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ORIGINAL RESEARCH

Control of citrus pathogens by protein extracts from *Solanum tuberosum* tubers

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Abstract A national program for citrus certification was started in Argentina in 2005 in order to provide healthy fruits free of toxic residues. In line with this goal, the aim of this study was to evaluate the efficacy of natural products, protein extracts obtained from potato tubers for the control of fungi responsible for disease in post-harvest citrus fruits. Different protein fractions were obtained from *Solanum tuberosum* tubers (IF25, IF50, SF25 and SF50) and their effect were evaluated on *Penicillium digitatum* and *Geotrichum candidum*, two citrus-pathogenic fungi. All fractions showed antifungal activity against both fungi species, the intensity of this activity being dependent on the type of fungus and extract. The fraction IF25 was the most active as an antifungal agent: it inhibited the mycelia growth of both pathogens, the elongation of the germ tube of *P. digitatum* and the conidial isotropic growth of *G. candidum* as well as its polygalacturonase activity. None of the IF25 concentrations were mutagenic in *Salmonella typhimurium* TA98 or TA100 strains. The efficacy of protein extracts to control *P. digitatum* and *G. candidum* growth was tested in artificially inoculated citrus fruits. Extracts were tested built-in to the wax used in citrus industry to coat and protect the fruit. The IF25 extract was effective in inhibiting the growth and development of *P.digitatum* and *G. candidum*. Consequently, the IF25 extract plus the wax could be used preventively in controlling fungal infection on post-harvest citrus.

Keywords Potato protein extracts · Antifungal activity · Citrus fruit · *Penicillium digitatum* · *Geotrichum candidum*

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Introduction

About 25 % of economic loss in harvested fruits and vegetables is caused by pathogens during harvesting, processing, packing, and transporting of the commodity (Barkai-Golan 2001; Droby et al. 1991; Narayanasamy 2006; Wisniewski and Wilson 1992). Green mold caused by *Penicillium digitatum* Sacc. is an important post-harvest citrus disease (Eckert and Eaks 1989; Whiteside et al. 1993). This disease, as with other citrus diseases, is currently managed by using synthetic fungicides. However, there is a growing global concern about the continuous use of synthetic chemicals on food crops because of their potential effects on human health

and the environment (Barkai-Golan 2001; Norman 1988; Schirra et al. 2011). Pathogen resistance is another factor against the continuous use of synthetic fungicides (Brent and Hollomon 2007; Eckert and Wild 1983). These concerns have resulted in a renewed interest in the search for alternative control measures. Plant extracts are one of several non-chemical control options that have recently received attention. The potential of plant extracts for the control of plant diseases has long been recognized (Balestra et al. 2009; Deberdt et al. 2012; Mendes Andrade et al. 2010; Uppal et al. 2008). Nevertheless, the current use of these products for the control of plant diseases is still scarce, particularly for the control of post-harvest diseases (Gatto et al. 2011; Sayago et al. 2012; Sukorini et al. 2013).

Solanum tuberosum tubers are an important vegetable used in many food preparations. The tubers have bioactive compounds such as steroidal alkaloids, flavonoids and proteins having antioxidant and/or antimicrobial activities (Esteves-Souza et al. 2002; Kim et al. 1996; Kusano et al. 1987; Ordoñez et al. 2011; Rowan et al. 1983). In previous work we purified and characterized a protein called solamarine isolated from potato tubers (Isla et al. 1991, 1992, 1999). This protein is a single polypeptide (Mr 18 kDa) that has antimicrobial activity against phytopatogenic bacteria and fungi (Isla et al. 2002). Other antimicrobial proteins involved in plant defence mechanisms have been reported to be present in potato tubers, such as patatin, and snakin 1 and 2 (Andrews et al. 1988; Bártová and Bárta 2009; Berrocal-Lobo et al. 2002; Ordoñez et al. 2012; Segura et al. 1999). Also, a potential antimicrobial activity might be attributed to aqueous extracts from potato tubers.

Argentina is the major lemon-producing country in the world and 90 % of the production comes from the Province of Tucuman (1.25 million tons) (Federcitrus 2005). Total net area covered with citrus groves in 2012 was 37.440 ha (Fandos et al. 2012). In our region, the genus *Penicillium* causes various post-harvest diseases and, hence, economic losses. Other fungi such as *Geotrichum*, *Alternaria*, *Botrytis*, also cause citrus fruit diseases to a lesser extent (Asociación Tucumana de Citrus 2013). Synthetic fungicides currently used (imazalil, tiabendazol) produce many problems such as the development of resistance, environmental pollution and accumulation of toxic wastes to humans who consume and/or handle these foodstuffs. These factors account for the increased interest in our region in using non-toxic natural fungicides.

Recently, a national program for citrus certification was started in Argentina in 2005, and became compulsory in 2010 in order to provide healthy fruits free of toxic residues. So the aim of this study was to evaluate the efficacy of aqueous extracts enriched in protein obtained from potato tubers for the control of fungi responsible for post-harvest disease in citrus fruits.

