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In vitro and *in vivo* Antifungal Activities of Organic and Inorganic Salts Against Citrus Sour Rot Agent *Geotrichum candidum*

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Abstract: The aim of this study was to find an alternative to the chemical fungicide currently used in the control of postharvest citrus sour rot. Here we screened thirty-two salt compounds, considered as common food additives, for their activity against *Geotrichum candidum*, causal agent of citrus sour rot. The lowest Minimum Inhibitory Concentrations (MICs) values were obtained by ammonium carbonate and EDTA at a concentration of 0.1% (w/v) and boric acid, sodium carbonate and sodium metabisulfite at 0.25% (w/v). Over all, the medium-pH in the range of 4.0 to 12.0 did not influence the mycelial growth of the pathogen. The ten best salt compounds were tested for their ability to reduce the arthrospores germination of the fungus. The effect of salts varied significantly ($p<0.05$) between tested compounds and depended on their concentrations. The arthrospore germination was completely inhibited by EDTA, boric acid, sodium metabisulfite, sodium carbonate, sodium sulfate and sodium thiosulfate, both at 100 and 75 mM. The most active salts in *in vitro* studies were tested *in vivo* against sour rot on citrus fruit. Incidence of sour rot was lowered to 25.93 and 38.89%, when mandarin fruit where treated by sodium salicylate, boric acid and EDTA, compared with 100% in the control. However, only the application of boric acid at 3% (w/v) reduced disease severity by more than 70%. These results suggest that sodium salicylate, boric acid and EDTA may be useful and effective compounds for control of citrus sour rot. Such healthy products therefore represent a sustainable alternative to the use of guazatine mainly in organic production.

Key words: Citrus, sour rot, *Geotrichum candidum*, chemical fungicide, organic production

INTRODUCTION

Sour rot caused by the fungus *Geotrichum candidum* is one of the major postharvest diseases of citrus fruits (Kitagawa and Kawada, 1984). It is one of the most economically important postharvest diseases of citrus in arid growing regions of the world (Smilanick and Sorenson, 2001) and cause serious problems for harvested citrus fruits during handling, transportation, exportation and storage (El-Mougy *et al*., 2008). The organism is a wound pathogen, inflicted during harvest and subsequent handling and requiring injury into the albedo for entry (Brown, 1979). Besides the injuries, the aggressiveness of the fungus increases especially during fruit degreening, wet and rainfall seasons (Eckert, 1978; Brown and Eckert, 1988; Cohen *et al*., 1991; Liu *et al*., 2009).

The measures employed to manage postharvest citrus rot are not effective against *Geotrichum candidum.* This pathogen is not controlled by any of the fungicides (e.g., Imazalil and Thiabendazole) registered for use on citrus fruit (Eckert, 1978; Feng *et al*., 2011; Liu *et al*., 2009; Smilanick *et al*., 2008; Mercier and Smilanick, 2005; Brown, 1988; Suprapta *et al*., 1997; Kitagawa and Kawada, 1984). Guazatine is the only commercial fungicide that can control sour rot (Brown, 1988; Rippon and Morris, 1981). However, this fungicide is not authorized in several countries. The disease can be partially reduced by Sodium o-phenylphenate (SOPP) (Rippon and Morris, 1981; Feng *et al*., 2011) which is found to be carcinogenic and has promoting activity towards the urinary bladder (Kitagawa and Kawada, 1984). Therefore, alternative treatments have become an essential requirement for the control of this disease. Furthermore, concerns about public health risks associated to fungicide residues and environmental issues have increased the need for these alternatives. Use of organic and inorganic salts, Generally Recognized as Safe (GRAS) compounds, is an interesting alternative to control postharvest disease of citrus fruits. Many of these salts have several advantages such as low mammalian toxicity, favorable safety profile for humans and environment and a relatively low cost (Olivier *et al*., 1998; Hervieux *et al*., 2002; Deliopoulos *et al*., 2010).

