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# Effect of polyamine biosynthesis inhibitors on *in vitro* growth of *Phyllosticta antirrhini* Sydow and on the infection of *Antirrhinum majus* L. by the pathogen

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Summary

Polyamine biosynthesis inhibitors, DL-α-difluromethylornithine (DFMO) and DL-α-difluromethylarginine (DFMA) at concentrations of 1.0 and 3.0 mM greatly inhibited mycelial growth of *Phyllosticta antirrhini* on Czapek Dox Agar (CDA). Putrescine at the concentration of 1.0 mM completely reversed the inhibitory effect of DFMO but only partially that of DFMA.

Both polyamine biosynthesis inhibitors at concentrations 1.0 and 3.0 mM applied preventively and curatively strongly inhibited the development of the pathogen on snapdragon leaves.

Key words: *Phyllosticta antirrhini*, snapdragon, mycelium, leaf, putrescine, inhibitors of polyamine biosynthesis

## INTRODUCTION

Polyamines appear to play a crucial role in many cellular processes regulating growth in fungi, higher plants and animals (Tabor and Tabor, 1985; Smith, 1985). The most widely distributed polyamines are putrescine, spermidine and spermine. Specific inhibitors of polyamine biosynthesis are useful tools in understanding of the role of polyamines in the growth and development of phytopathogenic fungi, and many of them have great potential as fungicides (Rajam and Galston, 1985; Khan and Minocha, 1989; Garcia et al., 1991).

It was showed that inhibitors of polyamine biosynthesis gave very good control of plant infections by rust and powdery mildew fungi (Rajam et al., 1985; Walters, 1986; West and Walters, 1988; Weinstein et al., 1987; Walters and Kingham, 1990).

The aim of the present work was to examine an *in vitro* effect of polyamine biosynthesis inhibitors and polyamine (putrescine) on mycelial growth of *Phyllosticta antirrhini* and on the snapdragon (*Antirrhinum majus* L.) leaves infection by the pathogen (Kochman, 1938; Saniewska, 1995).

## MATERIAL AND METHODS

### *In vitro* growth of *Phyllosticta antirrhini* in the presence of polyamine biosynthesis inhibitors

*Phyllosticta antirrhini* Sydow (syn. *Phoma poolensis* Taub. var. *poolensis* - de Gruyter et al., 1993), isolate W-5, was used in all trials. Inhibitors of polyamine biosynthesis, DL-α-difluromethylornithine (DFMO) and DL-α-difluoromethylarginine (DFMA) at final concentration 1 and 3 mM and putrescine at concentration 1mM were added to Czapek Dox Agar (CDA) separately or as mixtures of DFMO with putrescine and DFMA with putrescine. Five mm diam. plugs taken from 7-day-old culture of *P. antirrhini* were placed in the middle of 90 mm Petri dishes containing CDA medium supplemented with the tested compounds. Control plates constituted the culture growing on CDA without any amendments.

The diameter of the fungal colony was measured within 8 day-incubation at 25°C in the dark. Five dishes were used for each treatment and the experiment was repeated 3 times.

### Influence of polyamine biosynthesis inhibitors, DFMO and DFMA, applied preventively and curatively in the control of *P. antirrhini* on snapdragon leaves

The experiments were conducted on *Antirrhinum majus* cv. Anna during 1994, 1996 and 1998 at the Research Institute of Pomology and Floriculture, Skierniewice.

Young plants in the stage of six leaves for greenhouse experiment and excised leaves taken from snapdragon growing in the field were used in laboratory test. Development of disease on intact and excised leaves of snapdragon in the presence of DFMO and DFMA at concentrations 1.0 and 3.0 mM was evaluated in boxes or in a greenhouse. The compounds were applied on leaves 2 h before or 24 h after inoculation with *P. antirrhini*. In the greenhouse experiment five leaves per plant from 20 plants (4 replicates with 5 plants) and in laboratory test 40 leaves (4 replicates with 10 leaves) in each treatment were used. Each leaf was inoculated with 10 μl of fungal inoculum at suspension $10^6$ spores/$10^{-3}$ $dm^3$. For comparison Penncozeb 80 WP 0.2% (80% of mancozeb) was used as a standard fungicide. The inoculated plants were kept in a small plastic tunnel on a greenhouse bench with high humidity inside or in plastic boxes covered with polyethylene in the laboratory test. In the laboratory test the diameter of necrotic spots was measured 6-days after inoculation, whereas in the greenhouse experiment 11- and 21-days after spraying number of infected leaves per plant was estimated. The trials were repeated 2 times at fortnight intervals.