Materials and methods

Plant material

Solanum tuberosum tubers var Kenebeck were obtained from a local supermarket.

Preparation and characterization of potato aqueous extracts (PAE)

Extraction of protein fractions

Potato tubers were washed with water, peeled and homogenized by using a high-speed blender to obtain slurry containing cell walls, juice and starch. In order to separate the solid components, the slurry was centrifuged at 10,000×*g* for 30 min. Soluble fraction (**SF**) and insoluble fraction (**IF**) were obtained. The **IF** was washed with distilled water, resuspended in 1 M NaCl and stirred overnight at 4 °C. It was, then, centrifuged at 10,000×*g* for 30 min and the supernatant was first taken up to 25 % saturation with solid ammonium sulphate and, then, up to 50 %. Precipitated fractions between 0 and 25 % and 25–50 % were recovered, dialyzed against 10 mM sodium phosphate buffer pH 7.3 and named IF25 and IF50, respectively. The SF was, thereafter, adjusted to pH 4.0 and centrifuged at 10,000×*g* for 30 min. The supernatant was discarded and the precipitate was resuspended in 0.2 M NaCl and stirred overnight at 4 °C. After that, the suspension was centrifuged at 10,000×*g* for 30 min and the supernatant was collected and fractioned with solid ammonium sulphate. The 0–25 % (SF25) and 25–50 % (SF50) fractions were recovered, dialyzed against 10 mM sodium phosphate buffer pH 7.3.

Soluble protein determination

Soluble protein content was determined by Bradford reagent (BIORAD), by using bovine serum albumin as standard (Bradford 1976).

SDS-PAGE

Samples (2 μg of protein) were treated and analyzed by electrophoresis as described by Laemmli (1970). Proteins were detected by $AgNO_3$ impregnation.

Agglutination assays

Human blood from healthy donors was collected in 10 mM EDTA. Erytrocytes (type A, O and B) were washed three times with 0.15 M NaCl (pH 7.0) and adjusted to 5 % (*w*/*v*). The agglutination assays were carried out in small glass tubes in a final volume of 250 μl containing 100 μl of each protein fraction, 50 μl of 5 % suspension of red blood cells and 100 μl of 0.15 M NaCl. Titre was recorded visually after 60 min at room temperature and was defined as the reciprocal of the highest dilution showing detectable agglutination in assay conditions.

Polygalacturonase inhibition assays

Polygalacturonase from *G. candidum* was obtained according to Torres et al. (2011).

The reaction mixture contained 20 μl of *G. candidum* polygalacturonase enzyme preparation (1.54 UE), a volume equivalent to 200 μg protein of each protein fraction, 40 μl of 0.2 M sodium acetate buffer pH 5.5 and distilled water. Enzyme reactions were started by the addition of the substrate, 10 μl of (4 %, *w*/*v*) sodium polygalacturonate (Na-PG), to the reaction mixtures that were then incubated at 37 °C for 30 min.

The amount of Na-PG hydrolyzed was determined by measuring the increase in reducing groups during the reaction course by using D-galacturonic acid as standard. The enzymatic reaction was stopped by the Cu alkaline reagent (Somogyi 1945) and reducing power was measured according to Nelson (1944). One enzyme unit was defined as the amount of enzyme required to release 1 μmol of reducing groups per minute under the standard assay conditions.

Mutagenicity of protein fractions

The mutagenicity assay with *Salmonella typhimurium* was performed as described by Maron and Ames (1983). In the Ames test, $His^- \rightarrow His^+$ mutations are visualized by plating *S. typhimurium* bacteria in a histidine poor growth medium. In this medium only His^+ mutants are able to form visible colonies. Strain TA98 gives an indication of frame-shift mutations, while a positive response from strain TA100 indicates base-pair substitution. Briefly, one hundred microlitres of an overnight culture of bacteria (cultivated for 16 h at 37 °C, approximate cell density of $2–5 \times 10^8$ cells/ml), different concentrations of protein fractions (500; 750 and 1000 μg protein/plate) and 500 μl of sodium phosphate buffer (0.2 M, pH 7.4) were added to 2 ml aliquots of top agar (containing traces of D-biotin and L-histidine). The resulting complete mixture was poured on minimal agar plates prepared as described by Maron and Ames (1983). The plates were incubated at 37 °C for 48 h and the revertant bacterial colonies of each plate were counted. An extract was considered mutagenic when the number of revertants per plate was more than twice the number of colonies produced on the solvent control plates (spontaneous revertant frequency). Samples were tested in duplicate with two replicates. Sodium phosphate buffer was used as a negative control, and 4-nitro-o-phenylenediamine (4-NPD), 10 μg/plate, were used as positive controls.

To discriminate citotoxicity, the number of surviving cells was determined by plating appropriate dilutions of treated bacterial suspension onto complete agar plates.

