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Wpływ wybranych herbicydów i zoocydów na wzrost akaropatogenicznych grzybów z rodzaju *Hirsutella*

The effect of selected herbicides and zoocides on the growth of acaropathogenic fungi from the *Hirsutella* genus

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Streszczenie

Badania grzybów entomopatogenicznych, które infekują roztocze w Polsce, a także w niektórych krajach europejskich wykazały, że stawonogi te, niezależnie od warunków, w których żerują, najczęściej atakowane są przez grzyby z rodzaju *Hirsutella*. Ich populacja w środowisku naturalnym, w największym stopniu ograniczana jest poprzez ciągłą intensyfikację produkcji rolniczej oraz nadmierne lub niewłaściwe stosowanie pestycydów. Celem badań było określenie wpływu wybranych środków ochrony roślin (herbicydów i zoocydów) na wzrost kolonii grzybów patogenów roztoczy należących do rodzaju *Hirsutella*. W warunkach laboratoryjnych zbadano wpływ dwóch herbicydów i czterech zoocydów na wzrost kolonii wybranych szczepów grzybów akaropatogenicznych: *H. thompsonii* var. *synnematosa*, *H. thompsonii*, *H. vandergeesti* i *H. danubiensis*. Zastosowane w doświadczeniu herbicydy zawierały następujące substancje czynne: quizalofop-P--etylowy i glifosat, a zoocydy: propargil, heksytiazoks, fenazachin i lambda-cyhalotrynę. Zostały one dodane do sterylnych pożywek SDA w dawce 10-krotnie wyższej od zalecanej (A), dawce zalecanej (B) i 10-krotnie niższej niż dawka zalecana (C). Przeprowadzone badania wykazały, że spośród testowanych w doświadczeniu herbicydów zastosowanych w zalecanej dawce (B), najmniej toksyczny dla badanych szczepów grzybów akaropatogenicznych był Roundup 360 SL, którego substancją czynną jest glifosat, a spośród zoocydów, Karate Zeon 050 CS zawierający w swoim składzie lambda-cyhalotrynę.

Słowa kluczowe: grzyby z rodzaju *Hirsutella*, herbicydy, zoocydy, toksyczność

Summary

Mites are pests commonly occurring on cultivated plants, grown both indoors and outdoors. So far relatively few mite pathogens have been identified and described, but fungi are the largest group of organisms infecting these arthropods and have a big potential in their biological control. The greatest impact on narrowing the species composition of acaropathogenic fungi in natural environment is exerted by human activity, associated with constant intensification of agricultural production and excessive or inappropriate use of pesticides. The aim of the research was to study the impact of selected pesticides (herbicides and zoocides) on the growth of a fungal colony of mite pathogens belonging to the *Hirsutella* genus. In a laboratory the effects of two herbicides and four zoocides on the growth of selected strains of acaropathogenic fungi: *H. thompsonii* var. *synnematosa*, *H. thompsonii*, *H. vandergeesti*, and *H. danubiensis* were examined. The herbicides used in the research were as follows: quizalofop-P-ethyl, glyphosate and the zoocides were as follows: propargyl, hexythiazox, fenazaquin and lambda-cyhalothrin. They were added to sterile SDA media in the 10 times higher than the recommended dose (A), recommended field dose (B), and 10 times lower than the recommended dose (C). Among the herbicides tested in the experiment recommended in the field dose (B), the least toxic to the tested strains of acaropatogenic fungi was a preparation containing glyphosate and the lambda-cyhalothrin among the zoocides.