### Effectiveness of DFMO and DFMA in the control of *P. antirrhini* on field grown, naturally infested snapdragon

Two-months-old seedlings were planted in the field in the middle of May 1994 and 1996. First preventive sprays with DFMO and DFMA at the concentration 2 mM

were done first week of July and the compounds were regulary applied at 7-day intervals during seven weeks. Baycor 300 EC 0.2% (27.8% of bitertanol) was used as a standard fungicide. One week after the final treatment number of infected leaves per plant was recorded for each experimental variant. The experiment was conducted upon the randomized block layout with 4 replicates with 5 plants each.

The results obtained for each experiment were subjected to analysis of variance, Duncan's multiple range t-test at P = 0.05 was used for means separation.

## RESULTS AND DISCUSSION

Polyamine biosynthesis inhibitors DFMO and DFMA at the concentrations 1mM and 3 mM greatly inhibited growth of *Phyllosticta antirrhini* on CDA medium (Tab. 1, Fig. 1). The inhibitory effect of DFMO applied at 1mM was completely reversed by 1mM of putrescine (Tab. 1, Fig. 1), but the effect of DFMO used at conc. 3mM was not only reversed by puterscine but even significantly increased the fungal growth above control values (Tab.1, Fig 1). The suppressive influence of DFMA at conc. 1mM and 3 mM on the growth of the pathogen was only partially reversed by putrescine at conc. 1mM (Tab. 1, Fig. 1). Such effect of both inhibitors on the growth of some fungi and reversed effect of polyamines was reported earlier by Rajam and Galston (1985), Khan and Minocha (1989), and Saniewska (1996).

Table 1

Influence of DFMO, DFMA and 1mM putrescine on mycelial growth of *Phyllosticta antirrhini* on Czapek Dox Agar

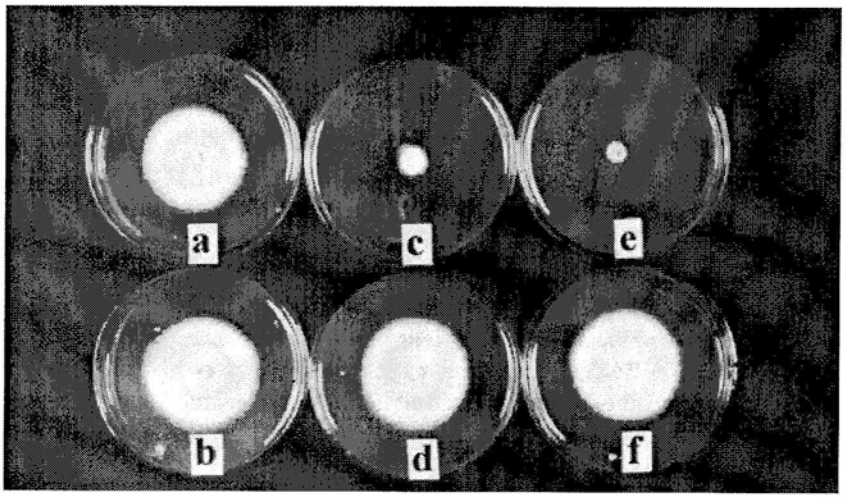
| Treatment | Diameter of mycelium in mm | | | | |
|---|---|---|---|---|---|
| | days of incubation | | | | |
| | 2 | 3 | 5 | 6 | 8 |
| Control | 17.3 d | 25.2 e | 41.8 e | 48.2 e | 69.1 e |
| Putrescine (Put) | 17.9 d | 26.9 f | 43.8 f | 50.1 e | 72.2 e |
| 1 mM DFMO | 7.3 a | 8.3 b | 12.4 b | 13.2 b | 22.9 b |
| 1 mM DFMO + Put | 17.7 d | 25.7 e | 43.7 f | 49.6 e | 69.3 e |
| 3 mM DFMO | 6.8 a | 7.4 a | 9.6 a | 8.9 a | 15.6 a |
| 3 mM DFMO + Put | 17.3 d | 26.9 f | 43.6 f | 50.2 e | 72.0 e |
| 1 mM DFMA | 7.0 a | 8.3 b | 12.8 b | 15.1 b | 21.2 b |
| 1 mM DFMA + Put | 12.9 c | 17.9 d | 30.6 d | 37.4 d | 59.4 d |
| 3 mM DFMA | 7.0 a | 8.8 b | 13.2 b | 14.9 b | 21.0 b |
| 3 mM DFMA + Put | 12.0 b | 16.6 c | 26.4 c | 31.0 c | 49.2 c |

Explanation: Mean in columns followed by the same letter do not differ significantly at 5% level (Duncan's multiple range test).