Antifungal activity

Fungal cultures

Two fungal strains (*Penicillium digitatum* Link IEV 548 and *Geotrichum candidum* Butler IEV 543) were used (Culture collection of the Instituto de Estudios Vegetales, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Argentina). Both pathogenic fungi were isolated from lemon fruit with green mold or sour rot diseases, respectively. Stock cultures were maintained on Sabouraud agar (SA: Merck) at 4 °C. SA and potato-glucose agar (P-GA) were used for routine fungus cultures. Pectin Agar (PA: 3 % citric pectin, 1 % beef peptone, 2.2 % agar, final pH 5.4–5.6) and Pectin Broth with citric pectin as

carbon source (PB: 3 % citric pectin, 1 % peptone, pH 5.4–5.6) were also used.

Effect of potato aqueous extracts on spore germination

P. digitatum and *G. candidum* spores from 7 days old cultures (SA, 28 °C) were harvested and suspended in sterile saline solution. Increasing concentrations of PAE (125–1000 μg of protein/ml) in a final volume of 1 ml of P-G Broth (1 % peptone and 3 % glucose) were prepared and then inoculated with 1×10^6 spores/mL. A control of spore inhibition (positive control with Metronidazol, 40 μg/ml) was also assayed. The whole set was incubated for 12 h at 28 °C. The percentage of germination at each PAE concentration was then evaluated by counting the germinated spores in a Neubauer chamber (400X, Zeizz Primo Star microscope).

The results were expressed as % germination inhibition, % *Inh.* = (*C-T*/ *C*) *x 100*, where *C* is the amount of germinated spores in 1 μl of the suspension without extract, and *T* is the amount of germinated spores in 1 μl of the suspension containing PAE, the results being the average of two independent experiments.

Determination of cellular viability

The assay was carried out as stated above. After incubation of spores with a germination inhibitory concentration (GIC, 1 mg/ml) of PAE for 12, 24 or 36 h at 28 °C, the suspensions were washed three times with sterile saline solution. Afterwards, the spore suspensions were used to inoculate P-G Broth and incubated at 28 °C for 12 h. Then, the spore germination for each treatment was evaluated.

Effect of potato aqueous extracts on spore size

The assay was carried out as previously described. The spore size was determined by using an optical microscope with a micrometer scale (40X, Zeizz Primo Star microscope). The spore was grouped according to the following scale: up to 5±1 μm, 7.5±1 μm, 10±1 μm or 12±1 μm for *P. digitatum* and up to 7.5±1 μm, 10±1 μm, 12.5±1 μm for *G. candidum*. More than 100 cells were counted per treatment.

Effect of protein aqueous extracts on mycelial growth

A plate macrodilution test was used to determine the minimal inhibitory concentration (MIC) of the most active extracts. Different amounts (100–1000 μg of protein/ml) of PAE were incorporated into Petri dishes (diameter, 5 cm) containing 5 ml of Pectin Agar. All plates were surface-inoculated with a volume of suspension containing 500 spores and incubated at 28±2 °C for 96 h. Plates without extract were used as growth control. MIC_{100} was defined as the lowest extract concentration with no visible growth after incubation, while MIC_{50} was defined as the extract concentration necessary to produce an inhibition of 50 % of mycelial growth. The mycelial growth inhibition or sporulation at different extract concentrations were calculated by comparing the mycelial growth or sporulation between treated and control plates.

Fruit protection assay

Assays of postharvest fruit protection (preventive effect of whole fruits previously infected with *P. digitatum or G. candidum*) were carried out according to Sayago et al. (2012).

A fixed volume of PAE was mixed with wax in order to get 1000 μg of proteins/ml. Then 1 ml of this mixture was applied onto the lemon surface. After 24 h, spore suspension (1×10^5 spores/ml) of *P. digitatum* (IEV 548 strain) or *G. candidum* (IEV 543 strain) was applied on the whole fruits. The assay included a set of fruits without treatment, a set of fruits inoculated with the fungus, a set of fruits treated with wax and inoculated with the fungus, a set of fruits treated with imazalil (200 ppm) and a set of fruits treated with wax containing the extract and inoculated with the fungus.

In all cases the treated fruits were maintained during 7 days at 25±2 °C in closed boxes. The macroscopic evaluation of the decay progress was carried out every 24 h up to 7 d. The test was performed in triplicate. In the assay with *P. digitatum*, the index of severity (S) was calculated as follows: $S=\sum F/N$; where N=total number of fruits, and $F=E\times$number of fruits with different severity grades; the severity grade being (E), may be from E_0 to E_4 according to Fig. 1. The index of incidence (I) was estimated in the assay with *P. digitatum* and *G. candidum*, as *I=number of infected fruits/ N*.

