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Effect of seventeen pesticides on mycelial growth of *Akanthomyces*, *Beauveria*, *Cordyceps* and *Purpureocillium* strains

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The effect of fungicides, herbicides and insecticides on mycelial growth of entomopathogenic fungi *Akanthomyces muscarius*, *Beauveria bassiana*, *Cordyceps fumosorosea* and *Purpureocillium lilacinum* were tested under laboratory conditions. Fungicides containing the active ingredients carboxin & thiram, dimethomorph & mancozeb, mancozeb & metalaxyl-M, boscalid & pyraclostrobin, mancozeb, metalaxyl-M & fludioxonil and herbicides with active ingredients pendimethalin, pethoxamid, chlorotoluron and pendimethalin & imazamox statistically significantly inhibited the mycelial growth of all tested fungi (20.4–100% and 14.9–100% inhibition). Insecticides did not significantly inhibit the mycelial growth of *Akanthomyces* and *Purpureocillium* strains but active ingredients tau-fluvalinate, pirimicarb and acetamiprid inhibited the mycelial growth of *Beauveria* strains (22.6–30% inhibition). The mycelial growth of *Cordyceps* strains was faster in the presence of insecticides than in controls.

Key words: entomopathogenic fungi, growth inhibition, *Lecanicillium*, *Isaria*, fungicides, insecticides, herbicides.

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Ondráčková E., Seidenglanz M., Šafář J. (2019): Vliv sedmnácti pesticidů na růst mycelií hub z rodů *Akanthomyces*, *Beauveria*, *Cordyceps* a *Purpureocillium*. – Czech Mycol. 71(2): 123–135.

V laboratorních podmínkách byl testován vliv vybraných fungicidních, insekticidních a herbicidních přípravků na růst entomopatogenních hub *Akanthomyces muscarius*, *Beauveria bassiana*, *Cordyceps fumosorosea* a *Purpureocillium lilacinum*. Fungicidy s účinnými látkami carboxin & thiram, dimethomorph & mancozeb, mancozeb & metalaxyl-M, boscalid & pyraclostrobin, mancozeb, metalaxyl-M & fludioxonil a herbicidy s účinnými látkami pendimethalin, pethoxamid, chlorotoluron a pendimethalin & imazamox významně inhibovaly růst všech kmenů testovaných entomopatogenních hub (inhibice v rozmezí 20,4–100 % a 14,9–100 %). Insekticidní přípravky významně neinhibovaly růst kmenů *Akanthomyces* a *Purpureocillium*, ale růst *Beauveria* byl inhibován účinnými látkami tau-fluvalinatem, pirimicarbem a acetamipridem v průměru o 22,6–30 %. Růst kmenů *Cordyceps* byl v přítomnosti insekticidů ve srovnání s kontrolou rychlejší.

Introduction

Entomopathogenic fungi of the order *Hypocreales* infect insect hosts of all orders. *Beauveria*, *Cordyceps*, *Metarhizium* and *Akanthomyces* belong to the most commonly used genera of fungi in biological control of plant pests. These fungi are part of a number of biological products. For instance, *Beauveria* strains are contained in Mycotrol, Nutri-Life and Myco-Force preparations, *Cordyceps* strains in Mycomite and NoFly preparations, and *Akanthomyces* strains in Mycotal, Mealikil, Vertalec and Ecocill preparations. The use of entomopathogenic fungi as mycopesticides has attracted interest in some parts of the world in recent years, mainly due to the limited effectiveness of insecticides against some pests (thrips, whiteflies, mosquitoes) and the discovery of insecticide tolerant populations (Avery et al. 2013). Moreover, there are many cases of acquired resistance to several groups of insecticides evolved in some insect pests, which have been confirmed throughout Europe (Derron et al. 2004, Wegorek et al. 2006, Ballanger et al. 2007, Djurberg & Gustafsson 2007, Eickermann et al. 2008, Philippou et al. 2011, Heimbach & Müller 2013). There are also other important reasons for wider incorporation of entomopathogenic fungi as biological pesticides into integrated pest management (IPM) strategies and into farming practices. Synthetic pesticides, on which most plant cultivation technologies are presently fully dependent in Europe, have shown (direct or indirect) adverse effects on human and animal health in many cases (Arcury et al. 2007, Michalakis et al. 2014). Surface and groundwater pollution due to pesticides is a worldwide problem (Gilliom et al. 2007). The use of synthetic pesticides decreases the general biodiversity in the soil (Johnston 1986). On the other hand, use of biopesticides results in higher soil quality (Lotter et al. 2003). Much valuable research is currently conducted into non-chemical methods, but only limited implementation occurs in farming practices. Growers need clear messages, and knowledge transfer should be improved (Skellern & Cook 2018).

