Plant Pathology Journal

ISSN 1812-5387

science alert

ANSInet
an open access publisher
http://ansinet.com

Plant Pathology Journal 10 (3): 99-107, 2011
ISSN 1812-5387 / DOI: 10.3923/ppj.2011.99.107

Effects of Organic Acids and Salts on the Development of *Penicillium italicum*: The Causal Agent of Citrus Blue Mold

Askarne Latifa, Talibi Idriss, Boubaker Hassan, Serghini Mohammed Amine,
Boudyach El Hassane and Ait Ben Aoumar Abdellah
Laboratoire De Biotechnologies et Valorisation Des Ressources Naturelles,
Équipe De Phytopathologie et Lutte Intégrée, Ibn Zohr University,
Faculty of Sciences, B.P 8106, Agadir, Morocco

Abstract: Control of Citrus blue mold, caused by *Penicillium italicum*, has been accomplished by postharvest application of synthetic fungicides. However, the development of resistant fungal strains and increasing public concern over food safety and the environment are driving a search for alternative disease control strategies. *In vitro* trials were conducted to evaluate the effect of several organic acid and salt compounds on mycelial growth, sporulation, spore germination and germ tube elongation of *Penicillium italicum*. Among 28 tested compounds, sodium carbonate, ammonium carbonate, copper sulfate, EDTA and sodium metabisulfite completely inhibited mycelial growth and sporulation of *Penicillium italicum* at only 0.02 M. The lowest Minimum Inhibitory Concentration (MIC), Minimum Fungicidal Concentration (MFC) and EC_{50} values were recorded in sodium metabisulfite treatment. All tested compounds, except Nicotinic acid, strongly inhibited spore germination and germ tube elongation in a dose-dependent manner. Results from this study provide an important basis for further study into the uses of salt compounds for the control of blue mold of Citrus fruit under semi-commercial conditions.

Key words: Citrus, *Penicillium italicum,* minimum inhibitory concentration, minimum fungicidal concentration, blue mold

INTRODUCTION

Citrus is a major fruit crop that is grown around the World (Smilanik *et al*., 2005). In most producing countries the crop is largely grown for fresh fruit consumption. It is the first exporting agricultural sector in Morocco, with the Souss-Massa-Draa (SMD) Valley as the main area of both production and exportation of fresh fruit (Boubaker *et al*., 2009).

The most important diseases that cause commercially significant losses, in Morocco (Elkhamass *et al*., 1994) and worldwide (Eckert and Eaks, 1989; Holmes and Eckert, 1999; Zhu *et al*., 2006) are green mold, caused by *Penicillium digitatum* (pers.:Fr.) Sacc. and blue mold, caused by *P. italicum* wehmer. These *Penicillium* species are strict wound pathogens, they are ubiquitous and produce profuse amount of asexual conidia that are readily disseminated by air current (Holmes and Eckert, 1995, 1999; Boubaker *et al*., 2009). Therefore they can infect the fruit in the grove, the packinghouses and marketing, through wounds occurred during harvest and subsequent handling (Brown and Miller, 1999; Boubaker *et al*., 2009). Blue mold is more harmful because it spreads in the box and healthy fruits are directly attacked, regardless of injury. This disease is, also, more important under cold storage conditions. Currently, measures employed to manage such fungal diseases involve the application of chemical fungicides usually incorporated into waxes (Smilanick and Sorenson, 2001; Boubaker *et al*., 2009). However, the use of fungicides is becoming increasingly restricted due to stringent regulation, pathogen resistance development and growing public concern about chemical residues in fruit (Zhang and Swingle, 2003; Palou *et al*., 2008). Therefore, alternative methods for decay control are needed. Various alternative measures such as the application of microorganisms (El-Ghaouth *et al*., 2000; Taqarort *et al*., 2008), plant extracts (Tripathi and Dubey, 2004; Ameziane *et al*., 2007; Rani and Devanand, 2011), Essential oils (Barrera-Necha *et al*., 2008, 2009) or the use of effective natural substances like food additives (Kazemi *et al*., 2011; Shirzadeh and Kazemi, 2011) have been developed. Food additives have minimal adverse effect on the environment and health (Arslan *et al*., 2009)

