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Differential antifungal activity of alfalfa (*Medicago sativa* L.) saponins originated from roots and aerial parts for some ornamental plant pathogens

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Summary

The total saponins isolated from aerial parts and roots of alfalfa (*Medicago sativa* L.) at the concentration of 0.01%, 0.05% and 0.1% showed differential influence on the mycelium growth of *Alternaria zinniae*, *Botrytis cinerea*, *Botrytis tulipae*, *Phoma narcissi*, *Phoma poolensis* and *Rhizoctonia solani*. A higher inhibitory effect on *in vitro* growth of mycelium of all tested pathogens indicated saponins from roots of alfalfa than from aerial parts.

Tested saponins from roots at the concentration of 0.1% totally inhibited linear growth of *Phoma narcissi*, and linear growth of *Alternaria zinniae* was limited about 67%, *Botrytis cinerea* about 74%, *Botrytis tulipae* about 68%, *Phoma poolensis* about 38%, and *Rhizoctonia solani* about 74% in comparison to the control.

The saponins of alfalfa from roots at the concentration of 0.1% and 0.2% applied as preinoculation sprays evidently inhibited the development of *Phoma narcissi* on *Hippeastrum* leaves. This dose of aerial saponins of alfalfa did not effect the development of necrotic spots on the leaves.

Key words: *Medicago sativa*, alfalfa saponins, fungal pathogens, mycelium growth, protective role

INTRODUCTION

Saponins (glycosylated triterpenoid or steroid molecules) are widely distributed in higher plants. Saponins occurring in alfalfa (*Medicago sativa* L.) are composed of a complicated mixture of triterpenoid glycosides (Price et al., 1987; Oleszek et al., 1992a; Oleszek, 1996; Biały et al., 1999).

According to the structure of aglycones, they can be divided into several groups: derivatives of medicagenic acid, oleanolic acid, zanhic acid, hederagenin, and soyasapogenols.

Many saponins have been shown to have potent antifungal activities and often occur in healthy plants at levels which are anticipated to be toxic to saponin-sensitive fungi. The fungistatic activity of saponins could be demonstrated both *in vivo* and *in vitro*.

Alfalfa saponins possess selective toxicity against *Trichoderma viride* (Jurzysta and Waller, 1996; Oleszek et al., 1990), several plant pathogenic fungi (Zehavi and Polacheck, 1996; Martyniuk et al., 1996, 1999; Zentmeyer and Thompson, 1967; Leatch et al., 1972; Pedersen et al., 1976; Levy et al., 1986), and medically important fungi (Zahavi and Polacheck, 1996; Polacheck et al., 1986; Śpiewak et al., 2000a,b).

Until now the response of the following plant pathogenic fungi to alfalfa saponins were tested *in vitro*: *Phytophthora cinnamomi* (Zentmeyer and Thompson, 1967), *Ascochyta imperfecta*, *Colletotrichum destructivum*, *Fusarium oxysporum* f. *medicaginis*, *Leptosphaerulina briosiana*, *Pythium irregulare*, *Pythium ultimum*, *Sclerotinia trifoliorum*, *Stemphylium botryosum*, *Phytophthora megasperma* (Leath et al., 1972), *Aspergillus niger*, *Fusarium oxysporum* f. sp. *lycopersici*, *Pythium aphanidermatum*, *Rhizoctonia solani*, *Sclerotium rolfsii*, *Rhizopus mucco* (Zehavi and Polacheck, 1996), *Cephalosporium gramineum*, *Gaeumannomyces graminis* v. *tritici* (Martyniuk et al., 1996, 1999).

The aim of the present work is determination *in vitro* of the fungitoxic activity of saponins originated from root and aerial parts of alfalfa to a few species of pathogenic fungi, which were not tested for saponins response.

MATERIAL AND METHODS

Chemicals

Total saponins have been isolated from aerial parts and roots of alfalfa (*Medicago sativa* L.) 'Radius', according to the known methods (Oleszek et al., 1992b; Biały et al., 1999) as follow: defatted plant material was extracted with boiling methanol. After removing of alcohol, the residue was dissolved in water. The solution was placed on LiChroprep RP-18 (25-40 mm) column preconditioned with water. The column was washed with water and 30% methanol successively. Total saponins were eluated with methanol and dried at 60°C.

