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The effect of chitosan on limitation of growth and development of some pathogenic fungi for ornamental plants

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Summary

The inhibitory effect of crab-shell chitosan, medium (200-800 cps) and high molecular weight (800-2000 cps) (purchased from Sigma-Aldrich Chemicals) toward *Alternaria alternata*, *Botrytis tulipae*, *Fusarium oxysporum* f. sp. *callistephi*, *Fusarium oxysporum* f. sp. *tulipae*, *Phoma narcissi* and *Phoma poolensis* was evaluated *in vitro* and *in vivo*. The chitosan evidently inhibited *in vitro* growth of all tested pathogens, with a marked effect at higher concentrations above 200 μg/cm³. Chitosan at a concentration of 1.25; 2.5 and 5.0 mg/cm³ didn't have inhibitory action in appearance of fungi growth on naturally contaminated *Callistephus chinensis* seeds. At the same concentrations, chitosan applied as bulb scales dressing of *Hymenocallis narcissiflora* bulbs, before inoculation or after inoculation with *Phoma narcissi*, inhibited the development of necrotic spots on scales. Chitosan used preventively or curatively at a concentrations of 1.25; 2.5 and 5.0 mg/cm³ indicated inhibitory effect on development of *Fusarium oxysporum* f. sp. *tulipae* on tulip bulbs. Chitosan at a concentration of 10 mg/cm³ applied preventively (first spray 12th June) was very effective in the control of *Puccinia antirrhini* on snapdragon in the field. The strongest inhibitory effect was observed on snapdragon treated 8 times at week intervals.

Key words: Chitosan, fungal pathogens, mycelium growth, protective role, *Hymenocallis narcissiflora*, *Antirrhinum majus*, *Tulipa gesneriana*

INTRODUCTION

Several models have been proposed to explain the biochemical basis of plant resistance against potential pathogens (E b e l and M i t h ö f e r, 1998). Elicitors are signaling compounds that stimulate any of such defence responses. Oligosaccharide elicitors were among the earliest to be characterized in greater details. Oligosaccharins are complex carbohydrates that can function in plants as molecular signals that regulate growth, development, and survival in the environment (R y a n and F a r m e r, 1991; A l d i n g t o n et al., 1991; D a r v i l l et al., 1992).

Four major classes of elicitors-active oligosaccharides have been identified: oligoglucan, oligochitin, oligochitosan of fungal origin and oligogalacturonide of plant origin (C ô t e and H a h n, 1994). Chitin, a linear polymer of (1,4)-linked N-acetyl-β-glucosamine, and chitosan, its de-N-acetylated chitin, are structural components of the cell wall of most higher fungi (E b e l and M i t h ö f e r, 1998; D a r v i l l et al., 1992). Oligosaccharide fragments of both of those polysaccharides elicit defence-related responses in various plants. Chitosan and chitosan-derived fragments can induce several defence responses in plant tissue, i.e. elicit the accumulation of phytoalexins in different plants, accumulation of defence-related proteinase inhibitors, induce the synthesis of defence-related β-(1→3)-glucan (callose), induced lignification of wall in suspension culture (D a r v i l l et al., 1992; C ô t e and H a h n, 1994; E b e l and M i t h ö f e r, 1998). Chitosan is known to be inhibitory to a number of pathogenic fungi (A l l a n and H a d w i g e r, 1979; S t ö s s e l and L e u b a, 1984).

The aim of the present work is to know the effect of chitosan in mycelium radial growth of some pathogens *in vitro* and its protective role in the development of disease symptoms induced by a few fungal pathogens.

MATERIAL AND METHODS

Crab-shell chitosan, medium molecular weight (200-800 cps) and high molecular weight (800-2000 cps) [poly(D-glucosamine)], were purchased from Sigma–Aldrich Chemicals. The chitosan solution in all trials was dispersed in distilled and sterilized water to which 2% of L-glutamic acid was added and heated to dissolve the chitosan and the pH was adjusted to value of control medium with 2N NaOH.

In experiment *in vitro* concerning the mycelium growth of all tested pathogens was used chitosan of medium and high molecular weight. For all others trials was used chitosan of high molecular weight only.

