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EFFECT OF GRAPEFRUIT EXTRACT ON DEVELOPMENT OF *PHYTOPHTHORA CRYPTOGEA* AND CONTROL OF FOOT ROT OF GERBERA

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Abstract: Amendment of potato-dextrose agar with grapefruit extract at dose 40μg/cm^3 inhibited linear growth of *Phytophthora cryptogea* about 50%. At such concentration of the product in soil leachate formation of zoosporangia was inhibited about 95%. Application of grapefruit extract as peat drench reduced population density of the pathogen about 80% and this high activity was observed at least 30 days. The product inhibited in at least 50% the development of *Phytophthora* foot rot of gerbera when applied at 165 μg/cm^3 of peat.

Key words: development, hyphae, zoosporangia, concentration, population, direct action

I. INTRODUCTION

Diseases caused by species of the genus *Phytophthora* continue to inflict significant losses on numerous important plants worldwide (Erwin and Ribeiro 1966). Among these diseases is foot rot of gerbera incited by *P. cryptogea* Pethybr. et Laff. (Orlikowski 1995). High losses attributed to that pathogen have been reduced through the use of furalaxyl and oxadixyl (Orlikowski 1984). Recently some plant products have been reported to have activity on *P. cryptogea*, including garlic and purple coneflower extracts (Orlikowski and Saniewska 1995; Orlikowski and Wolski 1998). Among many plant products extract from grapefruit (*Citrus* x *paradisi* Macfad.) posseses microorganisms control properties. Compounds extracted from seeds or/and pulps inhibited *in vitro* the growth of *Bacillus subtilis*, *Candida maltosa*, *Escherichia coli*, *Micrococcus flavus*, *Penicillium digitatum*, *P. italicum*, *Proteus mirabilis*, *Serratia marcescens* and *Staphyllococcus subtilis* (Angioni et al. 1998; Woedtke et al.1999). Grapefruit extract applied as plant spray reduced damage of passion-flower fruits caused by *Colletotrichum* sp. (Caceres et al. 1998) and *Botrytis* rot of table grapes (Esterio et al. 1992). The effectiveness of extract was similar like standard fungicides used for plant protection. Study of Orlikowski and Skrzypczak (2001) showed that grapefruit extract has wide range of activity. Applied as soil drench strongly decreased population density of *Fusarium oxysporum* f. sp. *dianthi* and *Pythium ultimum*. Application of grapefruit extract as plant spray inhibited the development of willow rust, *Myrothecium* leaf spot of dieffenbachia and grey mold of tulip (Orlikowski and Skrzypczak 2001). In lite-

rature there are no information concerned this product activity toward *Phytophthora* species. The objectives of the following study was to evaluate *in vitro* and *in vivo* impact of grapefruit extract on the growth and sporulation of *P. cryptogea* and its activity in the control of *Phytophthora* foot rot of gerbera (*Gerbera jamesonii* Bolus).

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 1.6 to 1000 $\mu g/cm^3$.

Fungus. *P. cryptogea*, isolated from rotted foot of gerbera was used . Stock culture was grown on potato-dextrose agar (PDA) at 25°C in the dark. For infestation of peat, inoculum of the pathogen was preparaed on Quick oats using procedure described by Orlikowski (1980/81).

***In vitro* trials.** Influence of GE on the growth of *P. cryptogea* was studied by placing 5 mm diam mycelial disk, taken from the edge of 7-day-old culture in centre of Petri dish (90 mm diam) filled with 10 cm^3 of PDA amended with GE at conc. from 0 (control) to 1000 $\mu g/cm^3$. After 5 and 7-day-incubation of Petri dishes at 25°C in the darkness diam of colonies was measured along 2 preset lines. Fungitoxicity of GE was expressed as percentage of mycelial growth inhibition.

Zoosporangia formation in the presence of GE was evaluated in soil leachate (Orlikowski 1979). Four mycelial disks, cut out from the edge of actively growing *P. cryptogea* on oatmeal agar, were placed in the Petri dish containing 10 cm^3 of soil leachate amended with GE to give the final concentration from 1.6 to 200 $\mu g/cm^3$. After 3 and 6-day-incubation of plates at 25°C the number of zoosporangia was counted on the edge of mycelial mats (4 observations on each disk). The obtained results were recalculated to a number of zoosporangia/mm of mycelium.

