JOURNAL OF PLANT PROTECTION RESEARCH
Vol. 41, No. 4 2001

BIOLOGICAL ACTIVITY OF GRAPEFRUIT EXTRACT IN THE CONTROL OF *FUSARIUM OXYSPORUM*

LESZEK B. ORLIKOWSKI[1], CZESŁAW SKRZYPCZAK[1], IZABELA HARMAJ[2]

[1]Research Institute of Pomology and Floriculture, Pomologiczna 18, 96-100 Skierniewice, Poland
e-mail: lorlikow@insad.isk.skierniewice.pl
[2]Cintamami Poland, Nefrytowa 16/54, 05-500 Piaseczno, Poland
e-mail: iharmaj@poczta.onet.pl

Accepted: July 16, 2001

Abstract: Influence of grapefruit extract (GE) on mycelial growth, spore germination, population density of *Fusarium oxysporum* f. sp. *cyclaminis* and *F. oxysporum* f. sp. *dianthi* and its effectiveness in the control of *Fusarium* wilt of carnation and *Fusarium* corm rot of gladiolus (*F. oxysporum* f. sp. *gladioli*) was studied. Amendment of PDA with 40 µg of GE/cm^3 inhibited mycelial growth about 50%. Drenching of carnation with 165 µg of GE/cm^3, immediately after planting, resulted in drastical decrease of colony forming units number of the pathogen and increased healthiness plant stand about 50%. Applied as gladiolus corm soak, GE at conc. 660 µg/cm^3 decreased development of *Fusarium* rot of gladiolus clones at least twice.

Key words: Biological control, *Fusarium*, carnation, gladiolus, population, healthiness

I. INTRODUCTION

Fusarium wilts or rot, caused by *Fusarium oxysporum* Schlecht. are some of the most widespread and destructive diseases of many major ornamental crops (Orlikowski and Skrzypczak 1997a). Some microorganisms and plant products have been reported to possess the pathogen control properties. Orlikowski and Skrzypczak (1997b) found that vermicompost and keratin – bark – urea extract inhibited the development of *Fusarium* wilt of carnation and *Fusarium* rot of tulip. Application of chitosan as substratum drench inhibited the spread of *F. oxysporum* f. sp. *dianthi* in carnation vessels (Orlikowski et al. 1997). Recently Orlikowski (2001) have found that grapefruit extract strongly inhibited the growth and sporulation of *Phytophthora cryptogea*. Amendment of peat, artificially infested with the pathogen, reduced population density of the fungus about 70% and increased healthy gerbera stand. In studies of Angioni et al. (1998) and Woedtke et al. (1999) compounds extracted from grapefruit seeds and pulp inhibited *in vitro* the growth of *Bacillus subtilis, Candida maltosa, Escherichia coli, Micrococus flavus, Penicillium digitatum, P. italicum, Proteus mirabilis, Serratia marcescens* and *Staphyllococcus subtilis*. Application of grapefruit extract as plant spray reduced damage of passion – flower fruits caused by *Colletotrichum* sp. (Caceres et al. 1998) and table grape incited by *Botrytis cinerea* (Esterio et al. 1992). In own studies the product controlled the development of *Botrytis tulipae,*

Melampsora epitea and *Myrothecium roridum* on tulip, willow and dieffenbachia, respectively (Orlikowski and Skrzypczak 2001).

The objectives of this study was to evaluate *in vitro* and *in vivo* impact of grapefruit extract on growth, spore germination, population dynamics of *F. oxysporum* f. sp. *cyclaminis*, *F. oxysporum* f. sp. *dianthi* and *F. oxysporum* f. sp. *gladioli* and some plants healthy stand.

II. MATERIALS AND METHODS

Grapefruit extract (GE). Biosept 33 SL, containing 33% of grapefruit extract, supplied by Cintamani Poland, was used. The product was applied at doses from 0 (control) to 1,320 μg/cm^3.

Fungi. Isolates C2 of *Fusarium oxysporum* Schlecht. f. sp. *cyclaminis* Gerlach (*Foc*), Gz 274 of *F. oxysporum* Schlecht. f. sp. *dianthi* (Prill. et Del.) Snyd. et Hans (*Fod*) and G 887 of *F. oxysporum* f. sp. *gladioli* (Massey) Snyd. et Hans. (*Fog*) were used. Stock cultures were maintained on potato-dextrose agar (PDA) at 25°C in darkness. For artificially peat infestation *Foc* and *Fod* were grown on Quick rolled oats using the procedure described by Orlikowski (1999).

