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THE EFFECT OF *MEDICAGO ARABICA*, *M. HYBRIDA* AND *M. SATIVA* SAPONINS ON THE GROWTH AND DEVELOPMENT OF *FUSARIUM OXYSPORUM* SCHLECHT F. SP. *TULIPAE* APT.

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S u m m a r y

In the present work it was shown that total saponins originated from *M. hybrida* and *M. sativa* substantially limited mycelium growth of *F. oxysporum* f. sp. *tulipae* and symptoms of fusariosis on tulip bulbs. Out of 15 individual tested saponins originated from *M. arabica*, *M. hybrida* and *M. sativa*, four compounds: 3-*O*-[*β*-D-glucopyranosyl (1→2)*α*-L-arabinopyranosyl] hederagenin, hederagenin 3-*O*-*β*-D-glucopyranoside, medicagenic acid, medicagenic acid 3-*O*-*β*-D-glucopyranoside had the strongest inhibitory effect on mycelium growth of *Fusarium oxysporum* f. sp. *tulipae* on PDA medium. The total saponins from *M. arabica*, *M. hybrida* and *M. sativa* inhibited the number of colony forming units of *Fusarium oxysporum* f. sp. *tulipae* in artificially infested substrate. The use of saponins originated from *Medicago* as a fungicide is suggested.

Key words: *Medicago arabica, M. hybrida, M. sativa, Fusarium oxysporum* f. sp. *tulipae*, saponins, mycelium growth

INTRODUCTION

The distribution of saponins in plant kingdom and their biological properties have been recently reviewed by S p a r g et al. (2004). Some saponins have antifungal, antibacterial, antiparasitic, molluscicidal, anti-inflammatory, cytotoxic and antitumor activities. Species of the genus *Medicago* are a rich source of different saponins with antibacterial and antifungal activities (A v a t o et al. 2006, H o u g h t o n et al. 2006). S a n i e w s k a et al. (2001) showed differential antifungal activity of alfalfa (*Medicago sativa*) total saponins originated from roots and aerial parts for *Alternaria zinniae*, *Botrytis cinerea*, *Botrytis tulipae*, *Phoma narcissi*, *Phoma poolensis* and *Rhizoctonia solani*. Next, it was found that out of ten tested saponins, isolated from the roots and leaves of *Medicago sativa*, the following four saponins greatly inhibited linear mycelium growth of *Botrytis tulipae* and *Phoma narcissi*: 3-*O*-*β*-D-glucopyranoside medicagenate, 3-*O*-[*β*-D-glucopyranosyl]-28-*O*-[*β*-D-xylopyranosyl (1→4)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside] medicagenate, 3-*O*-*β*-D-glucuronopyranosyl-28-*O*-[*β*-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside] medicagenate and medicagenic acid (Saniewska et al. 2003). Total saponins originated from shoots of *Medicago arabica* showed antifungal activity *in vitro* against the following 11 tested taxons of pathogenic fungi: *Alternaria alternata*, *Botrytis cinerea*, *Botrytis tulipae*, *Pestalotia* spp, *Phoma narcissi*, *Phoma poolensis*, *Pythium ultimum*, *Rhizoctonia solani*, *Fusarium oxysporum* f. sp. *callistephi*, *Fusarium oxysporum* f. sp. *tulipae*, *Fusarium oxysporum* f. sp. *narcissi* (S a n i e w s k a et al. 2005). The total saponins of *Medicago arabica* used preventively to treat tulip bulbs inhibited the development of *Fusarium oxysporum* f. sp. *tulipae* (S a n i e w s k a et al. 2005). Total saponins originated from the roots of *Medicago hybrida* substantially inhibited mycelium growth of *Botrytis cinerea*, *Botrytis tulipae*, *Fusarium oxysporum* f. sp. *callistephi*, *Fusarium oxysporum* f. sp. *narcissi*, *Phoma narcissi* (S a n i e w s k a et al. 2006). Eight individual major saponin glycosides isolated from *M. hybrida* roots were tested for their effect on mycelium growth of *Botrytis tulipae* and it was documented that hederagenin 3-*O*-*β*-D-glucopyranoside and medicagenic acid 3-*O*-*β*-D-glucopyranoside greatly inhibited the growth of the pathogen (S a n i e w s k a et al. 2006).

In the present study, the effect of total and individual saponins from *Medicago arabica*, *M. hybrida*, and *M. sativa* on growth and development of *Fusarium oxysporum* f. sp. *tulipae* is evaluated *in vitro* and *in vivo*.

MATERIALS AND METHODS

The study was conducted in 2005-2006 at the Research Institute of Pomology and Floriculture in Skierniewice. Four isolates of *Fusarium oxysporum* f. sp. *tulipae*, strongly pathogenic to tulip bulbs, were used.