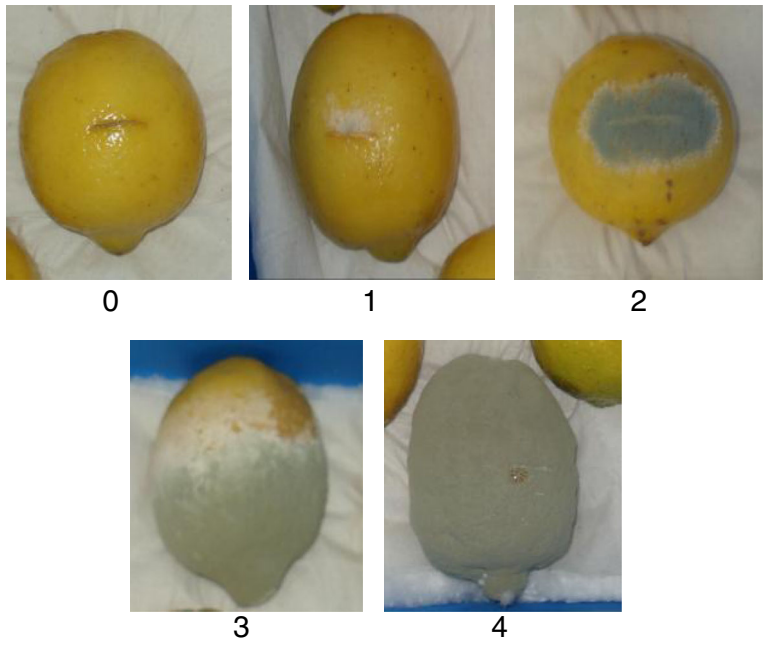

Fig. 1 Severity grade (*E*) of lemons infection with *Penicillum digitatum*. Severity grade was assessed according to the extent of the infection from 0 to 4

Statistical analysis

Data are represented as a mean±standard deviation. Analysis of variance (one-way ANOVA; Minitab® 16.1.0) was performed by using a probability level of less than 5 % ($p<0.05$) when appropriate. In the case of disease incidence assays, one-way ANOVA was applied to arcsin-transformed data. Multivariate analysis of variance (MANOVA) was performed in the case of assays on spores size and mycelial growth in the presence of IF25 extract (Hotelling´s test, $p=0.05$).

Results

Protein fraction characterization

Four protein fractions called SF25, SF50, IF25 and IF50 were prepared from fresh potato tubers. The yield of obtained fractions was around 10 to 120 mg of total protein/100 g potato, respectively. The protein profiles of the each extract were analysed by SDS-PAGE (Fig. 2). All fractions were enriched in low molecular weight proteins around 10 and 20 kDa. Moreover SF50 and IF50 showed protein profiles also enriched in proteins with molecular weight of 34 kDa. Furthermore, SF25 and IF25 showed agglutinating activity of red blood cells (data not shown).

Effect of protein extract fractions on polygalacturonase activity from *G. candidum*

Endo-polygalacturonases (endo-PG) would be one of the enzymes secreted by fungi pathogens in their invasive mechanism on plant tissue. We evaluated the effect of each fraction obtained from aqueous extract of potato tubers on polygalacturonase activity from *G. candidum*. The fractions SF25, IF25 and IF50 at the same

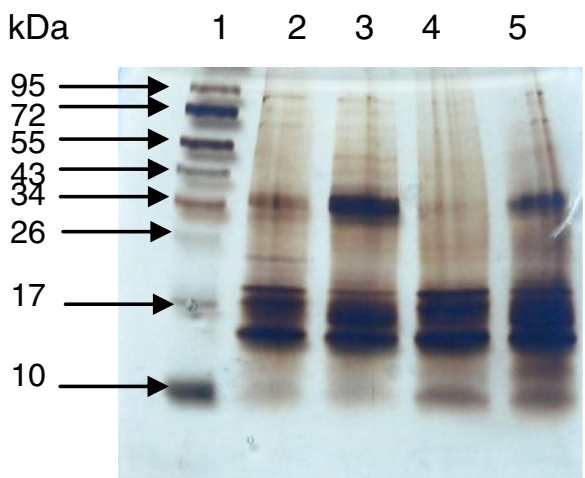

Fig. 2 SDS-PAGE of protein fractions (2 μg of protein) obtained from crude extracts of *S. tuberosum*. Proteins were stained using the silver staining procedure. Line 1: molecular mass standards. The *arrows* indicate de molecular weight of each marker; line 2: IF25; line 3: IF50; line 4: SF25; line 5: SF50

concentration (1 mg/ml) showed inhibitory effect on enzyme activity with no significant differences between them (50 and 60 %). The SF50 fraction did not show inhibitory activity (Table 1).

In vitro antifungal activity assay

Effects of extracts on spore germination

The antifungal activity of SF25, SF50, IF25 and IF50 was analysed by measuring their effect on the fungus spore germination. Figure 3 shows that all extracts assayed were effective against *G. candidum*, with values of germination inhibition between 30 and 60 %, IF25 being the most effective. On the other hand, IF25 also resulted in a greater germination inhibition of *P. digitatum* spores (60 %) followed by SF50 (both at 1 mg/mL). The behaviour of all the extracts against *P. digitatum* showed a dose–response relationship.