***In vitro* growth of some pathogenic fungi in the presence of saponins from *Medicago sativa*.** *Alternaria zinniae* Pope, *Botrytis cinerea* Pers. [= *Botryotinia fuckieliana* (de Bary) Whetzel], *Botrytis tulipae* (Lib.) Lind, *Phoma narcissi* Aderh. [syn. *Stagonospora curtisii* (Berk.) Sacc.], *Phoma poolensis* Taub. (syn. *Phyllosticta antirrhini* Syd.) i *Rhizoctonia solani* Kühn. [= *Tanatephorus cucumeris* (Frank) Donk.] were used in all trials. Saponins of *Medicago sativa* from aerial (foliage) parts and from roots at final concentration 0.01%; 0.05% and 0.1% were dissolved in 5 cm^3 distilled and sterilized water and added to potato-dextrose-agar (PDA – Difco) after sterilization at temperature of about 50°C. Five mm diam. plugs taken from 7-day-old culture of tested fungi, were placed in the middle of 90 mm Petri dishes containing PDA medium supplemented with tested compounds. Control plates constituted the culture growing on PDA without any amendments.

The diameter of the fungi colony was measured within an 8 day-incubation at 25°C in darkness. Five dishes were used for each treatment and the experiment was repeated 2 times.

Influence of saponins in the control of *Phoma narcissi* on *Hippeastrum hybr.* hort.

Development of red spot on intact leaves of *Hippeastrum* 'Jan' in presence of saponins from root and aerial parts at a concentration 0.1% and 0.2% was evaluated. The compounds were applied on leaves preventively. Intact leaves were sprayed with saponins 1h before inoculation with *Phoma narcissi*. Tween 80 was added to the solution to improve wettability. Five mm diam. plugs of mycelium taken from 5-day-old culture of *Phoma narcissi* grown on PDA were put on the surface of leaves in three places on each one. Control leaves were sprayed with distilled water containing Tween 80 but no saponins, and inoculated with disks of mycelium of *P. narcissi*.

All plants with inoculated leaves were kept in a small plastic tunnel on greenhouse bench with high humidity inside. Five pots plants of *Hippeastrum* with four leaves each were used for each treatments and experiment were repeated three times.

The data were subjected to an analysis of variance and Duncan's multiple range test at 5% of significance was used for means separation.

RESULTS AND DISCUSSION

Total saponins isolated from aerial and roots of alfalfa (*Medicago sativa* L.) at the concentration of 0.01%, 0.05%, and 0.1% showed differential influence on the mycelium growth of *Alternaria zinniae*, *Botrytis cinerea*, *Botrytis tulipae*, *Phoma narcissi*, *Phoma poolensis* and *Rhizoctonia solani* (Fig. 1 and 2). All tested pathogens indicated saponins from roots of alfalfa had a higher inhibitory effect on *in vitro* growth of mycelium than those from aerial parts (Fig. 1 and 2). The saponins from roots, used at the lowest concentrations, evidently inhibited *in vitro* growth, with a marked effect at higher concentrations (Fig. 1). After 6 days of incubation at the concentration 0.1%, linear growth of *Phoma narcissi* was totally inhibited, and linear growth of *Alternaria zinniae* was limited about 67%, *Botrytis cinerea* about 74%, *Botrytis tulipae* about 68%, *Phoma poolensis* about 38%, *Rhizoctonia solani* about 73% in comparison to the control (Fig. 1 and 2).

The total saponins from aerial parts of alfalfa showed a less inhibitory effect on the growth of tested pathogens (Fig. 1 and 2). These saponins had a strong inhibitory effect for mycelium growth of *Phoma narcissi* and *Botrytis tulipae*. Applied at a concentration of 0.1% inhibited the growth of the pathogens in 64% and 48%, respectively. The linear growth of *Alternaria zinniae* was limited about 33%, *Botrytis cinerea* about 14%, and *Phoma poolensis* about 17%. This dose of aerial saponins did not effect the mycelium growth of *Rhizoctonia solani*. It is well known that the high fungitoxic activity of alfalfa saponins is due to glycosides of medicagenic acid and hederagenin, which occur in higher concentrations in alfalfa roots than in shoots (O l e s z e k et al., 1992b).

Medicagenic acid 3-(O-glucopyranoside) isolated from alfalfa roots demonstrates high activity against *Trichodema viride* as well as against some important plant pathogens: *Sclerotium rolfsii*, *Rhizopus nucco*, *Aspergillus niger*, *Phytophthora cinnamomi*, *Fusarium oxysporum* f. sp. *lycopersici* (L e v y et al., 1986). The same compound was toxic to ten medically important yeasts (*Candida* sp., *Torulopsis* sp., *Geotrichum candidum*) (P o l a c h e c k et al., 1986).

Generally a positive relationship has been found between fungistatic and haemolytic activities of root tissue saponin extract of *Medicago* (J u r z y s t a and B i a ł y, 1999).