In vitro growth of six species of fungi in the presence of chitosan. The antifungal activity of chitosan against pathogens listed in Tab. 1 was evaluated *in vitro* on potato-dextrose-agar (PDA) medium. The solution of chitosan at final concentration 50, 100, 200, 400, 600, 800 and 1000 μg/cm^3 medium was added to autoclaved PDA. Five mm diam. plugs taken from 5-day-old culture of tested pathogens were placed in the middle of 90 mm Petri dishes containing PDA medium supplemented with the tested compounds. Control plates constituted the fungi culture growing on PDA without any amendments.

The diameter of the fungal colony was measured within 10 day-incubation at 23 ±2°C in the dark. Five dishes were used for each treatment and the experiment was repeated 2 or 3 times.

Table 1

Pathogens used for testing the effect of chitosan on the mycelium growth

Pathogen	Source of isolation
Alternaria alternata (Fr.) Kreisler	seeds of *Callistephus chinensis* (L.) Nees
Botrytis tulipae (Lib.) Lind	base of flower stalk of *Tulipa gesneriana* L.
Fusarium oxysporum Schl. f. sp. *callistephi* (Beach) Snyd. et Hans	roots of *Callistephus chinensis* (L.) Nees
Fusarium oxysporum Schl. f. sp. *tulipae*	bulbs of *Tulipa gesneriana* L.
Phoma narcissi (Aderh.)	bulbs of *Hippeastrum* x *hybr.* hort.
Phoma poolensis Taub.	leaves of *Antirrhinum majus* L.

Influence of chitosan on development of fungi contaminated *Callistephus chinensis* seeds. The seeds of 'Ewa' cv. were soaked 1h in sterilized water, dryed on filter paper and soaked 30 min. in chitosan in dose 1.25; 2.5 and 5.0 mg/cm^3 of solution. Control seeds were soaked only in sterilized and autoclaved water. Such treated seeds were placed on PDA medium in Petri dishes covered with empty plates. The seeds were incubated at 25°C in the dark. One hundred seeds (10 seeds/plate) were used for each treatment and experiment was repeated three times. Number of contaminated seeds with fungi were calculated after 3, 6 and 9-day incubation. The microscopic observation of isolated fungi was done also.

Influence of chitosan in the control of *Phoma narcissi* on *Hymenocallis narcissiflora*. Development of red spot caused by *Phoma narcissi* on excised bulb scales of *Hymenocallis narcissiflora* in presence of chitosan at concentration of 1.25; 2.5 and 5 mg/cm^3 was evaluated. The chitosan prepared as described above was applied on scales dressing preventively and curatively. The excised bulb scales were soaked 15 and 30 min. in solution of chitosan 2h before inoculation with pathogen or 20h after inoculation with *Phoma narcissi*. Five mm diam. plugs of mycelium taken from 7-day-old culture of *P. narcissi* grown PDA were put on scales surface. After inoculation, the excised scales were kept in plastic boxes covered with polyethylene. Control bulb scales were inoculated with disks of mycelium of *P. narcissi* only. Forty bulb scales were used for each treatment and experiment were repeated twice. The size of spots developed on inoculated scales of *Hymenocallis* were measured during 7-day incubation.

Influence of chitosan, applied preventively and curatively in the control of *Fusarium oxysporum* f. sp. *tulipae*. The experiments were conducted on tulip bulbs 'Apeldoorn'. The each of tulip bulb was damaged by corkborer (5mm diam. and 1 mm depth) near the basal plate. The damaged places of the bulbs were inoculated with pathogen 2h after soaking (preventively) or 10, 24 and 48h before soaking them

during 30 min. (curatively) in chitosan at three concentrations 1.25; 2.5 and 5 mg/cm^3 or only in distilled water (control). Each bulb was inoculated with 10 µl of fungal inoculum at suspension 8.1 x 10^5 spores/cm^3. The control bulbs were inoculated 2h after damaged and their soaking in water (in the case of preventively experiment) and directly after damage in curatively experiment. The inoculated bulbs were placed on plastic net in a tray lined wet filter paper and incubated at 23-25°C in the laboratory room. During 18 days of incubation a size of necrosis on inoculated bulbs was measured. Twenty bulbs were used for each treatment and the experiment was repeated three times.