Entomopathogenic fungi are commonly found in soils, although these fungi are mainly isolated from insects. Some of them can endophytically colonise plants or act as mycoparasites on phytopathogenic fungi (Ownley et al. 2010).

Of the *Beauveria* genus, the species *Beauveria bassiana* (Bals.-Criv.) Vuill. is commercially available and often investigated and used in the biological control of pests, such as larvae and pupae of beetles occurring in the soil, e.g. cockchafers (*Melolontha*), the Colorado beetle (*Leptinotarsa decemlineata*), *Otiorhynchus*, etc. (Koubová 2009, Ropek & Kołodziejczyk 2019). *Beauveria bassiana* has also been used to control bark beetle (*Ips typographus*) populations in the Šumava mountains (Landa et al. 2007). Moreover, this fungus has the ability to endophytically colonise plants, which can be used for controlling

corn borer (*Ostrinia nubilalis*) larvae (Koubová 2009). In the Czech Republic *B. bassiana* has been available as the Boverol preparation.

One representative of the genus *Cordyceps* in biological control is *Cordyceps fumosorosea* (Wize) Kepler, B. Shrestha & Spatafora, formerly *Isaria fumosorosea*. Most research is focused on testing *C. fumosorosea* in the control of silverleaf whitefly (*Bemisia tabaci*). However, its host range includes other pests such as aphids, thrips, whiteflies, leafhoppers, representatives of some weevils, beetles, butterflies, etc. (Hunter et al. 2011, Matter & Sabbour 2013, Kavallieratos et al. 2014).

Akanthomyces species (widely known in the genus *Lecanicillium*, closely related to *Verticillium*) have a broad host range, including insects (mainly whiteflies and aphids), phytopathogenic fungi (agents causing powdery mildew, e.g. *Sphaerotheca fuliginea*, or rusts, e.g. *Hemileia vastatrix*) and plant-parasitic nematodes, e.g. *Heterodera glycines* (Askary & Yarmand 2007, Ramanujam et al. 2011). *Akanthomyces muscarius* (Petch) Spatafora, Kepler & B. Shrestha and *A. lecanii* (Zimm.) Spatafora, Kepler & B. Shrestha are most often investigated and partly used in the biological control of insect pests (Koubová 2009, Grent 2011, Upadhyay et al. 2014).

The fungus *Purpureocillium lilacinum* (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson is more widely known as *Paecilomyces lilacinus*. It is an egg-pathogenic fungus and one of the most extensively tested soil fungi for the biological control of plant-parasitic nematodes (Hajji et al. 2017).

Biopesticides based on entomopathogenic fungi can be used in integrated pest management (IPM). Although IPM prefers the use of non-synthetic plant protection strategies, it allows the use of synthetic pesticides. Entomopathogenic fungus hosts are mainly arthropods, therefore these fungi cannot protect against other harmful agents such as diseases, non-target pests and weeds. Hence there is still a need for synthetic pesticides, too. Entomopathogenic fungi can thus not replace synthetic insecticides in all commercial agricultural ecosystems. Insecticides are always required to suppress rapidly expanding insect pest populations. In general, pesticides are an anthropogenic factor with a synergistic or antagonistic effect not only on pests but also on their pathogens (entomopathogenic fungi) and through that on their efficiency. Furthermore, the efficacy of entomopathogenic fungi living in soil is affected by regular pesticide usage in agronomical practice, as a result of residue accumulation in the soil. Therefore, selecting and applying pesticides with less antagonistic effects can minimise their harmful effect on entomopathogenic fungi and improve their efficacy. Consequently, it is important to know and understand the compatibility of entomopathogenic fungi with pesticides in order to incorporate mycoinsecticides based on entomopathogenic fungi in integrated crop production (Celar & Kos 2012).

Our research focused on evaluating the influence of 17 selected pesticides (fungicides, herbicides and insecticides) on the radial growth of entomopathogenic species of the genera *Akanthomyces*, *Cordyceps*, *Beauveria*, and *Purpureocillium* under laboratory conditions. Practical consequences based on the results are discussed.