Isolation of total saponins and saponin glucosides

Total saponins and their individual glucosides were obtained from the aerial parts of *Medicago arabica* L. (B i a ł y et al. 2004), and from the roots *M*. of *hybrida* (Pourret) Trautv. (B i a ł y et al. 2006) and *M. sativa* L. (B i a ł y et al. 1999), according to the procedures described in the papers. Shortly afterwards, the ground plant material was defatted with chloroform, and then extracted with methanol under reflux. After removal of alcohol, the residue was dissolved in a small volume of water and the solution was placed on a LiChroprep RP-18 column. The column was washed with water and with diluted methanol until a colourless solution was obtained. Total saponins were eluted with methanol and dried. Then the obtained total saponins were fractionated on a silica gel column by eluting with n-butanol saturated with water, and individual saponin glycosides were separated from the fractions by means of reversed-phase chromatography on LiChroprep RP-18 columns eluting with aqueous methanol solutions. Saponin structures were established on the basis of hydrolysis and spectral evidence, including IR, optical rotations, NMR and FAB-MS analyses.

The total saponins and the following saponin glycosides isolated from *M. arabica*, *M. hybrida* and *M. sativa* were studied from the point of view of their effect on growth and development of *Fusarium oxysporum* f. sp. *tulipae*:

1. Hederagenin (*M. arabica*)
2. 3-*O*-(*α*-L-arabinopyranosyl), 28-*O*-(*β*-D-glucopyranosyl) hederagenin (*M. arabica*)
3. 3-*O*-[*β*-D-glucopyranosyl (1→2)-*α*-L-arabinopyranosyl], 28-*O*- *β*-D-glucopyranosyl hederagenin (*M. arabica*)
4. 3-*O*-[*β*-D-glucopyranosyl (1→2)*α*-L-arabinopyranosyl] hederagenin (*M. arabica*)
5. Hederagenin 3-*O*-*β*-D-glucopyranoside (*M. hybrida*)
6. Hederagenin 3-*O*-[*α*-L-rhamnopyranosyl(1→2)-*β*-D-glucopyranosyl(1→2)-*β*- D-glucopyranosyl]-28-*O*-*β*-D-glucopyranoside (*M. hybrida*)
7. Hederagenin 3-*O*-*β*-D-glucuronopyranosyl-28-*O*-*β*-D-glucopyranoside (*M. hybrida*)
8. Medicagenic acid (*M. sativa*)
9. Medicagenic acid 3-*O*- *β*-D-glucopyranoside (*M. hybrida*, *M. sativa*)
10. Medicagenic acid 3-*O*-*β*-D-glucopyranosyl-28-*O*-*β*-D-glucopyranoside (*M. hybrida*)
11. Medicagenic acid 3-*O*-*β*-D-glucuronopyranosyl-28-*O*-*β*-D- glucopyranoside (*M. hybrida*)
12. 3-*O*-[*β*-D-glucopyranosyl]-28-*O*-[*β*-D -xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-*α*-L-arabinopyranoside] medicagenate (*M. sativa*)
13. 3-*O*- *β*-D-glucuronopyranosyl-28-*O*-[*β*-D -xylopyranosyl(1→4)-α -L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside]medicagenate (*M. sativa*)
14. 2*β*,3*β*-dihydroxyolean-12ene-23-al-28-oic acid 3-*O*-*β*-D-glucuronopyranosyl- 28-*O*-*β*-D-glucopyranoside (*M. hybrida*)
15. Oleanolic acid 3-*O*-[*β*-D-glucuronopyranosyl(1→2)-*β*-D-galactopyranosyl]- 28-*O*-*β*-D-glucopyranoside (*M. hybrida*)

***In vitro* growth of *Fusarium oxysporum* f. sp. *tulipae* in the presence of saponins.** Four isolates of *Fusarium oxysporum* f. sp. *tulipae* (*F.ox.t*. 17, *F.ox.t*. 36, *F.ox.t*. 188 and *F*.ox.*t*. 218) were used for the investigations. The total amount of saponins, isolated from *Medicago hybrida* and *M. sativa* at final concentrations of 0.01%; 0.05%, 0.1%, was previously dissolved in 5 cm^3 sterile distilled water and added to potato-dextrose-agar (PDA-Merck) after sterilization at about 50°C. Single saponins isolated from of *Medicago arabica, M. hybrida and M. sativa* at final concentrations of 8 – 140 $\mu g \times cm^{-3}$ were dissolved in 3 cm^3 of 75% methanol and added to 100 cm^3 of PDA after sterilization. 5-mm diameter plugs, taken from 7-day-old culture of the tested isolates, were placed in the middle of 90 mm Petri dishes containing PDA medium supplemented with the tested compounds and the control without saponins. The plates were incubated at 25ºC in darkness. The diameter of the colonies was measured after 4, 6 and 8 days of incubation, depending on the fungus tested. Five dishes were used for each treatment and the experiment was repeated twice. To analyse the differences between mean values, Duncan's test was used, with a significance level of $\alpha = 0.05$.

