EFFECT OF SELECTED FUNGICIDES ON THE GROWTH OF ACAROPATHOGENIC FUNGI FROM THE GENUS *HIRSUTELLA*

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Abstract. The aim of the research was to study the impact of selected fungicides on the growth of a fungal colony of mite pathogens belonging to the *Hirsutella* Pat. genus. In a laboratory bioassay effects of five fungicides on the growth of selected strains of acaropathogenic fungi: *H. thompsonii* F.E. Fisher, *H. thompsonii* var. *synnematosa* Samson, C.W. McCoy and O'Donnell, *H. vandergeesti* Bałazy, Mietkiewski et Tkaczuk and *H. danubiensis* Tkaczuk, Bałazy et Wegensteiner were examined. Fungicides used in the research were as follows: sulphur, cyprodinil and fludioxonil, mancozeb, copper oxychloride and azoxystrobin. They were added to sterile SDA media in the recommended field dose as well 10 and 100 times lower doses than the recommended one. The effect of fungicides on the growth of acaropathogenic fungi varied depending on the applied substance and its concentration in the culture medium. Of all fungicides tested the fungal growth was most strongly limited by mancozeb and a mixture of cyprodinil and fludioxonil while sulphur had the weakest effect.
Keywords: *mites, mite-pathogenic fungi, pesticides, toxicity, mycelial growth, inhibition*

Introduction

Mites are pests commonly found on crops growing both in the field and under cover (Boczek, 1999). So far relatively few pathogens of mites have been identified, but fungi constitute the most numerous group infecting these arthropods (van der Geest et al., 2000). Fungal pathogens are a permanent component of mite natural habitats. For the most part, they represent *Ascomycota* anamorphs, grouped in the *Hirsutella* and *Lecanicillium* W. Gams and Zare genera (van der Geest et al., 2000; Bałazy et al., 2008). *Hirsutella* (Patouillard, 1892) includes over 70 species of asexually-reproducing pathogens of insects, mites, and nematodes that mainly belong to within Ophiocordycipitaceae G.H. Sung, J.M. Sung, Hywel-Jones and Spatafora (Kepler et al., 2013; Quandt et al., 2014), though the genus is usually considered to be associated with the genus *Ophiocordyceps* typified by a sexual morph (Sung et al., 2007). *Hirsutella* infects hosts by using conidia born at the tip of phialides (Lipa, 1971), and infection quickly leads to a death of the hosts (McCoy, 1981).

The greatest impact on the depletion of species composition of acaropathogenic fungi is exerted by human activity, by the intensification of agricultural production and the use of chemical plant protection products in particular. In commercial production systems, the need for chemical pesticides persist, despite many efficient introductions of biological control agents, including entomopathogenic fungi. These compounds, especially fungicides, applied against plant pathogens might also negatively affect the populations of entomopathogenic fungi, and thus reducing a pest regulation potential as a consequence (Miętkiewski et al., 1997; Hummel et al., 2002; Meyling and Eilenberg, 2007).

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Research conducted in laboratories shows a negative impact of pesticides, in particular fungicides, on entomopathogenic and acaropathogenic fungi. Those products may restrict their growth, germination, and intensity with which fungi infect potential hosts (Majchrowicz and Poprawski, 1993; Miętkiewski et al., 1996; Andalo et al., 2004; Li et al., 2004; Tkaczuk and Miętkiewski, 2005; Fiedler and Sosnowska, 2007, 2017; Tkaczuk et al., 2012; Celar and Kos, 2016; Perez-González and Sańchez-Pena, 2017).

The task of modern plant protection is to provide effective methods and solutions to combat pests - with the smallest possible pressure on the environment. The biological method based on products containing entomopathogenic fungi is one of the most environment-friendly ones (Lipa, 2000; Sosnowska, 2013). Because of their properties, in integrated plant protection programs acaropathogenic fungi can be used to limit the populations of herbivorous mites.

The aim of the study was to analyse an impact of selected fungicides on the growth of a colony of *Hirsutella* fungi, in laboratory conditions.