Key words: fungi of the *Hirsutella* genus, herbicides, zoocides, toxicity

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Wstęp / Introduction

As crop pests, mites are vectors of many dangerous viral, bacterial and fungal diseases commonly found on plants grown both outdoors and indoors (Boczek 1999; Tkaczuk *et al.* 2004). So far, relatively few mite pathogens have been identified and described, but the most numerous group of such organisms feeding on these arthropods are fungi (van der Geest *et al.* 2000). The first report on Entomophthoromycota fungi found on mites was published by Petch (1940, 1944), and the infection of *Panonychus citri*, a herbivorous mite, caused by a species of the *Neozygites* genus was first described by Fisher (1951). Mite pathogens occupy the same taxonomic ranks as entomopathogenic fungi. For the most part, they represent anamorphic fungi (Ascomycota) grouped mostly in the *Hirsutella* and *Cephalosporium* genera (van der Geest *et al.* 2000; Bałazy *et al.* 2008). Fungi of the *Hirsutella* genus infect mites by using conidial spores produced at the tips of their phialides (Lipa 1971), which quickly leads to the death of the host (McCoy 1981). In the course of infection, a series of enzymes is produced. They perforate tissue covering the host to let germ tubes invade the body cavity of the host (van der Geest *et al.* 2000). Studies on the species composition of entomopathogenic fungi that infect mites in Poland, and in some European countries have Bałazy and Wiśniewski (1986), and Bałazy *et al.* (2008) shown that these arthropods, regardless of the conditions in which they prey, are most frequently attacked by *Hirsutella* species.

The greatest impact on narrowing the species composition of acaropathogenic fungi in the natural environment is exerted by human activity, associated with the constant intensification of agricultural production and excessive or inappropriate use of pesticides. Most studies conducted in laboratories indicate a negative effect of the commonly used plant protection products, including herbicides and zoocides, on entomopathogenic and acaropathogenic fungi. These products may restrict their growth or their spore germination, and thus adversely affect the process of infection and development of fungal diseases in the pest population (Majchrowicz and Poprawski 1993; Miętkiewski *et al.* 1995, 1996; Andalo *et al.* 2004; Oliveira and Neves 2004; Tkaczuk and Miętkiewski 2005; Tkaczuk *et al.* 2012; Celar and Kos 2016; Fiedler and Sosnowska 2017; Perez-González and Sańchez-Pena 2017).

The guidelines on integrated plant protection underline the need for limiting the use of synthetic pesticides to a minimum, and increasingly replacing them with biological and agronomic methods. As one of the most environmentally friendly, the biological method, protecting plants against pests by using microorganisms, is based, among others, on entomopathogenic fungi (Lipa 2000; Sosnowska 2013).

Entomopathogenic fungi have been found to be able to colonise plants and exist in the form of endophytes which offer a long-term preventive measure for pests and diseases (Vega *et al.* 2009; Parsa *et al.* 2013). The fungal endophytes, aside from their role in pests and diseases prevention, also act as plant growth promoters (Lopez and Sword 2015; Jaber and Enkerli 2017). Fungal endophytes have so far been reported in corn (Bing and Lewis 1991, 1993), common bean (Parsa *et al.* 2018), tomato (Qayyum *et al.* 2015), soybeans and wheat (Russo *et al.* 2015), cotton (Lopez *et al.* 2014) and in many other economically important crops.

Acaropathogenic fungi are a constant component of naturally occurring habitats of herbivorous mites, including pests of crops which are important from the economic point of view (Bałazy *et al.* 2008). Mycoses caused by those fungi often take the form of epizootics and reduce the number of pests to an insignificant level and, therefore, they should be used in the integrated plant protection programmes. The aim of the research was to study the impact of selected herbicides and zoocides under laboratory conditions on the growth of acaropathogenic fungi from *Hirsutella* genus.

Materiały i metody / Materials and methods

In the laboratory experiment, two herbicides and four zoocides were used. Detailed characteristics of the tested pesticides are shown in Table 1.

The fungal material was obtained from stock collections maintained at the Department of Plant Protection and Breeding, Siedlce University of Natural Sciences and Humanities, Siedlce, Poland. Tests were performed with four fungal species isolated from mites. The characteristics of the fungal isolates are shown in Table 2. Prior to treatments, isolates were applied to Petri-plates with sabouraud dextrose agar (SDA) medium and maintained at 20°C ±2°C for 7 days in total darkness. The fungi isolated from mites were identified with standard keys (Hodge 1998). Moreover, molecular studies were conducted to confirm the proper identification of the fungal isolates. The ITS marker was chosen for identification as it has been proposed as a universal DNA bar code marker for fungi (Schoch *et al.* 2012).