The influence of saponins on the colony forming units (cfu) of *Fusarium oxysporum* f. sp. *tulipae*. Peat-sand (2:1) was artificially infested with *F. oxysporum* f. sp. *tulipae* (*F. ox.t*. 218) using the procedures of T r a m i e r et al. (1983) and O r l i k o w s k i and S a n i e w s k a (1988). After two-week incubation of inoculated substrate at 25ºC in darkness, twenty pots (100 cm^3) were filled with 60 g of inoculated substratum samples (4 x 5 reps). Then, water suspension of total saponins from the used species of *Medicago* at a concentration 5.0% was added to the inoculated substrate (three times every 7 days). After 7, 14 and 21 days of incubation of the substratum inoculated and treated with saponins, the population of the pathogen was determined using K o m a d a (1975) medium. The

inoculated substratum watered only without saponins served as the control. From each sample, 10 g of soil was taken, diluted with 90 cm^3 of sterile distilled water and mixed in a magnetic blender for 30 minutes. One millilitre of the suspension was mixed with 50 cm^3 of K o m a d a (1975) medium at a temperature of 40 – 45ºC and poured into 90 mm Petri dishes. After a 3-day-long incubation of the plates at 25ºC in the dark and 2-day-long incubation in daylight, the number of *F. oxysporum* f. sp. *tulipae* colonies was counted as mean values from ten Petri dishes (ø 90 mm), and population densities were converted to the number of propagules per gram of air dry soil. The experiment was repeated twice. Duncan's multiple range t-test at 5% level of significance was used for mean separation.

The effect of total saponins originated from *Medicago sativa* and *M. hybrida* on the development of fusariosis on tulip bulbs. 'Apeldoorn' tulip bulbs at the flower bud stage, uncooled, after removal of the first outer scale, were surface disinfected in 50% ethanol for 5 min., and then rinsed 3 times in sterile distilled water. Then, the bulbs were cut at the basal plate with a cork borer to a depth 1-2 mm and at a diameter of 3 mm. After cutting the scale, the bulbs were soaked for 60 min. in saponin solutions at concentrations of 0.1% and 0.5%. Thereafter, 20 µl of spore suspension of *F.ox.t.* 218 with a density of 1.4 x 10^6 spores×cm^3 of the inoculum was put in the place of the bulb scale damage. The bulbs soaked for 60 min. in distilled water and, inoculated with the suspension of the pathogen spores, were the control. The inoculated bulbs were placed in trays on damp absorbent paper lined with aluminium foil. The trays with the bulbs were put into film bags in order to increase humidity. After 10 days of incubation of the bulbs at a temperature of 25ºC, the length of infection spots was measured.

The comparative fungicide was Sportak Alpha 380 EC (300 g prochloraz + 80 g carbendazim×dm^3). In each combination, 10 bulbs were tested. The experiments were carried out in two series on the bulbs obtained in the study years.

RESULTS AND DISCUSSION

***In vitro* growth of *Fusarium oxysporum* f. sp. *tulipae* in the presence of saponins**

The total amount of saponins originated from the roots of *M. hybrida* and of alfalfa (*M. sativa*) at concentrations of 0.01%, 0.05% and 0.1% showed strong fungitoxic effect against *F. oxysporum* f. sp. *tulipae* (*F.ox.t.*). However, there were variable effects on the growth of all the studied isolates. The growth inhibition of *F.ox.t.* isolates was generally proportional to the concentration of saponins. The linear growth of the mycelium of the tested pathogen isolates, treated with a 0.1% solution of saponins isolated from *M. hybrida* and *M. sativa* was inhibited in 73% – 80% and 46% – 69%, respectively, in relation to the control culture (Figs 1, 2). In earlier studies (S a n i e w s k a et al. 2005), it was documented that total saponins from *M. arabica* at a concentration of 0.1% limited the mycelium growth of *F.ox.t.* 17 *in vitro* at 63%.