In the next trial mycelial disks were transferred from soil leachate after 2-day-incubation into plates amended with GE at concentrations mentioned above. In the 3rd trial mycelial disks incubated 2 days in soil leachate amended with GE were transferred into clean leachate. After 3 and 6-day-incubation zoosporangia number was counted. In all trials experimental design was completely randomised with 4 replications (4 Petri dishes) and tests were repeated at least twice.

Influence of GE on population density of *P. cryptogea* in peat and development of foot rot of gerbera. One dm^3 pots were filled with peat infested with *P. cryptogea* and gerbera plants at stage of 4–5 leaves were planted. Immediately after planting gerbera was drenched with GE at doses 165 and 330 $\mu g/cm^3$ (50 cm^3/pot). Fongarid 25 WP (25% of furalaxyl) was used as the standard fungicide. Plants were grown on greenhouse bench at temperature range from 17° to 25°C. Population density of the pathogen was assayed before gerbera planting (initial population) and next 6 times at 5-day-intervals using gallic acid medium (Flowers and Hendrix 1969) and procedure described by Orlikowski (1980/81). The incidence of *Phytophthora* foot rot of gerbera was evaluated weekly during 3 months.

Experimental design was completely randomised with 4 replications (5 Petri dishes or 10 plants in each rep) and trials were repeated 3 times.

III. RESULTS AND DISCUSSION

***In vitro* growth of *P. cryptogea* colony on PDA amended with grapefruit extract.** The significant ability of GE to inhibit the colony growth was observed at concentrations from 1.6 to 1000 μg/cm^3 (Fig.). About 40 μg of GE/cm^3 was necessary for 50% inhibition of mycelial growth whereas the pathogen did not develop at 1000 μg /cm^3 (Fig.).

***In vitro* formation of zoosporangia of *P. cryptogea* in the presence of grapefruit extract.** On mycelial disks incubated 3 days in soil leachate amended with 1.6 μg of GE/cm^3 zoosporangia formation was not inhibited. Increase of GE dose to 40 μg/cm^3 resulted in decrease of zoosporangia number about 98%. The pathogen did not produce zoosporangia at 200 μg of GE/cm^3. After the next 3-day-incubation, sporulation of the tested fungus was not inhibited only in the presence of 1.6 μg of GE/cm^3. At dose 40 μg/cm^3 the pathogen sporulated only sporadically (Tab. 1).

Transferring of mycelial disks incubated 2 days in soil leachate to GE solution (40 and 200 μg/cm^3) resulted, both, after the next 3 and 6 days in reduction of sporulation at least 88% (Tab. 1). When mycelial disks were incubated 2 days in GE solution with concentra-