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Moreover, these compounds have a broad-spectrum antimicrobial activity (Corral *et al*., 1988; Olivier *et al*., 1998; Deliopoulos *et al*., 2010) and are usually used in the food industry for controlling pH, taste and texture (Smilanick *et al*., 1999; Hervieux *et al*., 2002; Arslan *et al*., 2009). Furthermore, several studies have reported the effectiveness of salts to control various pathogens of many crops. Potassium sorbate (KS) was shown to reduce the incidence of sour rot under laboratory conditions (Kitagawa and Kawada, 1984; Smilanick *et al*., 2008; El-Mougy *et al*., 2008). Sodium benzoate and KS were used to control postharvest decays caused by many fungi (Al-Zaemey *et al*., 1993; Olivier *et al*., 1998; El-Mougy *et al*., 2008; Palou *et al*., 2001). Palou *et al*. (2009) and Smilanick *et al*. (1999) demonstrated that sodium carbonate reduced the incidence of citrus green and blue mold. Moreover, salts compounds also improve their performance when used in combination with other treatments like microbial antagonists (Nunes *et al*., 2002; El-Ghaouth *et al*., 2000; Zhang *et al*., 2008; Sharma *et al*., 2009), fungicides (Smilanick *et al*., 2008) or hot water (Palou *et al*., 2001; Porat *et al*., 2002; Smilanick *et al*., 2008). Although most researches have focused on controlling green and blue mold, little has been published about the control of sour rot.

The present study was performed to evaluate the efficacy of a wide range of organic acids and salts, for *in vitro* and *in vivo* control of *G. candidum,* the causal agent of citrus sour rot.

MATERIALS AND METHODS

Pathogen culture and chemicals: *Geotrichum candidum* was isolated from a decayed Clementine fruit and was one of the most aggressive isolates in our collection. The fungus was maintained on PDA plates at 5°C, with periodic transfers through citrus fruit to maintain its aggressiveness. The pathogen inoculum that consisted of aqueous arthrospores suspensions obtained from 7-day-old culture plates incubated at 25°C. Arthrospores were harvested by flooding plates with 5 mL of sterile distilled water containing 0.05% (v/v) Tween 80 and passing the suspension through two layers of sterile cheesecloth to remove hyphal fragments. The arthrospores concentration was determined with the aid of a heamacytometer and adjusted to 10^6 arthrospores mL^{-1} with sterile distilled water.

A total of 32 salt compounds (Table 1) considered as common food additives were used in this study to evaluate their effectiveness against *G. candidum*.

Table 1: Salt compounds tested and their minimum inhibitory concentrations against *Geotrichum candidum*

Compounds	Chemical formula	Molecular weight	MIC (w/v)
Ammonium acetate	$C_2H_7NO_2$	77.08	>2
Ammonium carbonate	$(NH_4)_2CO_3$	96.09	0.1
Ammonium dihydrogen phosphate	$NH_4H_2PO_4$	115.03	>2
Ammonium molybdate	$(NH_4)6Mo_7O_{24}$, $4H_2O$	1235.86	0.5
Ammonium sulfate	$(NH_4)_2SO_4$	132.14	>2
Ascorbic acid	$C_6H_8O_6$	176.13	>2
Aspartic acid	$C_4H_7O_4N$	133.11	>2
Boric acid	H_3BO_3	61.83	0.25
Calcium carbonate	$CCaO_3$	100.09	>2
Calcium chloride	$CaCl_2$	110.98	>2
Calcium nitrate	$Ca(NO_3)_2$•$4H_2O$	236.14	>2
Citric acid	$C_2H_8O_7$, H_2O	210.14	2
Dipotassium hydrogen phosphate	K_2HPO_4	174.18	>2
EDTA	$C_{10}H_{14}N_2Na_2O_8$, $2H_2O$	372.24	0.1
Glutaric acid	$C_5H_8O_4$	132.12	>2
Magnesium chloride	$MgCl_2$	203.31	>2
Magnesium sulfate	$MgSO_4$	246.48	>2
Potassium acetate	$C_2H_3KO_2$	98.14	>2
Potassium carbonate	K_2CO_3	138.21	0.5
Potassium chloride	KCl	74.55	>2
Potassium phosphate dibasic	K_2HPO_4	174.18	>2
Potassium sodium tartrate	$C_4H_4KNaO_6$, $4H_2O$	282.23	>2
Sodium acetate	$C_2H_3O_2Na$	82.03	>2
Sodium bicarbonate	$NaHCO_3$	84.01	0.75
Sodium carbonate	Na_2CO_3	105.99	0.25
Sodium chloride	NaCl	58.44	>2
Sodium metabisulfite	$Na_2S_2O_5$	190.1	0.25
Sodium molybdate	Na_2MoO_4	241.95	2
Sodium salicylate	$C_7H_5NaO_3$	160.11	>2
Sodium sulfate	Na_2SO_3	126.04	0.75
Sodium thiosulfate	$Na_2S_2O_3$, $5H_2O$	248.18	0.5
Sodium salicylate	$CuSO_4$, $5H_2O$	249.68	0.5