F o c k e (1970) reported that fungi pathogenic to alfalfa showed a greater tolerance for saponins than did fungi not pathogenic to alfalfa and postulated that saponins may have a function in a non-specific type of disease resistance.

Saponins obtained from roots and aerial parts of alfalfa had the most inhibitory effect to *Phoma narcissi* among other tested pathogens in our study.

In studies by S z c z e p a n i k et al. (2001) no significant differences in activity against Colorado potato beetle larvae (*Leptinotarsa decemlineata*) have been found between root and shoot saponins of alfalfa. Similar results have been obtained by T a v a and O d o a r d i (1996) in their studies on the insecticidal activity of saponins from the roots and the leaves of alfalfa towards the European grape moth (*Lobesia botrana*). On the other hand, the results of studies carried out by N o z z o l i l l o et al. (1997) and A d e l et al. (2000) point to a higher activity of root saponins compared with shoot ones.

The principle of the fungitoxic activity of saponins is an interaction with fungal membrane sterols, and probably with proteins and phospholipids (G r u i z and B i a c s, 1989; B i a c s and G r u i z, 1984; G r u i z, 1996; A s s a et al., 1972, 1975). The attact of saponins on various membrane constituents caused changes in membrane permeability and lysis. Differential fungitoxic of alfalfa saponins to different species of pathogenic fungi is possibly dependent on membrane compositions of pathogens.

Total saponins, isolated from roots of alfalfa applied at a concentration of 0.1% and 0.2% as preinoculation sprays, had 4 days later inhibited the development of necrotic spots on *Hippeastrum* leaves (tab. 1). After an 8-day-incubation, the length of necrotic spots was at least 43% smaller than of controls. This dose of aerial saponins of alfalfa did not effect the development of *Phoma narcissi* on *Hippeastrum* leaves, and at higher concentrations (0.2%) aerial saponins gave a stimulatory effect to the development of *Phoma narcissi* on the leaves.

Table 1

The effect of total saponins isolated from aerial parts and roots of alfalfa (*Medicago sativa* L.) on the development of *Phoma narcissi* on *Hippeastrum* leaves: spraying 1h before inoculation

Saponins source and conc. in %	Length of necrotic spots (mm) after days of incubation	
	4	8
Control untreated	9.4 b	17.4 b
Root 0.1	4.1 a	10.2 a
Root 0.2	4.7 a	11.0 a
Aerial 0.1	8.9 b	16.8 b
Aerial 0.2	12.6 c	24.6 c

Explanation: Means in columns followed by the same letters are not significantly different at 5% level.

Thus, the root saponins of alfalfa, in a much smaller degree, inhibited the development of disease induced by *Phoma narcissi* on *Hippeastrum* than the mycelium growth of the pathogen *in vitro*. It is possible that glycosides of medicagenic acid and hederagenin are degradated to free aglycones by glycosyl hydrolases of *Hippeastrum* tissues which remove sugar molecules from glycosyl chain. Fungitoxic activities of aglycones are weaker to fungal growth than glycosides of the compounds. This kind of detoxification of saponin is known by some plant pathogenic fungi (Osburn et al., 1996). It is well known also that fungitoxic activity of saponins can be strongly modified by the structure of sugar chain (Osburn et al.,1996; Biały and Jurzysta, 2000).