Corresponding Author: Boubaker Hassan, Laboratoire De Biotechnologies et Valorisation Des Ressources Naturelles, Équipe De Phytopathologie et Lutte Intégrée, Ibn Zohr University, Faculty of Sciences, B.P 8106, Agadir, Morocco

and exhibit a broad-spectrum antifungal activity (Corral *et al*., 1988). These alternative measures have been applied in combination or in a replacement for fungicide in order to reduce the input of fungicides and to prolong the shelf life of citrus fruit, particularly in a market where transport from producer to consumers may take several weeks (Boubaker *et al*., 2009). Several studies have dealt with the use of different salt compounds to control various post-harvest diseases of citrus and other crops (Arslan *et al*., 2006; Nigro *et al*., 2006; Arslan *et al*., 2009). Treatment of fruit with carbonate or bicarbonate salts was shown to reduce the incidence of post-harvest diseases of citrus fruit caused by *Penicillium digitatum*, *Penicillium italicum* or *Geotrichum candidum* (Zhang and Swingle, 2003; Smilanick *et al*., 2006; Smilanick *et al*., 2008). They have also been used to control the blue mold caused by *Penicillium expansum* and the gray mold caused by *Botrytis cinerea* in apple fruit (Droby *et al*., 2003; Palou *et al*., 2009). Sodium metabisulfite was shown to reduce potato silver scurf caused by *Helminthosporiun solani* (Olivier *et al*., 1998; Hervieux *et al*., 2002; Mills *et al*., 2006) and potato dry rot caused by *Fusarium sambucinum* (Mecteau *et al*., 2002). EDTA was used to control *P. digitatum* on oranges (Valencia-Chamorro *et al*., 2008) and *B. cinerea* on apple fruit (Droby *et al*., 2003).

The present work was performed to evaluate the efficacy of a range of organic acids and salts, for *in vitro* control of the mycelial growth, sporulation and spore germination of *Penicillium italicum,* the causal agent of blue mold of citrus fruit.

MATERIALS AND METHODS

Pathogen culture: *P. italicum* was isolated from naturally infected lemon fruit after storage of several weeks. This isolate was the most aggressive one in our collection and produced the largest lesions on inoculated fruit. This fungus was purified and maintained on Potato Dextrose Agar (PDA) and stored at 4°C, with periodic transfers through citrus fruit to maintain its aggressiveness.

***In vitro* screening of chemicals:** The inhibitory effects of 28 Organic acids and salts (Table 1) on mycelial growth of *Penicillium italicum* were tested *in vitro* using the agar dilution technique. An aqueous solution of each compound was prepared in sterile distilled water and was added aseptically to molten (50°C) sterile PDA to achieve a final concentrations of 0.02 and 0.2 M before pouring the medium into petri plates (15 mL PDA/plate). Chemicals unamended plates served as control. 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 PDA medium supplemented with chemicals. The plates were sealed with parafilm and incubated in the dark at 25°C. Radial growth was measured daily at two perpendicular colony diameters until the growth in the control plates reached the edge of the Petri plates. The antifungal activity was expressed in terms of percentage of Mycelial Growth Inhibition (MGI) and calculated according to the following formula:

$$\text{MGI (\%)} = \frac{\text{(Unamended-Chemical amended)}}{\text{Unamended}} \times 100$$

Compounds that allowed more than 50% of mycelial growth inhibitions in the first screening were further retained for determination of the effective concentration causing a 50% reduction (EC_{50}) in the linear growth on PDA using probit analysis (POLO software). The Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) were also determined in parallel experiment.