The individual saponin glucosides exerted different effects on the mycelium growth of the isolates of *Fusarium oxysporum* f. sp. *tulipae*. Four of them: **(4)** 3-*O*-[*β*-D-glucopyranosyl (1→2)*α*-L-arabinopyranosyl] hederagenin, **(5)** hederagenin 3-*O*-*β*-D-glucopyranoside, (**8)** medicagenic acid, (**9)** medicagenic acid 3-*O*-*β*-D-glucopyranoside had the strongest inhibitory influence on the mycelium growth of *F.ox.t.* on PDA medium (Figs 3,4,5,6). The growth inhibition of the pathogen isolates was generally proportional to the concentration of saponin glucosides (Figs 3, 4, 5 and 6). **(1)** Hederagenin, (**2)** 3-*O*-(*α*-*L*-arabinopyranosyl), 28-*O*-(*β*-D-glucopyranosyl) hederagenin, (**3)** 3-*O*-[*β*-D-glucopyranosyl (1→2)-*α*-L-arabinopyranosyl], 28-*O*-*β*-D-glucopyranosyl hederagenin, (**6)** hederagenin 3-*O*-[*α*-L-rhamnopyranosyl(1→2)-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranosyl]-28-*O*-*β*-D-glucopyranoside, (**7)** hederagenin 3-*O*-*β*-D-glucuronopyranosyl-28-*O*-*β*-D-glucopyranoside, **(10)** medicagenic acid 3-*O*-*β*-D-glucopyranosyl-28-*O*-*β*-D-glucopyranoside, **(11)** medicagenic acid 3-*O*-*β*-D-glucuronopyranosyl-28-*O*-*β*-D-glucopyranoside, **(12)** 3-*O*-[*β*-D-glucopyranosyl]-28-*O*-[*β*-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside] medicagenate, **(13)** 3-*O*-*β*-D-glucuronopyranosyl-28-*O*-[*β*-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside]medicagenate, (**15)** oleanolic acid 3-*O*-[*β*-D-glucuronopyranosyl(1→2)-*β*-D-galactopyranosyl]- 28-*O*-*β*-D-glucopyranoside showed slight fungitoxic activity against mycelium growth of the pathogen (Tab. 1). (**14)** 2*β*,3*β*-dihydroxyolean-12ene-23-al-28-oic acid 3-*O*-*β*-D-glucuronopyranosyl- 28-*O*-*β*-D-glucopyranoside did not limit but stimulated the growth of the tested pathogen (Table 1). (**6)** Hederagenin 3-*O*-[*α*-L-rhamnopyranosyl(1→2)-*β*-D-glucopyranosyl(1→2)-*β*- D-glucopyranosyl]-28-*O*-*β*-D-glucopyranoside, **(7)** hederagenin 3-*O*-*β*-D-glucuronopyranosyl-28-*O*-*β*-D-glucopyranoside, **(10)** medicagenic acid 3-*O*-*β*-D-glucopyranosyl-28-*O*-*β*-D-glucopyranoside, **(11)** medicagenic acid 3-*O*-*β*-D-glucuronopyranosyl-28-*O*-*β*-D- glucopyranoside, slightly stimulated mycelium growth of *F. oxysporum* f. sp. *tulipae* at low concentrations and showed slight fungitoxic activity at higher concentrations (Tab. 1). The individual saponins from *Medicago* which greatly inhibited mycelium growth of *F. oxysporum* f. sp. *tulipae* were also most active against other pathogens of ornamental plants (S a n i e w s k a et al. 2003, 2006).

Table 1
The effect of individual saponins indicated in Material and Methods as number **1, 2, 3, 6, 10, 11, 12, 13, 1**4 and **15** on the mycelium growth of *Fusarium oxysporum* f. sp. *tulipae* (F. *ox. t.* 17) after eight days of incubation (% of control).

Conc. μg×cm^{-3}	Saponin **1**	Saponin **2**	Saponin **3**	Saponin **6**	Saponin **7**	Saponin **10**	Saponin **11**	Saponin **12**	Saponin **13**	Saponin **14**	Saponin **15**
10	-13.6	-6.4	-0.8	+2.3	+19.5	-2.3	-1.2	-7.7	-2.0	+24.3	-13.5
25	-24.5	-17.5	-7.2	-6.2	+16.5	+0.5	+0.2	-8.3	-7.1	+18.8	-25.3
50	-26.6	-16.0	-11.6	-1.6	-20.3	-4.8	-6.0	-9.5	-10.5	+8.5	-30.4
140	-31.2	-29.6	-14.3	-7.0	-24.6	-5.6	-1.2	-25.5	-37.5	+2.7	-15.4

"+" – stimulatory effect , "-" – inhibitory effect

Table 2
Influence of saponin solutions on colony forming units (cfu) of F. *oxysporum* f. sp. *tulipae*.

Treatments	Number of cfu/gram of air dry soil after days of incubation		
	7 (1st – treatment)	14 (2nd – treatment)	21 (3rd – treatment)
Control	8539 d	5579 d	3684 d
M. arabica 5.0%	5278 c	1052 b	1388 b
M. hybrida 5.0%	1677 b	1777 c	2217 c
M. sativa 5.0%	0,0 a	179 a	279 a

Means in colums followed by the same letters are not significantly different at P=0.05 according to Duncan's test.

Table 3
Effect of preventively used saponins on the development of fusariosis on tulip bulbs cv. Apeldoorn.

Treatments	Necrosis diameter (mm)	Depth of necrosis (mm)	Presence (+) or absence (-) of gum
Control	18.9 c	6.4 c	(+)
Saponins from *M. hybrida*			
0.1%	3.2 b	1.0 b	(-)
0.5%	0.0 a	0.0 a	(-)
Saponins from *M. sativa*			
0.1%	3.2 b	1.0 b	(-)
0.5%	0.0 a	0.0 a	(-)
Prochloraz + carbendazim (Sportak Alpha 380 EC)			
0.4%	0.0 a	0.0 a	(-)

