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THE EFFECT OF FUNGICIDES APPLIED IN APPLE ORCHARDS ON ENTOMOPATHOGENIC FUNGI *IN VITRO*

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ABSTRACT

The experiment tested the following entomopathogenic fungi isolated by the method of bait insects: *Beauveria bassiana (Bb), Metarhizium anisopliae (Ma)* and *Paecilomyces fumosoroseus (Pf)*, which were obtained from a cultivated field (*Bb I, Ma I, Pf I*) and from a herbicide fallow belt (*Bb II, Ma II, Pf II*) in an apple orchard with a full program of plant protection. The fungi were inoculated on Sabouraud's medium with an addition of fungicides: Atemi C 76 WG, Delan 700 WG, Captan 50 WP and Rubigan 12 EC in the following doses: one recommended in agricultural practice – (A), 10 times lower than the recommended one – (B) and 100 times lower than the recommended one (C). The studies showed that the effect of fungicides on entomopathogenic fungi was related to the active substance of the preparations while the toxic effect was most frequently directly proportional to their concentration in the soil. Fungus *M. anisopliae* was the most sensitive to the applied fungicides while *P. fumosoroseus* was the most resistant. Rubigan 12 EC inhibited the growth of the tested entomopathogenic fungi in the strongest way. That preparation – depending on its concentration – limited or inhibited the formation of spores. Atemi C 76 WG, like Rubigan 12 EC, limited the sporulation of cultures. The reaction of entomopathogenic fungi to Captan 50 WP varied. However, with low doses the growth of the majority of isolates was similar to the control. Delan 700 WG turned out to be the least toxic preparation towards the studied fungi. The studies showed that the changes in sporulation and morphology of fungi cultures growing on the subsoils infected with fungicides were short-lasting and they disappeared after the causative factor was removed.

Key words: fungicides, entomopathogenic fungi, growth and morphology of colonies, germination of spores

INTRODUCTION

Entomopathogenic fungi, besides bacteria and viruses, belong to the most commonly found pathogens of insects and mites. Their effect is related to a number of factors such as the host's susceptibility, population density, outside conditions as well the commonly used pesticides [6, 7].

Fungicides found wide application in agricultural cultivations and in pomology very early. Their negative effect to various elements of the eco-system, including entomopathogenic fungi, was observed early as compared to other pesticides. As early as in 1913 Fron and Feytans proved that Bordeaux mixture, which was very popular then, strongly inhibits the development of fungus *Spicaria farinose* var. *verticilloides* Fron when the exposition is long enough [13]. When the use of fungicides was periodically ceased for the protection of citrus trees in Brazil during World War II, it contributed to greater frequency of infection of scale insect *Aonidiella aurantii* (Mask.) by entomopathogenic fungi Lepage 1943, after Müller-Kögel [11]. The most frequent studies on the effect of fungicides on entomopathogenic fungi are conducted in the conditions of nutrient medium cultures [1, 2, 3, 7, 10, 12]. These studies include first of all observations of the effect of fungicides on the germination of entomopathogenic fungi spores or the sporulation of entomopathogenic fungi [4, 5, 15, 16].

The effect of fungicides on entomopathogenic fungi is principally negative and it is related to the kind and concentration of the active substance of the applied preparation. The reactions of particular species of entomopathogenic fungi sometimes vary, for example, chlorotalonil does not inhibit the development of entomopathogenic *Hyphomycetales* [6], while completely limiting the infectiousness of *Erynia neoaphidis* conidia [5].

It seems purposeful to continue the studies on the effect of fungicides on entomopathogenic fungi, especially in commercial apple orchards where synthetic fungi constitute the basis for plant protection.

The present paper determined the effect of fungicides Atemi C WG, Delan 700 WG, Captan 50 WP and Rubigan 12 EC on the growth and morphology of entomopathogenic *Hyphomycetales* isolated from the soil of apple orchards and cultivated fields. Besides, the studies established the consecutive effect of fungicides on the sporulation of germs and the growth of the tested fungi.