To determine whether compounds have fungistatic or fungicidal effect on *Penicillium italicum,* Plugs from treatment with no growth were transferred to chemical

Table 1: Chemicals used in this study

Chemicals	Chemical formula	Molecular weight
Ammonium acetate	$C_2H_7NO_2$	77.08
Ammonium carbonate	$(NH_4)_2CO_3$	96.09
Ammonium molybdate	$(NH_4)_6Mo_7O_{24}$, $4H_2O$	1235.86
Ascorbic acid	$C_6H_8O_6$	176.13
Aspartic acid	$C_4H_7O_4N$	133.11
Boric acide	H_3BO_3	61.83
Calcium carbonate	$CaCO_3$	100.09
Calcium chloride	$CaCl_2$	147.02
Calcium hypochlorite	$CaCl_2O_2$	142.99
Citric acid	$C_2H_8O_7$, H_2O	210.14
Copper sulfate	$CuSO_4$, $5H_2O$	249.68
Disodium succinate	$C_4H_4Na_2O_4$	162.06
EDTA	$C_{10}H_{14}N_2Na_2O_8$, $2H_2O$	372.24
Glutaric acid	$C_5H_8O_4$	132.12
Nicotinic acid	C_5H_4NCOOH	123.11
Potassium acetate	$C_2H_3KO_2$	98.14
Potassium carbonate	K_2CO_3	138.21
Potassium Phosphate, dibasic	K_2HPO_4	174.18
Sodium acetate	$C_2H_3O_2Na$	82.03
Sodium bicarbonate	$NaHCO_3$	84.01
Sodium carbonate	Na_2CO_3	105.99
Sodium metabisulfite	$Na_2S_2O_5$	190.10
Sodium molybdate	Na_2MoO_4	241.95
Sodium phosphate, dibasic	Na_2HPO_4, $2H_2O$	177.99
Sodium salicylate	$C_7H_5NaO_3$	160.11
Sodium Sulfite	Na_2SO_3	126.04
Sodium thiosulfate	$Na_2S_2O_3$, $5H_2O$	248.18
Trisodium citrate	$Na_3C_6H_5O_7$, $2H_2O$	294.10

unamended PDA, treatment in which mycelial growth did not occur after additional 9 days of incubation were considered fungicidal (Hervieux *et al*., 2002; Mecteau *et al*., 2002). The experiments were performed twice.

Effect of chemicals on sporulation: Sporulation was assessed using chemical amended PDA as described above, only for compounds with more than 50% of mycelium growth inhibition. Once fungal colony in control reached the edge of the plate, amended plates as well as control were flooded with 10 mL of sterile distilled water containing 0.05% (w/v) tween 80 and spores were gently dislodged from the medium using a bacteriological loop. The resulted suspension was filtered through a Buchner funnel and the spores were determined using a heamacytometer. The result was expressed as a percent sporulation inhibition using the following formula:

$$\frac{\text{No. of spores in unamended- No. of spores in amended}}{\text{No. of spores in unamended}} \times 100$$

Three replicates were made for each chemical.

Effect of chemicals on spore germination and germ tube elongation: A spore-suspension was obtained from one-week-old culture of *Penicillium italicum,* grown on PDA at 25°C, by flooding the culture with 10 mL^{-1} of sterile distilled water containing 0.05% (v/v) Tween 80. The spores were gently dislodged from the surface and the suspension was filtered through two layers of sterile cheesecloth to remove hyphal fragments. The spore concentration of the suspension was adjusted to 2.10^5 spores mL^{-1} with the aid of a heamacytometer (Gabler and Smilanick, 2001). The germination and germ tube elongation of conidia of *P. italicum* were determined in concentrations of 0.002, 0.005, 0.01, 0.02 and 0.04 M for the compounds that allowed a mycelial growth inhibition superior to 50%. Aliquots (40 μL) of spore suspensions were aseptically transferred in triplicate to sterile depression slides containing 40 μL of 2% sterile and filtered oranges juice amended with different concentrations of salts (Droby *et al*., 2003). Inoculated slides were placed on moist filter paper in Petri plates and then incubated at 25°C for 18 h. Each slide was then fixed with acid fuchsine solution to stop further germination (Smilanick *et al*., 1999). Spore germination and germ tube elongation was determined in three microscopic fields using 10×40 ocular micrometers. At least 100 conidia within each replicate were observed. A spore was scored as germinated when the germ tube extended to at least twice the length of the spore itself (Punja and Gaye, 1993; Mills *et al*., 2004; Amiri and Bompeix, 2011). Germination and germ tube elongation were assessed microscopically at various concentrations of tested chemicals compared with control treatment. The results were expressed as percent spore germination or germ tube elongation inhibition. Each treatment included three replicates and the test was conducted twice.