Effect of IF25 on size and viability of phytopathogenic spores

Since the IF25 fraction was a good inhibitor of polygalacturonase activity, and of spore germination from both *G. candidum* and *P. digitatum*, this fraction was selected for further studies. The IF25 extract showed no effect on *P. digitatum* spore size (Fig. 4a). Otherwise, the spores of *G. candidum* when exposed to the protein extract were affected in terms of their size, showing a marked difference as compared with the control, in which smaller spores prevailed (5 μm) (Fig. 4b). The same behaviour was observed in the presence of the synthetic fungicide metronidazol.

The viability recovery by pathogenic spores from both fungi (*P. digitatum* and *G. Candidum*) after a 12 h exposure to IF25 (up 1 mg/ml) was affected. When different exposure times were assayed (12, 24 and 36 h) the percentage of spore viability at 36 h was observed to be between 0.6 and 3.5 % for *P. digitatum* and *G. candidum*, respectively.

Table 1 Effect of fractions obtained from potato tubers (1 mg/mL) on polygalacturonase activity from *G. candidum*

Soluble (SF) and Insoluble (IF) fractions obtained from potato tubers	% of residual activity[a]
SF 25	42.53±0.6
SF 50	93.26±3.8
IF 25	52.36±1.8
IF 50	41.37±1.5

[a] The results are expressed as mean±S.D

In vitro effect of IF25 on mycelial growth

The IF25 fraction was able to inhibit 75 % of *P. digitatum* mycelial growth at 1 mg/ml and 36 h, while at the same concentration and incubation time *G. candidum* showed 32 % of mycelial growth inhibition (Fig. 5).

Postharvest fruit protection using IF25 (in vivo tests)

Based on in vitro assays, the IF25 preventive effect on fresh lemon fruits was assayed under laboratory conditions. The extract was applied before the artificial inoculation with *P. digitatum* or *G. candidum* spores. Aqueous extracts (IF25) significantly reduced the incidence of green mold caused in citrus fruits by *P. digitatum*. The percentages of lemons with the disease symptoms decreased with the treatment with 1 mg IF25/ml compared with control (Fig. 6a), with an incidence values of 60 %. The severity index of lemons treated with IF25 and then inoculated with *P. digitatum* showed a significant difference with the infection control but not with the disinfection control and the imazalil control (Fig. 6b).

Furthermore, when the fruits treated with potato extract were inoculated with *G. candidum*, the percentages of lemons with sour rot symptoms decreased as compared with the control (Fig. 7), with an incidence value of 20 %. The incidence percentage was similar to a positive control prepared with a commercial antifungal (imazalil).

The severity index in lemons treated with IF25 and inoculated with *G. candidum* was similar in all lemons with sour rot symptoms. The lemons showed a softening area around the infection site without apparent development of mycelium during 7 days.

Furthermore, no visible phytotoxicity symptom was detected on fruits treated with IF25 extracts.

Mutagenic activity

In this study, IF25 mutagenicity was evaluated by the Ames assay. In a series of experiments preceding the mutagenicity studies, the different amounts of protein added to the indicator bacteria was ascertained not to