Materials and methods

Fungal isolates

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 presented in *Table 1*. Prior to treatments, isolates were applied to Petri-plates with Sabouraud dextrose agar (SDA) medium and maintained at 20 ± 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 universal DNA barcode marker for fungi (Schoch et al., 2012).

Table 1. *Characteristics of fungal isolates used in the experiment*

Funngal species	Host mite species	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.

Preparation of media with fungicides

Five fungicides, that are commonly used to protect fruit crops against fungal diseases, were selected for the testing. Detailed characteristics of the fungicides are shown in *Table 2*. Fungicides were added to sterile SDA medium at about 40-50°C in the following doses:

A – recommended field dose,

B – dose 10 times lower than the recommended,

C – dose 100 times lower than the recommended.

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Table 2. *Characteristics of fungicides used in the experiment*

Brand name	Active ingredient	Recommended dose
Siarkol Extra 80 WP	sulphur - 80%	12.5 g/l
Switch 62,5 WG	cyprodinil - 375 g/l fludioxonil - 250 g/l	1.3 g/l
Dithane Neo Tec 75 WG	mancozeb - 750 g/l	6.0 g/l
Miedzian 50 WP	copper oxychloride - 50%	2.5 g/l
Amistar 250 SC	azoxystrobin - 250 g/l	1.2 ml/l

The media supplemented with fungicides were poured into 9 cm-diameter Petri dishes and inoculated with fungi after 24 hours. After inoculating the media with mycelium fragments, the dishes were incubated in an incubator at 22°C ± 1C°. Observation of colonies was carried out by measuring their diameter every 5 days until the 25th day. A fungi culture growing on SDA medium without fungicides was used as control. Every experimental combination was replicated four times. The results were presented as a colony diameter expressed as a percentage in relation to control.

Statistical analysis

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 the significance level of $\alpha = 0.05$. All the calculations were performed in STATISTICA®, version 12.0.

Results

The effect of the fungicides on the growth of tested species of acaropathogenic fungi was diverse, and their reaction was dependent on the applied product and its concentration in the culture medium. Of all tested fungicides, the growth of *Hirsutella thompsonii* var. *synnematosa* was the least inhibited by sulphur (*Fig. 1*).

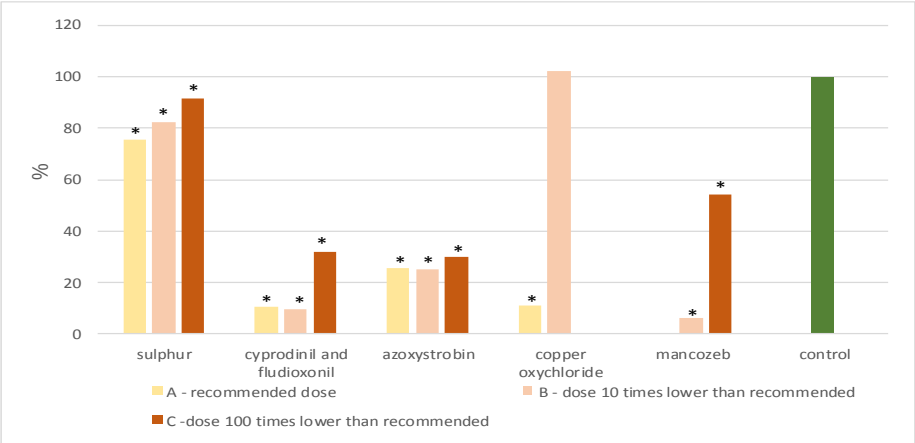

Figure 1. *Hirsutella thompsonii var. synnematosa colony size on media supplemented with investigated fungicides on the 25th day of observation (expressed in % relative to control)*
** - significance at the level α = 0.05 in relation to the control*

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After 25 days of cultivation, colonies growing on the medium with A fungicide concentration (recommended), B (10 times lower than the recommended), and C (100 times lower than recommended) constituted 75.6%, 82.2, and 91.4% of the control culture size.

A potent inhibitor of fungal testing colony growth turned out to be azoxystrobin. Cultures growing on the media with this fungicide were smaller, irrespective of the dose, than control colonies by an average of 75%. However, the strongest growth restriction of *H. thompsonii* var. *synnematosa* was caused by mancozeb. This fungicide, when added to the medium in the recommended dose (A), completely inhibited the development of this pathogen. Cultures with B and C concentrations reached 6.2% and 54% of the control colony size, respectively.