Influence of chitosan, applied preventively in the control of *Puccinia antirrhini* on field grown naturally infested snapdragon. The experiment were conducted on *Antirrhinum majus* 'Lawina'. Two-months-old seedlings were planted in the field in the first days of May 2000. Chitosan at concentration 10 mg/cm^3 and 20 mg/cm^3 was used for snapdragon leaves spraying. Tween 80 was added to the solution to improve wettability. A distilled water control contained Tween 80 but no chitosan. First preventive sprays with chitosan were done 12th June and following continued: a) one spray, b) two sprays at 14 days intervals, c) 8 sprays at weekly intervals, d) 4 sprays at 14 days intervals. One week after the final treatment number of infected plants was recorded for each trial. The next observation were done thirty days later, and then, number of infected leaves per plant, and number of urediniospores pustels per leaf (from 100 infected leaves) 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

***In vitro* growth of six species of fungi in the presence of chitosan.** The chitosan of medium and high molecular weight evidently inhibited *in vitro* growth of all tested pathogens, with a marked effect at higher concentrations (Tab. 2, 3). At the highest concentration, 1000 µg/cm^3, both, medium and high molecular weight of chitosan gave similar inhibitory effect on growth of tested pathogens. At the concentration of 400 µg/cm^3 radial growth of *Alternaria alternata* was inhibited in 84%, *Fusarium oxysporum* f. sp. *callistephi* in 87%, *Fusarium oxysporum* f. sp. *tulipae* in 65%, *Phoma narcissi* in 10% and *Phoma poolensis* in 83% (Tab. 2, 3, Fig. 1). It should be mentioned that chitosan of high molecular weight at a concentration 400 mg/cm^3 inhibited radial growth of *Botrytis tulipae* in 13%, and chitosan of medium molecular weight at the concentration from 50 to 400 µg/cm^3 gave a small stimulatory effect on *in vitro* growth of *B. tulipae* (Tab. 2, 3).

Table 2

Mycelial growth of six species of fungi on potato-dextrose-agar amendment with chitosan, a medium molecular weight (200-800 cps)

Chitosan, conc. in µg/cm³ PDA	Surface of mycelium (cm²) after 6 days incubation					
	Alternaria alternata	*Botrytis tulipae*	*Fusarium oxysporum* f. sp. *callistephi*	*Fusarium oxysporum* f. sp. *tulipae*	*Phoma narcissi*	*Phoma poolensis*
Check	29.3 e*	50.3 c	27.6 d	8.0 c	56.7 e	15.1 b
50	18.7 d	54.1 cd	25.6 cd	8.8 c	54.8 de	14.5 b
100	13.3 c	54.1 cd	22.8 c	7.2 c	55.4 de	14.8 b
200	14.4 c	53.8 cd	10.1 b	4.6 b	56.2 e	13.6 b
400	4.8 b	56.9 d	3.6 a	2.7 ab	53.5 d	1.9 a
600	2.1 a	15.4 b	2.7 a	1.8 a	16.6 c	1.4 a
800	1.4 a	8.0 a	1.8 a	1.3 a	3.6 b	1.7 a
1000	0.9 ab	6.6 a	1.1 a	1.3 a	1.7 a	1.6 a

*Explanation: Means in column followed by the same letter do not differ at 5% level of significance.

Table 3

Mycelial growth of six species of fungi on potato-dextrose agar amendment with chitosan, a high molecular weight (800-2000 cps)

Chitosan, conc. in µg/cm³ PDA	Surface of mycelium (cm²) after 6 days incubation					
	Alternaria alternata	*Botrytis tulipae*	*Fusarium oxysporum* f. sp. *callistephi*	*Fusarium oxysporum* f. sp. *tulipae*	*Phoma narcissi*	*Phoma poolensis*
Check	30.4 e	63.3 c	32.7 g	13.4 d	54.1 d	15.9 g
50	28.4 e	56.7 b	25.7 e	9.7 bc	53.4 d	12.8 f
100	21.8 d	60.8 bc	26.7 f	11.8 cd	53.4 d	9.7 e
200	13.6 c	59.5 bc	14.1 d	8.2 b	50.2 c	8.1 d
400	4.9 b	56.7 b	3.9 c	4.1 a	48.4 c	3.1 c
600	1.8 ab	58.1 bc	2.4 b	2.7 a	18.4 b	1.7 b
800	1.2 ab	15.2 a	2.2 b	1.8 a	1.6 a	0.7 a
1000	0.7 a	15.2 a	1.1 ab	1.8 a	1.6 a	0.5 a