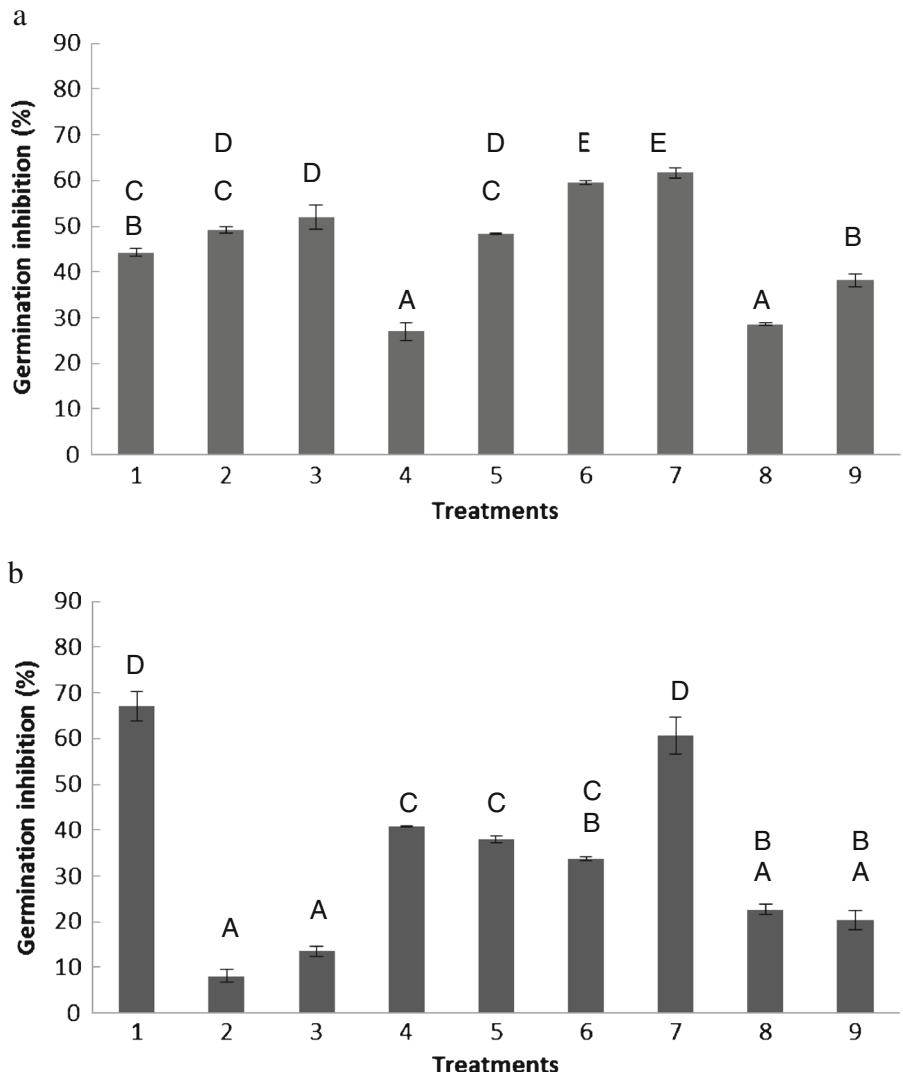

Fig. 3 Effect of potato aqueous extracts (PAE) on spore germination of *G. candidum* (**a**) and *P. digitatum* (**b**). Percentage of inhibition of spore germination was calculated by counting the germinated spores in presence and absence of PAE, according to the following equation: % *Inh*. = (*C-T*/ *C*) *x 100*, where *C* is the amount of germinated spores without PAE and *T* is the amount of germinated spores in presence of PAE. *1*, Positive control; *2*, SF25 (0.5 mg); *3*, SF25 (1 mg); *4*, SF50 (0.5 mg); *5*, SF50 (1 mg); *6*, IF25 (0.5 mg); **7**, IF25 (1 mg); *8*, IF50 (0.5 mg); *9*, IF50 (1 mg). Means with the same letter are not significantly different

influence their viability. Table 2 shows the number of revertants/plate after the treatments with IF25 in the two different *S. typhimurium* strains.

Discussion

Several antimicrobial proteins (with low molecular weight) were reported in potato tubers (Andrews et al. 1988; Bártová and Bárta 2009; Berrocal-Lobo et al. 2002; Isla et al. 2002; Segura et al. 1999). In this study, we evaluated for the first time the antifungal activity of aqueous extracts (enriched in proteins of low molecular weight) from potato tubers on citrus pathogens. Through a method of removing soluble and insoluble material and fractional saline precipitation, four fractions were obtained. SF25 and IF25 had a similar protein profile with low molecular weight proteins (20–15 kDa) and haemagglutinating activity while SF50 and IF50 was also enriched with protein of about 34 kDa.

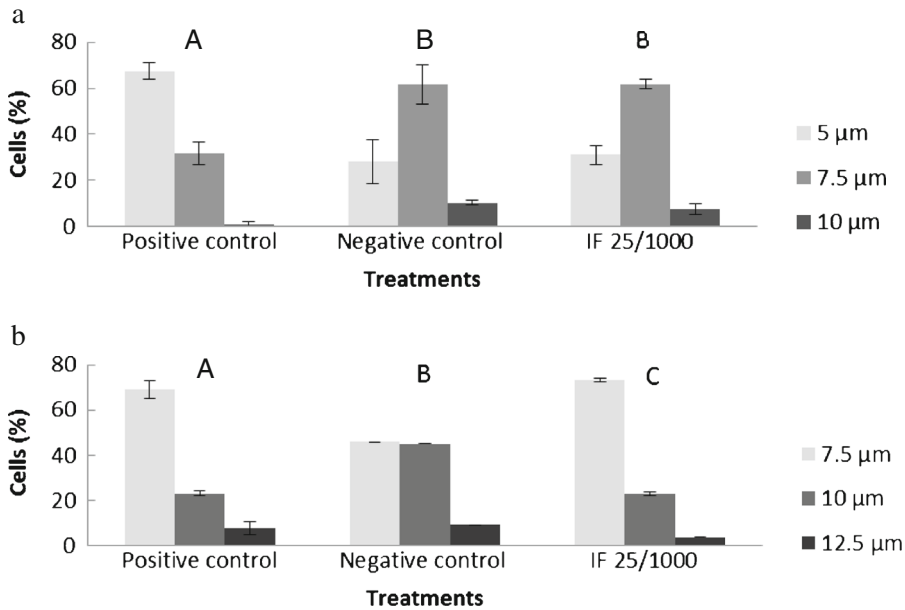

Fig. 4 Effect of extract IF25 (1 mg/mL) on the size of spores from *P. digitatum* (**a**) and *G. candidum* (**b**). The spores size was measured using an optical microscope with a micrometer scale ($n>100$). Spores were grouped according their sizes in: 5, 7.5, 10 and 12 µm for *P. digitatum* and 7.5, 10 and 12.5 µm in *G. candidum*. Means with the same letter are not significantly different