Material and methods

The effect of 7 fungicides, 5 insecticides, and 5 herbicides on the radial growth of entomopathogenic fungi was evaluated. Products and application rates tested are given in Tab. 1. Commercial pesticides were used. The pesticide dose was calculated according to recommended field application rate based on 1000 litres of spray fluid per hectare. The recommended doses followed the Register of Plant Protection Products in the Czech Republic (Central Institute for Supervising and Testing in Agriculture). Pirimor, Mospilan and Karate Zeon insecticides were also tested at half concentrations. The insecticides were used in half doses, too, to test their potential suitability for usage of lower rates in combination with biological preparations. This approach should have certain importance in future IPM practice. We supposed that a lower dose of synthetic insecticide could both weaken or/and kill the population of a target pest and allow entomopathogens to attack the host more easily.

The following fungus strains were used: *Akanthomyces muscarius* – strains OP1, CCF 3297, Ve6, *Cordyceps fumosorosea* – strains AGTA 7, CCF 2790, *Beauveria bassiana* – strain AGTA 5, and *Purpureocillium lilacinum* – strain CCF 3531. CCF fungal strains used in the present study were obtained from the Culture Collection of Fungi in Prague. Strains *C. fumosorosea* AGTA 7 and *B. bassiana* AGTA 5 were isolated from *Carabus* sp. (Šumperk, 2014), and *A. muscarius* OP1 was isolated from *Brassicogethes aeneus* (Bílovec, 2013). All the AGTA and OP1 strains are deposited in the Agritec Plant Research Ltd. Strain Ve6 was obtained from the commercial product Mycotal.

Each strain was cultured in Petri dishes on sterile Czapek Dox Agar (HiMedia Laboratories; autoclaved at 121 °C for 15 min.) medium in the dark at 23 °C. The pesticides were evaluated with the poisoned food technique (Amutha et al. 2010) in Czapek Dox Agar. Each pesticide with its respective concentration was added to sterile Czapek Dox Agar just before the medium was poured into sterile Petri dishes (9 cm in diameter). The controls consisted of sterile Czapek Dox Agar with no pesticide added. Mycelial plug inoculum was prepared by cutting 5 mm diameter discs with a sterile cork borer from the periphery of actively growing (14-day-old) Petri dish cultures and placed on the treated medium with different pesticides. Four replicates were used for each treatment. The plates were incubated

Tab. 1. Active ingredients and application rates of pesticides used in laboratory tests of the growth of entomopathogenic fungi.

Product name	**Product class**	**Active ingredient**	**Recommended field application rate** (g a.i./ha or t of seed*)	**Concentration** (g a.i./l of Czapek Dox Agar)
Acrobat MZ WG	Fungicide (cinnamic acid amides and dithio-carbamates and relatives)	Dimethomorph	1200	1.2
		Mancozeb	180	0,18
Ridomil Gold MZ Pepite	Fungicide (dithio-carbamates and relatives and acylalanines)	Mancozeb	1600	1.6
		Metalaxyl-M	100	0.1
Kuprikol 50	Fungicide (inorganic)	Copper oxychloride	4200	4.2
Signum	Fungicide (pyridine-carboxamides and methoxy-carbamates)	Boscalid	400.5	0.4005
		Pyraclostrobin	100.5	0.1005
Dithane DG Neotec	Fungicide (dithio-carbamates and relatives)	Mancozeb	1500	1.5
Vitavax 2000	Fungicide (oxathiin-carboxamides and dithio-carbamates and relatives)	Carboxin	500	0.5
		Thiram	500	0.5
Maxim XL 035 FS	Fungicide (phenylpyrroles and acylalanines)	Metalaxyl-M	10	0.01
		Fludioxonil	25	0.025
Mavrik 2 F	Insecticide (pyrethroids)	Tau-fluvalinate	48	0.048
Pirimor 50 WG	Insecticide (carbamates)	Pirimicarb	250	0.25 0.125**
SpinTor	Insecticide (spinosyns)	Spinosad	96	0.096
Mospilan 20 SP	Insecticide (neonicotinoids)	Acetamiprid	40	0.04 0.02**
Karate with Zeon technology	Insecticide (pyrethroids)	Lambda-cyhalothrin	10	0.01 0.005**
Stomp 400 SC	Herbicide (dinitroaniline) – pre- and post-emergent	Pendimethalin	1640	1.64
Somero	Herbicide – pre- and post-emergent	Pethoxamid	1200	1.2
Command 36 CS	Herbicide (isoxazolidinone) – pre-emergent	Clomazone	90	0.09
Lentipur 500 FW	Herbicide – pre- and post-emergent	Chlorotoluron	750	0.75
Escort New	Herbicide (imidazolinone and dinitroaniline) – post-emergent	Pendimethalin	750	0.75
		Imazamox	50.1	0.05

* g a.i./tonne of seed is applied for fungicides Vitavax 2000 and Maxim XL 035 FS; otherwise the rate is given in g a.i./ha
** values of half concentrations

in the dark at 23 °C in a laboratory Q-Cell 140 incubator with refrigeration (Pol-Lab, Wilkowice, Poland). The colony diameter was measured every day for 16 days.