By analyzing the growth of *Hirsutella thompsonii*, it turned out that azoxystrobin had the strongest toxic effect and when applied in all doses it completely stopped the growth of the fungus (*Fig. 2*). Fungicides Switch 62.5 WG with cyprodinil and fludioxonil and Dithane Neo Tec 75 with mancozeb as their active substance applied in the recommended dose (A) and 10 times less than recommended (B) completely inhibited the growth of the isolate too. Of all tested fungicides sulphur was the least effective in hindering the growth of *H. thompsonii* culture, and this growth limitation was statistically significant in relation to control.

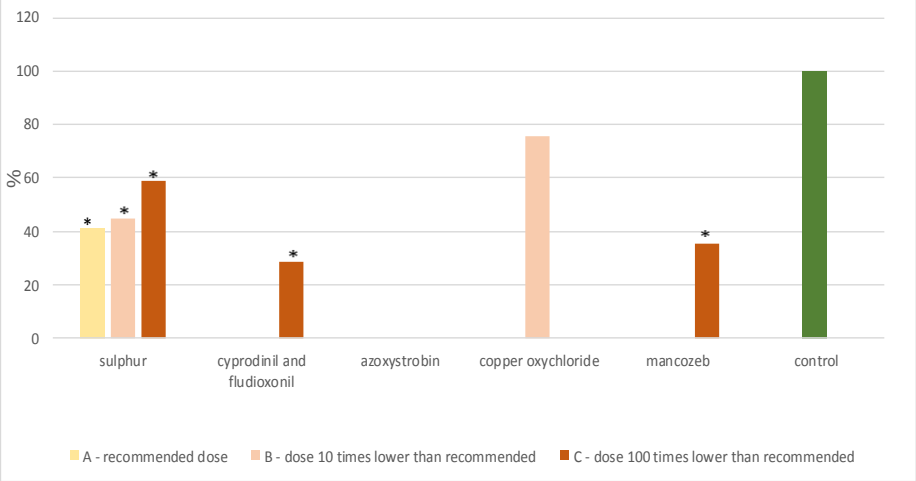

Figure 2. *Hirsutella thompsonii colony size on media supplemented with investigated fungicides on the 25th day of observation (expressed in % relative to control)*
** - significance at the level α = 0.05 in relation to the control*

The fungicides tested in this research significantly restricted the development of the colony of the *H. vandergeesti* fungal isolate (*Fig. 3*), with cyprodinil and fludioxonil having the most adverse effect on pathogen development. The fungicide with A (recommended) and B (10 times lower than the recommended) concentrations, completely prevented colony development. However, the culture of *H. vandergeesti* showed relatively high tolerance to sulphur. Colonies growing on media that contained the fungicide were smaller than control by 23.2% at A concentration, and 23.6 % and 13.9% at B and C concentrations. The tests showed that adding copper oxychloride and mancozeb to the medium according to the recommended dose (A) completely stopped the

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growth of *H. vandergeesti*. Mancozeb had its fungicidal effect on the growth of fungal colonies also at the concentration 10 times and 100 times lower than the recommended field dose.