MATERIALS AND METHODS

The experiment tested the following species of entomopathogenic fungi: *Beauveria bassiana* (Bals.) Vuill., *Metarhizium anisopliae* (Metsch.) Sorok and *Paecilomyces fumosoroseus* (Wize) Brown et Smith. The fungi were isolated from the soil using the method of bait insects [17, 18] onto the larvae of the last but one larval stage of *Galleria mellonella* L. Two fungi isolates were tested within each species. Isolates *Bb I, Ma I, Pf I* originated from the cultivated field adjoining an orchard, where the use of pesticides was small and isolated *Bb II, Ma II, Pf II* were obtained from the soil of the herbicide fallow, where the use of pesticides was high.

Four synthetic fungicides, which were most often applied in apple orchards from where entomopathogenic fungi came were selected for the studies ($\underline{tab. 1}$).

Commercial name of the preparation	Name and content of active substance in g or %	Recommend ed dose in g or ml/l	Class of toxicity	Producer
Atemi C 76 WG	Cyproconazol 1, Kaptan 75	2	IV	Novartis Agro S. A., F 92845 Rueil-Malmaison dla Novartis Crop Protection AG, Switzerland
Delan 700 WG	Ditianon 700	ianon 700 0.8		American Cyanamid Company, USA
Captan 50 WP	Captan 50 WP Kaptan 50		IV	Tomen Agro, Inc., USA
Rubigan 12 EC	Fenarimol 12	0.4	IV	Dow Agrosciences-Polska Sp. z o.o.

Table 1. Fungicides used in the experiment

The preparations were used in the following doses:

A – one recommended in practice

B – one that was 10 times as low as the recommended on

C - one that was 100 times as low as the recommended one.

Pesticides were added to the sterile Sabouraud's medium (SDA) cooled to the temperature of about 55°C. The subsequent concentrations were used by the method of dilutions. The medium prepared in such a way was poured out to Petri dishes. The fungi growing on SDA medium without an addition of pesticide constituted the control. The experiment was established in five repetitions. The culture was kept at the temperature of 22°C for 20 days. The diameter of fungi colonies was measures in 5-days' intervals and observations were carried out on the morphological features of the colonies.

When significant changes were found out in the appearance of the colonies, then after the experiment was finished the fungi were inoculated onto SDA medium without a pesticide addition in order to find the persistence of those changes.

When significant deviations were found in the sporulation of fungi growing on the media with an addition of pesticides, then the consecutive effect of pesticides on the germination of the spores was established. To this aim, the suspension of spores from the chosen combinations was placed on the basic glasses with a layer of 2% sterile agar. The experiment was set in three repetitions and it was conducted at the temperature of 22-23°C.

The germinating spores in the number of 100 in each repetition were counted under a microscope for three successive 24-hours' periods.

RESULTS AND DISCUSSION

It follows from the conducted studies that the toxicity of pesticides towards entomopathogenic fungi was related to the kind of the active substance, the concentration of the preparation and it was most frequently directly proportional to the fungicide concentration in the medium (<u>tab. 2</u>, <u>fig. 1</u>). Similar results referring to 16 out of 17 examined fungicides are presented by Keller et al. [4] while testing fungi *Beauveria brongniartii* (Sacc.) Petch.

Fig. 1. Sensitivity of entomopathogenic fungi isolates to fungicides (in % in relation to the control)



Fungicides used in the experiment were the strongest to inhibit the growth of *M. anisopliae* isolates while *P. fumosoroseus* proved to be the most resistant to this group of preparations ($\underline{\text{fig. 1}}$).

