Impact of some fungicides on growth and development of morphological structures of *Seimatosporium hypericinum* (Ces.) Sutton *in vitro*

BEATA ZIMOWSKA

Department of Plant Pathology, Agricultural University of Lublin
Leszczyńskiego 7, 20-069 Lublin, Poland
e-mail: beata.zimowska@ar.lublin.pl

S u m m a r y

The object of the research work was an isolate of *S. hypericinum* D 1224, as well as ten fungicides belonging to different chemical groups. The studies on the toxic effect were conducted *in vitro* by means of poisoning the media with the use of 1, 10 and 100 μg a.i./cm^3 of the tested preparations. The obtained results showed that the compounds of thiophanate-methyl and triadimephon caused complete inhibition of the growth of *S. hypericinum*, when the dose of 10 and 100 μg a.i./cm^3 was used, while in the case of mancozeb the effective dose was 100μg a.i./cm^3. These compounds, as well as trifloxystrobin, can be therefore regarded as a prospective means of limiting growth and development of *S. hypericinum* and protecting St. John's wort from this pathogen. The effect of the other compounds on *S. hypericinum* was less toxic.

Key words: activity, fungicides, Seimatosporium hypericinum, Hypericum perforatum

INTRODUCTION

The spread of cultivation of St. John's wort (*Hypericum perforatum* L.) both in Poland and worldwide in recent years, due to special properties of active substances contained in *Hyperici herba*, caused accumulation of pathogenic fungi in the cultivated environment [1, 2]. *Seimatosporium hypericinum* (Ces.) Sutton is a species commonly isolated from the above-ground parts of plants [3]. The pathogen, whose presence results in red spots occurring on the leaves and the stem necrosis of St. John's wort, lowers the quality and quantity of the raw plant material obtained from *Hyperici herba* [4].

The Program of Protecting Herbaceous Plants, worked out in recent years, does not include any recommendations concerning the control of *S. hypericinum* on production plantations of St. John's wort. In some European countries the satisfactory

health state of cultivations is maintained by means of using resistant cultivars, seed dressing, setting up plantations from seedlings and, possibly, by using fungicides [5, 6]. The author's own studies showed a considerable ability of some compounds, such as grapefruit extract and chitosan, to limit the growth and development of *S. hypericinum* [Zimowska, unpublished]. The present paper is concerned with *in vitro* studies, the aim of which was to determine the toxicity of fungicides from different chemical groups towards *S. hypericinum* and their effect on the formation of morphological structures of the pathogen.

MATERIAL AND METHODS

The tested material was an isolate of *S. hypericinum* D 1224 obtained from stems of St. John's wort with the symptoms of necrosis. The isolate was chosen at random from a collection of *S. hypericinum* cultures isolated during long-term studies concerning infection diseases of St. John's wort plants [2]. Ten fungicides were chosen to test the susceptibility of the fungus (Table 1). In the study, the PDA medium was used, and the method employed consisted in poisoning the media with doses of 1, 10 and 100 μg of active ingredient (a.i.) per 1 cm^3. This method enabled an approximate determination of the dose of ED_{50} and classifying the tested fungicides into one of the four categories according to their fungicidal activity [7]. The fungicidal activity was expressed as a percentage of the inhibition of the growth of the colonies on the medium with fungicide, when compared with the growth of the colonies grown on the control medium, determined on the basis of the formula presented by Kowalik and Krechniak [8]. Besides, during the studies the kind of toxic effect of fungicides (i.e. fungicidal, fungistatic or stimulating) in the case of *S. hypericinum* was established. As far as the fungistatic effect of the preparations is concerned, attention was paid to changes in the development of morphological and macroscopic structures, analysing the appearance of the colonies. The trials were repeated twice, with a 4-week interval. The obtained results were submitted to a variance analysis and the Tukey confidence intervals procedure was employed.

