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EFFECTS OF SELECTED PESTICIDES ON THE GROWTH OF FUNGI FROM *HIRSUTELLA* GENUS ISOLATED FROM PHYTOPHAGOUS MITES

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Abstract: The aim of this study was to determine *in vitro* effects of selected pesticides on the growth of fungi: *Hirsutella nodulosa*, *H. brownorum*, *H. kirchneri* and *H. necatrix*, isolated from phytophagous mites. Insecticides and herbicide were added to the medium at the following rates: 0.1; 1 and 10 times the field rate recommended by manufacturer. Fungicides were applied at the following rates: 0.01, 0.1 and 1 times the rate recommended by manufacturer. Pesticides tested in this experiment showed a strong inhibiting effects on the growth of fungi from *Hirsutella* genus – natural pathogens of phytophagous mites. Among all pesticides tested, fungicides showed the strongest inhibiting effect on the growth of *Hirsutella* species and especially flusilazole (Punch 400 EC). Amitraz (Mitac 20 EC) and phosalone (Zolone 350 EC) were the most toxic among tested insecticides. Fungi *H. necatrix* and *H. kirchneri* isolated from eriophyid mite *Abacarus hystrix* were the most resistant to toxic effect of tested pesticides but *H. nodulosa* isolated from *Tarsonemidae* mite seems to be the most susceptible.

Key words: pesticides, *Hirsutella* fungi, toxicity

INTRODUCTION

Our knowledge of fungal diseases of mites is still fragmentary, although an increasing number of reports on this subject have been published in recent years (Chandler et al. 2000; McCoy 1996; Miętkiewski et al. 2000; Miętkiewski et al. 2003; van der Geest 2004; van der Geest et al. 2000). The most frequently encountered fungal pathogens of mites belong to the genera *Neozygites* of the order *Entomophthorales* and *Hirsutella* of the order *Hyphomycetales*. Fungi from *Hirsutella* genus are very important factor in reduction of phytophagous mite population causing up to 60% of mortality (Miętkiewski et al. 2000; Miętkiewski et al. 2003). They are used in biological control, e.g. acaricide "Mycar" based on *H. thompsonii* (McCoy 1996).

The use of insect and mite pathogenic fungi as microbial control agents usually needs to be integrated with the use of different pesticides, which may have a direct impact on natural occurrence, infectivity and population dynamic of these pathogens. Many *in vitro* studies have demonstrated that some pesticides restrict or prevent growth, sporulation and germination of entomopathogenic fungi (Bajan and Kmitowa 1982; Ignoffo et al. 1975; Keller 1978; Majchrowicz and Poprawski 1993; Miętkiewski et al. 1997; Vanninen and Hokkanen 1988).

In the literature there are only few reports about the influence of pesticides on mycelial growth and sporulation of fungi from *Hirsutella* genus and they concern only two species - *Hirsutella thompsonii* (Sosa Gomez 1991; Sosa Gomez et al. 1984, 1987; Tkaczuk 2001) and *Hirsutella nodulosa* (Tkaczuk et al. 2004).There is no information about the effects of pesticides on development of other fungal species from the genus *Hirsutella*, isolated from mites.

The aim of this study was to determine *in vitro* effects of selected pesticides on the growth of four *Hirsutell*a species fungi, isolated from phytophagous mites in Poland.

MATERIAL AND METHODS

The four species of mite pathogenic fungi used in this experiment were: *Hirsutella nodulosa* Petch, *H. brownorum* Minter et Brady , *H. kirchneri* (Rostrup) Minter, Brady et Hall and *H. necatrix* Minter, Brady et Hall.

H. nodulosa and *H. brownorum* were isolated from *Tarsonemidae* mites feeding on *Potentilla anserina* L., while *Hirsutella kirchneri* and *H. necatrix* were isolated from eriophyid mite *Abacarus hystrix* (Nalepa) feeding on the grass species *Lolium perenne* L. The growth of these fungi was examined on Sabouraud dextrose agar (SDA) containing pesticides. Insecticides amitraz and hexythiazox chosen for the experiment are typical representatives of acaricides used against mites. Fungicides and herbicide taken for evaluation are commonly used in plant protection of arable crops and orchards. The characteristics of evaluated pesticides are presented in Table 1.