All test compounds were mixed with culture medium to the concentrations of 0.1, 0.25, 0.5, 0.75, 1, 1.75 and 2% (w/v)

Fruit: The fruit of mandarin (*Citrus reticulata* blanco) cv. Clementine was used. Fruits were harvested from orchards of the M'brouka cooperative which used standard culture practices, in Souss-Massa-Draa region, Morocco. Only healthy and commercially mature fruits were used in the *in vivo* test. Freshly harvested or briefly stored (no longer than a week) fruits were used in the experiment.

Determination of minimum inhibitory concentration (MIC): The Minimal Inhibitory Concentrations (MICs) of the 32 salts were determined by the broth dilution method using the Nutrient Yeast Dextrose Broth (NYDB: nutrient broth 8 g L^{-1}; yeast extract 5 g L^{-1}; dextrose 10 g L^{-1}) as culture medium. Each salt was tested at seven concentrations: 0.1, 0.25, 0.5, 0.75, 1, 1.75 and 2% (w/v). One millilitre of each aqueous solution at desired concentration was transferred to test tubes containing 9 mL of NYDB. The medium without salt compounds served as control. Each tube was inoculated wit 100 μL of a suspension of 10^6 arthrospores mL^{-1} of *G. candidum* and incubated at 25°C for 72 h with shaking. The MICs were recorded by reading the lowest salt concentration that allowed no visible growth of the pathogen. There were three replicates for each salt at each concentration and the experiment was conducted twice.

Effect of pH on mycelial growth of *G. candidum*: Since some of the salt compounds could affect the pH of NYDB medium, we tested the effect of pH alone on mycelial radial growth of *G. candidum*. The pH tested varied from 2 to 12 and were adjusted with 1 N HCl or NaOH. Hyphal plugs (5 mm diameter) were cut from the periphery of actively growing colonies (7 to 10 day-old) and transferred aseptically, mycelium down, to three replicate Petri plates containing NYDA at different pH. Radial growth was determined daily, by measuring colony size along two perpendicular axes. The experiment was performed with three replicate plates per treatment.

Effect of salts on arthrospore germination: To evaluate the impact of salt compounds on arthrospores germination, only salts which showed an MIC value equal or lower than 0.5% were tested. Aqueous solution of salt compounds was prepared in orange juice (2%) as nutrient medium. The germination of arthrospores of *G. candidum* was determined in concentrations of 25 mM, 50 mM, 75 mM and 100 mM of each salt. Aliquots (40 μL) of an arthrospore suspension (10^6 arthrospores mL^{-1}) were aseptically transferred in triplicate to sterile depression slides containing 40 μL of 2% sterile orange juice amended with different concentrations of salt (Droby *et al*., 2003). The pH of solution was not modified; it was determined by the salt and its concentration. Inoculated slides were placed on moist filter paper in Petri plates, sealed with Parafilm to avoid evaporation and then incubated at 25°C for 24 h. Each depression slide was then fixed with acid fuchsine solution to stop further germination (Smilanick *et al*., 1999). Arthrospore germination was estimated under a microscopec using a micrometer. At least 100 arthrospores within each replicate were observed. An arthrospore was scored as germinated if the germ tube length was equal or superior to the length of the spore body at least (Suprapta *et al*., 1997). The results were expressed as percent spore germination inhibition and calculated by using the following formula:

$$GI\ (\%) = \frac{Gc\text{-}Gt}{Gc} \times 100$$

Gc and Gt represent the mean number of germinated spores in control and treated slides, respectively (Soylu *et al*., 2010). Each treatment included three replicates and the experiment was conducted twice.