Plants. Carnation (*Dianthus caryophyllus* L.) 'Master' and gladiolus corms (*Gladiolus x hybridus* hort.) 'Oscar' were used.

***In vitro* trials.** Influence of GE on linear growth of *Foc* and *Fod* was evaluated on PDA. Five mm diam mycelial disks, taken from the rim of 7-day-old cultures, were placed in centre of 90 mm Petri dishes filled with 10 cm^3 medium containing different concentrations of GE (1.6, 8, 40, 200 and 1,000 μg/cm^3). After 5 and 7-day-incubation of plates in darkness at 25°C, diam of colonies was measured along 2 lines. After 7-day-incubation disks of mycelium were taken from cultures grown on PDA, amended with different concentration of GE, and transferred on the medium without of GE. After 5-day-incubation diameter of colonies was measured. Fungitoxicity of GE was expressed as percentage of mycelial growth inhibition. Treatments were replicated (one plate) four times and arranged in a completely randomised design. Means were separated by Duncan's multiple range test (P = 0.05). Trial was repeated 3 times.

Germination of microconidia of *Foc* was evaluated in distilled water and on PDA amended with GE (1.6, 8, 40, 200 μg/cm^3). Spores (10^5/cm^3) were transferred into media and their germination was observed under microscope after 24 hr incubation at 25°C. Additionally, a length of germ tubes of germinated spores were measured. Four 90 mm diam Petri dishes were used for each concentration of GE and germinated spores were counted in 4 places on each plate.

Influence of GE on population dynamic of *F. oxysporum* f. sp. *dianthi* and healthiness stand of carnation and gladiolus. One dm^3 pots were filled with peat, artificially infested with *Fod* (2,400 colony forming units/g of air dry substratum) and carnations were planted. Immediately after planting pots were drenched with GE at doses 165 and 330 μg/cm^3 once or twice at 2-week-interval (50 cm^3 of solution/pot). Carbendazim at dose 500 μg/cm^3 was used as the standard compound. Pots were placed on greenhouse bench and

plants were grown at temperature range from 13° to 22°C. Number of colony forming units of *Fod* was determined using Komada (1975) *Fusarium* selective medium and procedure described by Orlikowski (1999) before GE application (initial population) and during 35 days at 5-day-intervals. Development of *Fusarium* wilt symptoms on plants was recorded during 14 week – growth at weekly intervals. Additionally, after that time carnation stems were longitudinally cut and discoloration of vessels was measured. Biological activity of GE toward *F. oxysporum* f. sp. *gladioli* was evaluated on gladiolus corms. They were soaked in GE solution 15 min and after the next 2 hr inoculated with *Fog*. Corms were planted in field and after 5-month-growth number of healthy clones and yield of corms and cormels was estimated. Experimental design was completely randomised with 4 replications and 5 plates with selective medium or 10 plants or corms in each rep. Trials were repeated twice.

III. RESULTS AND DISCUSSION

***In vitro* trials.** Amendment of potato-dextrose agar with GE at dose 40 μg/cm^3 resulted in the decrease of *F. oxysporum* f.sp. *cyclaminis* and *F. oxysporum* f.sp. *dianthi* mycelial growth about 50%. Grapefruit extract only slightly inhibited the pathogens growth at 1.6 μg/cm^3 whereas at 1,000 μg/cm^3 development of both fungi was almost completely suppressed (Fig. 1). At doses of extract higher than 8 μg/cm^3 colonies of both fungi changed colour from bright violet on white and in case of 1,000 μg/cm^3 on yellow. The colonies were very compact and air mycelium grew up. After 7-day-incubation sporulation was not observed on colonies incubated on PDA amended with 200 and 1,000 μg of GE/cm^3 whereas