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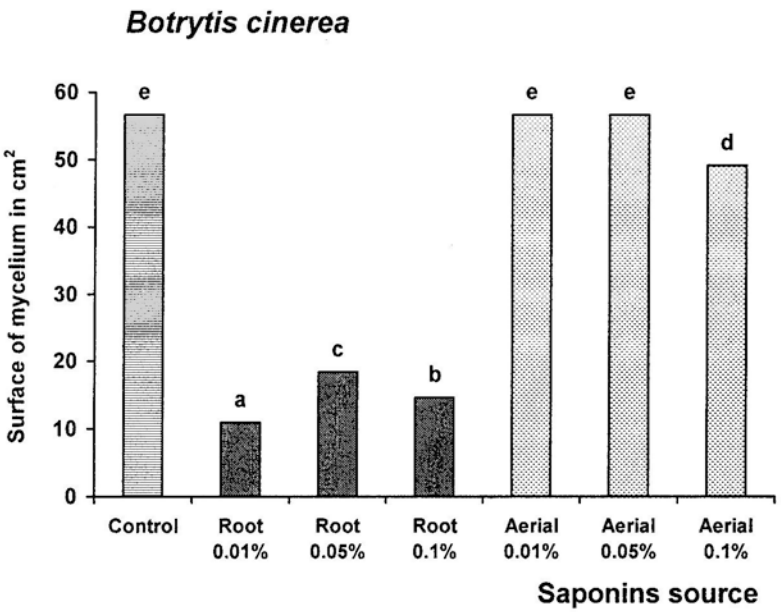
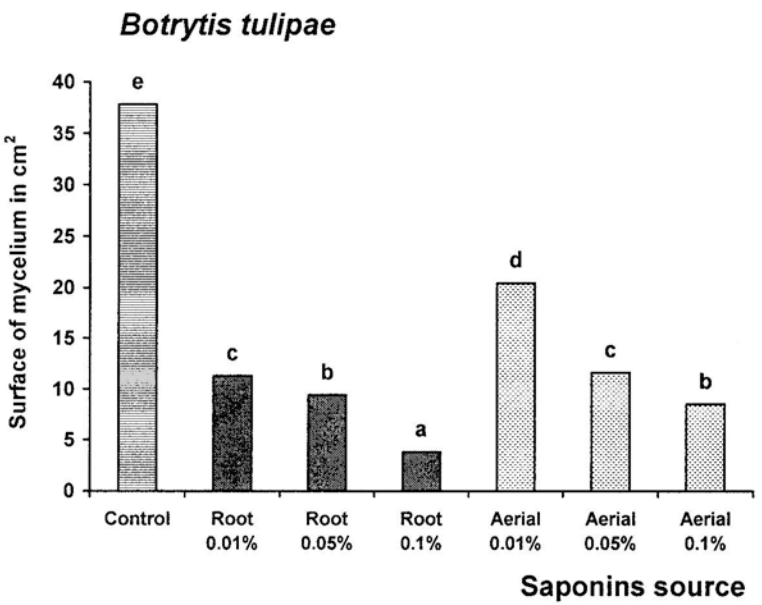
Zróżnicowana aktywność antygrzybowa saponin z korzeni i części nadziemnych lucerny siewnej (*Medicago sativa* L.) dla kilku patogenów roślin ozdobnych