Effects of salts on sour rot development in artificially inoculated and wounded fruit: Based on the *in vitro* antifungal activity, only salt compounds with a MIC equal or inferior to 0.5% were retained. Mandarin fruits were washed, disinfected with 0.1% (v/v) sodium hypochlorite, rinsed three times in sterile distilled water and then air-dried before wounding. One wound (2 mm deep and 4 mm wide) was made per fruit using a sterile needle at the equatorial side (Liu *et al*., 2009). The wounds were treated with 30 μL of salt solution at concentrations of 1, 2 and 3% (w/v). Controls were treated with the same volume of sterile distilled water under the same conditions. After two hours incubation at room temperature, each wound was inoculated with 20 μL of an aqueous suspension of arthrospores of *G. candidum* (10^6 arthrospores mL^{-1}). Treated fruits were placed on a plastic tray in cardboard boxes and incubated at 26°C and 95% Relative Humidity (RH). The number of the infected wounds and the lesion diameters of the overall treated fruit were determined daily. All treatments were arranged in a complete randomized block design. Eighteen fruits constituted a single replicate and each treatment was replicated three times. The experiment was conducted twice. The incidence and severity of disease were calculated as follows:

$$\text{Disease incidence } (\%) = \frac{\text{Number of rotten wounds}}{\text{Number of total wounds}} \times 100$$

$$\text{Disease severity (\%)} = \frac{\text{Average lesion diameter of treatment}}{\text{Average lesion diameter of control}} \times 100$$

In all experiments, the possible phytotoxic effect on mandarin fruit was examined.

Statistical analysis: All data were subjected to statistical Analysis of Variance (ANOVA) using STATISTICA software, version 6, France. Percentage values were subjected to arcsine-square root transformation before analysis of variance. Mean separation was performed following the Newman and Keels test at $p<0.05$.

RESULTS

Preliminary screening of salts (MICs): The *in vitro* antifungal activity of 32 organic acids and salts was first examined at different concentrations, varying from 0.1 to 2% (w/v) and showed a variable effects of tests compounds on *G. candidum* growth (Table 1). It was also noticed that the reduction in growth was correlated to the increase in compounds concentration. The lowest MIC values were recorded for ammonium carbonate and EDTA tested at 0.1%. Tested at 0.25% boric acid, sodium carbonate and sodium metabisulfite inhibited completely the mycelial growth of *G. candidum*. The third lowest MICs values were recorded for potassium carbonate, ammonium molybdate and sodium thiosulfate at 0.5%. At 0.75% (w/v), only sodium sulfate and sodium bicarbonate completely inhibited the mycelial growth of *G. candidum*. The highest MICs values (2%) were obtained for citric acid and sodium molybdate. The others tested salt compounds are not effective against *G. candidum* even at 2% (Table 1). The data show that *G. candidum* has differential sensitivity to salts, as demonstrated by its varying rates for complete inhibition of growth.

Effect of pH on mycelial growth of *G. candidum*: To determine the effect of pH on the growth of *G. candidum*, NYDA medium at different pH values was used. The obtained results demonstrate that the fungus grew both on acidic and basic pH (Fig. 1). The data indicate that pH from 4 to 12 does not significantly affect the growth of the fungus after 10 days of incubation at 25°C. Furthermore, only pH 2 reduced significantly the growth of the fungus in comparison with the control.

Effect of salt compounds on arthrospore germination: Based on the previous results, only salt compounds that inhibited the growth of *G. candidum* at concentrations lower than 0.5% (w/v), were selected and evaluated for their potential to inhibit the arthrospores germination of the fungus. It is evident from the Table 2, that the salt compounds tested against *G. candidum* showed a reduction or complete inhibition of arthrospore germination in a dose-dependent manner. The arthrospore germination was completely inhibited by EDTA, Boric acid, sodium metabisulfite, sodium carbonate, sodium sulfate and sodium thiosulfate both at 100 and 75 mM. Tested at 50 mM, sodium carbonate, EDTA, sodium sulfate, sodium metabisulfite and boric acid, strongly inhibited the arthrospore germination. At this concentration, the percentage of germination of arthrospore of *G. candidum* oscillates between 1%, for sodium carbonate and 8.33%, for boric acid. At 25 mM, only sodium metabisulfite, boric acid and EDTA strongly inhibited arthrospores germination of *G. candidum,* since percent germination ranged between 11.66 and 33.33%.