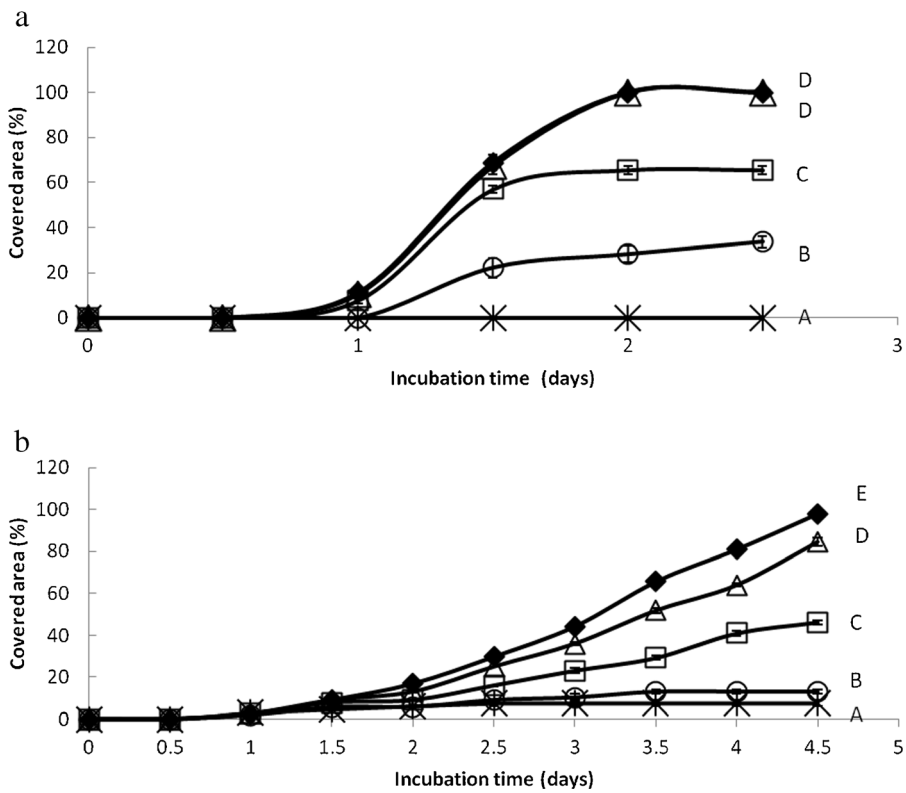

Fig. 5 Effect of different concentrations of IF25 (100–1000 µg of protein/ml) on the mycelial growth of *P. digitatum* (**a**) and *G. candidum* (**b**) determined by macrodilution agar method. The inhibition of mycelial growth was calculated by comparing the mycelial growth between treated and control plates. Negative control (◆), 250 µg/ml (△), 500 µg/ml (□), 750 µg/ml (○), 1000 µg/ml (×). Means with the same letter are not significantly different