The average daily increments were statistically analysed (Statistica 12, Factorial ANOVA, Tukey HSD test, $p = 0.05$) and the differences in mean levels of increments recorded at the treatments with pesticides were compared with the mean increments recorded in the related controls.

Results

Fungicides

All the fungicides tested, with the exception of Kuprikol 50 for both *Cordyceps* strains, suppressed the growth of entomopathogenic fungi mycelium statistically significantly ($p < 0.001$).

Ridomil Gold MZ Pepite decreased mycelium growth of all investigated strains by 40.7–97.5%, Acrobat MZ WG by 47.2–94.9%, Signum by 47.2–92.4%, Dithane DG Neotec by 57.1–100%, Maxim XL 035 FS by 85.6–100%, and Vitavax 2000 by 20.4–100% (Figs. 1–2). Kuprikol 50 inhibited statistically significantly the growth of *B. bassiana* by 44.4%, *P. lilacinum* by 44.1%, *A. muscarius* OP1 by 35.0%, *A. muscarius* CCF 3297 by 37.8%, and *A. muscarius* Ve6 by 81.4%. *Cordyceps* strains growth was not significantly inhibited by Kuprikol 50 ($p = 0.453$). *Cordyceps* strains were generally less susceptible to fungicides; the most susceptible were *A. muscarius* Ve6 and *P. lilacinum* strains. Of all the fungicides, Maxim XL 035 FS had the greatest inhibitive effect on mycelium growth, while Kuprikol 50 had the smallest (Tab. 2).

Insecticides

Insecticides, as compared to other pesticides, did not have a very damaging effect on mycelium growth of entomopathogenic fungi.

In almost all tests of the insecticides, the mycelium growth of *Cordyceps* strains was increased compared to the control. In the case of Karate with Zeon technology insecticide at the full rate [concentration of 0.01 g active ingredient (a.i.)/l], the growth stimulation was statistically significant. The colony size of the *C. fumosorosea* CCF 2790 strain was 55.8% higher in comparison with the related control, while the colony size of *C. fumosorosea* AGTA 7 was 47.8% higher. The insecticides Spintor, Mavrik, and Mospilan slightly suppressed the mycelium growth of *A. muscarius* strains. The inhibition was not statistically significant. The mycelium growth of the *B. bassiana* strain was suppressed by all the insecticides tested, with the exception of Karate with Zeon technology. The insecticides Mavrik 2 F, Pirimor at both full and half rates and Mospilan 20 SP at the full rate resulted in significant reductions by 22.6%, 31.3%, 24.5% and 30%, respectively, relative to the control (Tab. 3).

Herbicides

Most herbicides tested affected mycelium growth of all the investigated entomopathogenic fungi negatively.

Tab. 2. Mycelium growth inhibition of entomopathogenic fungi *Akanthomyces muscarius*, *Cordyceps fumosorosea*, *Beauveria bassiana* and *Purpureocillium lilacinum*, cultured on Czapek Dox Agar treated with fungicides.
Percentages are given as mean ± standard deviation. Values followed by different letters are significantly different from each other according to the Tukey HSD test at $p < 0.001$. Values in bold are significantly different from the control.