Statistical analysis: All data were subjected to statistical Analysis of Variance (ANOVA) using STATISTICA software, version 6, Stat- Soft, 2001, France. Percentage values of inhibition were subjected to arcsine-square root transformation before analysis of variance. Duncan multiple range tests were used to segregate treatments which were significantly different at $p<0.05$. The EC_{50} values were calculated for each compound by probit analysis using POLO software.

RESULTS AND DISCUSSION

Effects on mycelium growth and sporulation: The *in vitro* antifungal activities of organic acids and salts were first examined at the concentrations of 0.02 and 0.2 M and showed a variable effects of tests compounds on *P. italicum* mycelial growth and sporulation (Table 2). Results also showed that after 7 days incubation at 25°C, most tested compounds reduced both mycelial growth and sporulation of *P. italicum* on PDA medium. It was also noticed that the reduction in growth and sporulation were correlated to the increase in compounds concentrations. Complete inhibition of growth and sporulation was achieved by sodium carbonate, ammonium carbonate, copper sulfate, sodium metabisulfite and EDTA at the lower tested concentration (0.02 M). Ammonium molybdate reduced also the mycelial growth and sporulation of the pathogen, on PDA medium amended with 0.02 M of this salt.

Boric acid and potassium carbonate reduced the mycelial growth by more than 50%. However, potassium carbonate appears to be more effective than boric acid when sporulation inhibition percentages were compared (68.89% versus 30.05%). The remaining compounds inhibited mycelial growth by less than 50%. In contrast, sodium acetate at 0.02 M enhanced the mycelial growth of *P. italicum.*

Tested at 0.2 M, 14 out of 28 studied compounds completely inhibited the mycelial growth and sporulation of *P. italicum.* Among these chemicals, ammonium carbonate, ammonium molybdate, sodium phosphate, sodium sulfite, EDTA, sodium metabisulfite and sodium salicylate were, also, fungicidal. Indeed, mycelial Plugs transferred from chemical amended PDA to unamended

Table 2: *In vitro* effects of various test compounds on mycelial growth and sporulation of *Penicillium italicum*

	% inhibition			
	Mycelial growth		Sporulation	
Chemicals	0.02 M	0.2 M	0.02 M	0.2 M
Ammonium acetate	8.04±1.55k-m	61.16±1.77d	ND	50.15±6.58d
Ammonium carbonate	100±0a	100±0.00a	100±0a	100±0a
Ammonium molybdate	86.65±0.87b	100±0.00a	92.74±0.51b	100±0a
Ascorbic acid	16.90±1.99i	40.49±0.50g	ND	ND
Aspartic acid	10.87±2.17jk	20.77±1.82j	ND	ND
Boric acid	68.78±2.67c	100±0.00a	68.89±4.22c	100±0
Calcium carbonate	13.40±0.43ij	23.82±1.87ij	ND	ND
Calcium chloride	4.46±0.39mn	26.56±3.02i	ND	ND
Calcium hypochlorite	6.08±3.38l-n	56.31±0.39e	ND	71.50±0.44b
Citric acid	29.16±0.72g	55.181±1.45e	ND	38.14±8.36e
Copper sulfate	100±0a	100±0a	100±0a	100±0
Disodium succinate	2.66±2.33no	48.31±2.93f	ND	ND
EDTA	100±0a	100±0.00a	100±0a	100±0a
Glutaric acid	13.77±1.26ij	46.14±0.42f	ND	ND
Nicotinic acid	11.74±1.08jk	67.37±4.13c	ND	67.05±6.45b
Potassium acetate	9.36±0.43kl	42.12±1.71g	ND	ND
Potassium carbonate	55.66±5.22l d	100±0.00a	30.05±8.32d	100±0a
Potassium Phosphate dibasic	24.18±2.43h	45.84±3.05f	ND	ND
Sodium acetate	-0.24±1.11p	72.46±5.02b	ND	58.53±7.97c
Sodium bicarbonate	48.88±3.75k	100±0.00a	ND	100±0a
Sodium carbonate	100±0a	100±0.00a	100±0a	100±0a
Sodium metabisulfite	100±0a	100±0.00a	100±0a	100±0a
Sodium molybdate	10.10±0.43jk	100±0.00a	ND	100±0a
Sodium phosphate dibasic	15.87±4.30i	100±0.00a	ND	100±0a
Sodium salicylate	44.47±3.48f	100±0.00a	ND	100±0a
Sodium sulfite	42.41±2.01f	100±0.00a	ND	100±0a
Sodium thiosulfate	11.49±4.11jk	36.49±3.58h	ND	ND
Trisodium citrate	26.09±4.10gh	100±0.00a	ND	100±0a