Table 1

List of examined fungicides.

fungicide	name of active ingredient and its content in the fungicide tested	producer
Bayleton 5 WP	5% triadimephon	Bayer AG – Germany
Chorus 75 WG	75% cyprodinil	Novelties Crop Protection AG – Switzerland
Champion 50 WP	50% copper hydroxide	Agtrd Chemicals Product – USA
Dithane M-4580 WP	80% mancozeb	Rhom and Haas – USA
Euparen 50 WP	50% dichlofluanid	Bayer AG – Germany
Kaptan zaw. 50 WP	50% captan	Organika – Poland
Miedzian 50 WP	50% copper oxychloride	Organika – Poland
Sumilex 500 SC	500 g procymidone in 1 l	Sumito Chemic Co. – Japan
Topsin M 70 WP	70% thiophanate-methyl	Nippon Soda Co. – Japan
Zato 50 WP	50% trifloxystrobin	Novartis Crop Protection AG – Switzerland

RESULTS

The effects of fungicides on *S. hypericinum* were various. The growth of the fungus colony on the medium containing 10 μg and 100 μg a.i./cm^3 of thiophanate-methyl was inhibited in 100% after four and eight days of incubation (Table 2). A similarly high percentage of growth inhibition was observed in the colonies of the pathogen on the medium containing triadimephon and mancozeb. The former, when the concentrations of 10 μg and 100 μg a.i./cm^3 were used, made the growth of four- and eight-day-old colonies of the fungus impossible, while the other turned out to be equally effective when the concentration of 100 μg a.i./cm^3 was used (Table 2). Trifloxystrobin in the concentration of 10 μg and 100 μg a.i./cm^3 caused the inhibition of the colony growth after four days in 57.3% and 56.9%, respectively, while in the case of an eight-day-colony the figures were 52.3% and 50.0%, respectively (Table 2). Captan, dichlofluanid and cypronidil, used in concentrations of 100 μg a.i./cm^3, inhibited the growth of four-day-old colonies in 8.75%, 72.5% and 56.5%, respectively. The toxic effect of these compounds, except for dichlofluanid, in concentrations of 100 μg a.i./cm^3 gradually decreased (Table 2). With the content of 100 μg a.i./cm^3 in the culture medium, the lowest percentage of the growth inhibition of four-day-old colonies of *S. hypericinum* was observed when procymidone and copper oxychloride were used. After eight days of the culture the enumerated compounds showed a slight inhibitory effect (Table 2). Out of the tested preparations only copper oxychloride in the concentration of 1 μg a.i./cm^3 stimulated the growth of eight-day-old colonies of the pathogen (Table 2).

Table 2

Impact of the fungicides tested on the growth inhibition of *Seimatosporium hypericinum*.

	diameter of four-day-old colonies (mm) in relation to a.i. concentration (μg/cm^3)				diameter of eight-day-old colonies (mm) in relation to a.i. concentration (μg/cm^3)		
chemicals**	1	10	100	chemicals**	1	10	100
I				I			
trifloxystrobin	59.3 hij	57.3 hij	56.9 ij	trifloxystrobin	54.9 ef	52.3 e	50.0 e
thiophanate-methyl	77.3 ijk	100 k	100.0 k	thiophanate-methyl	80.3 g	100.0 g	100.0 g
II				II			
triadimephon	39.1 fgh	100 k	100.0 k	triadimephon	43.1 cde	87.5 g	100.0 g
mancozeb	15.5* abcdef	52.3 ghi	100.0 k	III			
III				mancozeb	16.4* ab	43.1 cde	100.0 g
cyprodinil	30.8 defg	20.9 abcdef	56.5 hi	dichlofluanid	17.0* ab	22.2 bc	78.0 fg
dichlofluanid	23.7 abcdef	35.4 efgh	72.5 ij	captan	9.5* ab	12.4* ab	81.7 g
captan	13.4* abcde	20.9 abcdef	87.5 jk	IV			
IV				copper hydroxide	–7.3* a	15.1* ab	24.5 bcd
copper hydroxide	3.9* ab	13.8* abcde	27.1 bcdef	cyprodinil	23.5 bc	14.1* ab	48.0 de
copper oxychloride	8.0* abcd	1.4* a	15.9* absdef	copper oxychloride	12.1* ab	5.6* ab	25.2 bcd
procymidone	29.1 cdefg	5.6* abc	8.0* abcd	procymidone	16.7* ab	7.1* ab	7.9* ab
control	0.0	0.0	0.0	control	0.0	0.0	0.0
	$LSD_{0.05}$=23.6926				$LSD_{0.05}$=23.4443		