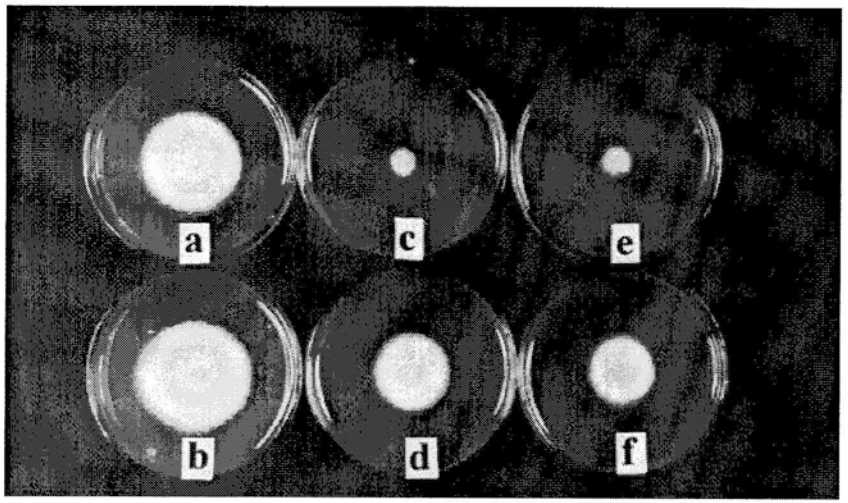
Fig. 1. Influence of DFMO (A) and DFMA (B) on mycelial growth of *Phyllosticta antirrhini* on Czapek Dox Agar and reversed effect of putrescine on the pathogen after 6-day incubation:

A) a - control, b - putrescine (Put.) 1mM, c - DFMO 1mM, d - DFMO 1mM + Put. 1mM, e - DFMO 3mM, f - DFMO 3 mM + Put. 1mM,

B) a - control, b - putrescine (Put.) 1mM, c - DFMA 1mM, d - DFMA 1mM + Put. 1mM, e - DFMA 3mM, f - DFMA 3mM + Put. 1mM.




A




B

In the laboratory test on excised leaves, both polyamine biosynthesis inhibitors DFMO and DFMA at the concentrations 1mM and 3 mM applied as preinoculation sprays completely inhibited infection of snapdragon leaves by *P. antirrhini* (Tab. 2).

When polyamine biosynthesis inhibitors were applied 24 h after inoculation they were effective only at the concentration 3 mM (Tab. 2).

Table 2

Influence of DFMO and DFMA used preventively and curatively on the control of *Phyllosticta antirrhini* in laboratory test on excised leaves of snapdragon; diameter of necrosis 6-day after inoculation; n=40 leaves

| Treatment | Preventively | Curatively |
|---|---|---|
| Control untreated | 15.1 b | 18.4 c |
| DFMO 1 mM | 0.0 a | 14.0 bc |
| DFMO 3 mM | 0.0 a | 1.1 a |
| DFMA 1 mM | 0.0 a | 14.8 c |
| DFMA 3 mM | 0.0 a | 0.0 a |
| Mancozeb (Penncozeb 80 WP 2 mg/ml) | 0.0 a | 7.9 b |

Explanation: see Table 1

In greenhouse tests on intact leaves, both, DFMO and DFMA at the concentration 3 mM, applied preventively and curatively, strongly inhibited the development of the pathogen. After 11- and 21-days from inoculation, affected leaves were found only sporadically (Tab. 3). The compounds ( DFMO and DFMA) and mancozeb (strong inhibitor of the pathogen) gave similar inhibitory effect in the development of *P. antirrhini* on snapdragon leaves (Tab. 3).