The specified doses of pesticides were added to the sterile SDA medium using an electronic pipette. Subsequent concentrations of herbicides and zoocides in the medium were obtained by the dilution method. The doses were calculated on the basis of the field dose recommended by the manufacturers, used in the protection of cereal crops, diluted in 300 l of water per ha. Herbicides and zoocides were added to a sterile SDA medium at about 40–50°C in the following doses:

A – dose 10 times higher than the recommended,

B – recommended field dose,

C – dose 10 times lower than the recommended.

Culture media prepared that way were put into 9 cm diameter Petri dishes.

Tabela 1. Charakterystyka herbicydów i zoocydów zastosowanych w doświadczeniu
Table 1. Characteristics of herbicides and zoocides used in the experiment

Nazwa handlowa Brand name	Substancja czynna Active substance	Zalecana dawka polowa Recommended field dose [ml/l]
Herbicydy – Herbicides		
Targa Super 05 EC	quizalofop-P-ethyl – 50 g/l	5 ml/l
Roundup 360 SL	glyphosate – 360 g/l	10 ml/l
Zoocydy – Zoocides		
Omite 570 EW	propargyl – 570 g/l	3 ml/l
Nissorun 050 EC	hexythiazox – 50 g/l	1.2 ml/l
Magnus 200 SC	fenazaquin – 200 g/l	0.6 ml/l
Karate Zeon 050 CS	lambda-cyhalothrin – 50 g/l	0.8 ml/l

Tabela 2. Charakterystyka izolatów grzybów wykorzystanych w doświadczeniu
Table 2. Characteristics of fungal isolates used in the experiment

Gatunek grzyba Fungal species	Gatunek roztocza Host mite species	Roślina żywicielska Host plant
Hirsutella thompsonii var. *synnematosa*	Pear-leaf blister mite, *Eriophyes piri* (Pgst.)	European pear, *Pyrus communis* L.
Hirsutella thompsonii	Two-spotted spider mite, *Tetranychus urticae* Koch.	Raspberry, *Rubus idaeus* L.
Hirsutella vandergeesti	*Amblyseius angulatus* Karg	Raspberry, *Rubus* sp.
Hirsutella danubiensis	Raspberry spider mite, *Neotetranychus rubi* Trag.	Raspberry, *Rubus ideaeus* L.

The media were inoculated with fungi after 24 hours. After inoculating the media with mycelium fragments, the dish was placed in an incubator at a temperature of 22°C ±1°C. Observation of the colonies' growth was carried out by measuring their diameter every 5 days until the 25th day. A fungal culture growing on an SDA medium without pesticides was used as the control. Every experimental combination was replicated four times. The results were presented as a colony diameter expressed as a percentage in relation to control.

The results obtained on the 25th day were statistically processed using two-factor analysis of variance for homogeneous groups ANOVA. To compare means, Tukey's test was used, assuming a significance level of $\alpha = 0.05$. All the calculations were performed in STATISTICA, version 12.0.

Wyniki i dyskusja / Results and discussion

The effect of the herbicides and zoocides used in the present studies on the growth of various species of acaropathogenic fungi was diverse, and their reaction was dependent on the applied product and its concentration in the culture medium (Table 3, 4).

Among the active substances found in the tested herbicides glyphosate, the principal ingredient of Roundup 360 SL, limited the growth of the colony *Hirsutella thompsonii* var. *synnematosa* to the lowest degree, but its effect was statistically significant (Table 3). On the 25th day after inoculation when the chemical had been applied at concentration A (10 times higher than the recommended one), B (recommended), C (10 times lower than the recommended one), the diameters of the fungal colonies constituted, respectively, 31.3%, 45.5% and 84.8% of the control one. Quizalofop-P-ethyl, as the active ingredient of Targa Super 05 EC, added to the medium at a dose 10 times higher than the recommended dose (A), it completely inhibited the growth of the fungal colony, while the use of the preparation at the recommended field dose (B) limited the increase of the fungal colony by more than 60% in relation to the control.