Insecticides and herbicide were applied at 0.1; 1 and 10 times the field rate recommended by manufacturer. Fungicides were applied at the following rates: 0.01, 0.1 and 1 times the rate recommended by manufacturer. Pesticides were added to

Table.1 Pesticides used in the experiment

Trade name	Active ingredients content [% or g l^{-1}]	Recommended rate (a.i.g/l)	Producer
Fungicides			
Punch 400 EC	flusilazole – 400g	0.4	Du Pont de Nemours S.A.S., France
Score 250 EC	difenconazole – 250g	0.1	Syngenta Crop Protec,, Switzerland
Syllit 65 WP	dodine – 65%	1.6	Chimac-Agriphar S.A., Belgium
Insecticides			
Mitac 200 EC	amitraz – 200g	0.6	Bayer CropScience S.A., France
Nissorun 050 EC	hexythiazox – 50g	0.5	Nippon Soda Co. Ldt., Japan
Zolone 350 EC	phosalone – 350g	0.1	Bayer CropScience S.A., France
Herbicides			
Stomp 330 EC	pendimethalin – 330g	0.2	BASF Agro B.V., The Netherlands

sterilized Sabouraud medium after cooling to approximately 60°C. Plates, after inoculation, were incubated at 21°C. Colony diameter was measured 10, 15, 20 and 25 days after inoculation. Control plates with no pesticide were also set up for each fungus. Each pesticide/concentration treatment and the control were replicated six times. Obtained results were expressed as a percentage of control value.

RESULTS

Among all pesticides used in this experiment, fungicides showed the strongest inhibiting effect on the growth of *Hirsutella* species (Table 2). None of the fungi were able to grow on medium containing flusilazole (Punch 400 EC)) used at the field rate, except *H. necatrix*, although its growth only began after 15 days and achieved 20% of the control value. At concentration 0.1 times the field rate flusilazole prevented the growth of *H. nodulosa* and strongly inhibited the growth of other species. Flusilazole at concentration 100-times lower than the field dose, strongly inhibited the growth of *H. nodulosa* but *H. kirchneri* and *H. necatrix* were the most resistant to toxic effects of this fungicide and their colonies achieved respectively 76.6% and 84.3% of control value.

None of the fungi were able to grow on medium containing difenconazole (Score 250 EC) used at the field rate. At concentration 0.1 times the field rate difenconazole prevented the growth of *H. nodulosa*. Colonies of other species achieved from 62.2 to 81.4% of the control growth on 25th day. The same tendency at 0.01 times the field concentration of difenconazole was observed.

The fungicide dodine (Syllit 65 WP) was considerably less toxic to investigated fungi. On a medium containing dodine at the field rate all fungi grew except *H. necatrix,* although the diameter of their colonies did not surpass 40% in relation to the check treatment. Colonies of *H. kirchneri* and *H. necatrix* growing on a medium containing dodine at 0.1 times the recommended rate reached only 30% of control value.

The tested fungi were unable to grow on media containing insecticides at 10 times the recommended field rate (Table 3). Acaricide amitraz (Mitac 200 EC) was the most toxic among insecticides used at the field rate, reducing colony size by 62.3–83.8%. *H. brownorum* was the most resistant to amitraz while *H. nodulosa* and *H. kirchneri* were the most susceptible. Amitraz at concentration 10 times lower than the field rate was less toxic and colonies of *Hirsutella* fungi achieved the growth from 53% to 85.3% of control values.

Hexythiazox (Nissorun 050 EC) was less toxic at the field rate than amitraz and reduced fungal growth on the level of 31.2–46.4% of the control, depending on the species. *H. necatrix* was the most resistant to hexythiazox at the field dose but *H. nodulosa* was the most sensitive. Colonies of *Hirsutella* fungi growing on media containing hexythiazox at 0.1 times the recommended rate reached more than 80% of the control value.

Phosalone (Zolone 350 EC) at the recommended rate showed a less inhibitory effect on the development of four tested species than armitraz, but was more toxic than hexythiazox at the same concentration. The most resistant to toxic effect of phosalone was *H. necatrix* and *H. nodulosa* was the most susceptible. Phosalone at

0.1 times recommended rate showed the most inhibiting effect on the growth of fungi among tested insecticides.

None of the fungi were able to grow on media containing herbicide pendimethalin at 10 times the field rate except *H. kirchneri* (Table 3). Pendimethalin (Stomp 330 EC) at the field and 0.1 times field rate strongly inhibited the growth of *H. nodulosa* and *H. brownorum* but *H. kirchneri* and *H. necatrix* were more resistant.