No. of isolate ti	Day of		Preparatus										
	observa	Atemi C 76 WG			Delan 700 WG		Captan 50 WP			Rubigan 12 EC			
	tion	А	В	С	A	В	С	A	В	С	A	В	С
Bb I	5	16.4±1.14	67.7±0.76	86.2±0.76	38.6±2.11	70.9±1.29	82.5±0.96	pw	44.6±0.58	88.1±1.14	bw	44.6±1.00	97.0±1.60
	20	23.4±2.08	76.6±2.27	76.3±1.26	71.3±3.28	75.8±0.95	85.9±2.89	48.7±2.12	65.3±0.48	87.6±1.04	pw	67.6±1.14	79.2±1.40
Bb II	5	16.8±0.74	57.1±0.79	92.4±1.00	45.7±0.25	76.6±1.02	100.0±1.25	pw	49.5±0.95	77.5±0.94	bw	39.5±0.74	68.2±1.04
	20	21.1±1.98	77.1±1.08	99.6±1.77	68.2±3.06	93.5±2.43	105.3±1.89	48.6±1.76	70.3±0.87	89.1±1.39	8.7±1.90	66.0±0.27	86.5±0.97
Ma I	5	23.8±0.48	53.7±1.04	81.1±0.65	30.4±0.63	62.1±0.96	83.7±1.46	pw	58.9±2.36	84.9±1.93	bw	pw	88.4±1.49
	20	22.3±0.41	59.6±6.36	92.4±3.28	52.2±1.53	81.9±2.48	93.3±0.76	28.9±0.94	82.8±1.71	101.0±1.31	bw	33.8±1.29	86.6±0.87
Ma II	5	21.6±0.71	57.7±1.78	94.3±1.47	38.9±2.10	70.7±0.57	98.6±0.94	41.6±0.67	64.8±1.55	102.2±1.58	bw	pw	83.9±1.06
	20	19.3±0.65	39.7±0.00	65.2±6.01	50.2±1.40	75.2±1.76	85.2±1.61	55.9±2.32	87.4±0.75	97.2±1.10	bw	60.6±0.57	77.2±1.47
PfI	5	17.6±2.00	76.7±1.85	85.0±1.60	85.9±1.50	83.3±1.14	90.7±2.01	39.4±0.48	60.0±0.76	88.5±0.58	bw	50.8±0.65	93.1±0.96
	20	34.8±2.08	78.6±2.50	91.4±0.97	80.2±1.15	89.2±1.88	97.3±1.61	86.1±1.11	93.2±0.42	94.1±3.20	16.3±3.14	81.5±3.03	95.8±3.19
Pf II	5	30.3±1.43	91.3±1.04	105.6±0.65	96.4±0.76	104.1±0.67	105.6±0.96	54.3±0.58	55.6±0.58	89.1±1.20	bw	36.2±1.06	99.3±0.76
	20	33.3±2.44	75.2±1.11	91.5±5.30	72.6±0.87	80.2±2.75	86.2±1.61	90.7±1.04	93.9±0.76	99.3±1.54	18.7±2.98	76.4±1.17	101.1±1.24

Table 2. Size of colonies of chosen isolates B. bassiana, M. anisopliae and P. fumosoroseus on media with fungicides (in % of the control)

Abbreviations:

Bb I, II – isolates of fungus Beauveria bassiana

Ma I, II – isolates of fungus Materhizium anisopliae Pf I, II – isolates of fungus Paecilomyces fumosoroseus

bw – no growth

pw – beginning of growth

 \pm – standard deviation

A – concentration of the preparation in the medium referring to the field dose

B – concentration of the preparation in the medium 10 times as low as the recommended one

C - concentration of the preparation in the medium 100 times as low as the recommended one