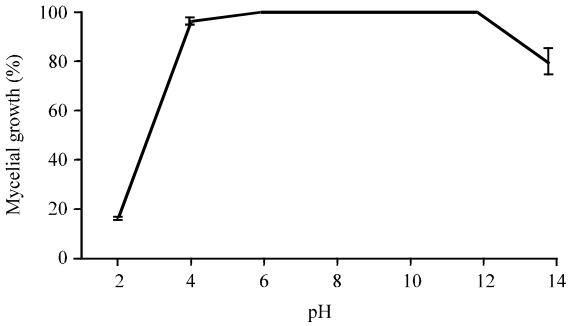

Fig. 1: Effect of pH on *in vitro* mycelial growth of *G. candidum*. Medium pH was adjusted with HCl or NaOH. Bars represent standard deviations of the means

Table 2: *In vitro* effect of salt compounds on arthrospore germination of *Geotrichum candidum*

	Arthrospore germination (%)			
	Concentration (mM)			
Salt	25	50	75	100
EDTA	33.33±2.22^{g}	2±1.33^{ab}	0±0^{a}	0±0^{a}
Sodium salicylate	100±0^{p}	87.33±3.56^{o}	73±2^{m}	61.33±2.44^{k}
Sodium metabisulfite	11.66±2.22^{d}	6±1.33b^{c}	0±0^{a}	0±0^{a}
Boric acid	22.66±2.44^{e}	8.33±2.22^{cd}	0±0^{a}	0±0^{a}
Sodium carbonate	54.64±0.61^{ij}	1±0^{ab}	0±0^{a}	0±0^{a}
Sodium sulfate	79.67±0.44^{n}	4.83±0.78^{abc}	0±0^{a}	0±0^{a}
Ammonium carbonate	99.23±0.51^{p}	95.37±1.71^{p}	86.58±2.88^{o}	56.63±5.79^{j}
Potassium carbonate	68.06±1.29^{l}	54.01±2.45^{ij}	50±1^{i}	50.63±1.04^{i}
Ammonium molybdate	99.33±0.44^{p}	80±1.33^{n}	42±1.33^{h}	12.66±1.55^{d}
Sodium thiosulfate	69.33±1.55l^{m}	28.33±3.55^{f}	0±0^{a}	0±0^{a}
Control	100±0^{p}	100±0^{p}	100±0^{p}	100±0^{p}

Means followed by different letter (s) in each column are significantly different at $p<0.05$

Table 3: Effect of salt compounds on sour rot incidence in infected mandarin fruits

	Disease incidence (%)		
	Concentration (% w/v)		
Salts	1	2	3
EDTA	83.33^{fghi}	50^{c}	38.89^{b}
Sodium salicylate	72.22^{ef}	46.3^{c}	25.93^{a}
Sodium metabisulfite	74.07^{ef}	66.67d^{ef}	68.52d^{e}
Boric acid	66.67^{de}	29.63^{a}	25.93^{a}
Sodium carbonate	72.22^{efg}	68.52^{def}	57.41^{cd}
Sodium sulfate	92.59^{ijk}	77.78^{efg}	66.67^{de}
Ammonium carbonate	83.33^{hij}	64.81^{de}	51.85^{c}
Potassium carbonate	90.74^{hijk}	83.33^{hij}	64.81^{de}
Ammonium molybdate	88.89^{hijk}	79.63^{ghi}	72.22^{efg}
Sodium thiosulfate	94.44^{ijk}	92.59^{ijk}	77.78^{fgh}
Control	100^{k}	100^{k}	100^{k}

Fruit were treated with different concentrations of salt (1, 2 and 3% w/v), inoculated with *G. candidum* and held for 10 days at 26°C. Means followed by different letter (s) in each column are significantly different at $p<0.05$

Table 4: Effect of salt compounds on disease severity

	Disease severity (%)		
	Concentration (% w/v)		
Salt	1	2	3
EDTA	80.97^{ijk}	50de	38.5^{bc}
Sodium salicylate	81.86^{ijk}	45.13^{cd}	30.97^{ab}
Sodium metabisulfite	65.93^{gh}	56.64^{efg}	54.42^{def}
Boric acid	63.72^{fg}	37.61^{bc}	22.574^{a}
Sodium carbonate	59.29^{efg}	55.75^{efg}	51.77^{de}
Sodium sulfate	96.9^{lm}	73.45^{hi}	59.73^{efg}
Ammonium carbonate	78.76^{ijk}	59.73^{efg}	53.1^{de}
Potassium carbonate	88.94^{kl}	83.19^{ijk}	60.62^{efg}
Ammonium molybdate	87.17^{k}	84.07^{hij}	74.34^{jk}
Sodium thiosulfate	99.12^{m}	98.67^{m}	87.61^{kl}
Control	100^{m}	100^{m}	100^{m}