When added to the medium in a concentration 10 times higher than recommended (A), the herbicides tested completely hampered the growth of the *H. thompsonii* fungus, isolated from *Tetranychus urticae*, the two-spotted spider mite. Inhibiting properties of quizalofop-P-ethyl were smaller than glyphosate. Colonies of the fungus in media containing the recommended concentration of the product (B) and 10 times less than recommended (C) were, respectively, 43.9% and 93% of the diameter of the control cultures.

The glyphosate, when applied to the culture medium at a dose A and recommended field dose (B), completely inhibited the growth of *H. vandergeesti*. In addition, qui-

Tabela 3. Wielkość kolonii grzybów z rodzaju *Hirsutella* na pożywkach z dodatkiem herbicydów w 25 dniu obserwacji (wyrażona w % w stosunku do kontroli)
Table 3. The colony size of *Hirsutella* fungi on media supplemented with investigated herbicides on the 25th day of observation (expressed in % relative to control)

Herbicydy Herbicides	Dawka Dose	Tempo wzrostu grzybów – Growth rates of fungi [%]			
		Hirsutella thompsonii var. *synnematosa*	*Hirsutella thompsonii*	*Hirsutella vandergeesti*	*Hirsutella danubiensis*
Quizalofop-P-ethyl	A	0*	0*	0*	0*
	B	36.4*	43.9*	65.8*	35.4*
	C	66.7*	93.0	69*	60.1*
Glyphosate	A	31.3*	0*	0*	0*
	B	45.5*	36.8*	0*	61.3*
	C	84.8*	86.0*	101.1	72.5*

A – dawka 10-krotnie wyższa od zalecanej – 10 times higher than recommended dose, B – zalecana dawka polowa – recommended dose, C – dawka 10-krotnie niższa od zalecanej – dose 10 times lower than recommended, * – istotność na poziomie α = 0,05 w stosunku do kontroli – significance at the level α = 0.05 in relation to the control

Tabela 4. Wielkość kolonii grzybów z rodzaju *Hirsutella* na pożywkach z dodatkiem zoocydów w 25 dniu obserwacji (wyrażona w % w stosunku do kontroli)
Table 4. The colony size of *Hirsutella* fungi on media supplemented with investigated zoocides on the 25th day of observation (expressed in % relative to control)

Zoocydy Zoocides	Dawka Dose	Tempo wzrostu grzybów – Growth rates of fungi [%]			
		Hirsutella thompsonii var. *synnematosa*	*Hirsutella thompsonii*	*Hirsutella vandergeesti*	*Hirsutella danubiensis*
Propargyl	A	11.8*	33.5*	0*	28.3*
	B	81.5*	66.6	50.6*	37.7*
	C	78.3*	76.4	54.9*	56.6*
Hexythiazox	A	10.9*	0*	0*	0*
	B	21.8*	44.7*	43.2*	65.4*
	C	95.2*	55.5*	51.7*	67.2*
Fenazaquin	A	30.5*	27.8*	32.7*	44.2*
	B	68.8*	71.7	57.0*	54.2*
	C	77.9*	60.5	66.5*	54.2*
Lambda-cyhalothrin	A	30.7*	28.8*	36.4*	21.2*
	B	82.0*	91.7	60.1*	74.3*
	C	102.5	98.1	86.5	86.6*

A – dawka 10-krotnie wyższa od zalecanej – 10 times higher than recommended dose, B – zalecana dawka polowa – recommended dose, C – dawka 10-krotnie niższa od zalecanej – dose 10 times lower than recommended, * – istotność na poziomie α = 0,05 w stosunku do kontroli – significance at the level α = 0.05 in relation to the control

zalofop-P-ethyl used in concentration A completely inhibited the growth of the studied fungus colony. In doses B and C, the preparation limited the growth of *H. vandergeesti* colonies in relation to the control by over 30%.

The herbicides tested in the experiment significantly limited the growth of *H. danubiensis*. Both preparations tested in the experiment, added to the culture medium in a concentration 10 times higher than the recommended field dose (A), completely inhibited the growth of the fungus colony. At concentration B – recommended field dose, quizalofop-P-ethyl limited the growth of the tested fungus more strongly than glyphosate. The *H. danubiensis* colonies reached 35.4% and 61.3% of the size, respectively, compared to the control colonies.