ND: Not determined. Values expressed are mean of three replicates. Values within each column followed by different letters are significantly different at $p<0.05$. Means followed by the same letter in each column do not differ significantly according to Duncan multiple range tests at $p<0.05$

Table 3: EC_{50}, Minimum inhibitory concentration (MIC)) and Minimum fungicidal concentration (MFC) values of various compounds tested against *P. italicum*

Chemicals	MIC (mM)	MFC (mM)	EC_{50} (mM)	Toxicity at 200 mM
Ammonium acetate	>200	>200	ND	-
Ammonium carbonate	10	20	3.43 (2.39- 4.78)	Fungicidal
Ammonium molybdate	200	200	1.94 (0.01- 8.99)	Fungicidal
Boric acid	50	>200	16.14 (14.38- 17.63)	Fungistatic
Calcium hypochlorite	>200	>200	ND	-
Citric acid	>200	>200	ND	-
Copper sulfate	10	20	3.69 (3.51-3.86)	Fungicidal
EDTA	10	150	2.67 (2.51-2.83)	Fungicidal
Nicotinic acid	>200	>200	ND	-
Potassium carbonate	150	>200	25.96 (20.58-30.76)	Fungistatic
Sodium acetate	>200	>200	ND	-
Sodium bicarbonate	75	>200	21.63 (18.53-24.34)	Fungistatic
Sodium carbonate	20	>200	12.07 (10.16-14.30)	Fungistatic
Sodium metabisulfite	5	5	1.69 (1.58-1.8)	Fungicidal
Sodium molybdate di hydrate	200	>200	95.36 (83.61-109.38)	Fungistatic
Sodium phosphate dibasique	150	200	52.81 (46.25-59.67)	Fungicidal
Sodium salicylate	150	200	27.29 (23.34-30.99)	Fungicidal
Sodium sulfite anhydrous	150	200	24.52 (21.21-27.61)	Fungicidal
Trisodium citrate dihydrate	75	>200	39.91 (34.23-45.57)	Fungistatic

PDA failed to grow after additional 9 days incubation at 25°C (Table 3). The remaining compounds reduced the pathogen growth; MGI values varied from 20.77% recorded with aspartic acid to 72.46% recorded with sodium acetate.

The obtained results demonstrate that several organic acids and salts can inhibit significantly the growth of *P. italicum* and reduce or completely inhibit its spore production. This is in agreement with the finding of Mills *et al.* (2004) and Hervieux *et al.* (2002) who reported complete inhibition of mycelial growth was generally associated with complete inhibition of sporulation. In this study, copper sulfate strongly inhibits mycelial growth and sporulation of *P. italicum.*

However, when tested against *Phytophthora erythroseptica,* this compound reduced mycelial growth without inhibiting sporulation (Mills *et al*., 2004).

Results of MIC and MFC study are shown in Table 3. The lowest MIC and MFC values were recorded for Sodium metabisulfite tested at 5 mM. The second lowest MIC value was recorded for EDTA, ammonium carbonate and copper sulfate treatments (10 mM). However, the MFC of EDTA is widely greater than the one of Copper sulfate and ammonium carbonate; since those salts showed a fungicidal activity at only 20 mM while EDTA at 150 mM. The highest MIC and MFC values were recorded for ammonium acetate, citric acid, sodium acetate, nicotinic acid and calcium hypochlorite.