Fruit were treated with different concentrations of salt (1, 2 and 3%), inoculated with *G. candidum* and held for 10 days at 26°C. Means followed by different letter (s) in each column are significantly different at $p<0.05$

In contrast, Sodium salicylate, ammonium carbonate and potassium carbonate that affect strongly the mycelial growth of the fungus, are less effective on arthrospore germination. The percentage of germination in salts amended medium varied between 50% for potassium carbonate at 75 and 100 mM and 100% for sodium salicylate at 25 mM (Table 2).

Effect of salt compounds on disease development: Data presented in Table 3 showed that all tested salt compounds significantly reduced the incidence of sour rot caused by *G. candidum* under the laboratory conditions. Percentages of rotted wounds were decreased by using all tested salts compared with control. Mandarin fruit treated 2 h before pathogen inoculation by sodium salicylate and boric acid at 3% resulted in the highest reduction in rot incidence compared with the control. EDTA and ammonium carbonate had a moderate effect on sour rot, the percentage of rot incidence varying between 38.89 and 51.85. In contrast, sodium thiosulfate and ammonium molybdate showed the least effect on reduction of sour rot incidence (Table 3). Also, all the salts tested did not reduce effectively the disease incidence at concentration of 1%.

On the other hand, data indicated that Boric acid, sodium salicylate and EDTA exhibited significant reduction of disease severity at 2 and 3% compared with the control. The disease severity for these salts ranged between 30.97% (sodium salicylate at 3%) and 50% (EDTA at 2%) (Table 4). Also, there was a significant reduction of the rot severity from 100% in non-treated fruit to 51.77 and 53.1%, respectively in citrus treated fruits, by sodium carbonate and ammonium carbonate at 3%.

Although, sodium salicylate was effective against citrus sour rot, it was phytotoxic to fruit rind at the three tested concentrations. A drying of the rind around the salt treated site was observed. The other salt compounds didn't lead any phytotoxic action on treated fruit at all tested concentrations.

DISCUSSION

The disease control strategies which have been studied as an alternative to currently used fungicides for the control of postharvest citrus fruits rots usually include the use of new fungicides (Smilanick *et al*., 2006; Kanetis *et al*., 2007), microorganisms (El-Ghaouth *et al*., 2000; Taqarort *et al*., 2008), plant extracts (Neri *et al*., 2006; Ameziane *et al*., 2007) and organic and inorganic salts (Biggs *et al*., 1997; Hervieux *et al*., 2002; Mills *et al*., 2004; Palou *et al*., 2009). Some workers combined more than one strategy (El-Ghaouth *et al*., 2000; Plaza *et al*., 2004). In this study, the antifungal activity of wide range of organic and inorganic salts was evaluated against *G. candidum* under both *in vitro* and *in vivo* conditions.

The results showed that among the 32 salts tested for their minimum inhibitory concentrations, ten were most active against *Geotrichum candidum*. EDTA, sodium salicylate, sodium metabisulfite, boric acid, sodium carbonate, sodium sulfate, ammonium carbonate, potassium carbonate, ammonium molybdate and sodium thiosulfate have a MICs values ranging between 0.1 and 0.5%.The present study are, therefore, consistent with those of Latifa *et al*. (2011), who showed that from 28 studied compounds tested against *Penicillium italicum*, causal agent citrus blue mold, sodium metabisulfite, EDTA, ammonium carbonate, sodium carbonate and boric acid were most active against the fungi, with MICs values ranged between 5 and 50 mM. Moreover, Palmer *et al*. (1997), showed that among

26 tested salts, ammonium carbonate, potassium carbonate, sodium carbonate and sodium metabisulfite were effective against the mycelial growth of *Botrytis cinerea.* However, the same authors reported that sodium sulfate and sodium thiosulfate did not affect the mycelial growth of the fungi. Olivier *et al.* (1998) and Hervieux *et al.* (2002) showed that sodium carbonate, potassium carbonate and sodium metabisulfite have completely inhibited the mycelial growth of *Helminthosporium solani,* causal agent of silver scurf on potato tubers. Also, Droby *et al.* (2003) demonstrates that EDTA has a distinct inhibitory effect on the radial growth of *Botrytis cinerea* and *Penicillium expansum in vitro.*