Zoocides tested in the experiment only slightly affected the growth of the *H. thompsonii* var. *synnematosa* colony (Table 4). The development of the culture of this strain of fungus was constrained the most strongly by hexythiazox

and by propargyl. Of all zoocides, lambda-cyhalothrin, as the active substance of Karate Zeon 050 CS, exerted the weakest inhibiting impact on the colony. It significantly reduced the growth of cultures when applied at concentrations A and B. Concentration C (10 times lower than recommended) of lambda-cyhalothrin slightly stimulated the increase of the fungal colony, which reached 102.5% of the size of the control group.

Zoocides had relatively low toxic effects on the *H. thompsonii* fungus. Hexythiazox added to the medium completely stopped fungus colony diameter growth only when applied in the highest concentration (A). In other concentrations, B and C, the fungus colonies reached 44.7% and 55.5% of the size of the control group. Lambda-cyhalothrin limited the growth of the *H. thompsonii* culture to the lowest degree, and on the last day of incubation fungal colonies with concentration A constituted 28.8% of the control, with 91.7% in the case of B concentration, and 98.1% with C concentration.

Zoocides applied in the experiment relatively poorly inhibited the development of cultures *H. vandergeesti*. Of this group of pesticides, hexythiazox proved to be the strongest inhibitor. At the highest concentration of the product (A) in the culture medium, there was no growth of the colony, while on the last day of incubation the culture of *H. vandergeesti* growing with concentrations B and C reached 43.2% and 51.7% of the size of the control. Lambda-cyhalothrin proved to be a zoocide relatively little toxic to this strain of the fungus.

Of the zoocides, acaricides with the active substance of propargyl and fenazaquin at the recommended field dose (B) were the most potent inhibitor of *H. danubiensis* colony growth. Lambda-cyhalothrin was the least toxic to this strain of fungus. Colonies growing on the medium with the recommended concentration and 10 times lower than recommended reached, respectively, 74.3% and 86.6% of the size of the control cultures.

The introduction of the integrated method of plant protection means reducing the use of synthetic products to a minimum, but today they are still applied on a wide scale (Sosnowska 2013). Mites are pests commonly occurring on cultivated plants, grown both indoors and outdoors (Boczek 1999). So far, relatively few mite pathogens have been identified and described, but fungi are the largest group of organisms infecting these arthropods (van der Geest 1985; McCoy 1996; van der Geest *et al.* 2000; Bałazy *et al.* 2008). 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 the natural occurrence, infectivity and population dynamic of these pathogens. Pesticides, especially systemic fungicides and herbicides, can negatively affect the endophytic development of entomopathogenic fungi within plant tissues.

Many reports indicate that the active substances of synthetic pesticides have a negative impact on entomopathogenic fungi *in vitro* (Ingoffo *et al*. 1975; Keller 1978; Bajan and Kmitowa 1982; Vänninen and Hokkanen 1988; Majchrowicz and Poprawski 1993; Miętkiewski *et al.* 1997).

In the literature, there are only a few reports on the impact of plant protection products (including herbicides and zoocides) on the growth of fungal colonies of the *Hirsutella* genus isolated from mites. They concern species such as *H. thompsonii* (Sosa-Gomez *et al.* 1984, 1987; Tkaczuk 2001), *H. nodulosa* (Tkaczuk *et al.* 2004, 2015), *H. kirchneri*, and *H. brownorum* (Tkaczuk and Miętkiewski 2005). Therefore, the results of the present research on the effect of different types of active substances present in herbicides and zoocides on acaropathogenic species of *H. vandergesti* and *H. danubiensis* are innovative. These species have been identified and described relatively recently (Bałazy *et al.* 2008), and so far have not been subjected to this kind of laboratory tests.