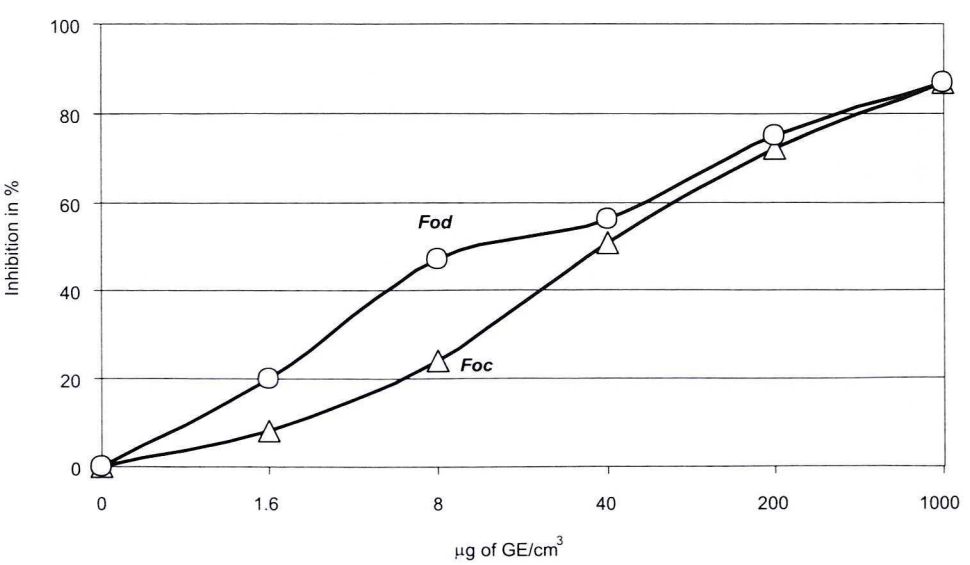

Fig. 1. Biological activity of grapefruit extract (GE) in the inhibition of linear growth of *Fusarium oxysporum* f.sp. *cyclaminis* (*Foc*) and *F. oxysporum* f.sp. *dianthi* (*Fod*)

at 40 μg of GE/cm^3 sporulation was very poor. Hyphae of both pathogens grown on nonamended PDA were straight without some branches whereas on PDA containing 40 and 200 μg of GE/cm^3 they were shorter with many branches. At dose of 1,000 μg of GE/cm^3 on very compact colonies hyphae were short with branches on main part and lateral branches as well. Transferring of mycelial disks, taken from cultures growing on PDA with different concentrations of GE, on PDA without GE and incubation of plates 4 days at 25°C resulted in only slightly inhibition of *Fod* obtained from disks amended with 1,000 μg of GE/cm^3. This data indicated that GE, even in high concentration, did not kill the pathogen nor had durable effect on its.

Grapefruit extract inhibited spore germination of *Foc* already at concentration 40 μg/cm^3 (34% of inhibition). Increase of GE dose to 200 μg/cm^3 caused complete suppression of spore germination (Fig. 2). The growth of germ tubes was already inhibited at 1.6 μg/cm^3 (about 40%) and tubes were not formed at 200 μg/cm^3 (Fig. 2).

Influence of GE on population dynamic of *F. oxysporum* f. sp. *dianthi* and healthiness stand of carnation and gladiolus. Five days after amendment of peat with grapefruit extract 165 μg/cm^3 number of colony forming units of *Fod* decreased about 40% (Fig. 3). Within one-month population density of the pathogen decreased and after 35 days reached the suppression level about 74%. Statistical analyse did not show significant differences between concentrations of grapefruit extract and its biological activity toward the pathogen. Application of GE as carnation drench, immediately after planting, resulted in significant decrease of *Fusarium* wilt spread (Tab. 1) during 14-week-growth of plants. After 10-week-growth – growth GE at conc. 165 μg/cm^3 completely suppressed the disease spread. Four weeks later *Fusarium* wilt symptoms occurred only sporadically. Drenching of