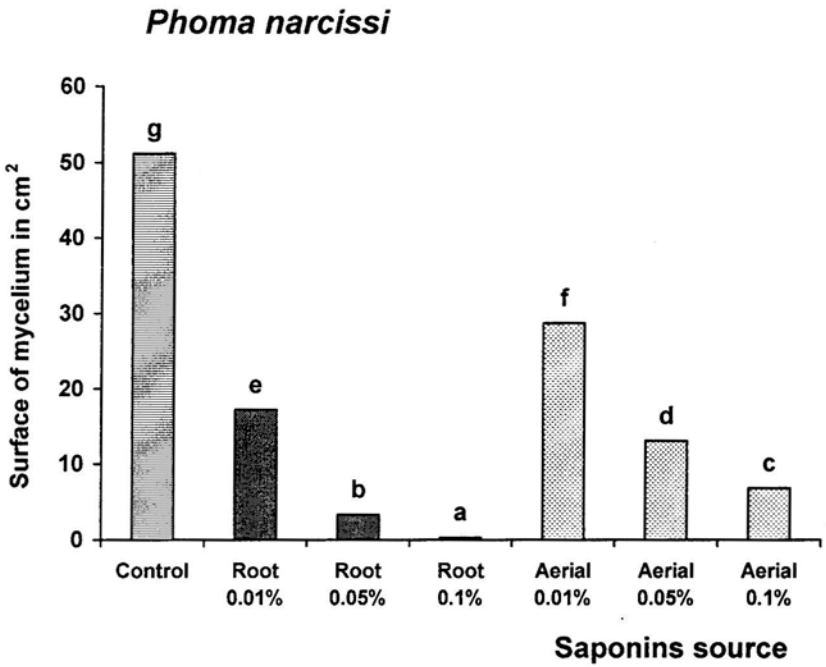
Streszczenie

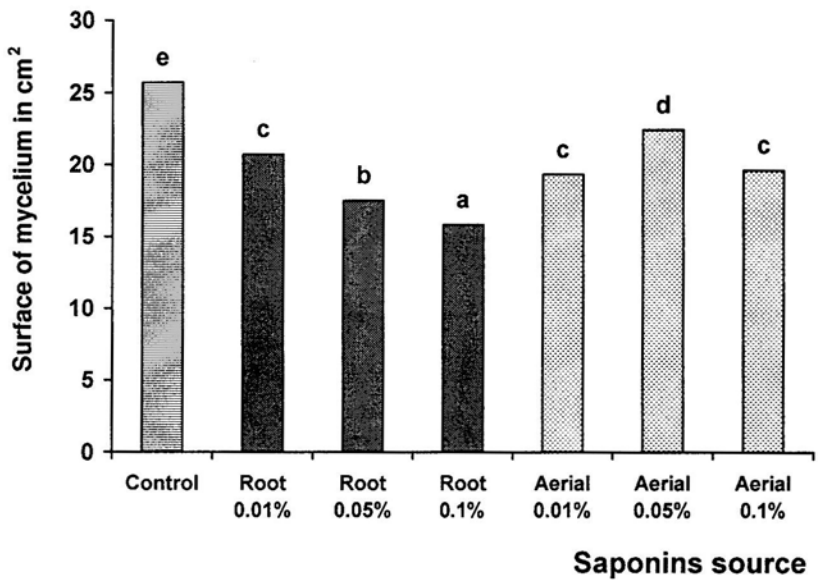
Badano fungitoksyczny wpływ sumy saponin, pozyskanych z części nadziemnych i korzeni lucerny siewnej (*Medicago sativa* L.), w stosunku do kilku gatunków grzybów chorobotwórczych dla roślin ozdobnych. Badane sumy saponin w stężeniu 0,01%; 0,05% i 0,1% wykazały zróżnicowany wpływ na wzrost *Alternaria zinniae*, *Botrytis cinerea*, *Botrytis tulipae*, *Phoma narcissi*, *Phoma poolensis* i *Rhizoctonia solani* w warunkach *in vitro* na pożywce ziemniaczano-glukozowej (PDA). Suma saponin pozyskana z korzeni, w najwyższym zastosowanym stężeniu (0,1%), całkowicie zahamowała wzrost grzybni *Phoma narcissi*, a wzrost grzybni pozostałych badanych gatunków był silnie ograniczony; *Alternaria zinniae* o 67%, *Botrytis cinerea* o 74%, *Botrytis tulipae* o 68%, *Phoma poolensis* o 38% i *Rhizoctonia solani* o 74% w porównaniu do kultur kontrolnych wzrastających na pożywce bez dodatku saponin. Suma saponin pozyskana z części nadziemnych wykazała słabszy fungitoksyczny wpływ na wzrost liniowy testowanych gatunków grzybów. Ta grupa saponin najsilniej hamowała wzrost *Phoma narcissi* i *Botrytis cinerea*. Wzrost liniowy grzybni tych gatunków był ograniczony, odpowiednio, o 64% i 48% w stosunku do kultury kontrolnej. Wzrost grzybni *Alternaria zinniae* był zahamowany o 33%, *Botrytis cinerea* o 14% i *Phoma poolensis* o 17%. Saponiny z części nadziemnych, w zastosowanych stężeniach, nie wpłynęły hamująco na wzrost grzybni *Rhizoctonia solani*. W warunkach *in vivo* suma saponin pozyskana z korzeni lucerny siewnej, zastosowana profilaktycznie do opryskiwania liści *Hippeastrum* w stężeniu 0,1% i 0,2% wpłynęła hamująco na rozwój *Phoma narcissi*. Zastosowane w tych samych stężeniach saponiny pozyskane z części nadziemnych nie wpłynęły ograniczająco na rozwój nekrotycznej plamistości na liściach *Hippeastrum*.

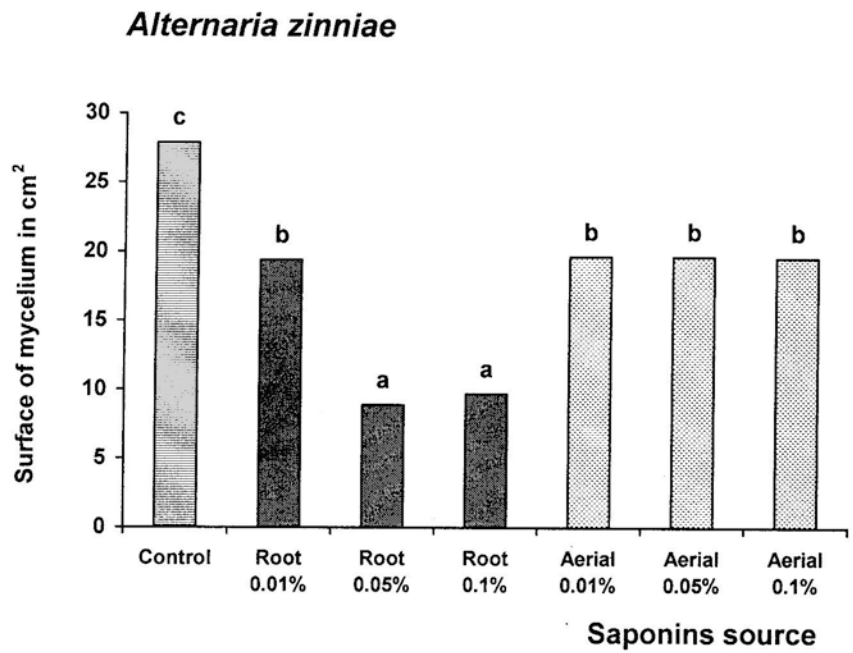
Fig. 1. Inhibitory effect of total saponins isolated from aerial parts and roots of alfalfa (*Medicago sativa*) on *in vitro* mycelium growth of *Alternaria zinniae*, *Botrytis cinerea*, *Botrytis tulipae*, *Phoma narcissi*, *Phoma poolensis* and *Rhizoctonia solani*.
Explanation: Means followed by the same letters are not significantly different at 5% level.