Explanation: see Table 2

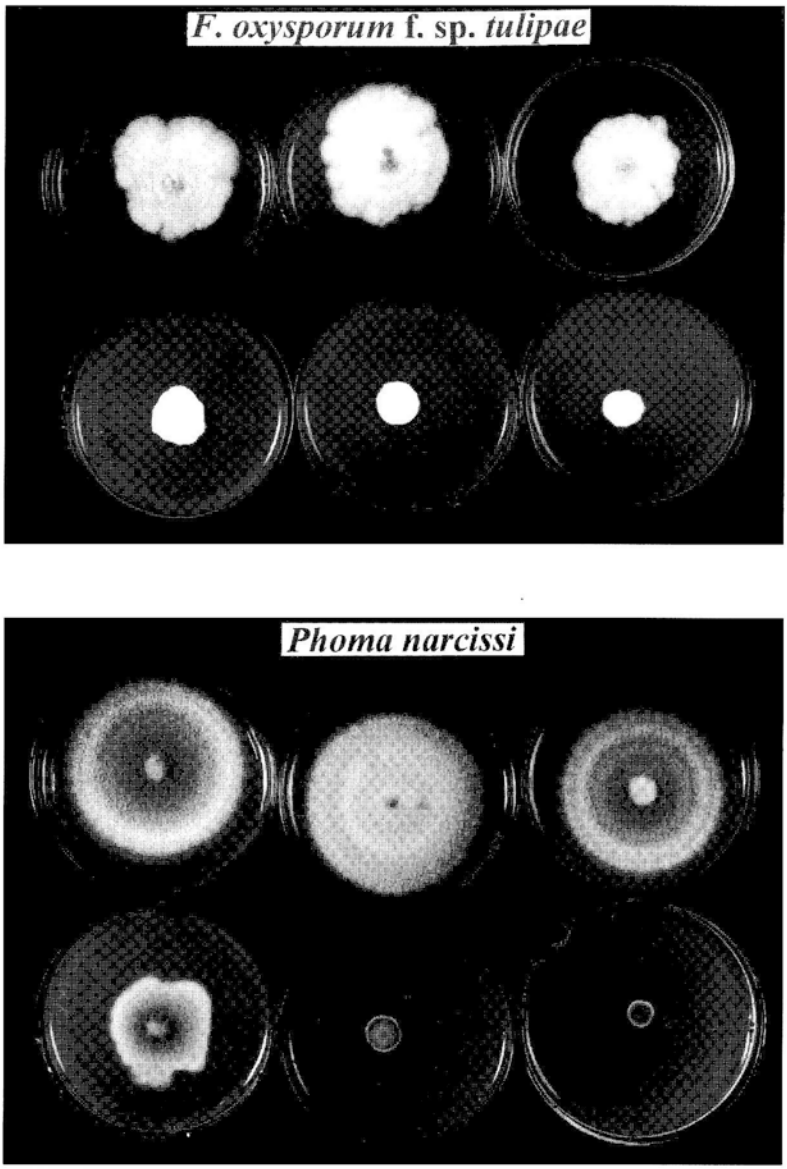

Fig. 1. Mycelial growth of *Fusarium oxysporum* f. sp. *tulipae* and *Phoma narcissi* on potato-dextrose agar amendment with chitosan.
upper row: control; 50; 100 μg/cm^3
lower row: 400; 800; 1000 μg/cm^3

The inhibitory effect of chitosan was also documented on the *in vitro* mycelium growth of many pathogenic fungi; *Colletotrichum lindemuthianum*, *Fusarium oxysporum* f. sp. *phaseoli*, *Fusarium solani*, *Rhizoctonia solani*, *Phytophthora megasperma*, *Pythium paroecandrum* (S t ö s s e l and L e u b a, 1984), *Botrytis cinerea*, *Rhizopus stolonifer* (E l G h a o u t h et al., 1992a), *Pythium aphanidermatum* (E l G h a o u t h et al., 1994), and others (A l l a n and H a d w i g e r, 1979). The mechanism of inhibitory action of chitosan on *in vitro* growth of pathogenic fungi is not yet known, but chitosan was shown to cause severe morphological, cellular and ultrastructural alterations in fungi (E l G h a o u t h et al., 1992b, 1994; B e n h a m o u, 1992).