The EC_{50} values for tested compounds were further determined (Table 3). The lowest values were obtained for sodium metabisulfite (1.69 mM), ammonium molybdate (1.94 mM), EDTA (2.67 mM), ammonium carbonate (3.43 mM) and copper sulfate (3.68 mM). The data show that *P. italicum* has differential sensitivity to bicarbonate and carbonate salts, as demonstrated by its varying rates for complete inhibition of colony growth and sporulation. Sodium carbonate and sodium bicarbonate are common food additives permitted with no restrictions for many applications in North American regulation including organic agriculture (Smilanick *et al*., 1999). According to their MIC and EC_{50} values, ammonium and sodium carbonate were more toxic than the other tested carbonic salts. Olivier *et al.* (1998) reported that sodium carbonate and potassium carbonate were more effective than the respective bicarbonate salts, as sodium and potassium carbonate completely inhibited radial growth of *Helminthosporium solani* at 0.1 and 0.2 M. This finding is similar to the level of inhibition reported by Hervieux *et al.* (2002) and five to ten time higher than the level of inhibition observed in this study. The present results are consistent with previous studies that demonstrate better disease control with carbonate salts than with bicarbonate salts (Biggs *et al*., 1994; Palmer *et al*., 1997). Palou *et al.* (2001) demonstrated that sodium carbonate and sodium bicarbonate had fungistatic rather fungicidal activity against *P. italicum* which is consistent with present data. Whereas, ammonium carbonate exhibited a fungicidal activity at 200 mM. Ammonium salts displayed also an inhibitory effect on *P. italicum* which concurs with the finding of Hervieux *et al.* (2002) on *H. solani.* Palmer *et al.* (1997) demonstrated that ammonium salts were effective under alkaline conditions where ammonia gas NH_3 (additional active compounds) is favored over the ineffective NH_4^+ form. Montesinos-Herrero *et al.* (2011) reported that postharvest green mold and blue mold, caused by *Penicillium digitatum* and *Penicillium italicum*, respectively, were effectively controlled by fumigation of lemons and oranges with ammonia gas. Sodium metabisulfite, a compound with antimicrobial activity (Hervieux *et al*., 2002; Mills *et al*., 2004), has been shown to completely inhibit *in vitro* mycelial growth and sporulation of *H. solani* (Hervieux *et al*., 2002), *Fusarium sambucinum* (Mecteau *et al*., 2002) and a wide range of potato postharvest pathogens (Mills *et al*., 2004) at a concentration of 0.2 M. Present results indicate that Sodium metabisulfite completely inhibited mycelial growth and sporulation of *P. italicum* at a concentration as low as 0.02 M. At biochemical level, the mode of action of this compound lies in the inhibition of cellular intermediary metabolism, energy production, protein biosynthesis, DNA replication and membranes synthesis (Mills *et al*., 2004).

Effect of chemicals on spore germination and germ tube elongation: It is evident from the Table 4 that out of 28 salt compounds tested against *P. italicum*, 27 compounds showed a reduction or complete inhibition of spore germination and germ tube elongation in a dose-dependent manner. In contrast, nicotinic acid allowed spore germination but slightly reduced germ tube elongation. The toxicity of ammonium molybdate, copper sulfate, calcium hypochlorite, sodium metabisulfite and sodium carbonate to spores was higher to that of the other test compounds. Since percent germination in salts amended medium (diluted oranges juice) ranged between 96.27 and 100% at only 2 mM. Results revealed that the compounds which inhibited spore germination also inhibited the mycelial growth of *P. italicum.* In contrast, calcium hypochlorite affects slightly the mycelial growth of *P. italicum* at 0.02 M (Table 2) but it strongly inhibited the spore germination and germ tube elongation at low concentration (Table 4). Mills *et al.* (2004) showed that copper sulfate completely inhibited the spore germination of various potato pathogens. This result agrees with the level of inhibition observed in the present study. While 5 mM concentration of sodium metabisulfite and copper sulfate was required for complete inhibition of spore germination and germ tube elongation, 20 mM concentration was required for complete inhibition of mycelial growth. This confirms that mycelium growth *in vitro* is less sensitive to these compounds compared with spore germination.