Phoma narcissi
Surface of mycelium in cm2
60
50
40
30
20
10
0
g
e
b
a
f
d
c
Control
Root 0.01%
Root 0.05%
Root 0.1%
Aerial 0.01%
Aerial 0.05%
Aerial 0.1%
Saponins source


Phoma poolensis
Surface of mycelium in cm2
30
25
20
15
10
5
0
e
c
b
a
c
d
c
Control
Root 0.01%
Root 0.05%
Root 0.1%
Aerial 0.01%
Aerial 0.05%
Aerial 0.1%
Saponins source


Alternaria zinniae
Surface of mycelium in cm2
30
25
20
15
10
5
0
c
b
a
a
b
b
b
Control
Root 0.01%
Root 0.05%
Root 0.1%
Aerial 0.01%
Aerial 0.05%
Aerial 0.1%
Saponins source

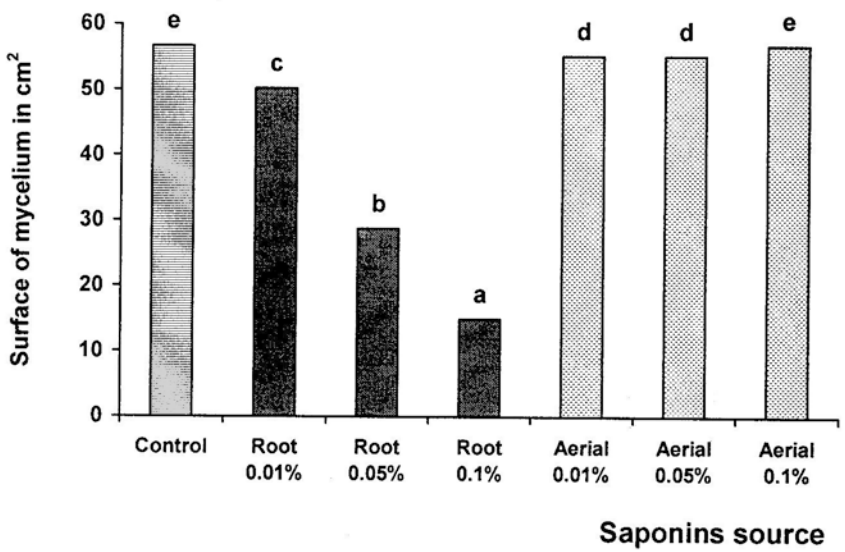
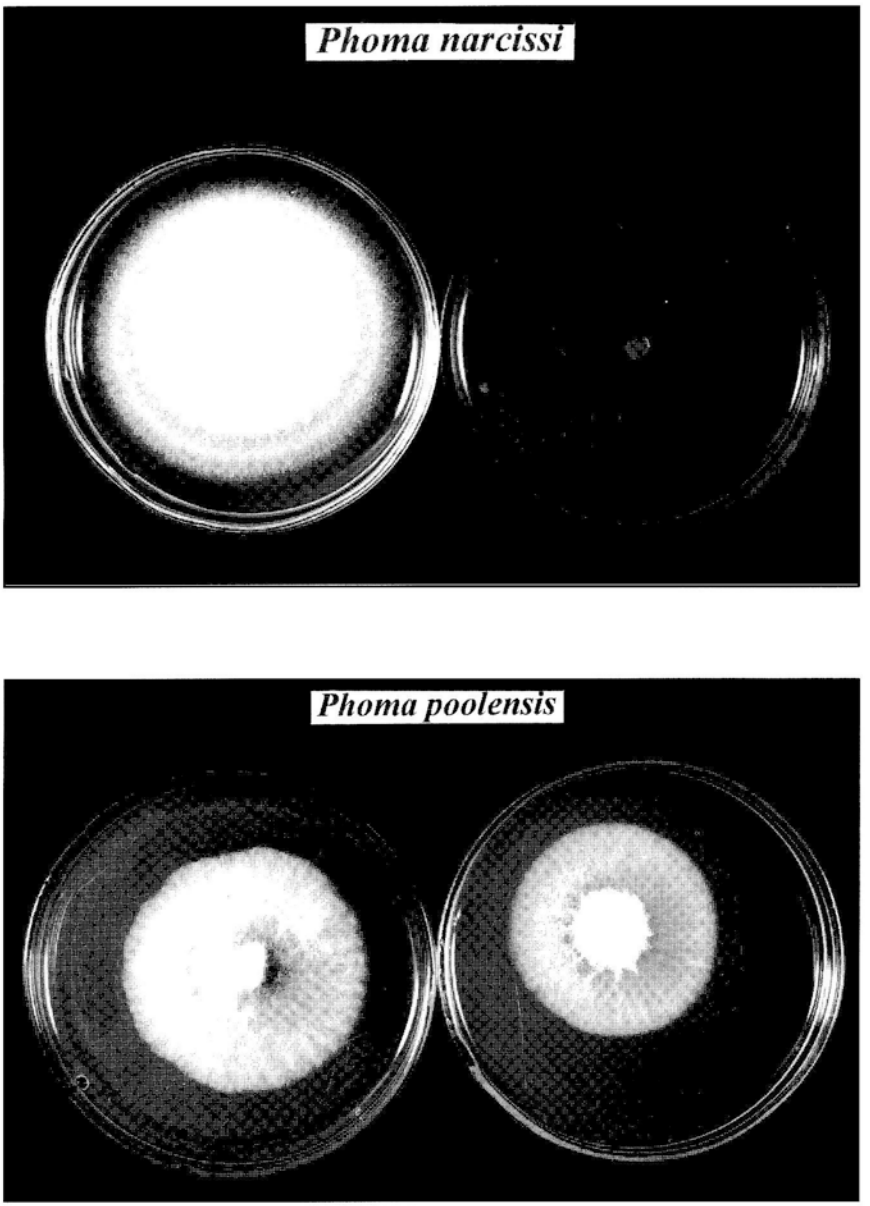
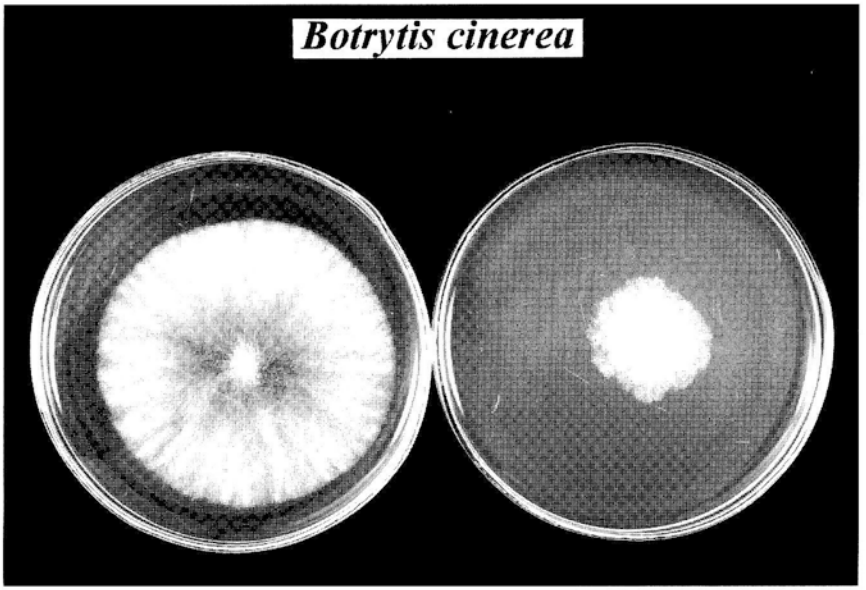
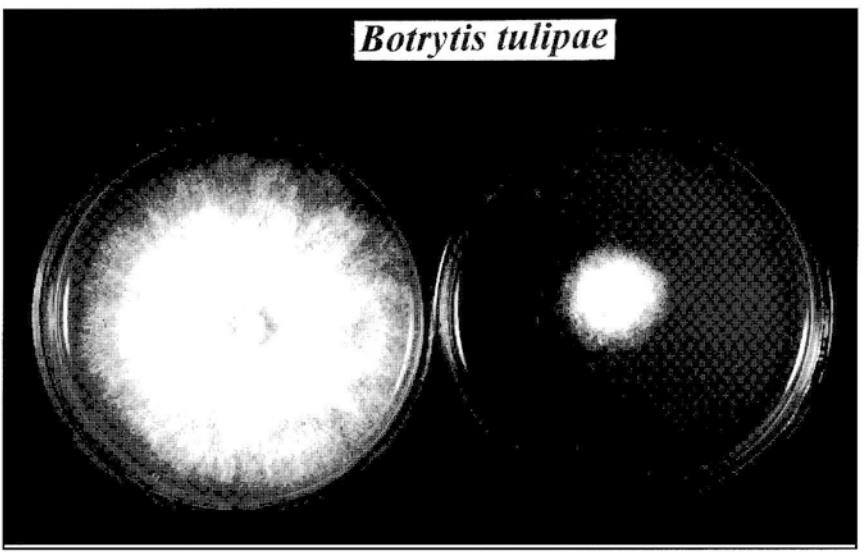
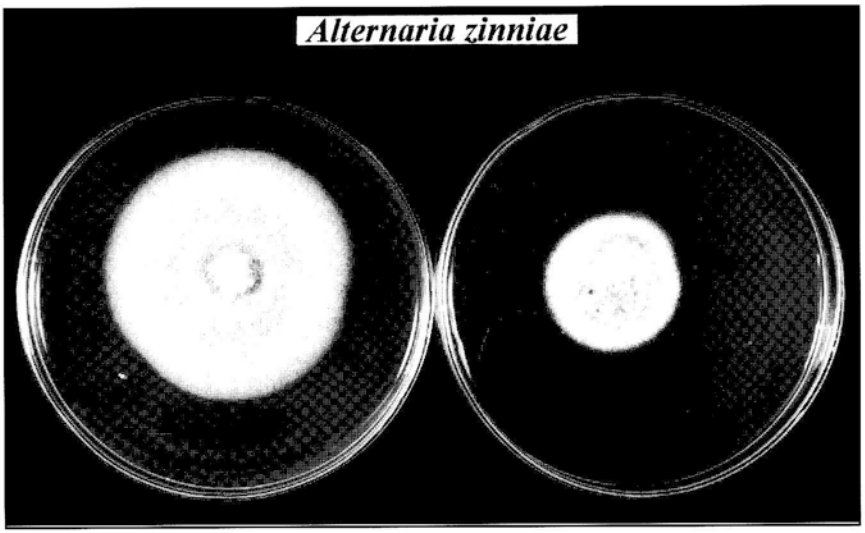