See Table 2

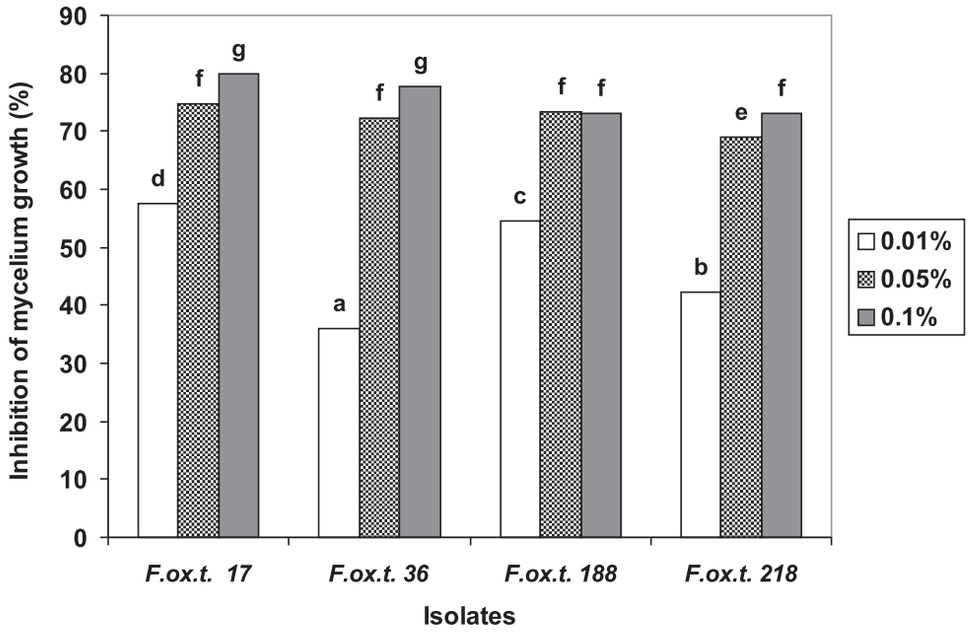

Fig. 1. Inhibitory effect of total saponins originated from roots of *Medicago hybrida* on *in vitro* mycelium growth of four isolates of Fusarium *oxysporum* f. sp. *tulipae* (*F.ox.t.* 17, *F.ox.t.* 36, *F. ox.t.* 188, *F.ox.t.* 218) after eight days of incubation; means followed by the same letters are not significantly different at P=0.05 according to Duncan's test.

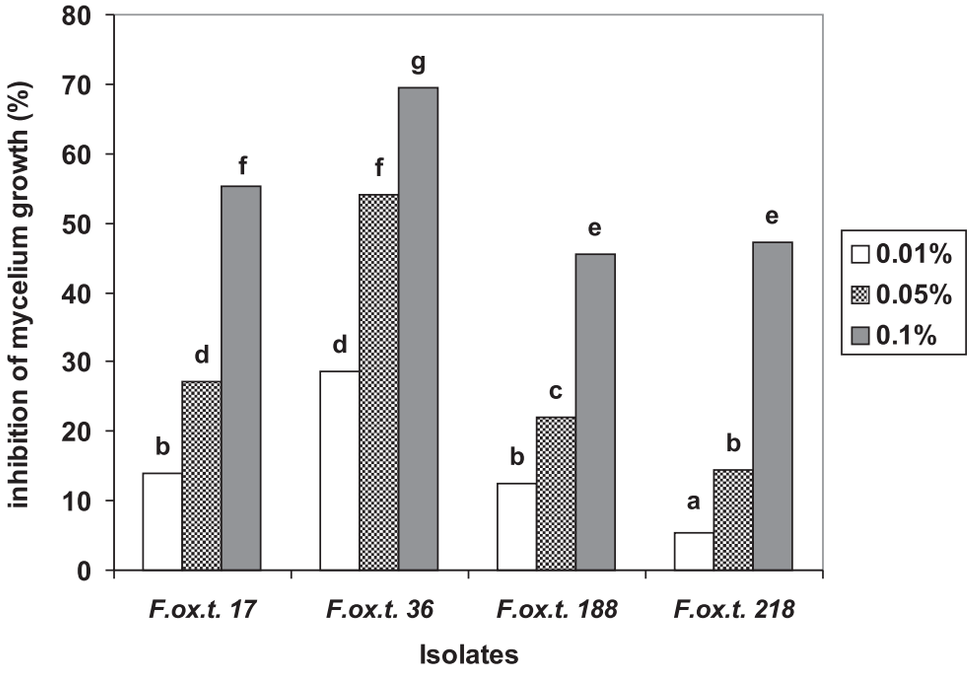

Fig. 2. Inhibitory effect of total saponins originated from roots of *Medicago sativa* on *in vitro* mycelium growth of four isolates of *Fusarium oxysporum* f. sp. *tulipae* (*F.ox.t.* 17, *F.ox.t.* 36, *F. ox.t.* 188, *F.ox.t.* 218) after eight days of incubation; means followed by the same letters for each isolate are not significantly different at P=0.05 according to Duncan's test.