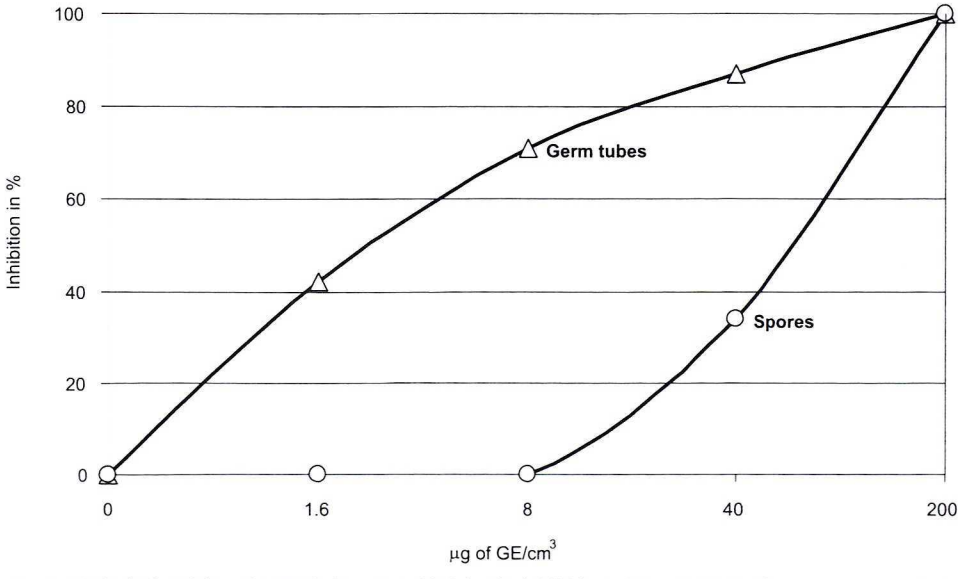

Fig. 2. Biological activity of grapefruit extract (GE) in the inhibition of germination of spores and germ tube growth of *Fusarium oxysporum* f.sp. *cyclaminis*

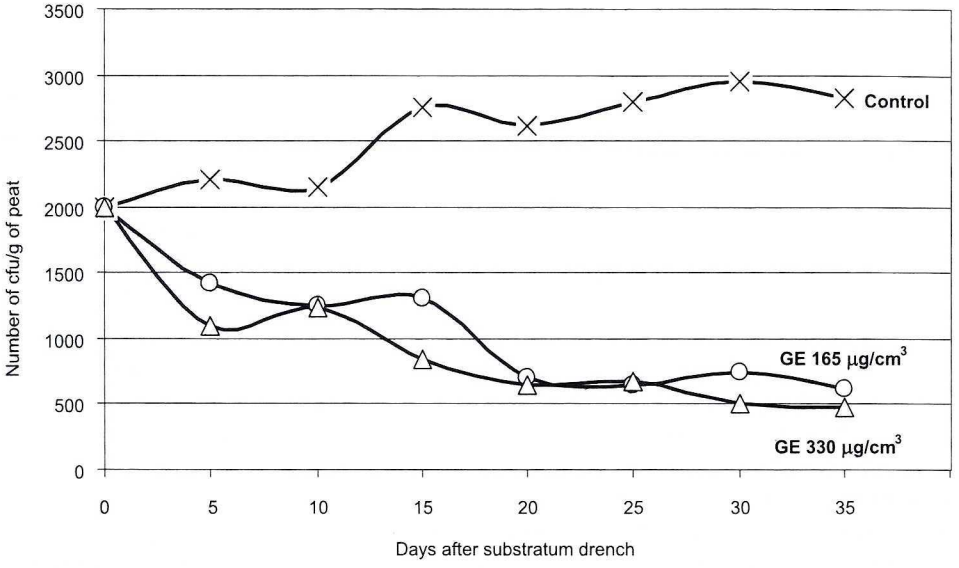

Fig. 3. Influence of grapefruit extract (GE) on the number of colony forming units (cfu) of *Fusarium oxysporum* f.sp. *dianthi* in peat

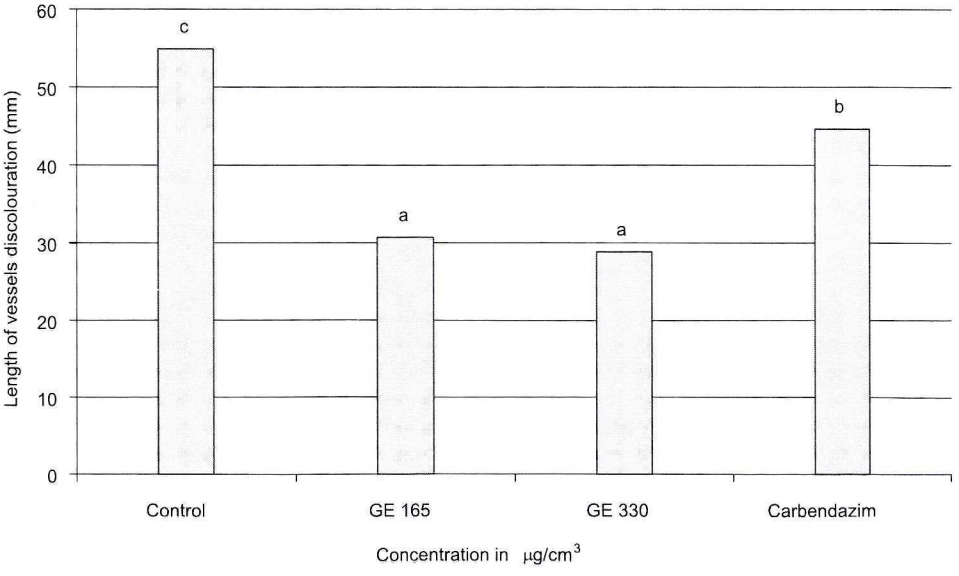

Fig. 4. Influence of grapefruit extract (GE) on carnation vessels discoloration caused by *Fusarium oxysporum* f.sp. *dianthi*

plants with GE at dose 660 µg/cm^3 once or twice gave similar effect in the pathogen control (Tab. 1). Treatment of plants with GE caused the decrease of the pathogen spread in carnation vessels. Discoloration of vessels on protected plants was at least 40% lower than on