Rubigan 12 EC had the strongest effect on the studied fungi. In the recommended dose (A) the preparation completely inhibited the growth of *M. anisopliae* while isolate *Bb I* showed only traces of growth (tab. 2, photo 1). Isolate *Bb II* and fungus *P. fumosoroseus* on the media with dose A of Rubigan 12 EC began growth only in the final stage of the experiment but it was found out that the mycelium of *Bb II* did not sporulate whereas the sporulation of both isolates of *P. fumosoroseus* was remarkably limited. Both isolates of *M. anisopliae* had the weakest growth on the media containing the dose that was 10 times as low as the recommended one (B) (fig. 2). On the fifth day of the culture the studies observed only traces of the colonies' growth. Later on the intensity of the growth of both isolates was clearly differentiated; the isolate from the cultivated fields formed colonies that were the size of about 33.8% in relation to the control. Fungi *B. bassiana* and *P. fumosoroseus* developed more intensively than *M. anisopliae* from the very beginning, reaching the colonies smaller than the control by about 20-30% on the 20th day of the experiment. These results are confirmed by Machowicz-Stefaniak [7], who found out that Rubigan 12 EC in the recommended concentration, besides *B. Bassiana* and *M. anisopliae*, inhibited the growth of *Verticillium lecanii* (Zimm.) Viegas at the initial stage of the experiment. Miętkiewski et al. [9] also point to strong inhibition of the growth of fungus *B. bassiana* by the recommended dose of Rubigan.





Fig. 2. Size of colonies of isolates *B. bassiana (Bb I, Bb II), M. anisopliae (Ma I, Ma II)* and *P. fumosoroseus (Pf I, Pf II)* on the medium with fungicides on the 20th day of the culture (in % in relation to the control)



The Rubigan 12 EC dose 10 times as low as the field one strongly limited the formation of spores or made it completely impossible. The colonies of isolate Ma I created a white mycelium of compact structure, while the colonies of Ma II were covered with the hyphae of white air mycelium in the central part. Rubigan 12 EC in the dose 100 times as low as the recommended one (C) did not have any significant effect on the growth of the tested fungi (tab. 2, fig. 2). However, the studies observed reduction or complete lack of culture sporulation with an exception of isolate Pf II, which formed spores in the manner comparable to the control.

Preparation Atemi C 76 WG in the recommended dose strongly limited the growth of all the studied fungi throughout the experiment (tab. 2, photo 2). The colonies of particular species were smaller than the control at the end of the experiment by about 70-80%. Atemi 70 WG, like Rubigan, had a negative effect on the sporulation and morphology of the fungi. Isolate Bb I formed colonies with raised hyphae, while Bb II formed a protuberant colony. In neither case was fruiting observed, like in both isolates of M. anisopliae. The colonies of P. fumosoroseus, especially of isolate Pf II, sporulated weakly. Atemi C 76 WG in the dose 10 times as low as the field one had a smaller effect on the tested entomopathogenic fungi (tab. 2). An exception was M. anisopliae, whose isolates – despite the initially intensive growth – had increasingly more limited growth in the course of the experiment, which especially concerned isolate Ma II from the herbicide fallow, the colonies of which reached the size of only 39.7% of the control on the 20th day (fig. 2). Generally, the B dose of the preparation, except isolate Bb I, strongly limited the sporulation of fungi colonies. The greatest morphological changes were observed in reference to both isolates of *M. anisopliae* which formed colonies of intensively yellow colour, clearly different from the green control colonies. Those changes were also observed after applying the dose that was 100 times as low as the recommended one although poor sporulation took place in the case of isolate Ma I. The fungi growth on the medium with the smallest C dose of preparation Atemi 76 WG was close to the control. An exception was *M. anisopliae* from the herbicide fallow the colonies of which were as much as 35% smaller than the control on the 20^{th} day (fig. 2). The literature lacks information on the effect of Atemi C 76 WG on entomopathogenic fungi. Keller et al. [4] report that the recommended dose of fungicides Systhane C and Topas C, which like Atemi c 76 WG belong to triasoles, made the growth of *B. brongniartii* impossible.

