lasting and they disappeared after removing the factor that had caused them. Similar observations are reported by Miętkiewski et al. [15] and Sapieha [22].

Application of herbicide Chwastox Ex. 300 SL both in the field dose (B) and the one 10 times lower (C) had no negative consecutive effect on the growth of the selected fungi isolates (tab. 4). Isolate *Bb II* from the bedding with the field dose of MCPA considerably exceeded the size of control colonies already on the 5th day of the culture. Isolate *Pf I* showed a similar reaction. In the course of the experiment the rate of fungi growth decreased and on the 20th day of the culture the studied isolates reached the size comparable to the control.

In the case of Roundup, isolate *Bb I*, from the bedding with the dose 10 times as high as the recommended one (A) at the beginning grew much more intensively than the control, while isolate *Ma I* on the day of incubation formed colonies by about 30% smaller than the control. However, like in the case of Chwastox Ex. 300 SL, the colonies of all fungi isolates on the bedding with Roundup at the end of the experiment reached the size comparable to the control (tab. 4).

Although Roundup limited the sporulation of fungi growing on the bedding with an addition of it, it did not cause any changes in the intensity of germination of the spores which germinated in a similar way, and even more intensively than the control (tab. 5). The results are comparable to the studies presented by Wojciechowska et al. [24], where it was found out that herbicides Gramoxone and Gesagard – although inhibiting the growth and decreasing the weight of the mycelium of entomopathogenic *Hyphomycetales* and *Mycophyta* – neither completely destroyed the mycelium nor lowered the pathogenicity. Wojciechowska et al. [25] and Miętkiewski et al. [13] reported that application of herbicides in a pot experiment did not eliminate entomopathogenic fungi from the soil.

The studied fungi from the beddings containing insecticide Zolone 35 EC in the recommended (B) and the lowest (C) doses developed after they were transferred without this preparation (tab. 4). Only isolate *Ma II* from the bedding with the recommended dose of phosalone at the beginning formed the colonies as much as by 67% smaller than the control. However, during the further culture, the intensity of the mycelium growth was similar to the control.

Table 5. The consecutive effect of selected pesticides on germination of spores *B. bassiana. M. anisopliae* and *P. fumosoroseus* (%)

No. of isolate	Day of obser-vation	Control	Preparation							
			Sanmite 20 WG			Zolone 35 EC		Roundup		
			A	B	C	B	C	A	B	C
Bb I	1	6.0	6.0	X	6.0	8.0	5.7	X	X	X
	2	46.7	49.0	X	51.0	38.3	38.3	X	X	X
	3	98.3	97.3	X	90.7	92.0	98.3	X	X	X
Bb II	1	7.0	6.3	9.0	X	8.7	7.7	5.7	X	7.3
	2	52.3	56.7	55.0	X	40.0	48.3	40.0	X	43.0
	3	100.0	98.3	97.7	X	92.3	91.7	100.0	X	95.7
Ma I	1	4.7	5.7	X	X	4.0	5.3	X	6.7	5.3
	2	60.0	52.3	X	X	52.7	52.3	X	53.3	56.3
	3	98.3	94.0	X	X	92.7	93.3	X	95.0	98.3
Ma II	1	3.7	4.0	X	5.3	4.3	5.7	X	4.3	4.0
	2	67.3	61.3	X	59.3	57.7	58.3	X	55.0	58.7
	3	98.7	96.7	X	98.3	98.3	100.0	X	100.0	98.3
Pf I	1	11.7	13.0	X	X	6.7	X	X	X	X
	2	39.0	26.3	X	X	28.3	X	X	X	X
	3	85.0	100.0	X	X	95.0	X	X	X	X
Pf II	1	11.0	7.0	7.7	14.0	10.7	X	15.3	X	X
	2	34.3	22.3	32.7	35.7	31.3	X	37.7	X	X
	3	89.0	92.7	98.3	99.3	95.7	X	100.0	X	X

Abbreviations:
BbI, II – isolates of fungus *Beauveria bassiana*
MaI, II – isolates of fungus *Metarhizium anisopliae*
PfI, II – isolates of fungus *Paecilomyces fumosoroseus*
A – concentration of the preparation in the medium 10 times higher than the recommended one
B – concentration of the preparation corresponding to the field dose
C – concentration of the preparation in the medium 10 times lower than the recommended one
X – combination not studied in respect of the spores' germination

Fungi spores from the cultures growing on beddings with Zolone 35 EC germinated in a similar or more intensive way as compared to the control (tab. 5). This does not confirm the results obtained by Bajan et al. [1], who report that photoorganic preparations can lower the pathogenicity level of entomopathogenic *Hyphomycetales* and *Mycophyta*.

Results of studies on the consecutive effect of Sanmite 20 WG on the germination of entomopathogenic fungi spores were similar to those obtained for Zolone 35 EC. The fungi conidia germinated comparatively or more intensively than in the control (tab. 5).

CONCLUSIONS

1. Among the studied pesticides, herbicides were more toxic to entomopathogenic fungi that insecticides. Herbicide Chwastox Ex. 300 SL and insecticide Zolone 35 EC strongly inhibited the growth of the mycelium of the studied fungi species.
2. Herbicides proved to be the least toxic towards *B.bassiana* , and insecticides turned out to be the least toxic towards *P. fumosoroseus*.
3. Isolates *Bb II, Ma II* and *Pf II* , which had a contact with herbicides in the natural conditions, were more resistant to their toxic abilities than the same isolates from the arable land. The resistance of those isolates under the effect of insecticides varied. Isolates *Bb II* and *Ma II* proved to be more resistant to this group of preparations.
4. None of the studied pesticides had a clear negative effect on the consecutive growth of fungi or germination of the spores.

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