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Impact of some fungicides on mycelium growth of *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc.

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Abstract: The object of the research work were an isolate of *Colletotrichum gloeosporioides* causing anthracnose on *Hypericum perforatum* L. (St. John's Wort) as well as three fungicides belonging to different chemical groups: Sarfun 500 SC, Amistar 250 SC and Gwarant 500 SC. The studies on the inhibiting mycelium growth effect were carried out *in vitro* on the media amended with the fungicides at concentrations corresponding to field recommended doses for control of anthracnose of many vegetables and ornamental plants and additionally in values double increased and decreased them. The results showed that Sarfun 500 SC even at the twice reduced dose (0.05%) concentration was most effective against this pathogen. Amistar 250 SC at the lower concentration (0.05%) demonstrated low antifungal activity but the effect of 0.1% concentration was significantly higher. In the case of the fungicide Gwarant 500 SC the effective concentration was 0.4% whereas recommended dose is 0.2%.
The Sarfun 500 SC and Amistar 250 SC can be therefore regarded as a prospective means of limiting growth and development of *Colletotrichum gloeosporioides* and protecting St. John's Wort from this pathogen.

Keywords*:* activity, fungicides, *Colletotrichum gloeosporioides*

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].

Anthracnose caused by *Colletotrichum gloesporioides* (Penz.) Penz. & Sacc. is prevalent in most plantations of St. John's Wort. Symptoms were characterized by brown sunken lesions 2 to 15 mm in diameter, that girdled the stems and often killed the plant. First description in Europe of anthracnose appearances on *Hypericum perforatum* L. was published in Germany in 1949 by Wollenweber and Hochapfel [3]. In Switzerland the disease was first observed in 1995. In 1999, most of the 20 ha of St. John's-Wort planted in this country were destroyed because of anthracnose in the first year of cultivation [4]. In 1997 a high incidence of severe anthracnose symptoms was observed in plantation of Saint John`s Wort in Hungary [3].

The first report in Poland about this disease and its pathogen was published by Mikołajewicz and Filoda in 1999 [5].

The program of protecting herbaceous plants, worked out in recent years, does not include any recommendations concerning the control of *Colletotrichum gloeosporioides* on production plantations of St. John's Wort. In Poland and some other European countries the satisfactory health state of cultivations is maintained by means of using resistant cultivars [6, 7] seed dressing [8], setting up plantations from seedlings and, possibly, by using fungicides [9].

The present paper is concerned with *in vitro* studies, the aim of which was to determine the antifungal activity of fungicides from different chemical groups against *Colletotrichum gloeosporioides*.

MATERIALS AND METHOD**S**

Isolate. The tested material was an isolate of *Colletotrichum gloeosporioides* obtained from stems of St. John's Wort cv. 'Topaz' with the symptoms of necrosis. The isolate was chosen at random from a collection of *C. gloeosporioides* cultures isolated during 1998-2000 studies concerning infection diseases of St. John's Wort plants cultivated on 3 plantations in the region of Poznań [10].

Fungicidal activity test. Three fungicides were chosen to test the susceptibility of the fungus (Table 1).

Table 1. List of examined fungicides

Fungicide name	Group of fungicides	Range of effectiveness	Active ingredient	Recommended commercial doses
Sarfun 500 SC	benzimidazole	**systemic**	carbendazim	0.1%
Gwarant 500 SC	chloronitrile	**contact**	chorothalonile	0.2%
Amistar 250 SC	strobilurin	**systemic**	azoxystrobin	0.1%

In the study, the PDA medium (potato dextrose agar) without and with fungicides was used:

- Sarfun 500 SC – 0.2%; 0.1%; 0.05%
- Gwarant 500 SC – 0.4%; 0.2%; 0.1%
- Amistar 250 SC – 0.2%; 0.1%; 0.05%

Agar 5 mm diameter with actively growing mycelium of 7-day-old colony of *Colletotrichum gloeosporioides* were cut out with sterile cork borer and placed on central point of 90 mm Petri dishes. Four plates were inoculated for each fungicide concentration. The plates were incubated at 25 °C in the darkness and, after appropriate time intervals (three days), two diagonal measurements of mycelium growth were made on each plate. Final measurements were taken when fungal growth in the control (medium without fungicide) was finished (after 21 days of incubation) [10]. Means of the colony diameter were used to calculate the percentage of growth inhibition of mycelium in relation to the control on the basis of the formula presented by Borecki [11].

$$S = \frac{A-B}{A} x100$$

S – % of the mycelium growth inhibition

A – mean diameter of fungus colonies on a medium without fungicide

B – mean diameter of fungus colonies on a medium with fungicide

The mean data from measurements obtained from test conducted twice were statistically analysed. The significant differences between treatments were calculated on the basis of t-Student test (P=0.05).