Strain	Vitavax 2000	Maxim XL 035 FS	Kuprikol 50	Acrobat MZ WG	Ridomil Gold MZ Pepite	Signum	Dithane DG Neotec	Control
Akanthomyces muscarius OP1	**75.4** ± 1.6$^{n–t}$	**93.7** ± 0^{wxyz}	**35.0** ± 3.3^{c}	**70.0** ± 6.0$^{l–p}$	**66.7** ± 3.8$^{j–n}$	**57.0** ± 3.8$^{fh–k}$	**60.0** ± 5.7$^{h–l}$	0.0 ± 3.2^{a}
Akanthomyces muscarius Ve6	**100.0** ± 0^{z}	**100.0** ± 0^{z}	**81.4** ± 8.8$^{r–v}$	**94.9** ± 1.7^{wxyz}	**91.2** ± 1.6$^{u–z}$	**92.4** ± 1.7$^{v–z}$	**92.1** ± 2.0$^{u–z}$	0.0 ± 1.6^{a}
Akanthomyces muscarius CCF 3297	**68.2** ± 1.8$^{k–p}$	**96.4** ± 0^{xyz}	**37.8** ± 9.4^{cd}	**78.1** ± 2.5$^{o–t}$	**74.4** ± 2.1$^{m–s}$	**50.0** ± 2.5^{efgh}	**82.9** ± 4.9^{rw}	0.0 ± 2.0^{a}
Cordyceps fumosorosea AGTA 7	**67.7** ± 1.4$^{k–p}$	**85.6** ± 2.8$^{s–y}$	10.1 ± 1.6^{ab}	**55.4** ± 3.3^{fhij}	**97.5** ± 5.0^{yz}	**62.9** ± 2.9$^{i–m}$	**57.1** ± 2.9$^{fh–k}$	0.0 ± 3.8^{a}
Cordyceps fumosorosea CCF 2790	**20.4** ± 13.7^{b}	**93.5** ± 1.9^{wxyz}	9.2 ± 1.6^{ab}	**47.2** ± 5.6^{defg}	**55.5** ± 1.8$^{f–j}$	**47.2** ± 5.6^{defg}	**73.2** ± 5.4$^{m–r}$	0.0 ± 5.2^{a}
Beauveria bassiana AGTA 5	**91.3** ± 2.5$^{u–z}$	**100.0** ± 0^{z}	**44.4** ± 3.3^{cdeg}	**79.5** ± 1.5$^{o–u}$	**40.7** ± 6.3^{cde}	**86.9** ± 3.3$^{t–y}$	**100.0** ± 0^{z}	0.0 ± 4.9^{a}
Purpureocillium lilacinum CCF 3531	**97.4** ± 2.9^{yz}	**100.0** ± 0^{z}	**44.1** ± 1.3^{cdeg}	**84.4** ± 1.3$^{s–x}$	**49.2** ± 3.2$^{d–i}$	**65.9** ± 2.7$^{j–n}$	**79.6** ± 2.1$^{o–u}$	0.0 ± 6.7^{a}

Tab. 3. Mycelium growth inhibition (positive values) or increased growth (negative values) of entomopathogenic fungi *Akanthomyces muscarius*, *Cordyceps fumosorosea*, *Beauveria bassiana* and *Purpureocillium lilacinum*, cultured on Czapek Dox Agar treated with insecticides.
Percentages are given as mean ± standard deviation. Values followed by different letters are significantly different from each other according to the Tukey HSD test at $p = 0.05$. Values in bold are significantly different from the control.