Hwang and Klotz (1938) indicated that the *in vitro* activity of several carbonate salts against germinated or

Table 4: *In vitro* effects of various test compounds on spore germination and germ tube elongation of *Penicillium italicum*

		% inhibition	
Chemicals	Concentration (mM)	Germination	Germ tube elongation
Control		0.00	0.00
Ammonium acetate	2	8.57 no	48.49p
	5	9.98 no	71.79j-l
	10	42.04h-k	73.35h-k
	20	59.85d-f	81.77d-f
	40	83.19bc	88.93c
Ammonium carbonate	2	37.10jk	71.02j-l
	5	37.73i-k	72.86i-l
	10	53.62e-i	80.52e-g
	20	57.82e-h	74.57g-j
	40	58.81e-g	79.22f-i
Ammonium molybdate	2	100.00a	100.00a
	5	100.00a	100.00a
	10	100.00a	100.00a
	20	100.00a	100.00a
	40	100.00a	100.00a
Boric acid	2	0.00o	14.54st
	5	0.00o	54.46o
	10	31.38k-m	76.50f-j
	20	100.00a	100.00a
	40	100.00a	100.00a
Calcium hypochlorite	2	100.00a	100.00a
	5	100.00a	100.00a
	10	100.00a	100.00a
	20	100.00a	100.00a
	40	100.00a	100.00a
Citric acid	2	14.03no	19.12rs
	5	50.74f-j	63.92mn
	10	98.58ab	100.00a
	20	100.00a	100.00a
	40	100.00a	100.00a
Copper sulfate	2	100.00a	100.00a
	5	100.00a	100.00a
	10	100.00a	100.00a
	20	100.00a	100.00a
	40	100.00a	100.00a
EDTA	2	89.22ac	90.79bc
	5	100.00a	100.00a
	10	100.00a	100.00a
	20	100.00a	100.00a
	40	100.00a	100.00a
Nicotinic acid	2	0.00o	-0.94u
	5	0.00o	-0.48u
	10	0.00o	10.37t
	20	0.00o	22.10r
	40	0.00o	38.34q
Potassium carbonate	2	59.69d-f	67.04l-m
	5	100.00a	100.00a
	10	100.00a	100.00a
	20	100.00a	100.00a
	40	100.00a	100.00a
Sodium acetate	2	18.58mn	64.07mn
	5	20.24mn	68.26k-m
	10	21.28l-n	70.57j-l
	20	36.47j-l	76.43f-i
	40	58.18e-h	78.26f-i
Sodium bicarbonate	2	60.35d-f	58.94no
	5	100.00a	100.00a
	10	100.00a	100.00a
	20	100.00a	100.00a
	40	100.00a	100.00a
Sodium carbonate	2	96.27ab	94.68ab
	5	100.00a	100.00a
	10	100.00a	100.00a
	20	100.00a	100.00a

Table 4: Continued

Chemicals	% inhibition Concentration (mM)	Germination	Germtube elongation
	40	100.00a	100.00a
Sodium metabisulfite	2	98.58ab	96.16ab
	5	100.00a	100.00a
	10	100.00a	100.00a
	20	100.00a	100.00a
	40	100.00a	100.00a
Sodium molybdate	2	88.16a-c	76.02f-j
	5	100.00a	100.00a
	10	100.00a	100.00a
	20	100.00a	100.00a
	40	100.00a	100.00a
Sodium phosphate, dibasic	2	19.90mn	70.99j-l
	5	31.23k-m	71.12j-l
	10	51.93e-j	79.16f-i
	20	94.57b	95.45ab
	40	100.00a	100.00a
Sodium salicylate	2	0.00o	22.95r
	5	74.88cd	78.32f-i
	10	100.00a	100.00a
	20	100.00a	100.00a
	40	100.00a	100.00a
Sodium sulfite	2	43.24g-k	75.07g-j
	5	98.94ab	100.00a
	10	100.00a	100.00a
	20	100.00a	100.00a
	40	100.00a	100.00a
Trisodium citrate	2	45.16f-k	79.72e-h
	5	46.32f-k	85.36c-e
	10	67.58de	87.30cd
	20	90.71ab	87.89c
	40	100.00a	100.00a