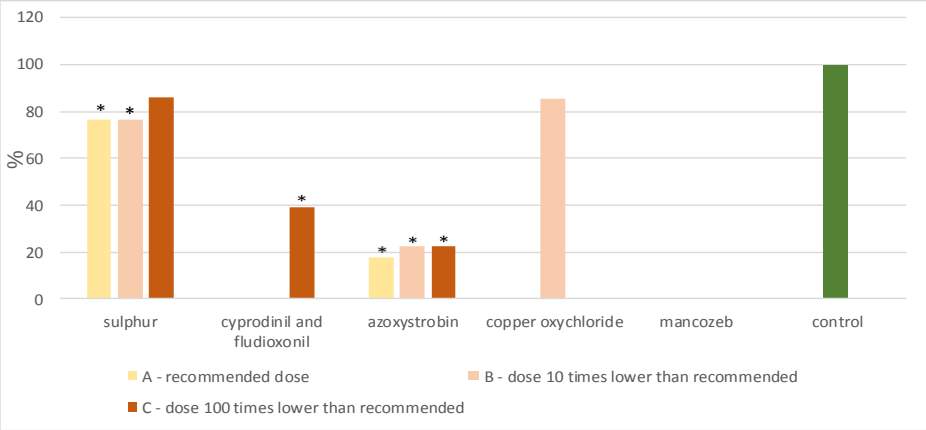

Figure 3. *Hirsutella vandergeesti colony size on media supplemented with investigated fungicides on the 25th day of observation (expressed in % relative to control)*
** - significance at the level α = 0.05 in relation to the control*

The present studies showed that applied fungicides strongly limited the growth of the fungal colonies of *H. danubiensis* (*Fig. 4*).

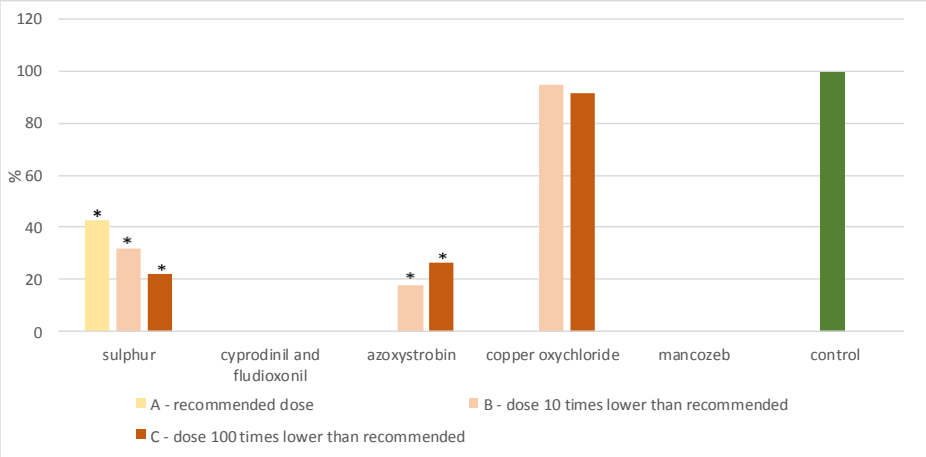

Figure 4. *Hirsutella danubiensis colony size on media supplemented with investigated fungicides on the 25th day of observation (expressed in % relative to control)*
** - significance at the level α = 0.05 in relation to the control*

There was no growth of colonies on media containing cyprodinil and fludioxonil and mancozeb, irrespective of the dose. The other tested fungicides, except for sulphur, added to the medium at the recommended field dose (A) also prevented the development of the colonies of this fungus. Sulphur proved to be relatively little toxic to the tested strain. The size of *H. danubiensis* colonies growing on solid medium with that fungicide in A, B, and

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C concentrations constituted 42.5%, 31.6% and 22.0% of the control diameter, respectively.

Discussion

Fungi that infect mites in natural conditions are constantly exposed to pesticides used to protect crops against other pests. Numerous reports (Miętkiewski et al., 1997; Tkaczuk et al., 2012; Celar and Kos, 2016; Fiedler and Sosnowska, 2017; Perez-González and Sańchez-Pena, 2017) indicate that chemical substances in pesticides may affect the natural occurrence of such fungi, their growth, sporulation, as well as their pathogenicity.

In the literature there are only a few reports on the impact of pesticides (fungicides) on the growth of fungal colony of the *Hirsutella* genus isolated from mites. They deal with species such as *H. thompsonii* (Sosa Gomez et al., 1987; Sosa Gomez, 1991), *H. nodulosa* (Tkaczuk et al., 2004, 2015), *H. kirchneri* and *H. brownorum* (Tkaczuk and Miętkiewski, 2005). Therefore, the results of tests on the effect of different types of active substances present in the fungicides carried out on the acaropathogenic species of *H. vandergeesti* and *H. danubiensis* are innovative. These species have been described relatively recently, and so far have not been subjected to this kind of laboratory tests.