Strain	Mavrik	Spintor	Pirimor	Pirimor half	Mospilan	Mospilan half	Karate Zeon	Karate Zeon half	Control
Akanthomyces muscarius OP1	**16.2** ± 3.4^{stuv}	13.6 ± 7.6$^{p–u}$	–5.4 ± 2.0$^{d–m}$	–5.4 ± 10.3$^{d–m}$	–0.9 ± 1.8$^{e–p}$	0.9 ± 3.4^{gs}	–12.5 ± 18.5$^{c–h}$	0.0 ± 0$^{f–r}$	0.0 ± 5.9$^{f–r}$
Akanthomyces muscarius CCF 3297	10.1 ± 0.9$^{h–u}$	10.3 ± 2.3$^{i–u}$	**–16.2** ± 2.2^{bcde}	–15.3 ± 5.7$^{b–f}$	11.5 ± 1.9$^{n–u}$	13.4 ± 1.9$^{p–u}$	2.9 ± 4.9$^{h–s}$	9.6 ± 4.8$^{k–u}$	0.0 ± 2.5$^{f–r}$
Akanthomyces muscarius Ve6	12.2 ± 2.8$^{o–u}$	11.4 ± 2.3$^{n–u}$	11.7 ± 5.8$^{n–u}$	9.2 ± 3.2$^{k–u}$	5.0 ± 2.0$^{i–s}$	3.3 ± 0^{hs}	7.5 ± 5.0$^{j–t}$	7.5 ± 10.4$^{j–t}$	0.0 ± 4.7$^{f–r}$
Cordyceps fumosorosea AGTA 7	**–21.4** ± 2.4^{bc}	–8.6 ± 3.7$^{c–i}$	–12.1 ± 1.5$^{c–h}$	–7.1 ± 5.5$^{c–j}$	–4.3 ± 1.6$^{d–n}$	5.7 ± 0^{is}	**–47.8** ± 8.5^{a}	**–30.7** ± 4.3^{b}	0.0 ± 4.0$^{f–r}$
Cordyceps fumosorosea CCF 2790	**–30.7** ± 3.3^{b}	–6.2 ± 4.1$^{d–k}$	**–19.5** ± 3.4^{bcd}	–14.2 ± 3.4$^{e–g}$	0.0 ± 4.5$^{f–r}$	–8.9 ± 8.9$^{c–i}$	**–55.8** ± 10.4^{a}	–11.5 ± 4.6$^{c–h}$	0.0 ± 6.5$^{f–r}$
Beauveria bassiana AGTA 5	**22.6** ± 4.9^{tuv}	12.0 ± 11.7$^{o–u}$	**31.3** ± 3.4^{v}	**24.5** ± 2.6^{uv}	**30.0** ± 1.4^{v}	15.6 ± 2.2$^{r–v}$	–5.4 ± 2.6$^{e–m}$	–2.7 ± 2.6$^{e–o}$	0.0 ± 5.8$^{f–r}$
Purpureocillium lilacinum CCF 3531	2.8 ± 2.8$^{h–s}$	–5.2 ± 1.6$^{d–km}$	**23.4** ± 3.5^{tuv}	15.7 ± 6.4$^{r–v}$	9.1 ± 7.9$^{k–u}$	4.9 ± 3.2$^{i–s}$	–7.5 ± 5.7$^{c–j}$	–15.7 ± 5.7$^{b–f}$	0.0 ± 7.4$^{f–r}$

The herbicides Escort New and Somero suppressed the mycelium growth of all the tested strains of entomopathogenic fungi most strongly throughout the cultivation period. Escort New decreased the mycelium growth by 87.6–100%, Somero by 80.6–100%, Stomp by 22.1–62.7%, and Lentipur by 12.9–55.5%. Contrary to that, the growth of the majority of the tested entomopathogenic fungi was not negatively affected by herbicide Command. This was the only herbicide included in the experiment which did not show negative effects on most of the tested fungi. The *B. bassiana* strain was most susceptible to the tested herbicides (Tab. 4).

Tab. 4. Mycelium growth inhibition (positive values) or increased growth (negative values) of entomopathogenic fungi *Akanthomyces muscarius*, *Cordyceps fumosorosea*, *Beauveria bassiana* and *Purpureocillium lilacinum*, cultured on Czapek Dox Agar treated with herbicides.
Percentages are given as mean ± standard deviation. Values followed by different letters are significantly different from each other according to the Tukey HSD Test at p = 0.05. Values in bold are significantly different from the control.

Strain	Command	Lentipur	Stomp 400 SC	Eskort New	Somero	Control
Akanthomyces muscarius OP1	–2.7 ± 5.3^{ab}	**45.2** ± 11.2^{gh}	**29.9** ± 12.4^{f}	**95.9** ± 0^{klm}	**82.1** ± 2.2^{jk}	0.0 ± 3.3^{abc}
Akanthomyces muscarius CCF 3297	–2.3 ± 2.7^{ab}	**22.2** ± 4.3^{ef}	**32.0** ± 2.7^{fg}	**100.0** ± 0^{lm}	**80.6** ± 3.8^{j}	0.0 ± 5.5^{abc}
Akanthomyces muscarius Ve6	–2.5 ± 2.1^{ab}	**33.4** ± 2.5^{fg}	**32.6** ± 6.3^{fg}	**96.5** ± 0^{klm}	**85.8** ± 2.8^{jkm}	0.0 ± 2.2^{abc}
Cordyceps fumosorosea AGTA 7	3.9 ± 4.8^{abcd}	12.9 ± 2.0^{cde}	**24.7** ± 5.9^{ef}	**100.0** ± 0^{lm}	**100.0** ± 0^{l}	0.0 ± 3.0^{abc}
Cordyceps fumosorosea CCF 2790	–6.9 ± 6.7^{a}	**14.9** ± 6.7^{de}	**22.1** ± 3.3^{ef}	**100.0** ± 0^{lm}	**90.2** ± 2.9^{jklm}	0.0 ± 2.4^{abc}
Beauveria bassiana AGTA 5	**14.0** ± 14.2^{de}	**55.5** ± 3.5^{hi}	**62.7** ± 5.5^{i}	**100.0** ± 0^{lm}	**100.0** ± 0^{l}	0.0 ± 9.6^{abc}
Purpureocillium lilacinum CCF 3531	6.8 ± 8.1^{bcd}	**32.7** ± 2.7^{fg}	**44.4** ± 7.9^{gh}	**87.6** ± 1.7^{jklm}	**89.4** ± 1.1^{jklm}	0.0 ± 2.5^{abc}