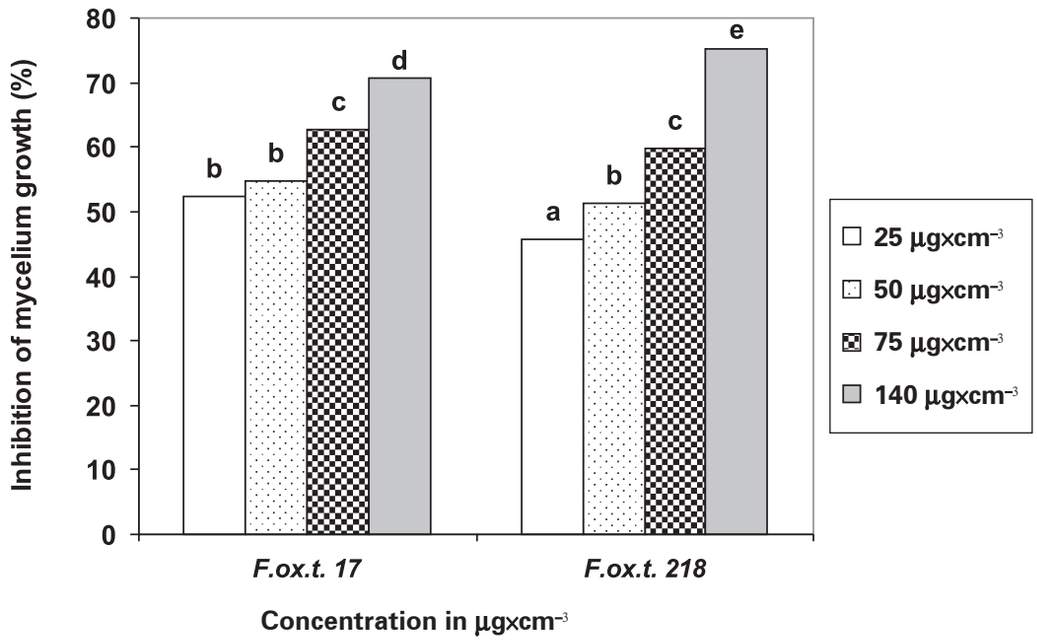

Fig. 3. Inhibitory effect of **(4)** 3-O-[*β*-D-glucopyranosyl(1→2)*α*-L-arabinopyranosyl] hederagenin at different concentrations on in vitro mycelium growth of two isolates of *Fusarium oxysporum* f. sp. tulipae (*F. ox. t.* 17 and *F.ox. t.* 218) after 8 days of incubation.

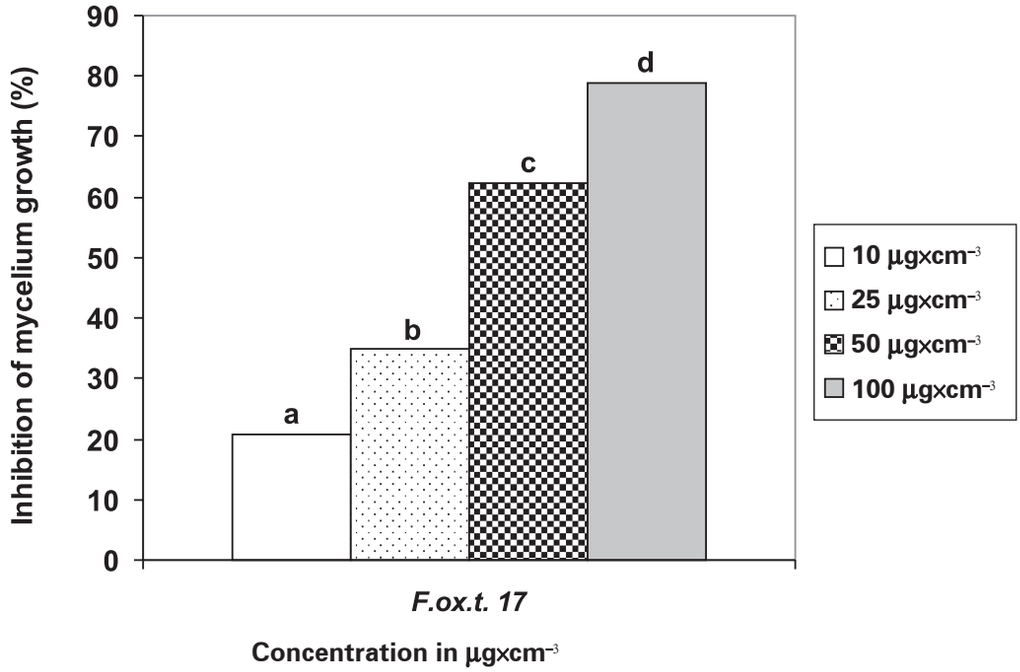

Fig. 4. Inhibitory effect of **(5)** hederagenin 3-*O*-*β*-D-glucopyranoside at different concentrations on *in vitro* mycelium growth of *Fusarium oxysporum* f. sp. *tulipae* (*F. ox. t.* 17) after 6 days of incubation.