The present research has shown that fungicides added to media adversely affect the growth of acaropathogenic fungi. Tkaczuk and Miętkiewski (2005) came to similar conclusions in their studies when they demonstrated that difenconazole was the most toxic to the strains of *Hirsutella* genus fungi. Exploring the effects of synthetic pesticides on *H. nodulosa* Petch, Tkaczuk et al. (2004) stated that fungicide iprodion added to the medium at the concentration 100 times lower than the recommended field dose, to a large extent reduced the growth of the fungi. Klingen and Westrum (2007) carried out an experiment to estimate the impact of pesticides on the development of the *Neozygites floridana* (J. Weiser and Muma) Remaud. and S. Keller mite-pathogenic entomophthoralean fungus. On the basis of the results they concluded that the fungicides strongly limited its survivability and potential infectiousness against the two-spotted spider mite (*T. urticae*).

Of the fungicides tested in the experiment mancozeb limited growth of the acaropathogenic fungi strains the strongest. Studies carried out by Tkaczuk and Miętkiewski (2001) found that this substance also had strong toxic effects on a strain of the *Hirsutella aphidis* Petch fungus, a pathogen of aphids. Additionally, research conducted by Tkaczuk et al. (2013) as well as by Todorova et al. (1998) and Jaros-Su et al. (1999), confirmed high toxicity of mancozeb to other species of entomopathogenic fungi.

Applied to the medium at a dose recommended by the manufacturer Miedzian 50 WP with the active substance of copper oxychloride strongly limited the growth of the isolates of investigated fungi. It was observed that this product inhibited the growth of the tested acaropathogenic fungi more at C concentration (0.01 of recommended dose) than B (0.1 of recommended dose). The colonies of the *H. thompsonii* strain growing on the medium with the dose10 times lower than the recommended dose reached diameter bigger than control. Examining the impact of copper hydroxide, and elemental sulphur on the vegetative growth of the *Hirsutella citriformis* Speare fungus Hall et al. (2012) observed that these fungicides administered in the highest dose inhibited its growth. This suggested that such chemical control regimens may have a negative impact on biological control of *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) by the fungus in citrus orchards.

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Spray oils and other chemical treatments to citrus might negate biological control by reducing infectivity and growth of *H. citriformis* as well as the longevity of mummies on leaves.

In our studies, sulphur limited the growth of the acaropathogenic fungal isolates less than other fungicides, but copper oxychloride, when added to the medium with recommended concentration, often prevented or significantly inhibited the development of the fungi. The results of this research confirm the studies of Sosa Gomez et al. (1987) and Sosa Gomez (1991), who found that copper oxychloride constrained the growth of *H. thompsonii* fungal colonies much more strongly than sulphur powder applied in the form of dust. It is worth noting that both of the above fungicides based on copper and sulphur are currently authorised for use in organic crops.

It should be, however, emphasized that the laboratory research on fungal susceptibility to fungicides do not necessarily reflect the complex situation in field, where the interactions between fungi and pesticides could be modified by a number of biotic and abiotic factors. Keller et al. (1993) suggested that the non-target effect of chemical pesticides on arthropod-pathogenic fungi applied as a microbial control agent might not be significant under practical conditions. For the use of *Beauveria brongniartii* (Sacc.) Petch in orchards, for example, the fungus is applied at a soil depth of some centimeters so that a direct contact with fungicides is avoided, thereby preventing adverse effects. In contrast to fungicides, soil herbicides penetrate several centimeters into the soil, which directly affects entomopathogenic fungi, i.e. *Beauveria bassiana* (Bals.-Criv.) Vuill.

Conclusions

The effect of fungicides on the growth of acaropathogenic fungi from the genus *Hirsutella* varied depending on the applied substance and its concentration in the culture medium. Of all fungicides tested the fungal growth was most strongly limited by mancozeb and a mixture of cyprodinil and fludioxonil. Of all the tested fungicides, sulphur showed the least adverse effect and is therefore probably compatible with fungi from the genus *Hirsutella* in the field. However, extensive field studies complemented by parallel pot experiments should consider assessing the interaction between fungicides and *Hirsutella* isolates to evaluate their ecological impact in crop environments.

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