Table 3

Influence of DFMO and DFMA used preventively and curatively on the control of *Phyllosticta antirrhini* in greenhouse snapdragon; n=25 leaves from 5 plants

| Treatment | Number of infected leaves per plant | |
|---|---|---|
| | days after last spraying | |
| | 11 | 21 |
| Control – inoculated | 2.3 b | 4.8 c |
| **Preventively** | | |
| DFMO 3 mM | 0.4 a | 0.6 ab |
| DFMA 3 mM | 0.1 a | 0.1 a |
| Mancozeb (Penncozeb 80 WP 2 mg/ml) | 0.0 a | 0.0 a |
| **Curatively** | | |
| DFMO 3 mM | 0.5 a | 1.0 b |
| DFMA 3 mM | 0.1 a | 0.2 ab |
| Mancozeb (Penncozeb 80 WP 2 mg/ml) | 0.0 a | 0.2 ab |

Explanation: see Table 1

In the field experiment with naturally infested snapdragon leaves, the polyamine biosynthesis inhibitors DFMO and DFMA at the concentration 2 mM applied weekly

seven times evidently inhibited the development of *P. antirrhini* (Tab. 4). No symptoms of disease on snapdragon leaves at the end of vegetation period were observed.

Table 4

Influence of DFMO and DFMA applied preventively on the control of *Phyllosticta antirrhini* on naturally infested field grown snapdragon; 7 sprays at week intervals; n=5 plants

| Treatment | Number of infected leaves per plant |
|---|---|
| Control untreated | 14.0 b |
| DFMO 2 mM | 0.0 a |
| DFMA 2 mM | 0.0 a |
| Bitertanol (Baycor 300 EC 2 µl/ml) | 0.0 a |

Explanation: see Table 1

Similar observations have been also made by several researchers who have also reported the remarkable efficacy of DFMO in controlling many plant diseases of fungal origin. Rajam and Sawhney (1996) noticed the complete protection of wheat seedlings (*Triticum aestivum* L) against *Puccinia recondita* following foliar spray with 0.5 mM DFMO. The compound was effective in controlling infection of *Phaseolus vulgaris* by *Uromyces phaseoli* (Rajam et al. 1985) and *Vicia fabae* by *Uromyces vicia-fabae* (Walters 1986).

These observations provide evidences that endogenous polyamines are necessary compounds for the growth and development of *Phyllosticta antirrhini* and that polyamine biosynthesis inhibitors (DFMO and DFMA) may be used as antifungal agents to prevent diseases caused by the pathogen.

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## Wpływ inhibitorów biosyntezy poliamin na wzrost *in vitro* *Phyllosticta antirrhini* i infekcję wyżlinu przez tego patogena

### Streszczenie

Szara plamistość liści i pędów powodowana przez *Phyllosticta antirrhini* jest groźną chorobą wyżlinu w uprawach pod osłonami, a ostatnio coraz częściej spotykana w uprawach reprodukcyjnych.

W warunkach *in vitro* na pożywce Czapek Dox Agar badano wpływ dwóch inhibitorów biosyntezy poliamin, DL-α-diflurometyloornityny - DFMO (inhibitor dekarboksylazy ornityny) i DL-α-diflurometyloargininy - DFMA (inhibitor dekarboksylazy argininy) na wzrost grzyba *P. antirrhini*. Na pożywce Czapek Dox Agar DFMO i DFMA w stężeniu 1 mM i 3 mM silnie hamowały wzrost grzybni *P. antirrhini*. Ten efekt inhibitorów był odwracany całkowicie lub częściowo przez putrescynę 1mM dodaną razem z inhibitorami do pożywki. Po dodaniu do pożywki DFMO w stężeniu 3 mM i putrescyny w stężeniu 1 mM wzrost liniowy grzybni był nawet większy niż w kontroli.

W warunkach sztucznej infekcji na liściach odciętych i całych roślinach wyżlinu badano wpływ inhibitorów DFMO i DFMA na rozwój grzyba.

DFMO i DFMA w stężeniu 1 mM zastosowane 2 godziny przed inokulacją, całkowicie hamowały infekcję liści przez *P. antirrhini*, a zastosowane po 24 godzinach od inokulacji ograniczały rozwój grzyba tylko w stężeniu 3 mM.

W warunkach naturalnej infekcji w polu, DFMO i DFMA w stężeniu 2 mM, zastosowane 7-krotnie do opryskiwania w odstępach tygodniowych, całkowicie chroniły wyżlin

przed infekcją *P. antirrhini*, podobnie jak bitertanol (Baycor 300 EC 0.2%). Należy sądzić, że endogenne poliaminy są związkami niezbędnymi do wzrostu i rozwoju grzyba *Phyllosticta antirrhini*.

Specyficzne inhibitory biosyntezy poliamin są użyteczne w określaniu roli poliamin we wzroście i rozwoju grzybów patogenicznych i mogą stać się nową grupą fungicydów.