Influence of chitosan on development of fungi contaminated seeds. Chitosan at a concentration 1.25; 2.5 and 5.0 mg/cm^3 didn't have inhibitory action in appearance of fungi growth on naturally contaminated *Callistephus chinensis* seeds (data not presented). Mycological analysis showed that *Alternaria alternata* (Fr.) Kreisler and *Botrytis cinera* Pers. dominated among isolated fungi. Thus, it seems that the use of chitosan in control of pathogens of *Callistephus chinensis* seeds is questionable. It is possible that reason is that treatment of *Callistephus chinensis* seeds with chitosan was too short time (only 30 min).

Influence of chitosan in the control of *Phoma narcissi* on *Hymenocallis narcissiflora* scales. In the laboratory test on excised scales of *Hymenocallis narcissiflora*, chitosan applied as bulb scales dressing, before inoculation or after inoculation with *Phoma narcissi* inhibited the development of necrotic spots on scales (Tab. 4, 5, Fig.2). Different time, 15 and 30 min. of scales dressing and different concentration of applied chitosan gave similar inhibitory effect in development of necrosis. Inoculation of scales after 2h and 20h from applied of chitosan gave the same inhibitory effect on development of *Phoma narcissi* (Tab. 4). After week-incubation diameter of spots on scales dressing with chitosan was at least 50% smaller than controls (Tab. 4, 5).

Table 4

Influence of chitosan, applied preventively (30 min. of dressing) in the control of *Phoma narcissi* on *Hymenocallis narcissiflora* scales; laboratory test

Chitosan, conc. in mg/cm^3	Length of necrosis in mm after days of incubation			
	A		B	
	4	7	4	7
Check	9.9 b	13.3 b	10.2 b	13.7 c
1.25	3.4 a	7.1 a	6.4 a	7.7 b
2.5	1.5 a	6.2 a	4.4 a	5.9 ab
5.0	1.2 a	7.3 a	3.8 a	5.6 a

Explanation: see Table 2

A - inoculation with pathogen 2h after applied of chitosan

B – inoculation with pathogen 20h after applied of chitosan

Table 5

Influence of chitosan, applied preventively (A) and curatively (B) in the control of *Phoma narcissi* on *Hymenocallis narcissiflora* scales; laboratory test

Chitosan, conc. in mg/cm^3	Length of necrosis in mm after days of incubation			
	A		B	
	4	7	4	7
Check	12.5 b	15.6 b	14.2 c	17.5 b
1.25	7.1 a	8.5 a	9.2 ab	11.9 a
2.5	6.0 a	6.1 a	8.4 a	10.0 a
5.0	5.6 a	6.3 a	9.6 b	9.7 a