The MIC test has allowed us to select the best salts that are effective against *G. candidum*. The ten best salts were further tested for their ability to reduce or inhibit the arthrospore germination of the pathogen. The results indicated that the arthrospore germination was completely inhibited by EDTA, boric acid, sodium metabisulfite, sodium carbonate, sodium sulfate and sodium thiosulfate both at 100 and 75 mM. This finding corroborates with those of Latifa *et al.* (2011) showing that EDTA, boric acid, sodium metabisulfite and sodium carbonate completely inhibited spore germination of *Penicillium italicum.* Furthermore, sodium carbonate and sodium metabisulfite were demonstrated to inhibit completely the spore germination of *Helminthosporium solani* (Hervieux *et al*., 2002). Also Smilanick *et al.* (1999) reported that sodium carbonate inhibit the spore germination of *P. digitatum*, causal agent of citrus green mold. Mills *et al.* (2004) reported that Sodium metabisulfite reduced significantly the spore germination of several phytopathogenic fungi. By contrast, among the tested salts, sodium salicylate, ammonium carbonate and potassium carbonate are not effective on the arthrospore germination of the pathogen.

Considering that several salt compounds influenced medium pH, the effect of pH on *G. candidum* growth was determined. The results showed that mycelial growth was not strongly affected by pH modification in the range of 4.0 and 12.0. This result agrees with those of Hervieux *et al.* (2002) and indicates that the inhibition obtained in the salt-amended medium cannot be due only to a direct effect of pH on pathogen growth.

Although, *in vitro* tests of salts compounds are an important first step in selecting salts with antifungal potential against postharvest citrus pathogens, *in vivo* tests are needed to check whether the positive results of the *in vitro* tests can be obtained too. The citrus fruits were treated with different concentrations of salt compounds (1, 2 and 3% w/v), inoculated with *G. candidum* and held for 10 days at 26°C. The results showed variable effects of tested compounds on disease incidence and severity. Sodium salicylate, boric acid and EDTA were the most effective against *G. candidum* in *in vivo* conditions. In most commercial packing houses in SMD, pre-storage chemical control with guazatine is commonly applied, as postharvest drench, to reduce the incidence of sour rot in citrus fruit that are stored in cold before processing. However, this fungicide is not registered in several countries. According to Hao *et al.* (2010), the lack of registered fungicide in many countries for sour rot control is becoming a serious problem. At concentration of 3%, the disease incidence was reduced to 25.93% in fruits treated with sodium salicylate or boric acid and to 38.89% in fruits treated with EDTA. The present results are consistent with previous studies which demonstrated that EDTA was effective to control *P. digitatum* on oranges (Valencia-Chamorro *et al*., 2008) and *B. cinerea* on apple fruit (Droby *et al*., 2003). Moreover, Smilanick and Sorenson (2001), reported that immersion of citrus fruits in boric acid solution reduced significantly the incidence of citrus green mold. Also of interest, we found that ammonium carbonate and sodium carbonate have significantly reduced the incidence of sour rot to 51.8 and 57.41%, respectively. This result is in agreement with the finding of Palou *et al.* (2009) who reported that the same salts reduced the incidence of sour rot of stone fruits.

To understand the mechanisms by which fungi are tolerant or sensitive to salt compounds, several studies were carried out. It was found that inhibition of microorganisms by salts might be caused by reducing of the cell turgor pressure with collapse and shrinkage of hyphae and spore (Fallik *et al*., 1997) or by alteration of cell-transport function and inhibition of enzymes involved in the glycolytic pathway (Sofos *et al*., 1986). However, the mechanisms by which salts inhibit *G. candidum* are not well understood.

CONCLUSION

In conclusion, this study is a part of an overall study that aims to determine the antifungal activity of salt compounds, against major postharvest citrus fungal pathogens. Among the 32 compounds tested sodium salicylate, boric acid and EDTA showed high antifungal activities against *Geotrichum candidum* both *in-vitro* and *in-vivo*. These compounds possess potent antifungal activities with potential practical applications in the treatment of postharvest sour rot of citrus fruits and should be tested in future under degreening conditions.

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