Table 1

Biological activity of grapefruit extract in the control of *Fusarium* wilt of carnation 'Master'; number of diseased plants (n = 10)

Treatment	µg of a.i/cm^3	Frequency of application	Weeks after planting	
			12	14
Control – infested (without GE)	–	–	4.5c	8.5c
Control – noninfested	165	1	0.5a	1.0a
Grapefruit extract	165	2	1.5a	2.5a
Grapefruit extract	330	1	1.0a	1.5a
Grapefruit extract	330	2	2.0ab	3.0ab
Carbendazim	500	1	3.5bc	5.0b

Note: Means in columns followed by the same letter do not differ at 5% level of significance (Duncan's multiple range test)

nontreated carnation (Fig. 4). GE was more effective in *Fusarium* wilt control than carbendazim (Tab. 1).

Prior inoculation soaking of gladiolus corms with GE at conc. 660 µg/cm^3 resulted in the strong decrease of *Fusarium* rot spread on clones. Number of healthy clones was about 2 times higher than on nontreated gladiolus (Tab. 2). GE at concentration 1,320 µg/cm^3 decreased the *Fusarium* rot spread in degree similar to its lower concentrations. The dose 660 µg of GE/cm^3 was also the most profitable for corms growth (Tab. 2).

The data obtained indicate on 2 mechanisms of grapefruit extract action on different formae speciales of *Fusarium oxysporum*. Inhibition of mycelial growth and germination of microconidia already at 8 µg/cm^3 in the *in vitro* study indicated on direct action of tested product on the pathogens. This activity was especially seen on potato-dextrose agar at concentration grapefruit extract 40 µg /cm^3 and higher. The pathogen hyphae were inhibited in the growth, they formed lateral branches but did not produce spores on mycelium. Application of grapefruit extract as peat drench reduced number of colony forming units of *F. oxysporum* f. sp. *dianthi* in the substratum. It is possible that peat absorbed some part of grapefruit extract and molecules influenced the development of the pathogen hyphae as well as spores during at least one-month. Probably very low population of the pathogen in

Table 2

Biological activity of grapefruit extract in the control of *Fusarium* corm rot of gladiolus 'Oscar'

Treatment	µg of a.i./cm^3	Number of healthy clones (n=10)	Yield of clones (g)
Control – noninfested	–	–	90.5c
Control – infested	–	3.4a	33.4b
Grapefruit extract	330	3.7a	23.2a
Grapefruit extract	660	7.7b	36.9b
Grapefruit extract	1320	5.4ab	23.0a
Carbendazim	2000	5.8ab	23.0a

Note: see Table 1

treatments with GE resulted in slower than in check development of *Fusarium* wilt symptoms on carnations. Decrease of vessels discoloration in carnation, treated with grapefruit extract, indicate on plant defence responses on the pathogen. Accumulation of chitinases, synthesis of proteinase inhibitors, lignification of some plant organs and induction of callose synthesis was observed when plants were treated with chitosan (El Ghaouth et al. 1992, Orlikowski et al. 1997). It is possible that grapefruit extract may induce similar processes in carnation and gladiolus corm. It is already known that several extracts of different plant species effectively reduce soil population of *F. oxysporum* and increase symptomless plant stand (Bowers and Locke 2000). Some of them in trials of Bowers and Locke (2000) did not reduce *F. oxysporum* population but suppressed disease development. The authors concluded that such effect is probably connected with selectivity of plant extracts for *Fusarium* and related fungi. Fungal species that are not affected increase in numbers and could suppress disease development in the presence of the pathogen. Further research with biological activity of grapefruit extract has the potential to extend its usefulness as the biopreparate.