Explanation: see Table 2

A - chitosan was applied 2h before inoculation with pathogen

B - chitosan was applied 28h after inoculation with pathogen

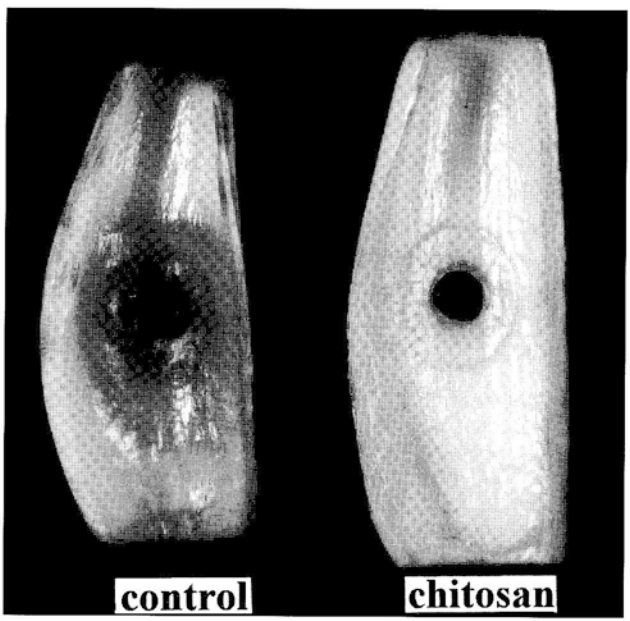

Fig. 2. Influence of chitosan at a concentration of 1.25 mg/cm^3 applied preventively on the development of *Phoma narcissi* on *Hymenocallis narcissiflora* scales.

Influence of chitosan, applied preventively and curatively in the control of *Fusarium oxysporum* f. sp. *tulipae* on tulip bulbs. Chitosan used 2h before inoculation or 10h, 24h and 48h after inoculation with pathogen inhibited development of necrosis on tulip bulbs (Tab. 6, 7). Different concentrations of chitosan used before inoculation with pathogen gave similar fungistatic inhibitory effect in

development of necrotic spots (Tab. 6, 7, Fig.3). Chitosan at highest concentration 5,0 mg/cm^3 used after inoculation gave largest inhibitory effect in the development of *F. oxysporum* f. sp. *tulipae* on tulip bulbs.

Table 6

The effect of preventively used of chitosan on the development of *Fusarium oxysporum* f. sp. *tulipae* on tulip bulbs

Chitosan, conc. in mg/cm^3	Diameter of necrosis (mm) after days of incubation				Depth of necrosis after 14 days of incubation
	7	9	11	14	
Check	10.0 b	15.1 b	20.7 b	23.3 b	3.6 b
1.25	0.0 a	1.8 a	2.8 a	3.3 a	1.0 a
2.5	0.0 a	2.5 a	3.4 a	3.9 a	0.7 a
5.0	0.0 a	1.1 a	3.0 a	3.4 a	0.6 a

Explanation: see Table 2

Table 7

Influence of chitosan used curatively as bulb dressing in reduction of *Fusarium oxysporum* f. sp. *tulipae* on tulip bulbs; bulb dressing 10h (A), 24h (B) and 48h (C) after inoculation with pathogen

Chitosan, conc. in mg/cm^3	Diameter of necrosis (mm) after days of incubation			Depth of necrosis (mm) after 21 days of incubation
	8	13	18	
A				
Check	10.8 b	16.4 e	22.1 d	4.0 d
1.25	0.8 a	1.0 ab	1.2 a	1.6 a-c
2.5	0.7 a	4.6 a-c	6.1 a-c	1.5 a-c
5.0	0.0 a	0.0 a	0.8 a	0.5 a
B				
1.25	0.0 a	9.3 cd	9.8 bc	1.9 bc
2.5	0.0 a	10.1 d	11.4 c	2.4 c
5.0	0.0 a	2.5 ab	2.1 a	0.7 ab
C				
5.0	0.7 a	5.5 b-d	5.8 ab	0.7 ab

Explanation: see Table 2

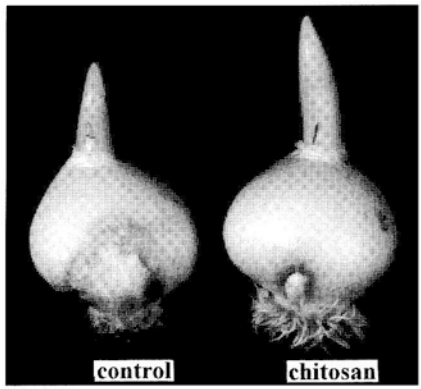

Fig. 3. Influence of chitosan at a concentration of 1.25 mg/cm^3 applied preventively on the development of *Fusarium oxysporum* f. sp. *tulipae* on tulip bulbs: observation after 11 days from inoculation.