DISCUSSION

Pesticides used for pest control in agroecosystems can affect the growth and thus the effectiveness of entomopathogenic fungi. In general, the results indicate that fungicides are highly inhibitory to the entomopathogens followed by herbicides. Insecticides were found to be less or non-toxic to fungi.

Fungicides

Fungicides are often required to control plant diseases, but many of them have a broad spectrum of activity and can affect the efficacy of entomopathogenic fungi adversely. Sterk et al. (2003) tested the colony growth of *C. fumosorosea*, *Akanthomyces lecanii* and *B. bassiana* in the presence of twelve active fungicide ingredients. They found the lowest toxicity in the active substances mepanipyrim, trifloxystrobin, sulphur and kresoxim-methyl. In their research,

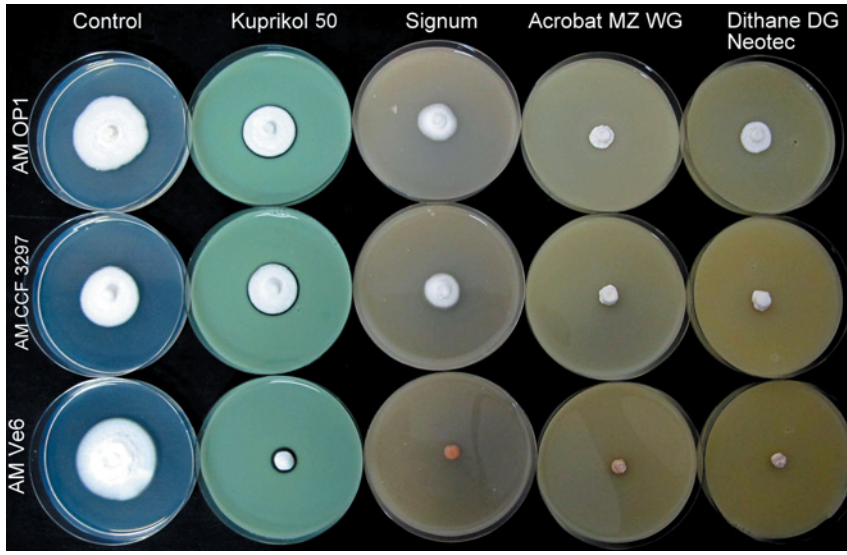

Fig. 1. Radial mycelium growth of *Akanthomyces muscarius* strains on Czapek Dox Agar plates treated with fungicides Kuprikol, Signum, Acrobat and Dithane after 14 days of incubation.