Mycelium development and forming of *C. gloeosporioides* conidia were examined both macroscopically and microscopically.

RESULTS

Colletotrichum gloeoesporioides grew the best on fresh PDA medium and the conidia were profusely produced. Inhibition of mycelium growth by added fungicides was significant (Figures 1-3).

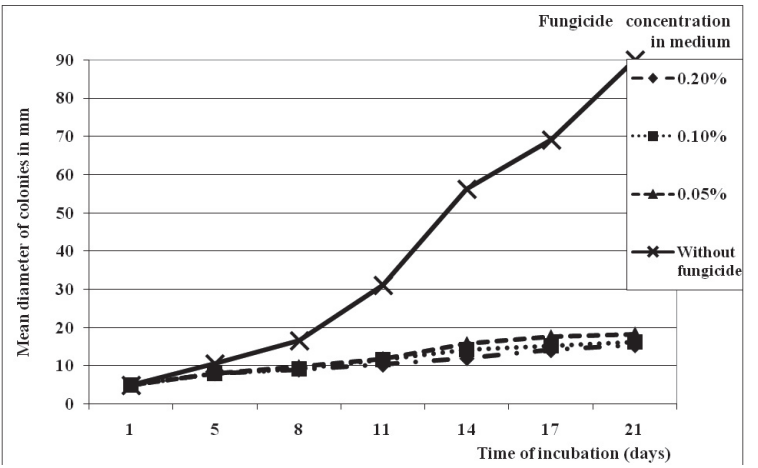

Figure 1. Effect of Sarfun 500 SC on linear mycelium growth of *C. gloeosporioides* on plates with PDA medium.

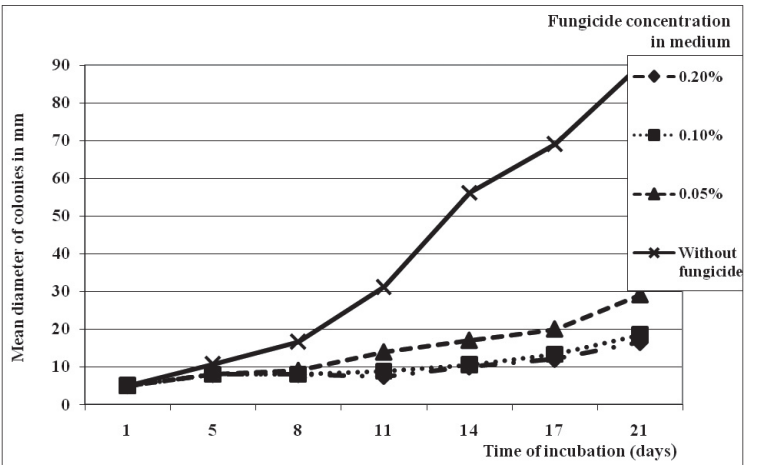

Figure 2. Effect of Amistar 250 SC on linear mycelium growth of *C. gloeosporioides* on plates with PDA.

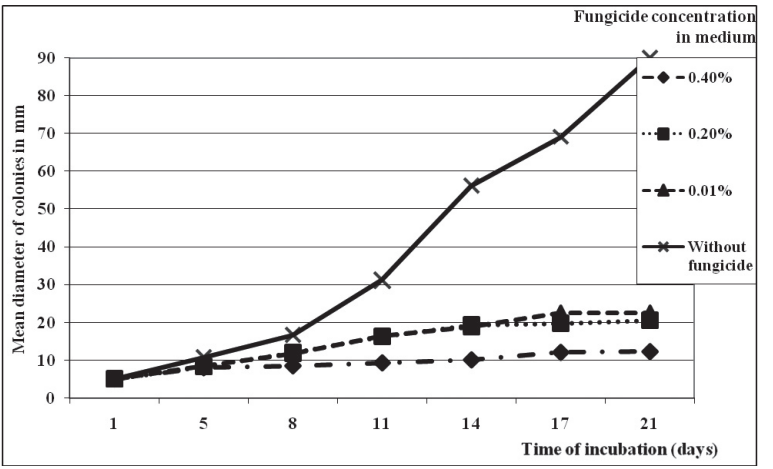

Figure 3. Effect of Gwarant 500 SC on linear mycelium growth of *C. gloeosporioides* on plates with PDA medium.