Photo 2. 20-days' colonies of *Beauvaria bassiana*, *Metarhizium anisopliae* and *Paecilomyces fumosoroseus* on the media with fungicide Atemi C 76 WG: K – control, A – concentrations referring to the field dose, B – concentration 10 times as low as the recommended one, C – concentration 100 times as low as the recommended one, *Bb I*, *II* – isolates of *B. bassiana* fungus, *Ma I*, *II* – isolates of *M. anisopliae* fungus, *Pf I*, *II* – isolates of *P. fumosoroseus* fungus



When Captan 50 WP, one of the most commonly used fungicides in orchards, was added to the medium in the recommended A dose, it had varied effects on particular fungi species (tab. 2, photo 3). The growth of both isolates of *P. fumosoroseus* was quite intensive, and on the 20th day of the culture they reached the size similar to the control (fig. 2). In the case of *M. anisopliae*, isolate Ma II grew more intensively, reaching 55.9% of the size of control colonies, while *Ma I* began its growth only on the 5th day of the culture reaching only 29.9% of the size of the colonies. High susceptibility to Captan 50 WP was also characteristic of both *B. bassiana* isolates, which grew very slowly at the beginning, reaching an identical size, namely 48% of the control on the 20th day of the culture. These studies are confirmed by the results obtained by Olmert and Kenneth et al. [12], who found out that preparations based on the active substance Captan are highly toxic towards *B. bassiana* and *B. brongniartii*. Captan 50 WP in the field dose significantly affected the macroscopic picture of the colonies of *B. bassiana* and *B. bassiana* and *M. anisopliae*. *B. bassiana* colonies were formed by the poorly sporulating mycelium with crawling huphae (photo 4). The mycelium of *Bb II* had strongly folded surface and it sporulated intensively. The colonies of *Ma I* were flat in the central part and had the sided covered with fluffy raised hyphae of white colour. Both isolates sporulated poorly (photo 5).

Photo 3. 20-days' colonies of *Beauvaria bassiana, Metarhizium anisopliae* and *Paecilomyces fumosoroseus* on the media with Captan 50 WP: K – control, A – concentrations referring to the field dose, B – concentration 10 times as low as the recommended one, C – concentration 100 times as low as the recommended one, *Bb I, II* – isolates of *B. bassiana* fungus, *Ma I, II* – isolates of *M. anisopliae* fungus, *Pf I, II* – isolates of *P. fumosoroseus* fungus



Photo 4. Morphology of 20-days' colonies of *Beauvaria bassiana*: K – control, A – Captan 50 WP in the concentration referring to the field dose, *Bb I*, *II* – isolates of *B. bassiana*





Bb II

Photo 5. Morphology of 20-days' colonies of *Metarhizium anisopliae*: K – control, A – Captan 50 WP in the concentration referring to the field dose, *Bb I*, *II* – isolates of *M. anisopliae*



Captan 50 WP in the doses 10 times – B, and 100 times – A as low as the field one – C, practically did not limit the growth of *P. fumosoroseus* and *M. anisopliae*. The fungus *B. bassiana* reacted by decreasing the growth rate in the case of C dose and the colonies of both isolates on the 20th day of the culture were smaller than the control by 30-35% (fig. 2). Doses B and C of Captan 50 WP did not have any effect on the macroscopic picture of fungi cultures. However, it was observed that both isolates of *B. bassiana* and isolate *Ma I* sporulated very intensively.

In the literature on the effect of fungicides on entomopathogenic fungi the information can be found that certain preparations do not have a negative effect on the mycelium growth but rather stimulate it [1, 6, 8]. Such an effect was shown by the fourth of the tested fungicides – Delan 700 WG, (tab. 2, photo 6). The fungus *P. fumosoroseus* turned out to be most tolerant towards the presence of Delan 700 WG in the subsoil. Independently of the applied dose, the colonies of both isolates developed intensively, reaching the size comparable to the control.