Rhizoctonia solani
Surface of mycelium in cm2
60
50
40
30
20
10
0
e
c
b
a
d
d
e
Control
Root 0.01%
Root 0.05%
Root 0.1%
Aerial 0.01%
Aerial 0.05%
Aerial 0.1%
Saponins source

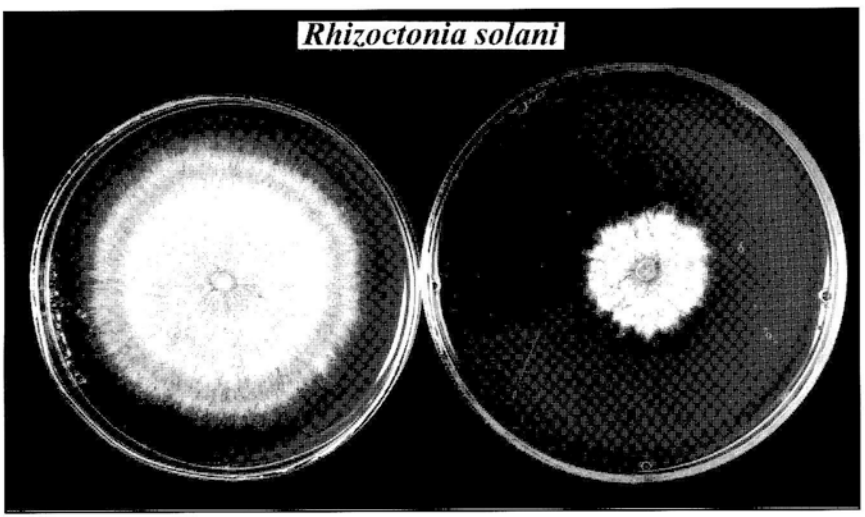
Fig. 2. Mycelium growth of *Alternaria zinniae*, *Botrytis cinerea*, *Botrytis tulipae*, *Phoma narcissi*, *Phoma poolensis* and *Rhizoctonia solani* on potato-dextrose-agar supplemented with total saponins from roots of alfalfa (*Medicago sativa*) on left – control; on right – saponin at conc. of 0.1%




Botrytis cinerea


Botrytis tulipae


Alternaria zinniae


Rhizoctonia solani