Values marked with the same letter do not differ significantly
*Values do not differ significantly from the control at P≤0.05
**Chemicals compared according to fungicidal activity group

In the group of the tested compounds, trifloxystrobin and thiophanate-methyl turned out to be very toxic towards *S. hypericinum* – group I of fungicidal activity, since their ED_{50} was below 1 μg a.i/cm^3 (Table 2). Preparations highly toxic – group II of fungicidal activity towards the discussed pathogen – included triadimephon and mancozeb, since their ED_{50} ranged from 1 μg to 10 μg a.i./cm^3. The substances of medium degree of toxicity – group III of fungicidal activity towards *S. hypericinum* – included cyprodinil, dichlofluanid and captan, as their ED_{50} ranged from 10 μg to 100 μg a.i./cm^3. The other tested compounds belong to group IV, i.e. the compounds of weak fungicidal activity, since their ED_{50} in the case of the studied pathogen exceeds 100 μg a.i./cm^3 (Table 2).

S. hypericinum on the medium with an addition of some compounds did not form any spores or the pace of that process was much slower than in the control culture. Lack of conidia, degeneration and browning of the hyphae, as well as the formation of dark swellings in four- and eight-day-old colonies of *S. hypericinum* were observed when 10 μg and 100 μg a.i./cm^3 of trifloxystrobin, thiophanate-methyl, mancozeb and triadimephon were applied. When 100 μg a.i./cm^3 of procymidone and captan were applied, numerous thick-walled swellings were observed on hyphae what were considerably shortened. The hyphae of air mycelium in the presence of the above mentioned compounds formed a compact, leathery structure. The toxic activity of the tested compounds was varied. The fungicidal activity towards *S. hypericinum* was shown by mancozeb and thiophanate-methyl when the concentration of 100 μg a.i./cm^3 was used. Copper hydroxide in the concentration of 1 μg a.i./cm^3 proved to be a preparation stimulating the pathogen's growth. The effect of the other preparations on *S. hypericinum* was fungistatic (Table 3).

Table 3

The kind of toxic activity of chemicals on *Seimatosporium hypericinum* (Ces.) Sutton.

chemicals	concentration of active ingredients in μg/cm^3		
	1	10	100
triadimephon	+	+	+
cyprodynil	+	+	+
copper hydroxide	++	+	+
mancozeb	+	+	–
dichlofluanid	+	+	+
captan	+	+	+
copper oxychloride	+	+	+
procymidone	+	+	+
thiophanate-methyl	+	+	–
trifloxystrobin	+	+	+