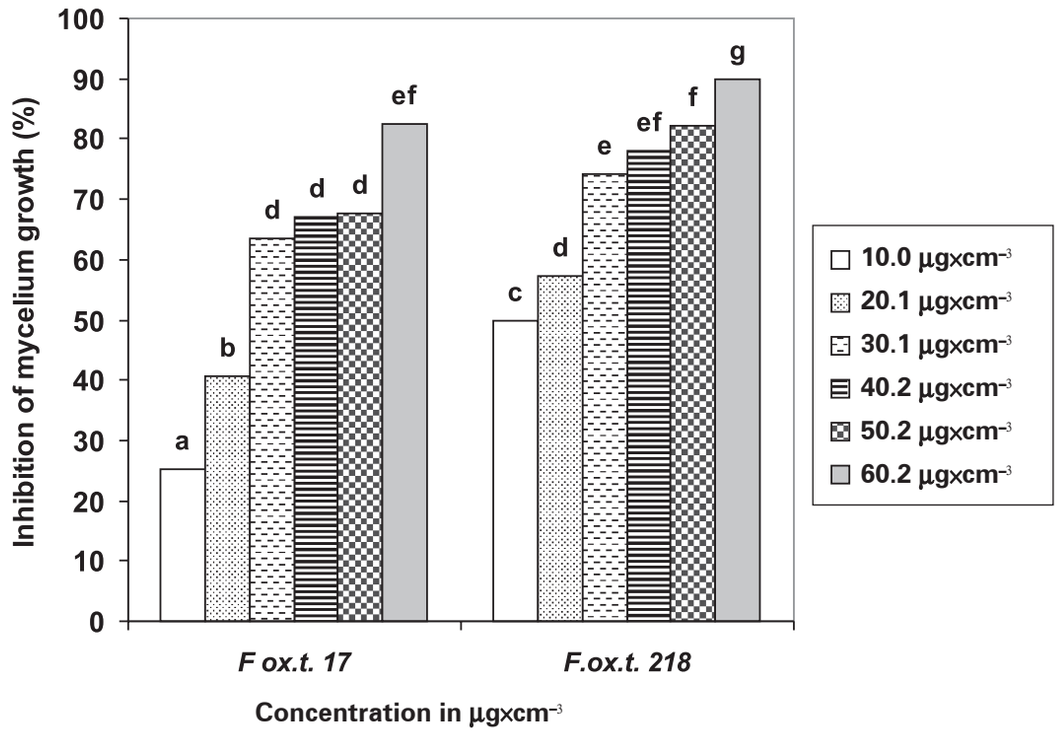

Fig. 5. Inhibitory effect of **(8)** medicagenic acid at different concentrations on in vitro mycelium growth of two isolates of *Fusarium oxysporum* f. sp. *tulipae* (*F. ox. t.* 17 and *F.ox.t.* 218) after 8 days of incubation.

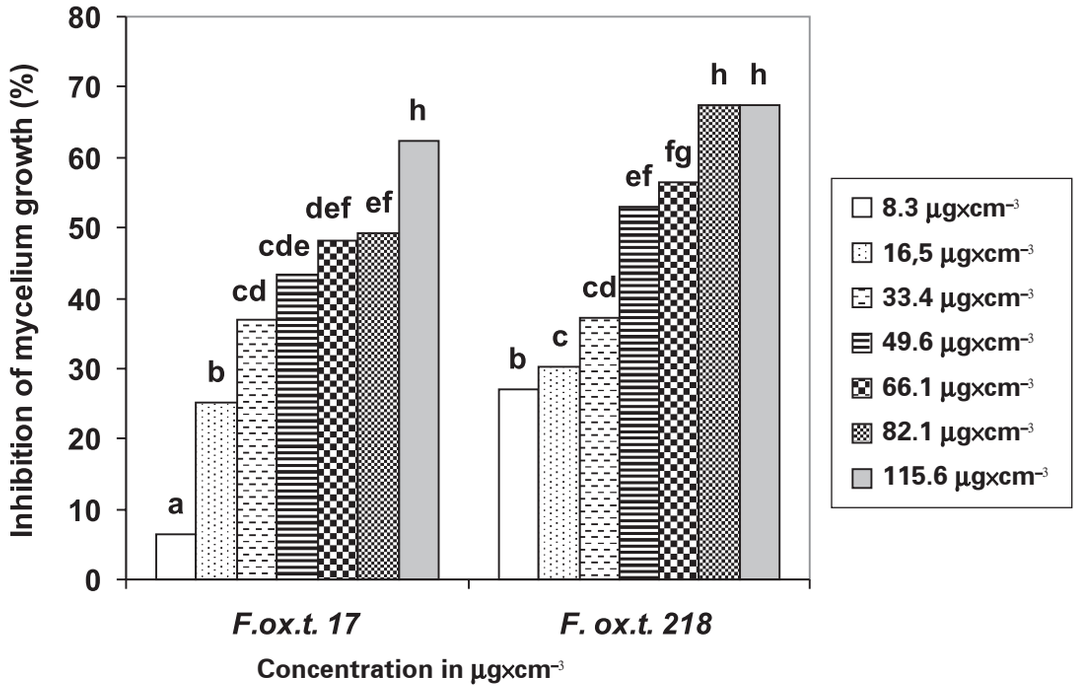

Fig. 6. Inhibitory effect of **(9)** medicagenic acid 3-O-β-D-glucopyranoside at different concentrations on *in vitro* mycelium growth of two isolates of *Fusarium oxysporum* f. sp. *tulipae* (*F. ox. t.* 17 and *F.ox.t.* 218) after 8 days of incubation.