In the experiment, the herbicides added to the medium in the highest concentration completely stopped the growth of the cultures of acaropathogenic fungi. The exception was the strain of *H. thompsonii* var. *synnematosa* isolated from the pear-leaf blister mite (*E. piri*), which developed colonies in the medium with the addition of glyphosate at the concentration 10 times higher than recommended. The results confirmed the previous studies of Tkaczuk and Miętkiewski (2001, 2005) and Tkaczuk *et al.* (2004), who observed a strong limitation of the growth of entomopathogenic fungi colonies incubating on synthetic media with herbicides of the above concentration. According to Tkaczuk and Miętkiewski (2001), 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 *et al.* (2004) observed that herbicide pendimethalin, added to the medium at the 10 times field rate, prevented the growth of the cultures of the *H. nodulosa* fungus.

Miętkiewski *et al*. (1990) found that the impact of herbicides on entomopathogenic fungi in a pot experiment, determined as the number of fungi colonies reisolated from the soil, was not as unequivocal as in colonies on cultures with herbicides added to the media, with the impact in the latter case determined by the size of the colony. The authors claimed that the weaker impact of herbicides on entomopathogenic fungi in the pot experiment than in media cultures was caused by the fact that, in soil, there are many abiotic and biotic factors that could modify the development of fungus as well as the metabolism of the herbicide. According to Alves *et al.* (1998), the high toxicity of chemical substance in *in vitro* conditions does not always suggest its high toxicity in field conditions, but only indicates such possibility.

In the present studies, there was a strong inhibiting influence of the zoocides added to the medium in the highest (A) concentration on the growth of some acaropathogenic fungi. A product containing hexythiazox as the active substance

stopped the growth of three out of four of the fungal isolates tested. Studying the effects of zoocides on *Hirsutella* fungi, Tkaczuk and Miętkiewski (2005) found that hexythiazox added to the medium in a concentration 10-times higher than recommended completely stopped the growth of fungal cultures.

The zoocides tested in this study, added to the culture media at the recommended field rate, were less toxic to *Hirsutella* fungi than herbicides. Tkaczuk *et al.* (2013) found that insecticides tested in a laboratory experiment, added to the medium at the recommended concentration, only slightly limited the growth of entomopathogenic fungi colonies. The *H. nodulosa* had the greatest sensitivity to the presence of insecticides than entomopathogenic fungi from *Metarhizium*, *Lecanicillium* or *Isaria* genus. Many authors indicate a relatively small effect of insecticides on the growth and germination of entomopathogenic fungi compared to fungicides and herbicides (Miętkiewski *et al.* 1997; Tkaczuk *et al.* 2012, 2013).

Investigating the impact of pesticides on the growth and production of *H. thompsonii* conidia, Filho *et al.* (2001) found that thiamethoxam added to the medium at the minimum and maximum recommended amounts decreased the production of fungal conidia even with a minimal concentration of the substance. On the other hand, vegetative growth did not change, but it was significantly higher than in the control group and similar to that obtained with the maximum concentration of the substance. In their studies, McCoy *et al.* (1982) found no negative effects of an insecticide containing miticide avermectin on the growth and pathogenic abilities of the *H. thompsonii*.

In the present experiment, significant differences were observed between the reactions of two strains of *H. thompsonii* to the pesticides added to the culture media. The phenomenon of a differentiated response of different strains of the same entomopathogenic fungi species to the presence of pesticides in culture media has been noted in many studies (Miętkiewski *et al.* 1990; Bajan and Kmitowa 1997; Tkaczuk 2008; Tkaczuk *et al.* 2012). According to Roberts and Campbell (1977), the susceptibility of entomopathogenic fungi to chemical products varies considerably depending on the pesticides applied and on the fungi species.

Wnioski / Conclusions

1. The side effect of the herbicides and zoocides used in the present studies on the growth of acaropathogenic fungi was diverse, and their reaction was dependent on the applied product and its concentration in the culture medium.
2. Among all the tested pesticides, herbicides showed the strongest inhibiting effect on the growth of the *Hirsutella* species.
3. Hexythiazox was the most potent inhibitor among the insecticides, but lambda-cyhalothrin proved to be only mildly toxic to these strains of the fungi.

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