Influence of chitosan, applied preventively, in the control of *Puccinia antirrhini* on field grown naturally infested snapdragon. The first symptoms of rust appeared on snapdragon leaves at the end of July. Therefore, it may be assumed that the spray treatments (12th June) were started before stage of fungal incubation. Chitosan at high molecular weight at concentration of 10 mg/cm^3 as well as 20 mg/cm^3 was very effective in the control of *Puccinia antirrhini* on snapdragon in the field (Tab. 8). In the middle of August no symptoms of rust was observed on snapdragon leaves. One month later chitosan continually strongly inhibited development of the pathogen on snapdragon (Tab. 8, Fig. 4). The strongest inhibitory effect was observed on snapdragon treated 8 times at weekly intervals (Tab. 8). Both concentrations of chitosan used in experiment gave similar inhibitory effect in the control of pathogen on snapdragon.

Table 8

Effectiveness of chitosan preventively used in the control of *Puccinia antirrhini* on field grown snapdragon; n=5 plants

Check	Chitosan 10 mg/cm^3				Chitosan 20 mg/cm^3			
	1 spray only	2 sprays at 14 days intervals	8 sprays at 7 days intervals	4 sprays at 14 days intervals	1 spray only	2 sprays at 14 days intervals	8 sprays at 7 days intervals	4 sprays at 14 days intervals
Number of infected plants in April 14								
4.0 b	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
Number of infected leaves per plant in September 15								
100.0 c*	3.2 ab	8.4 b	0.0 a	4.2 ab	2.2 ab	5.0 ab	0.6 a	2.8 ab
Number of urediniospore pustels per infected leaf in September 18								
29.1 b	1.0 a	4.2 a	0.0 a	1.6 a	1.2 a	1.9 a	1.8 a	1.4 a

Explanation: see Table 2

*Control plants had much more infected leaves

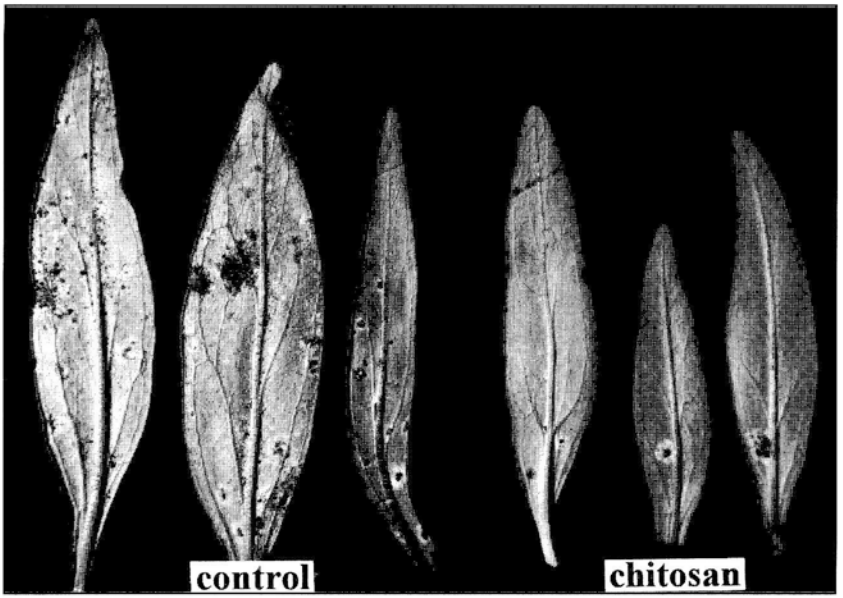

Fig. 4. Influence of chitosan (10 mg/cm^3) applied preventively on the control of *Puccinia antirrhini* on naturally infected field grown snapdragon; 2 sprays from 12th June at 14 days intervals – photographed in September 24.

Recently the inhibitory effect of chitosan on development of disease induced by *Fusarium oxysporum* f. sp. *radicis-lycopersici* on tomato plants was documented by Benhamou and Thériault (1992). El Ghaouth et al. (1994) showed that chitosan substantially reduced disease symptoms induced by *Pythium aphanidermatum* in cucumber plants. The marked reduction by chitosan of symptoms disease induced by *Phoma narcissi* on *Hymenocallis narcissiflora* scales, *Fusarium oxysporum* f. sp. *tulipae* on tulip bulbs, and *Puccinia antirrhini* on snapdragon, may be caused by direct inhibitory effect of chitosan on the pathogens or by triggering the plants' natural defense mechanisms and consequently helping the tissue restrict fungal colonization. It is well know that chitosan can induce many of biological processes in plant tissues, as stimulation of chitinases (El Ghauth at al., 1994), synthesis of proteinase inhibitors, accumulation of phytoalexins, increased lignification and others (Darvill et al., 1992; Côte and Hahn, 1994; Ebel and Mithöfer, 1998).