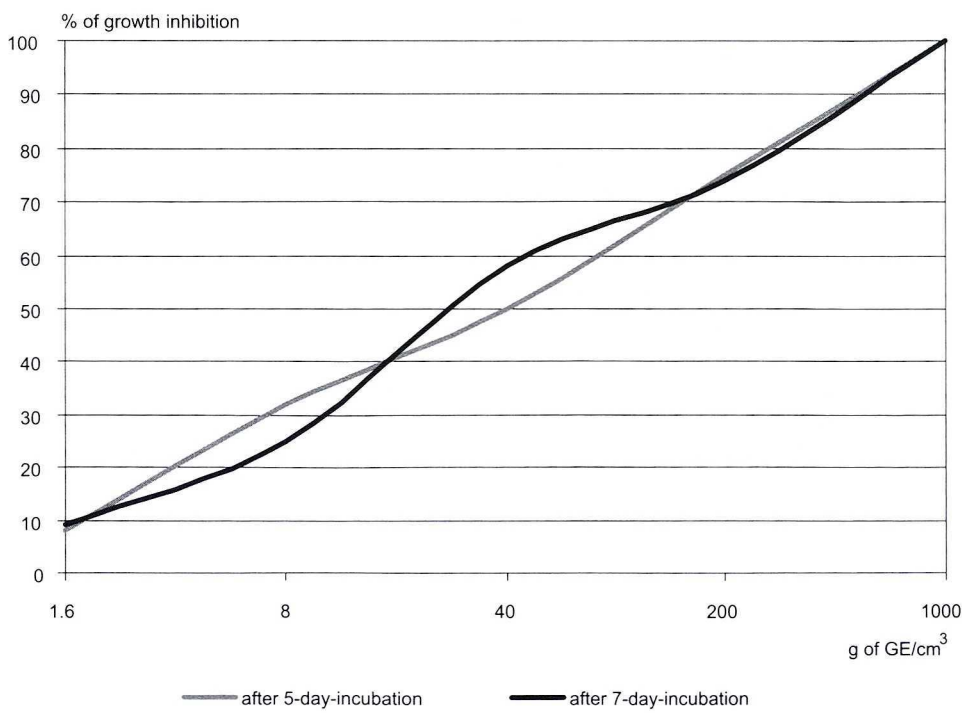

Fig. Influence of grapefruit extract (GE) on the growth of *Phytophthora cryptogea in vitro*

Table 1

Influence of grapefruit extract (GE) on sporulation of *Phytophthora cryptogea*; number of zoosporangia / mm^2 of mycelium after 6-day-incubation

µg of grapefruit extract / cm^3	Soil leachate	After 2-day-incubation transferring of mycelial disks	
		From soil lechate to GE solution	From GE solution to soil lechate
0	90.3 b	78.6 b	97.0 bc
1.6	90.0 b	72.8 b	94.5 b
8	13.5 a	71.5 b	14.6 a
40	2.5 a	9.4 a	2.7 a
200	0 a	2.8 a	0 a

* Within column means followed by the same letter are not significantly different at P = 0.05 according to Duncan's test.

tions higher than 1.6 µg/cm^3 and transferred to soil leachate, formation of zoosporangia was reduced at least 85%. Sporulation of the pathogen was observed only sporadically at 40 µg of GE/cm^3 and zoosporangia were not noticed at dose 5 times higher (Tab. 1).

Using of GE solution as the medium for zoosporangia formation during the first 2 days resulted in an increase of *P. cryptogea* swelling number already at dose 8 µg/cm^3. At this concentration and higher doses zoosporangia were strongly deformated. Their cryptogamous shape changed on excessively long with small vesicle on the base. At 40 and 200 µg of GE/cm^3 disintegration of haphae was observed. In liquid medium containing GE already at dose 40 µg/cm^3 development of other fungal species like *Alternaria*, *Gliocladium*, *Mucor* or *Fusarium* was not observed.

Influence of grapefruit extract on population density of *P. cryptogea* in peat and development of foot rot of gerbera. Initial population of the pathogen in tested peat was at least 3 times higher than in naturally infested substrates. Higher propagule numbers, however, was necessary for studying of population changing using gallic acid selective medium. Application of GE in peat, immediately after planting of gerbera, caused significant decrease of colony forming units number during at least one month (Tab. 2). Fungal population in peat amended with GE decreased at least 70% whereas in nontreated substratum propagule densities was stabile within 25 days and increased during the next 5 days (Tab. 2). The dose of GE had no significant influence on colony forming units number in peat.

In 3 trials with the control of *Phytophthora* foot rot of gerbera, GE was applied as peat drench at 2 concentrations once or twice at 14-day-interval. Frequency of biological control agent application had no any influence on its activity so in Tab. 3 results with only one treatment are presented. GE significantly inhibited the development of foot rot symptoms within 9 weeks of gerbera growth. After 7 weeks of growth 6/10 of unprotected gerbera plants showed discoloration of leaves and wilt symptoms whereas in pots treated with GE only 1/4 of plants were diseased (Tab. 3). After the next 2 weeks most of the control (untreated) plants showed wilt symptoms or their leaves were greengrey or brown. Application of GE protected about 6 from 10 of gerbera plants. Dose of GE had no significant influence on its

Table 2

Activity of grapefruit extract in the control of *Phytophthora cryptogea* in peat; number of colony forming units of air dry substratum
Initial population = 730 cfu/g

Days after application of grapefruit extract	µg of grapefruit extract / cm^3		
	0 (control)	165	330
5	722 a	127 ab	85 a
10	810 ab	212 b	42 a
15	670 a	0 a	42 a
20	790 a	0 a	40 a
25	820 ab	0 a	120 a
30	1010 b	135 ab	120 a

Note: See Table 1.

activity in foot rot control. Drenching of gerbera with furalaxyl was more effective than application of GE (Tab. 3).