No changes in the morphology of cultures or their sporulation were observed (<u>tab. 2</u>, <u>photo 6</u>). The fungi *B. bassiana* and *M. anisopliae*, growing on the subsoils with the recommended dose of Delan 700 WG created the colonies smaller than the control ones by about 30% in the case of *B. bassiana* and 50% in the case of *M. anisopliae* (<u>fig. 2</u>). It was also found out that under the effect of the dose 10 times lower, B, *M. anisopliae* colonies formed a flat mycelium radiantly folded, of cream colour, with poorly visible sporulation (<u>photo 6</u>). The development of *M. anisopliae* and *B. bassiana* on the subsoils with dose B (10 times lower) and C (100 times lower than the recommended one) of Delan 700 WG was intensive, comparable to the control (<u>tab. 2</u>). No greater changes in the morphology of the cultures of the tested fungi were observed. Different information on the effect of Delan SC is given by Keller et al. [4]. They found out that its concentrations of 0.05% and 10 times as low caused that the fungus *B. brongniartii* formed colonies that were significantly smaller than the control.

The studies on the consecutive effect of fungicides on entomopathogenic fungi chose two fungicides that inhibited the growth most strongly, i.e. Rubigan 12 EC and Captan 50 WP, which were applied in the field dose (A). The fungi forming colonies on the subsoils containing fungicides were inoculated onto the SDA medium without an addition of fungicide in order to find the possible changes in the growth intensity of fungi cultures or the persistence of morphological changes of their colonies. The studies found out that the changes in the growth rate of the colonies or the macroscopic picture of the cultures caused by Rubigan 12 EC and Captan 50 WP did not persist and disappeared after the causative factor was removed (fig. 3). After being inoculated onto SDA medium without the fungicide, the majority of the tested isolates at the beginning grew more intensively than the control colonies (fig. 3). At the initial stage the most intensive growth was characteristic of the mycelium of both isolates of *M. anisopliae* obtained from the medium with an addition of Captan 50 WP, exceeding the size of control colonies by about 43%. During the further incubation, however, the growth rate of fungi decreased and all of them formed the colonies of the size similar to that of the control.

Photo 6. 20-days' colonies of *Beauvaria bassiana*, *Metarhizium anisopliae* and *Paecilomyces fumosoroseus* on the media with fungicide Delan 700 WG: K – control, A – concentrations referring to the field dose, B – concentration 10 times as low as the recommended one, C – concentration 100 times as low as the recommended one, *Bb I*, *II* – isolates of *B. bassiana* fungus, *Ma I*, *II* – isolates of *M. anisopliae* fungus, *Pf I*, *II* – isolates of *P. fumosoroseus* fungus



Fig. 3. Size of colonies of selected fungi isolates inoculated from the media containing fungicides Captan 50 WP and Rubigan 12 EC onto the medium without fungicide (% in relation to the control)



No. of isolate	24-hour observation	Control	Fungicide							
			Ca	aptan 50 V	/P	Rubigan 12 EC				
			A	В	С	А	В	С		
Bb I	1	6.0	4.7	6.7	7.3	Х	7.3	4.5		
	2	46.7	36.7	41.7	43.3	Х	34.7	31.0		
	3	98.3	83.3	81.0	91.7	Х	86.7	89.0		
Bb II	1	7.0	5.0	6.0	Х	Х	Х	Х		
	2	52.3	40.0	47.7	Х	Х	Х	Х		
	3	100.0	83.0	85.0	Х	Х	Х	Х		
Ma I	1	4.7	6.0	Х	5.0	Х	5.3	4.7		
	2	60.0	54.0	Х	50.7	Х	57.3	55.7		
	3	98.3	98.3	Х	98.3	Х	100.0	100.0		
Ma II	1	3.7	2.7	Х	4.7	Х	5.3	Х		
	2	67.3	55.7	Х	55.3	Х	56.0	Х		
	3	98.7	100.0	Х	97.0	Х	100.0	Х		
Pf I	1	11.7	7.3	Х	7.0	Х	Х	Х		
	2	39.0	20.7	Х	22.3	Х	Х	Х		
	3	85.0	84.3	Х	87.3	Х	Х	Х		
Pf II	1	11.0	4.7	Х	6.0	9.7	Х	Х		
	2	34.3	17.0	Х	36.0	36.0	X	Х		
	3	89.0	87.0	Х	100.0	100.0	Х	Х		