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Wpływ chitozanu na ograniczenie wzrostu i rozwoju niektórych gatunków grzybów chorobotwórczych dla roślin ozdobnych

Streszczenie

W warunkach *in vitro* i *in vivo* badano wpływ chitozanu (firmy Sigma-Aldrich Chemicals), o średniej masie cząsteczkowej (200-800 cps) i wysokiej masie cząsteczkowej (800–2000 cps) na wzrost grzybni *Alternaria alternata*, *Botrytis tulipae*, *Fusarium oxysporum* f. sp. *callistephi*, *Fusarium oxysporum* f. sp. *tulipae*, *Phoma narcissi* i *Phoma poolensis*. Chitozan, o dwóch różnych masach cząsteczkowych, zastosowany w stężeniu 50, 100, 200, 400, 600, 800 i 1000 μg/cm^3 pożywki

ziemniaczano-glukozowej (PDA) wykazał (z wyjątkiem *Botrytis tulipae*) podobnie ograniczający wpływ na wzrost liniowy grzybni testowanych gatunków. Badane gatunki grzybów, wykazały zróżnicowanie w stopniu zahamowania wzrostu liniowego grzybni na pożywce z dodatkiem zastosowanych koncentracji chitozanu. W obecności 400 mg chitozanu/cm^3 pożywki wzrost grzybni w stosunku do kultury kontrolnej był zahamowany dla *Alternaria alternata* w 84%, *Fusarium oxysporum* f. sp. *callistephi* w 87 %, *Fusarium oxysporum* f. sp. *tulipae* w 65%, *Phoma narcissi* w 10% i *Phoma poolensis* w 83%. Chitozan, o wysokiej masie cząsteczkowej, w stężeniu 400 µg/cm^3 pożywki, hamował wzrost grzybni *Botrytis tulipae* w 12%, a chitozan, o średniej masie cząsteczkowej, w tej samej koncentracji (400 mg/cm^3) wpłynął w małym stopniu stymulująco na wzrost liniowy tego gatunku. Nie stwierdzono całkowitego zahamowania wzrostu grzybni, testowanych gatunków, przy najwyższej badanej koncentracji chitozanu (1000 µg/cm^3 PDA).

Chitozan w stężeniu 1,25; 2,5 i 5,0 mg/cm^3 zastosowany do zaprawiania nasion astra chińskiego (*Callistephus chinensis*) nie wpłynął ograniczająco na wzrost grzybów zasiedlających nasiona. Chitozan zastosowany w koncentracji 1,25, 2,5 i 5,0 mg/cm^3, do zaprawiania łusek *Hymenocallis narcissiflora* przed inokulacją lub po inokulacji grzybnią *Phoma narcissi* wykazał fungistatyczny wpływ na rozwój patogena w tkance łusek cebuli. Średnica nekrotycznych plam na łuskach cebuli zaprawianych w chitozanie (2.5 mg/cm^3) była około połowę mniejsza w porównaniu do plam infekcyjnych na łuskach kontrolnych nie zaprawianych. Podobnie, wykazano hamujący wpływ chitozanu (stosowanego w tych samych stężeniach), na rozwój *Fusarium oxysporum* f. sp. *tulipae* na cebulach tulipanów zaprawianych profilaktycznie lub interwencyjnie (10, 24 i 48 godzinach od inokulacji zarodnikami patogena). Chitozan w stężeniu 10 mg/cm^3 zastosowany profilaktycznie do opryskiwania silnie ograniczał rozwój *Puccinia antirrhini* na wyżlinie w uprawie polowej. Najsilniej ograniczany był rozwój rdzy wyżlinu na roślinach opryskiwanych od 12 czerwca 8-krotnie w odstępach tygodniowych.