Table 2. Effects of different concentration of fungicides on the growth (% in relation to the control) of *Hirsutella* species *in vitro*

Pesticide	Dose	Days of growth	Fungal species			
			H. nodulosa	*H. brownorum*	*H. kirchneri*	*H. necatrix*
Punch 400 EC (flusilazole)	A	10	0	0	0	bg
		15	0	0	0	18.6± 0.8
		20	0	0	0	20.3± 0.6
		25	0	0	0	20.3± 0.6
	B	10	0	bg	bg	bg
		15	0	20.3 ± 0.7	24.5 ± 0.8	33.0 ± 1.2
		20	0	19.3 ± 0.7	19.3 ± 0.4	34.1 ±1.0
		25	0	20.1 ± 0.9	17.2 ± 0.6	33.1 ± 0.8
	C	10	0	bg	bg	bg
		15	bg	38.2 ± 0.3	71.6 ± 1.2	79.4 ± 0.5
		20	bg	44.2 ± 0.6	74.5 ± 0.5	81.2 ± 0.9
		25	21.4	52.6 ± 0.8	76.6 ± 0.8	84.3 ± 1.1
Score 250 EC (difenconazole)	A	10	0	0	0	0
		15	0	0	0	0
		20	0	0	0	0
		25	0	0	0	0
	B	10	0	bg	bg	bg
		15	0	24.4 ± 0.9	Mg	30.9 ± 1.2
		20	0	26.1 ± 0.9	23.4 ± 1.3	38.4 ± 1.0
		25	0	71.6 ± 1.0	71.4 ± 2.2	83.1 ± 1.6
	C	10	0	bg	Bg	bg
		15	bg	24.4 ± 1.8	63.7 ± 0.9	67.0 ± 0.7
		20	bg	55.8 ± 1.0	71.0 ± 0.6	85.5 ± 0.4
		25	21.4 ± 1.7	62.2 ± 0.9	72.9 ± 0.6	81.4 ± 0.5
Syllit 65 WP (dodine)	A	10	0	bg	0	0
		15	bg	40.7 ± 1.4	bg	0
		20	31.4 ± 1.8	38.2 ± 1.0	bg	0
		25	37.9 ± 1.3	38.1 ± 0.8	26.0 ± 1.0	0
	B	10	0	bg	bg	bg
		15	56.1 ± 0.7	54.5 ± 1.0	39.2 ± 0.7	33.0 ± 0.6
		20	51.1 ± 0.4	48.7 ± 0.4	34.5 ± 0.7	30.4 ± 0.5
		25	64.3 ± 0.4	53.2 ± 0.6	31.3 ± 0.4	30.8 ± 0.3
	C	10	bg	71.1 ± 1.1	90.9 ± 0.7	93.8 ± 2.4
		15	53.1 ± 1.2	75.6 ± 0.9	81.4 ± 1.2	69.1 ± 0.9
		20	53.3 ± 0.8	75.8 ± 1.2	84.1 ± 1.0	85.5 ± 2.3
		25	80.7 ± 0.9	72.7 ± 0.8	72.9 ± 1.2	85.5 ± 1.7

Note: bg – beginning of growth, A – field rate, B – 0.1 field rate, C – 0.01 field rate

Table 3. Effects of different concentration of insecticides and herbicide on the growth (% in relation to the control) of *Hirsutella* species *in vitro*