 Table 3. The consecutive effect of selected fungicides on the germination of spores of fungi
 B. bassiana, M. anisopliae and P. fumosoroseus

Abbreviations

Bb I, II – isolates of fungus Beauveria bassiana

Ma I, II – isolates of fungus Materhizium anisopliae

Pf I, II – isolates of fungus Paecilomyces fumosoroseus

X – combination not tested on spore germination

A - concentration of the preparation in the medium referring to the field dose

 $B-\mbox{concentration}$ of the preparation in the medium 10 times as low as the recommended one

C - concentration of the preparation in the medium 100 times as low as the recommended one

Studying the effect of fungicides on the germination of spores it was found out that the preparations used in the experiment limited the germination of the conidia formed on the infected subsoils only in a small degree (tab. 3). It was found out that the spores of both isolates of *B. bassiana* that were obtained from the subsoils infected with Captan 50 WP in the recommended dose (A) and one 10 times as low (B) germinated like in the control or slightly more poorly. The isolate *Bb I* from the cultivated field showed a similar reaction to Rubigan 12 EC in the concentrations B and the smallest one C (tab. 3). Sapieha [14] also reports lack of a negative effect of fungicides Dithane M-45 and Ridomil Mz on the germination of spores formed on the subsoils containing these preparations.

CONCLUSIONS

- 1. The effect of fungicides to entomopathogenic fungi was related to the active substance of the preparation and its concentration.
- 2. Out of the tested fungicides, Rubigan 12 EC inhibited the growth of entomopathogenic fungi in the strongest manner, while Delan 700 WG turned out to be the least toxic towards the examined fungi.
- 3. The fungus *M. anisopliae* proved to be the most susceptible to the tested fungi, while *P. fumosoroseus* was most resistant.
- 4. The fungicides used in the experiment did not have any permanent effect on the growth and the morphological properties of the mycelium or the germination of the spores.