Percentages of the inhibition of the *Colletotrichum gloeosporioides* growth colonies were various (Table 2). Among of tested fungicides Sarfun 500 SC was the most effective, showing a strong inhibition even at the lowest dosage, of both mycelium growth and colony development. The growth of the 21 days old colonies on the medium containing 0.2; 0.1 and 0.05% of Sarfun 500 SC was inhibited in 83; 82; 80% respectively. A similarly high percentage of growth inhibition was observed when Amistar 500 SC (azoxystrobin) was used at concentrations 0.1 and 0.2% (80 and 82%). Only lowest concentrations (0.05%) was not satisfactory and the inhibition was 68% only. Gwarant 500 SC at concentration 0.4 % showed good efficacy in limiting the fungal growth (86%) while in the case of lower concentrations 0.2 and 0.1% was significantly poorer and the retardations were 77 and 75% respectively.

Thus except of Gwarant 500 SC the other two fungicides turned out to be equally effective when the recommended concentrations were used.

Table 2. Impact of the tested fungicides on the colony growth of *Colletotrichum gloeosporioides*

No.	Treatments	Concentration of fungicides in medium (%)	Mean diameter of twenty one-day-old colonies (mm)		Inhibition effect (%)
1	Control	-	90.0	a	-
2	Sarfun 500 SC	0.05	18.3	e	80
		0.10	16.3	f	82
		0.20	15.4	g	83
3	Gwarant 500 SC	0.10	22.5	c	75
		0.20	20.5	d	77
		0.40	12.3	h	86
4	Amistar 250 SC	0.05	29.1	b	68
		0.10	18.1	e	80
		0.20	16.1	f	82

Values marked with the same letter do not differ significantly at P = 0.05 , t-Student test

Colletotrichum gloeosporioides on the medium with an addition of fungicides at low concentrations formed considerably less spores and the pace of that process was much slower than in the control culture. The fungus did not form any spores and hyphae of air mycelium formed a compact, leathery structure of colony on PDA medium with the highest concentration of fungicides (Figure 5).

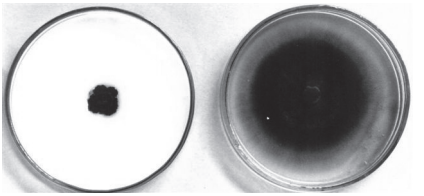

Figure 4. Influence of the highest concentration of fungicides on the mycelium growth after 21 days of incubation.

DISCUSSION AND CONCLUSIONS

The studies showed that out of three fungicides belonging to different chemical groups Sarfun 500 SC (carbendazim – benzimidazole group) was worth mentioning due to the fact that even at twice reduced recommended

dose did not significantly decrease fungicidal activity against *Colletotrichum gloeosporioides*. Benzimidazole fungicides (benomyl) also were most effective against this pathogen in the investigations conducted with the same method by Adaskavey and Forster [12]. In Poland benzimidazole fungicides (tiophanat methyl) were recommended for use in the plantations of St. John's Wort against *Erysiphe hyperici* and *Septoria hyperici* [13] and Sarfun 500 SC is registered in Poland for control of anthracnose on lupine [14]. Using this fungicide one should assure a simultaneous control of foliar and stem base diseases control of St. John's Wort, having in mind the danger that fungi can quickly acquire resistance to the preparations from the group of benzimidazole [11].

Amistar 250 SC showed good activity in limiting mycelial growth and conidia forming of *Colletotrichum gloeosporioides.* The active ingredient of this fungicide azoxystrobin is characterized by its ability to limit the growth of a wide spectrum of pathogens and at the concentration 0.1% is recommended for use in many vegetables like: onion, bean, pumpkin plants and tomato for control of anthracnose [15]. In Germany Amistar 250 SC is applied for limitation of St. John's Wort anthracnose occurrence on plantations [16]. In the case of chlorotalonil (Gwarant 500 SC) the sensitivity of *Colletotrichum gloesporioides* to this fungicide was not so significant although is recommended for protection onion, bean and tomato against anthracnose [15].

The obtained results point out that Sarfun 500 SC and Amistar 250 SC can be taken into consideration in the studies on their efficacy in the control of *C. gloeosporioides*, as well as in the tests concerning an integrated protection of St. John's Wort on production plantations. To ensure crop safety before widespread application there is need to test treatments of small batch of plants. Fungicide treatment might have the unwanted effects of reducing plant size and development. The fungicides we used had broad-spectrum effects, it seems likely that the fungicide treatment killed both pathogenic fungi as well as beneficial mycorrhizae [17].

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