Pesticide	Dose	Days of growth	Fungal species			
			H. nodulosa	*H. brownorum*	*H. kirchneri*	*H. necatrix*
Mitac 200 EC (amitraz)	A	10	0	0	0	0
		15	0	0	0	0
		20	0	0	0	0
		25	0	0	0	0
	B	10	bg	73.3 ± 1.1	bg	bg
		15	24.7 ± 1.1	40.0 ± 0.8	25.8 ± 0.8	27.2 ± 1.7
		20	20.0 ± 0.8	41.7 ± 0.8	23.1 ± 0.4	23.9 ± 1.2
		25	16.2 ± 0.4	37.7 ± 0.6	22.4 ± 0.4	23.1 ± 0.9
	C	10	82.1 ± 1.7	100.0 ± 2.3	100.0 ± 1.3	91.4 ± 0.9
		15	57.0 ± 1.5	78.8 ± 0.9	70.1 ± 1.1	66.0 ± 1.2
		20	55.7 ± 0.7	79.2 ± 0.8	85.5 ± 0.7	76.9 ± 0.7
		25	53.0 ± 0.7	76.6 ± 0.6	85.3 ± 0.8	70.1 ± 0.7
Nissorun 050 EC (hexythiazox)	A	10	0	0	0	0
		15	0	0	0	0
		20	0	0	0	0
		25	0	0	0	0
	B	10	0	bg	bg	bg
		15	48.9 ± 0.9	51.2 ± 1.2	44.1 ± 1.3	59.8 ± 0.8
		20	43.8 ± 0.8	68.3 ± 0.8	53.8 ± 1.4	70.3 ± 1.0
		25	53.6 ± 0.9	62.1 ± 0.6	62.5 ± 1.0	69.8 ± 1.2
	C	10	bg	60.5 ± 1.2	81.8 ± 0.4	100.0 ± 2.1
		15	71.4 ± 0.9	78.9 ± 1.0	83.3 ± 0.6	96.9 ± 0.8
		20	83.9 ± 0.8	83.2 ± 0.8	82.8 ± 0.7	86.9 ± 0.3
		25	117.9 ± 0.9	80.3 ± 0.7	83.3 ± 0.7	87.2 ± 0.5
Zolone 350 EC (phosalone)	A	10	0	0	0	0
		15	0	0	0	0
		20	0	0	0	0
		25	0	0	0	0
	B	10	bg	bg	bg	bg
		15	35.5 ± 0.3	47.5 ± 0.6	36.1 ± 1.4	32.0 ± 1.3
		20	35.7 ± 0.6	43.3 ± 0.4	48.7 ± 1.2	59.8 ± 1.0
		25	30.3 ± 1.2	37.1 ± 0.4	59.0 ± 1.0	63.3 ± 0.8
	C	10	96.4 ± 0.7	90.0 ± 2.3	78.6 ± 2.0	42.9 ± 1.3
		15	45.2 ± 1.0	62.5 ± 1.4	56.7 ± 1.0	51.5 ± 1.4
		20	44.3 ± 1.2	64.2 ± 1.0	81.2 ± 0.8	70.1 ± 1.0
		25	37.8 ± 1.0	58.7 ± 0.7	80.1 ± 0.9	63.3 ± 0.8
Stomp 330 EC (pendimethalin)	A	10	0	0	bg	0
		15	0	0	bg	0
		20	0	0	25.6 ± 1.6	0
		25	0	0	25.6 ± 1.2	0
	B	10	bg	bg	bg	bg
		15	43.0 ± 1.5	41.3 ± 1.7	53.6 ± 2.1	36.9 ± 1.7
		20	35.7 ± 0.6	33.3 ± 1.2	70.1 ± 1.6	58.1 ± 0.7
		25	29.7 ± 0.4	32.9 ± 0.9	65.4 ± 1.0	53.1 ± 0.8
	C	10	bg	bg	bg	bg
		15	50.5 ± 1.1	62.5 ± 1.8	68.0 ± 0.7	55.3 ± 1.4
		20	47.9 ± 1.0	68.3 ± 2.2	74.8 ± 0.4	76.9 ± 1.2
		25	44.3 ± 0.9	58.7 ± 1.1	78.8 ± 0.5	68.0 ± 1.2

Note: bg – beginning of growth, A – 10 times field rate, B – field rate, C – 0.1 field rate

Hirsutella necatrix and *H. kirchneri* isolated from eriophyid mite *A. hystrix* were the most resistant to toxic effect of tested pesticides but *H. nodulosa* isolated from *Tarsonemidae* mite seems to be the most susceptible.

DISCUSSION

Among all pesticides used in this experiment, fungicides showed the strongest inhibiting effect on the growth of fungi from *Hirsutella* genus, especially flusilazole. The strongest toxic effect of fungicides towards *Hirsutella aphidis* was reported by Tkaczuk and Mietkiewski (2001). Flusilazole completely inhibited the growth of *H. aphidis* at all concentrations. Strong toxic effect of flusilazole on *Hirsutella thompsonii* cf. var. *synnematosa* isolated from pear leaf blister mite was stated by Tkaczuk (2001).

Sosa Gomez (1991) and Sosa Gomez et al. (1987), found that fungicide based on copper oxychloride suppressed completely conidial production of *H. thompsonii*, although mycelial growth was only reduced. Sulphur as wettable powder also caused reduction in conidiogenesis and mycelial growth of this fungus, but the effect was less pronounced than for copper oxychloride.

The tested fungi were unable to grow on media containing insecticides at concentrations 10 times the recommended field rate. The acaricide amitraz (Mitac 200 EC) was the most toxic among insecticides at the field rate. Strong toxic effect of amitraz to *H. nodulosa* isolated from strawberry mite (*Phytonemus pallidus* ssp. *fragariae* Zimm.) was reported by Tkaczuk et al. (2004). Sosa Gomez et al. (1987) observed a considerable reduction in the growth and sporulation of *H. thompsonii* on media containing acaricides chlorobenzileat, dicofol and tetradiphon.