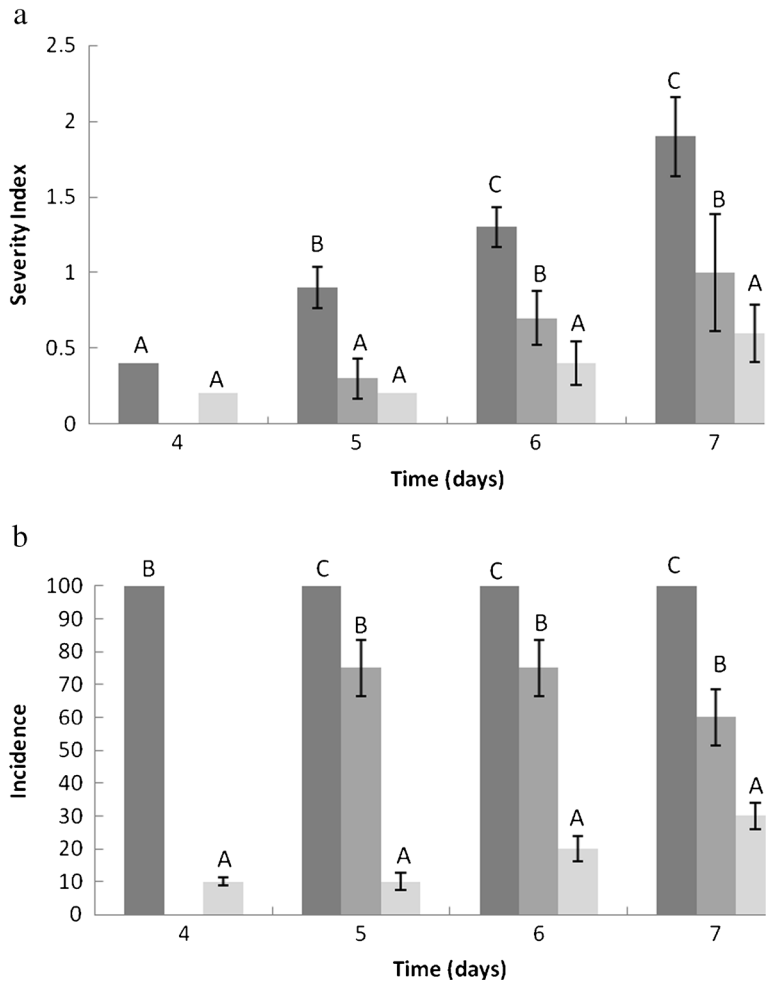

Fig. 6 Index of severity "*S*" (**a**) and incidence "*I*" (**b**) of the disease (*green mold*) caused by *P. digitatum* on harvest lemon fruits. One (1) ml of wax containing 1000 μg proteins/ml was applied on the lemon surface. After 24 h, 1×10^5 spores/mL of *P. digitatum* (IEV 548 strain) were applied on the whole fruits which were maintained 7 days at 25 °C. $S=\sum F/N$; *N*=total number of fruits, and *F=E x number of fruits with different severity grade*; the severity grade "*E*", may be from E_0 to E_4 according to Fig. 1. *I= number of infected fruits/N*. Controls of protein extracts, disinfection, wax and wax plus inoculum were performed. Treatment: (■) wax plus extracts plus inoculum; (■) inoculum; (□) imazalil plus inoculum. Means with the same letter are not significantly different

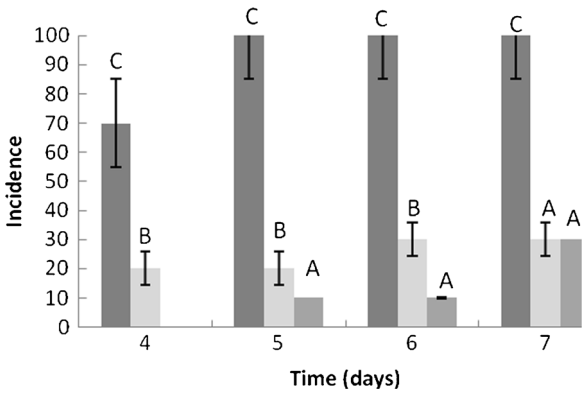

Fig. 7 Incidence "*I*" of the disease caused by *G. candidum* on harvest lemon fruits. One (1) ml of wax containing 1000 μg proteins/ml was applied on the lemons surface. After 24 h, 1×10^5 spores/ml of *G. candidum* were applied on whole fruits, which were maintained 7 days at 25 °C. Controls of protein extracts, disinfection, wax and wax plus inoculum were performed. Treatments: (■) wax plus extracts; (■) inoculum; (□) imazalil plus inoculum

Table 2 Revertant strains of TA98 and TA100 *Salmonella typhimurium* after treatment with various doses of proteic extracts of *Solanum tuberosum* tuber

IF25 (μg/plate)	Revertant number/plate[a]	
	TA98	TA100
500	23±1 A	135±15 A
750	17±1 A	131±11 A
1000	21±1 A	139±2 A
Positive control[b]	1078±89 B	963±62 B
Negative control[c]	21±4 A	133±11 A

[a] Mean number of revertants [Mean of four plates±S.D.]

[b] Mean number of revertants induced by reference mutagen 4-NPD, 4-nitro-o-phenylenediamine (10 μg/plate) positive control

[c] The number of spontaneous revertants was determined in assays without protein samples

Soluble and insoluble fractions exhibited different antifungal effects. IF25, SF25 and SF50 showed approximately 50 % of inhibitory activity on *G. candidum* polygalacturonase, one of the hydrolytic enzymes responsible for the invasion mechanism on plant tissue. IF25 was able to affect the swelling and germination of *G. candidum* spores. Based on these results we proposed that the IF25 extract may act by inhibiting the conidial isotropic growth and germ tube emergence of *G. candidum* spores. In addition, IF25 was able to inhibit the mycelial growth of the fungus.

The protein fraction IF25 was able to inhibit the *P. digitatum* spores germination (60 %) and mycelial growth without affecting the spore size and cellular viability. Since the spore viability did not recover after contact with an inhibitory germination concentration and subsequent incubation at different times, we can infer that the extract has a fungicidal effect and may act by inhibiting the germ tube elongation.

None of the IF25 concentrations were mutagenic in TA98 or TA100 strains under the conditions used in this assay, which indicated the non-existence of mutagens that cause base pair substitution (detected in TA100) and frameshift (detected in TA98) mutations. The absence of mutagenicity for the protein preparation in the *Salmonella* tested strains indicates that DNA did not seem to be a relevant target for IF25.

By in vivo assays, we observed that extracts reduced significantly disease produced by *P. digitatum* and *G. candidum* on fresh fruits, with their action being preventative. Some features should be considered in order to analyze the possible IF25 action mechanisms on pathogenic fungi. Their antifungal activity might be due to protein direct action on fungal growth and/or cellular structure plus the inhibitory effect on fungal polygalacturonase, one of the hydrolytic enzymes responsible for the invasion mechanism on plant tissue.

The fungicidal activity of potato aqueous extract provides a new opportunity to improve control of different citrus diseases that cause postharvest losses in citrus fruits, in particular through a preventive effect by using a natural product obtained from an abundant and economic resource such as potato tubers.

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