– fungicidal activity
\+ fungistatic activity
++ stimulatic activity

DISCUSSION

The studies showed that out of ten compounds belonging to different chemical groups thiophanate-methyl and trifloxystrobin were worth mentioning due to the fact that they belong to group I of fungicidal activity towards *S. hypericinum in vitro*. Moreover, a strong toxic effect of these substances persisted despite the longer period of cultivating the colony. Trifloxystrobin belonging to the group of strobilurine fungicides is characterized by its ability to limit the growth of a wide spectrum of pathogens [9, 10]. A big advantage of the listed preparations is their strong anti-sporulation effect, which is accompanied by an inhibitory development of the mycelium, observed in the present studies. As far as limiting the growth and sporulation of *S. hypericinum* is concerned, a similarly high effectiveness should be ascribed to thiophanate-methyl. Numerous swellings on the hyphae, which in the case of *S. hypericinum* are formed in the conditions unfavourable for its growth, suggest a negative effect of the above-mentioned compounds [11]. Thiophanate-methyl is one of the fungicides recommended for use in the plantations of St. John's wort against *Erysiphe hyperici* and *Septoria hyperici* (Recommendations for Plant Protection 2004/2005 – ornamental and herbaceous plants). Positive effects of the studies point to the usefulness of testing this compound against *S. hypericinum*, at the same time having in mind the danger that fungi can quickly acquire resistance to the preparations from the group of benzymidazoles [12]. Mancozeb seems to be worth considering when the aspect of limiting the growth and occurrence of *S. hypericinum* on the plantations of St. John's wort is important, due to its fungicidal effect in certain concentrations. This ability of mancozeb was observed earlier, for example it was tested against *Botrytis cinerea* Pers. and *Monilia coryli* Schellenberg [9, 13]. Similarly, triadimephone, belonging to one of the more important groups of systemic fungicides, can prove useful in limiting the occurrence of *S. hypericinum,* due to the fact that it is included in group II of fungicidal activity towards this pathogen. On the other hand, cyprodinil, considered to strongly limit the growth of different species of fungi [14], inhibited the growth of *S. hypericinum* rather poorly, especially at a longer period of its application. A similarly insignificant inhibiting effect on the growth of *S. hypericinum* was shown by dichlofluanid and captan, commonly used in the protection of a number of plant species. Similar results were achieved by Montealegre and Rustom (1989) in Chile, who studied the effect of the above-mentioned compounds in the case of *Seimatosporium lichenicola*, the pathogen causing the dying out of raspberry shoots [15]. Procymidone, as well as copper preparations, which were the first ones used in plant protection, turned out to be practically harmless to *S. hypericinum*.

The obtained results point out that methyl thiophanate-methyl, mancozeb and triadimephone can be taken into consideration in the studies on their effect in the control of *S. hypercinum*, as well as in the tests concerning an integrated protection of St. John's wort on production plantations.

CONCLUSIONS

1. Trifloxystrobin and thiophanate-methyl belong to the most toxic compounds (group I) towards *S. hypericinum*.
2. Thiophanate-methyl and triadimephon, used at concentrations of 10 μg and 100 μg a.i./cm^3, and mancozeb at the concentration of 100 μg a.i./cm^3 inhibited growth of *S. hypericinum* in 100%, and only mancozeb and thiophanate-methyl in the concentrations of 100 μg a.i./cm^3 showed fungicidal properties towards *S. hypericinum*.
3. The effect of the other compounds, except for copper hydroxide, which showed a strong stimulating effect, was toxic only to a slight degree.

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WPŁYW AKTYWNOŚCI WYBRANYCH FUNGICYDÓW NA WZROST I TWORZENIE STRUKTUR MORFOLOGICZNYCH *SEIMATOSPORIUM HYPERICINUM* (CES.) SUTTON *IN VITRO*

B. ZIMOWSKA

Katedra Fitopatologii, Akademia Rolnicza
ul. Leszczńskiego 7, 20-069 Lublin

S t r e s z c z e n i e

Obiektem badań był izolat *S. hypericinum* D 1224 oraz dziesięć fungicydów należących do różnych grup chemicznych. Badania toksycznego oddziaływania przeprowadzono *in vitro*, stosując metodę zatruwania podłoży przy użyciu 1, 10 i 100 μg s.a./cm^3 badanych preparatów. Uzyskane wyniki wskazały, że tiofanat metylu oraz triadimefon powodowały całkowite zahamowanie wzrostu *S. hypericinum* przy zastosowaniu 10 i 100 μg s.a./cm^3, mankozeb natomiast przy użyciu 100 μg s.a./cm^3. Związki te, a ponadto trifloksystrobinę, uznano za perspektywiczne w wypadku ograniczania wzrostu i rozwoju *S. hypericinum*, a być może także w celu ochrony dziurawca zwyczajnego przed tym patogenem. Oddziaływanie pozostałych związków na *S. hypericinum* było mniej toksyczne.

Słowa kluczowe: aktywność, fungicydy, Seimatosporium hypericinum, Hypericum perforatum