REFERENCES

- Bajan C., Fedorko A., Kmitowa K., 1992. Grzyby owadobójcze w integrowanej ochronie roślin. Mat. Konf. "Zwalczanie biologiczne szkodników w programach metod integrowanych" [Entomopathogenic fungi in integrated protection of plants. Materials from the Conference "Biological control of pests in the programs of integrated methods"]. E. Niemczyk (Red.), Skierniewice, 32-39 [in Polish].
- 2. Clark R. A., Casagrande R. A., Wallace D. B., 1982. Influence of pesticides on *Beauveria bassiana*, a pathogen of the Colorado potato beetle. Environ. Entomol. 11, 67-70.
- 3. Gardner W. A., Sutton R. M., Noblet R., 1979. Evaluation of the effects of six selected pesticides on the growth of *Nomuraea riley* and *Beauveria bassiana* in broth cultures. J. Georgia, Entomol. Soc. 14(2), 106-113.
- 4. Keller S., Pärl B., Lujan M., Schweizer C., 1993. Der einfluß von Fungiziden auf den insektopathogen Pilz *Beauveria brongniartii* (Sacc.) Petch. Anz. Schädlingskde., Pflanzensch., Umweltsch. 66, 108-114.
- 5. Latteur G., Jensen J. P., 2002. Effects of 20 fungicides on the infectivity of conidia of the aphid entomopathogenic fungus *Erynia neoaphidis*. Bio Contr. 47, 435-444.
- 6. Machowicz-Stefaniak Z., 1980. Wpływ wybranych fungicydów stosowanych w ochronie sadów na rozwój grzybów owadobójczych [The effect of selected fungicides used in the protection of orchards on the development of entomopathogenic fungi]. Roczn. Nauk Roln. E 10 (1-2), 187-199 [in Polish].
- Machowicz-Stefaniak Z., 1981. Wpływ fungicydów systemicznych stosowanych w ochronie sadów na rozwój strzępcza-ków owadobójczych (*Hyphomycetales, Mycophyta*) [The effect of systemic fungicides used in the protection of orchards on the development of entomopathogenic (*Hyphomycetales, Mycophyta*)]. Roczn. Nauk Roln. E 11 (1-2), 63-75 [in Polish].
- Majchrowicz I., 1967. Wpływ niektórych środków chemicznych ochrony roślin na rozwój kilku grzybów w czystych kulturach [The effect of certain chemical crop protection preparations on the development of a few fungi in clear cultures]. Zesz. Nauk. WSR. Szczecin, 24, 179-184 [in Polish].
- 9. Miętkiewski R., Sapieha A., Miętkiewska Z., 1989/90. Wzrost grzybów owadobójczych na pożywkach zawierających herbicydy stosowane w sadownictwie [Growth of entomopathogenic fungi on the media containing herbicides applied in pomology]. Acta Mycol. 25(2), 35-49 [in Polish].
- Miętkiewski R., Pell J. K., Clark S. J., 1997. Influence of pesticide use on the natural occurrence of entomopathogenic fungi in arable soils in the UK: field and laboratory comparisons. Bioc. Sci. Technol. 7, 565-575.
- 11. Müller-Kögler E., 1965. Pilazkrankheiten bei Insekten. Berlin, 444 pp.
- 12. Olmert I., Kenneth R. G., 1974. Sensitivity of the entomopathogenic fungi, *Beauveria bassiana*, *Verticillium lecanii* and *Verticillium* sp. to fungicides and insecticides. Environ. Entomol. 3(2), 33-38.
- Sandner H., Bajan C., Kmitowa K., 1971. Wykorzystanie zjawiska synergizmu w kombinowanych zabiegach ochrony roślin [Using the phenomenon of synergism in combined treatments of plant protection]. Wiadomości Ekol. 27(2), 104-117 [in Polish].
- 14. Sapieha A., 1994. Łączne stosowanie inhibitorów syntezy chityny i insektycydów biologicznych w zwalczaniu stonki ziemniaczanej (*Leptinotarsa decemlineata* Say.), ze szczególnym uwzględnieniem grzybów owadobójczych. Praca doktorska WSR-P Siedlce [Combined application of inhibitors of chitine synthesis and biological insecticides in controlling potato beetle (*Leptinotarsa decemlineata* Say.), with particular regard to entomopathogenic fungi. Doctoral dissertation of WSR-P Siedlce] [in Polish].
- 15. Vänninen I., Hokkanen H., 1988. Effect of pesticides on four species entomopathogenic fungi *in vitro*. Ann. Agric. Fenn. 27, 345-353.
- 16. Zimmermann G., 1975. Über die Wirkung systemischer Fungizide auf verschiedene insektenpathogene Fungi imperfecti *in vitro*. Nachrichtenbl. Deut. Pflanzenschutzdie. 27, 113-117.
- Zimmermann G., 1986. "Galleria bait method" for detection of entomopathogenic fungi in soil. J. Appl. Ent. 102, 213-215.
- 18. Zimmermann G., 1998. Suggestion for a standardized method for reisolation of entomopathogenic fungi from soil using the bait method. "Insect pathogens and insect parasitic nematodes". IOBC Bull. 21(4), 289.

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Anna Sapieha-Waszkiewicz, Ryszard Miętkiewski Chair of Plant Protection Podlasie University in Siedlce, Poland 14 B. Prusa Street, 08-110 Siedlce tel. (+48 25) 643 13 02 e-mail: <u>mietkier@ap.siedlce.pl</u> Barbara Marjańska-Cichoń Laboratory of Pomology Podlasie University in Siedlce, Poland 14 B. Prusa Street, 08-110 Siedlce tel. (+48 25) 643 13 62 <u>Responses</u> to this article, comments are invited and should be submitted within three months of the publication of the article. If accepted for publication, they will be published in the chapter headed 'Discussions' in each series and hyperlinked to the article.