Another insecticide, phosalone, also showed strong inhibitive influence on the growth of *Hirsutella* species tested in experiment. Phosalone is one of phosphoroorganic insecticides and this class of insecticides is highly toxic to entomopathogenic fungi (Bajan et al. 1977; Olmert and Kenneth 1974). Phosalone strongly inhibited the growth of entomopathogenic fungi such as *Metarhizium anisopliae, M. flavoviride* and *Paecilomyces fumosoroseus* (Mietkiewski and Sapieha 1995) as well as aphid pathogen, *H. aphidis* (Tkaczuk and Mietkiewski 2001). Phosalone strongly inhibited the growth of *H. thompsonii* cv. var. *synnematosa* (Tkaczuk 2001) isolated from pear blister mite and *H. nodulosa* isolated from strawberry mite (Tkaczuk et al. 2004). Methidathion, another phosphoroorganic insecticide was highly toxic to *H. thompsonii* isolated from eriophyid mite feeding on soya bean in Brazil (Sosa Gomez et al. 1984).

Herbicide Stomp 330 EC (pendimethalin) also negatively influenced the growth of *Hirsutella* fungi. Pendimethalin and glyphosate completely inhibited the growth of *H. aphidis* at 10 times field rate; pendimethalin was more inhibitory than glyphosate at 0.1 times the recommended rate (Tkaczuk and Miętkiewski 2001).

Effects of pesticides on entomopathogenic fungi *in vitro* do not necessarily reflect field effects. Laboratory tests performed on artificial media may not give more than tentative answers concerning the influence of pesticides in living arthropod-fungal pathogen systems (Poprawski and Majchrowicz 1995). Many factors may be responsible for differences observed between laboratory and field experiments. Con-

centrations of pesticides that affect fungi developing in a host body or in the soil are likely to be lower than those used in a laboratory experiment although locally the concentration may be much higher and exceed the normal dose.

Miętkiewski et al. (2003) ascertained highly significant differences in participation of mycoses, caused by *Hirsutella* fungi in eriophyid mite (*A. hystrix)* populations between the Polish and French sites. In opinion of the authors higher mortality level in Poland results most probably from lower agrotechnological pressure (mainly limited use of chemicals) and differentiated landscape structure abounding in mid-field afforestations and other semi-natural associations that favour maintenance and distribution of entomopathogens, contrary to uniform big fields of the Parisian Basin.

CONCLUSIONS

1. Pesticides tested in this experiment showed strong inhibiting effects on the growth of fungi from *Hirsutella* genus – natural pathogens of phytophagous mites.
2. Among all tested pesticides, fungicides and especially flusilazole (Punch 400 EC) showed the strongest inhibiting effect on the growth of *Hirsutella* species.
3. Amitraz (Mitac 200 EC) and phosalone (Zolone 350 EC) were the most toxic among insecticides.
4. Fungi *H. necatrix* and *H. kirchneri* isolated from eriophyid mite *A. hystrix* were the most resistant to toxic effect of tested pesticides but *H. nodulosa* isolated from *Tarsonemidae* mite seems to be the most susceptible.

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POLISH SUMMARY

WPŁYW WYBRANYCH ŚRODKÓW OCHRONY ROŚLIN NA WZROST GRZYBÓW Z RODZAJU *HIRSUTELLA* WYIZOLOWANYCH Z FITOFAGICZNYCH ROZTOCZY

W doświadczeniu zbadano wpływ wybranych środków ochrony roślin na wzrost czterech gatunków grzybów: *Hirsutella nodulosa, H. brownorum, H. kirchneri* i *H. necatrix,* będących naturalnymi patogenami fitofagicznych roztoczy. Fungicydy dodawano do podłoża hodowlanego w trzech dawkach: zalecanej, 10-krotnie niższej od zalecanej i 100-krotnie niższej od zalecanej; zoocydy i herbicyd zastosowano w dawkach: 10-krotnie wyższej od zalecanej, zalecanej i 10-krotnie niższej od zalecanej. Stwierdzono, że testowane pestycydy silnie hamowały

wzrost badanych gatunków grzybów. Najbardziej toksyczne okazały się fungicydy, a wśród nich zwłaszcza Punch 400 EC (flusilazol). Spośród zoocydów najsilniej wzrost kolonii grzybów- patogenów roztoczy hamowały Mitac 200 EC (amitraz) i Zolone 350 EC (fozalon). Grzyby *H. kirchneri* i *H. necatrix* wyizolowane z *A. hystrix* okazały się najbardziej odporne na działanie pestycydów użytych w doświadczeniu. Z kolei gatunek *H. nodulosa* wyizolowany z roztocza z rodzaju *Tarsonemidae* wykazał największą wrażliwość na obecność pestycydów w pożywce.