IV. REFERENCES

1. Angioni A., Cabras P., Hallewin G., Pirisi F.M., Reniero F., Schirra M. 1998. Synthesis and inhibitory activity of 7 – geranoxycoumarin against *Penicillium* species in *Citrus* fruit. Phytochemistry 47 (8): 1521–525.
2. Bowers J.H., Locke J.C. 2000. Effect of botanical extracts on the population densities of *Fusarium oxysporum* in soil and control of *Fusarium* wilt in the greenhouse. Plant. Disease 84: 300–305.
3. Caceres M.I., Martinez E., Torres F. 1998. Efecto de tiabendazol y extractos de semilla de toronja en el control de enfermedades de curuba en pre y poscosecha en Nuevo Colon. Fitopatologia – Colombiana 22: 1–2: 35–38.
4. Esterio M.A., Auger J.G., Vazquez E., Reyes M., Scheelje F., Verhoeff K. 1992. Efficacy od grapefruir extract BC – 1000 for control of *Botrytis cinerea* on table grapes in Chile. Proc. of the 10th Int. *Botrytis* Symposium, Heraklion, Crete, Greece, 1992.04.05–10: 211–214.
5. Ghaouth El, Arul J., Grenier J., Asselin A. 1992. Antifungal activity of chitosan on two postharvest pathogens of strawberry fruits. Phytopathology 82: 398–402.
6. Komada H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Rev. Plant Prot. Res., 8: 114–124.
7. Orlikowski L.B. 1999. Selective media for the evaluation of biocontrol agents efficacy in the control of soil-borne pathogens . Bull. Pol. Acad. Sci., Biolog. Sci., 2–4: 167–172.
8. Orlikowski L.B. 2001. Effect of grapefruit extract on development of *Phytophthora cryptogea* and control of foot rot of gerbera. J. Plant Protection Res., 41(3): 276–282.
9. Orlikowski L.B., Skrzypczak Cz. 1997a. Effectiveness of Fusaclean in the control of five formae speciales of *Fusarium oxysporum* Schlecht. on some ornamental plants. Phytopathol. Pol., 13: 49–57.
10. Orlikowski L.B., Skrzypczak Cz. 1997b. Wapń, Antifung i wyciąg keratyno-koro-mocznikowy w ochronie niektórych roślin przed fuzariozą naczyniową i fytoftorozą. Prog. Plant Protection/Post. Ochr. Roślin 37 (1): 151–156.
11. Orlikowski L.B., Skrzypczak Cz. 2001. Biopreparat z wyciągu z grejpfruta – postęp w biologicznej ochronie roślin przed chorobami. Ann. Univ. Mariae Curie-Skłodowska, sec. EEE, IX Suppl.: 261–269.
12. Orlikowski L.B., Skrzypczak Cz., Niekraszewicz A. 1997. Effectiveness of chitosan in the control of *Fusarium* wilt of carnation. ANPP – 5th Conference Intern. sur les maladies des plantes., Tours, France II: 1015–1020.
13. Woedtke Th., Schluter B., Pfleger P., Lindequist U., Julich U.D. 1999. Aspects of the antimicrobial efficacy of grapefruit seed extract and its relation to preservative substances contained. Pharmacie 54 (64): 52–456.

V. POLISH SUMMARY

AKTYWNOŚĆ BIOLOGICZNA WYCIĄGU Z GREJPFRUTA W ZWALCZANIU *FUSARIUM OXYSPORUM*

Badano oddziaływanie wyciągu z grejpfruta *in vitro* na wzrost i rozwój grzybni *Fusarium oxysporum* f. sp. *cyclaminis* i *F. oxysporum* f. sp. *dianthi*. Ponadto analizowano wpływ wyciągu, zastosowanego doglebowo w dawkach 165 i 330 µg/cm^3, na dynamikę liczebności populacji *F. oxysporum* f. sp. *dianthi*. W trzecim etapie oceniano skuteczność wyciągu w hamowaniu rozwoju fuzariozy naczyniowej goździka i zgnilizny bulw mieczyka.

Dodatek wyciągu z grejpfruta do agaru ziemniaczano-glukozowego w dawce 40 µg/cm^3 ograniczał wzrost obu gatunków grzybów o około 50%. Wprowadzenie wyciągu do substratu torfowego już w dawce 165 µg/cm^3 spowodowało spadek liczebności populacji *F. oxysporum* f. sp. *dianthi* o 74% w ciągu 35 dni oraz ograniczenie rozwoju fuzariozy naczyniowej goździka o około 50%. Zwiększenie dawki wyciągu nie miało istotnego wpływu na poprawę zdrowotności goździków. Wyciąg z grejpfruta, zastosowany do zaprawiania bulw mieczyków, spowodował około 2-krotne ograniczenie fuzaryjnej zgnilizny na nowo tworzących się bulwach.