Several plant extracts were shown to effectively reduce soil-borne pathogen population, especially *Fusarium oxysporum* and increase symptomless plant stand (Bowers and Locke 2000; Orlikowski and Saniewska 1995). In Bowers and Locke (2000) trials different of *F. oxysporum* react in similar manner to tested paper/mustard, cassia and clove treatment. The authors concluded that results obtained in one system might be applicable to another.

Grapefruit extract was developed for foliar application and little data exist for its activity in soil or substrata. As such, using of soil leachate as the medium for *P. cryptogea* growth *in vitro* it was possible to determine if grapefruit extract affected the pathogen. *In situ* approach, closer to production conditions in greenhouse, an assay carried out *in vitro* was confirmed. The product studied applied at dose 40 µg of a.i./cm^3 inhibited the mycelial growth in 50% but zoosporangia formation about 95%. It indicates that zoosporangia are much more affected by grapefruit extract than hyphae. Even when mycelial disks were incubated for 2 days in clean soil leachate and sporulation of the pathogen was already visible, their transferring to solution containing 40 µg of GE/cm^3, suppressed zoosporangia formation

Table 3

Activity of grapefruit extract in the control of *Phytophthora* foot rot of gerbera; number of diseased plants (n = 10)

Treatment	µg of a.i./cm^3	Weeks after planting			
		2	5	7	9
Control	–	1.25 b	3.0 c	6.25 c	8.75 c
Grapefruit extract	165	0.25 a	0.5 ab	2.5 b	4.0 b
Grapefruit extract	330	0.5 a	1.25 b	2.5 b	3.25 b
Furalaxyl	125	0 a	0.25 a	0.5 a	0.75 a

Note: See Table 1.

about 88%. Data obtained indicated on direct action of grapefruit extract on the pathogen by inhibition of its growth and sporulation, deformation of zoosporangia and disintegration of hyphae. Drastical reduction of colony forming units number of *P. cryptogea* in peat, amended with grapefruit extract, confirmed that hypothesis. Liu et al. (1990) concluded, that limonin and 5 of its derivatives, extracted from grapefruit seeds, are microbial inhibitory compounds. In trials of Woedtke et al. (1999) benzethonium chloride, methyl parabene and triclosan, obtained from grapefruit seeds, suppressed development of 6 species of bacteria. Recently Angioni et al. (1999) found that 7-geranoxycoumarin also extracted from grapefruit, strongly affected the development of *Penicillium*. It is possible that some of those compounds or their complex are inhibitory agents for *P. cryptogea*.

In greenhouse experiments grapefruit extract affected *P. cyptogea* forming units present in amended peat. Drastical decrease of the pathogen population within 5 days and its very low level in the next 25 days resulted in the inhibition of *Phytophthora* foot rot symptomless gerbera stand. Additionally, 7-geranoxycoumarin, present in grapefruit extract, may induce resistance of gerbera on *P. cryptogea*. Further research in this area has the potential to extend the usefulness of grapefruit extract in crop protection.

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WPŁYW WYCIĄGU Z GREJPFRUTA NA ROZWÓJ *PHYTOPHTHORA CRYPTOGEA* I ZDROWOTNOŚĆ GERBERY

STRESZCZENIE

Celem badań było określenie aktywności biologicznej wyciągu z grejpfruta na wzrost i zarodnikowanie *Phytophthora cryptogea* oraz rozwój fytoftorozy gerbery (*Gerbera jamesonii*). Doświadczenia przeprowadzono w laboratorium i w szklarni. Dodatek do agaru ziemniaczano-glukozowego 40 µg wyciągu z grejpfruta/cm^3, powodował zahamowanie wzrostu *P. cryptogea* o około 50%. Jeżeli taką samą ilość wyciągu dodawano do ekstraktu glebowego, a następnie wprowadzano grzybnię patogenu, formowanie się zarodni pływkowych było hamowane w około 95%. W tymże stężeniu oraz dawce 5-krotnie wyższej wyciąg z grejpfruta powodował deformację tworzących się zarodni pływkowych oraz rozpad strzępek grzybni. Podlanie torfu sztucznie zakażonego przez *P. cryptogea* roztworem wyciągu z grejpfruta (165 µg/cm^3) powodowało spadek liczebności patogenu o co najmniej 70% i stan ten utrzymywał się przez 30 dni. Badany produkt chronił około 50% roślin gerbery przed *P. cryptogea*, gdy zastosowano go doglebowo, bezpośrednio po sadzeniu roślin.