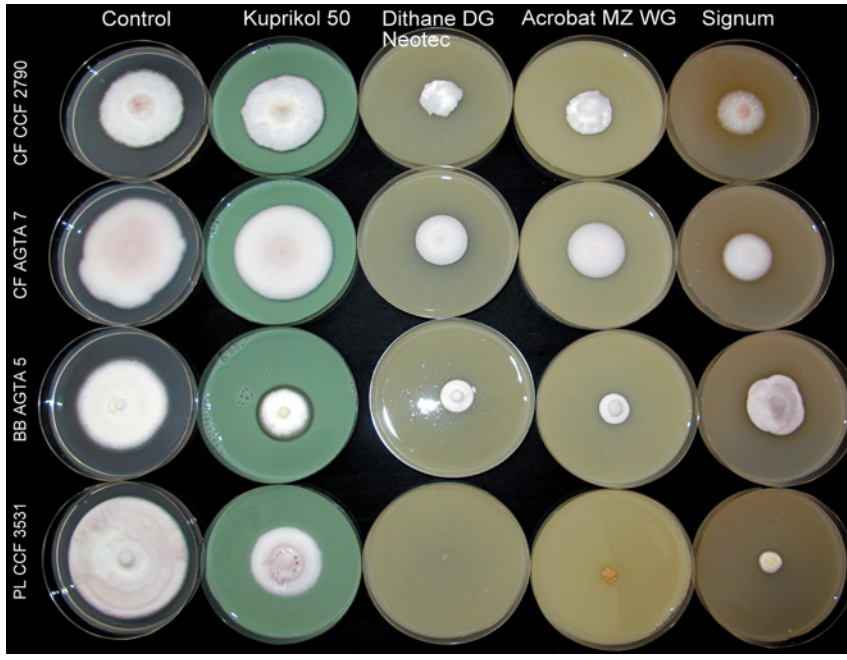

Fig. 2. Radial growth of *Cordyceps fumosorosea*, *Beauveria bassiana* and *Purpureocillium lilacinum* strains on Czapek Dox Agar plates treated with fungicides Kuprikol, Signum, Acrobat and Dithane after 14 days of incubation. Photos E. Ondráčková.

thiram (concentration 1.6 g a.i./l) was moderately harmful (51–75% growth inhibition) to *Akanthomyces lecanii* and *C. fumosorosea*, and slightly harmful (25–50% growth inhibition) to *B. bassiana*. We found that thiram (concentration 0.5 g a.i./l) together with carboxin (concentration 0.5 g a.i./l) strongly inhibited (68–100%) the mycelial growth of *A. muscarius*, *B. bassiana* and *P. lilacinum* strains, and *C. fumosorosea* strain AGTA 7. By comparing both results, it seems that the combination of thiram and carboxin inhibits the mycelial growth of the entomopathogens much more strongly than thiram alone.

Rachappa et al. (2007) also reported high toxicity (100% inhibition) of the fungicides, mainly those with azole active ingredients chlorthalonil and carbendazim, to *Metarhizium anisopliae*. Moreover, mancozeb inhibited colony growth of *M. anisopliae* by 83%. Similarly, we found that fungicides with mancozeb inhibited the colony growth of entomopathogenic fungi by 77.8% on average. Thus, fungicides based on active ingredient mancozeb or on a combination with other active ingredients will not be suitable for the use in IPM systems where the use of biological preparations based on entomopathogenic fungi is considered. By contrast, copper oxychloride was found to be comparatively safe for the tested entomopathogenic fungi (inhibition of 37.43% on average) in our study. Rachappa el al. (2007) reported 67.53% growth inhibition of *M. anisopliae* which roughly corresponds to our results.

Insecticides

Insecticides are often needed to suppress a rapidly expanding pest population or to control pests not targeted by entomopathogenic fungi. The majority of tested insecticides did not statistically significantly affect the growth of these fungi negatively, or even stimulated the growth of some strains. The growth of *C. fumosorosea* strains was increased by insecticides with pirimicarb, spinosad, and tau-fluvalinate, and *P. lilacinum* by insecticides with spinosad and tau-fluvalinate. These pesticides can therefore be used together with biological preparations containing the aforementioned fungi. Contrary to our findings, some authors report that insecticides inhibit mycelial growth of entomopathogenic fungi but some of them (e.g. imidacloprid, spinosad, chlorpyriphos 20 EC, dimethoate) were found to be safe for the fungi (Rachappa et al. 2007, Amutha et al. 2010, Asi et al. 2010).

Herbicides

Herbicides applied pre-emergently or early post-emergently enter the soil and can thus affect the growth of useful soil entomopathogenic fungi. The herbicides tested in this study, with the exception of active ingredients pendimethalin in combination with imazamox (included in Escort New), are commonly used for

pre-emergent applications. In our tests, most of them had a negative effect on the mycelium growth of entomopathogens. Only clomazone (included in Command 36 S) did not have an adverse impact on their growth. Similarly, Celar & Kos (2012) reported that *B. bassiana* is highly sensitive to five commonly used herbicides with active ingredients pyridate, fluazifop-P-butyl, foramsulfuron, tembotrione and metolachlor-S, applied in the full and lower doses (75–25% of recommended field dosage rate). Rachappa et al. (2007) also found that herbicides (attrazin, diuron, nitrofen, glyphosate, alachlor, pedimethalin and butachlor) appeared to be toxic, but the level of toxicity was low (10.26–26.07% inhibition). Pedimethalin inhibited mycelial growth of *M. anisopliae* by 23.08%, which corresponds to our finding (22.1–62.7% inhibition depending on the fungal strain).

In general, fungi may recover after some synthetic pesticides have decomposed in the soil or on plant leaves. A high toxicity in vitro does not always mean that the same effect will occur in the field, but it indicates the possibility that some negative effects may also appear under field conditions (Alves et al. 1998).

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