The influence of saponins on colony forming units (cfu) of *Fusarium oxysporum* f. sp. *tulipae*

The total saponins from M. arabica, M. hybrida and M. sativa decreased the number of colony forming units (cfu) of Fusarium oxysporum f. sp. tulipae in artificially infested substrate. However, there were variable effects on cfu of the tested saponins of different species of Medicago. The total saponins used at a concentration of 5.0% reduced the number of cfu of the pathogen of M. sativa in 92.5%, M. arabica in 60% and M. hybrida in 40%, in comparison to the control substrate without saponins (Tab. 2). It is the first report that saponins of Medicago decrease the number of colony forming units of Fusarium oxysporum f. sp. tulipae in substrate.

The effect of total saponins from *Medicago sativa* and *M. hybrida* on the development of fusariosis on tulip bulbs

Total saponins from *M. hybrida* and *M. sativa* used preventively to treat tulip bulbs, inhibited the development of *F. oxysporum* f. sp. *tulipae* (Tab. 3). The size of necrosis on the surface of scale of bulbs was smaller, being totally inhibited at a concentration of 0.1%, like in the case of prochloraz + carbendazim (Sportak Alpha 380 EC). Disease development was not observed in internal tissues or with a minimum increase during the incubation of the bulbs in comparison with the control bulbs which were not treated. The control bulbs showed the development of the mycelium and gummosis in the area of inoculation. Such sings of development of the mycelium of the pathogen were not observed on the bulbs treated with the saponis solution. Similar results, concerning the limitation of disease symptoms caused by *Fusarium oxysporum* f. sp. *tulipae* on tulip bulbs, were documented earlier for saponins isolated from *Medicago arabica* (Saniewska et al. 2005). It is suggested that saponins of these three species of *Medicago* which showed considerable fungitoxic activity against *Fusarium oxysporum* f. sp. *tulipae* may be used as a natural fungicide for limiting the spread of the pathogen on tulip bulbs.

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Oddziaływanie saponin z *Medicago arabica*, *M. hybrida* i *M. sativa* na wzrost i rozwój *Fusarium oxysporum* f. sp. *tulipae*

Streszczenie

Wykazano, że suma saponin pozyskana z korzeni *Medicago hybrida* i *M. sativa* w stężeniach 0,01%, 0,05% i 0,1% oddziaływała fungitoksycznie na wzrost grzybni 4 izolatów *Fusarium oxysporum* f. sp. *tulipae* (*F.ox.t.*) w warunkach *in vitro* na pożywce PDA. Najwyższe stężenie saponin (0,1%) najsilniej ograniczało wzrost grzybni badanych izolatów *F.ox.t.*; inhibicja wynosiła w granicach 73-80% dla saponin izolowanych z *M. hybrida* i 46–65% dla saponin z *M. sativa*.

Spośród badanych 15 poszczególnych pojedyńczych saponin otrzymanych z *M. arabica*, *M. hybrida* i *M. sativa* 4 następujące saponiny wykazały silne ha-

mujące działanie na wzrost grzybni izolatów *F.ox.t*: 3-*O*-[*β*-D-glukopiranozylo(1→2)α-L-arabinopiranozyd] hederageniny (*M. arabica*) – w stężeniu 140 μgxcm^{-3} 70% hamowanie, 3-*O*-*β*-D-glukopiranozyd hederageniny (*M. hybrida*) – w stężeniu 100 μgxcm^{-3} 80% hamowanie, kwas medikagenowy (*M. sativa*) – w stężeniu 60 μgxcm^{-3} 85% hamowanie, 3-*O*-*β*-D-glukopiranozyd kwasu medikagenowego (*M. hybrida*, *M. sativa*) – w stężeniu 115 μgxcm^{-3} 65% hamowanie.

Suma saponin pozyskana z *M. hybrida* i *M. sativa* zastosowana profilaktycznie do zaprawiania cebul tulipana silnie ograniczała rozwój fuzariozy. W stężeniu 0,1% suma saponin z każdego badanego gatunku lucerny ograniczała rozwój choroby w około 80%, oceniany długością i głębokością plam infekcyjnych i hamowała tworzenie się gum. Zastosowane sumy saponin w wyższym stężeniu 0,5% silniej ograniczały rozwój patogena.

Badane sumy saponin, zastosowane w stężeniu 5%, do podlewania zakażonego przez *F.ox.t*. 218 podłoża, wykazały inhibicyjny, jednakże zróżnicowany wpływ na rozwój patogena w podłożu. Najsilniej ograniczała liczbę jednostek tworzących kolonie *F.ox.t* 218, suma saponin z *M. sativa* (około 92%), słabsze wyniszczające oddziaływanie na patogena wykazała *M. hybrida* (około 60%) a najsłabiej ograniczała rozwój patogena suma saponin z *M. arabica* (około 40%) – w porównaniu do liczebności jednostek tworzących kolonie *F.ox.t*. 218 w podłożu kontrolnym nie traktowanym.