Germination of at least 100 conidia was assessed microscopically after 18 h incubation in the dark in dilute orange juice. Value was the mean of three replicates. Means followed by the same letter in each column do not differ significantly according to Duncan multiple range tests at $p<0.05$

ungerminated spores of *P. italicum* was higher than that of bicarbonate salts. In contrast, no significant difference was found in the inhibitory effect between the two salts. The inhibitory effect of salt compounds on spore germination and germ tube elongation of various fungal pathogens have been previously documented (Mecteau *et al*., 2002; Mills *et al*., 2004; Arslan *et al*., 2006). Fallik *et al*. (1997) reported that the inhibitory effect of sodium bicarbonate on microorganisms may be due to a reduction of cell turgor pressure with collapse and shrinkage of hyphae and spore. However, the mechanisms by which fungi are tolerant or sensitive to salt compounds are poorly understood.

Present results confirmed high antifungal activities of sodium metabisulfite and copper sulfate on various asexual developmental stages of the life cycle of *P. italicum in vitro*. According to Errampalli (2004), antifungal compounds that significantly inhibits germination of conidia, mycelial growth and subsequently, rot formation should reduce the ability of pathogen to cause disease. As citrus fruit are commonly contaminated by postharvest fungal pathogens during processing, a salt compound with strong effect on spore germination and germ tube elongation is highly desirable to reduce the initial load of spores and subsequent deterioration of fruits. To our knowledge, this is the first report on the *in vitro* use of sodium metabisulfite and copper sulfate to control *P. italicum* development.

CONCLUSION

The results of this study suggest that salt compounds may act as an alternative to synthetic fungicides. However, the potential use of organic acid and salts to control blue mold of citrus requires a detailed examination of their biological activity *in vivo* and the development of formulation which inhibits the growth of the pathogen at non-phytotoxic concentrations.

REFERENCES

Ameziane, N., H. Boubaker, H. Boudyach, F. Msanda, A. Jilal and A. Ait Benaoumar, 2007. Antifungal activity of Moroccan plants against citrus fruit pathogens. Agron. Sustainable Devel., 27: 273-277.

Amiri, A. and G. Bompeix, 2011. Control of *Penicillium expansum* with potassium phosphite and heat treatment. Crop Protection, 30: 222-227.

Arslan, U., K. Ilhan and O.A. Karabulut, 2006. Evaluation of food additives and low-toxicity compounds for the control of bean rust and wheat leaf rust. J. Phytopathol., 154: 534-541.

Arslan, U., K. Ilhan, C. Vardar and O.A. Karabulut, 2009. Evaluation of antifungal activity of food additives against soilborne phytopathogenic fungi. World J. Microbiol. Biotechnol., 25: 537-543.

Barrera-Necha, L.L., S. Bautista-Banos, H.E. Flores-Moctezuma and A.R. Estudillo, 2008. Efficacy of essential oils on the conidial germination, growth of *Colletotrichum gloeosporioides* (Penz.) penz. and sacc and control of postharvest diseases in papaya (*Carica papaya* L.). Plant Pathol. J., 7: 174-178.

Barrera-Necha, L.L., C. Garduno-Pizana and L.J. Garcia-Barrera, 2009. *In vitro* antifungal activity of essential oils and their compounds on mycelial growth of *Fusarium oxysporum* f. sp. *gladioli* (Massey) snyder and hansen. Pak. J. Nutr., 8: 17-21.

Biggs, A.R., M.M. El-Kholi and S.M. El-Neshawy, 1994. Effect of calcium salts on growth, pectic enzyme activity and colonization of peach twigs by *Leucostoma persoonii*. Plant Dis., 78: 886-890.

Boubaker, H., B. Saadi, E.H. Boudyach and A. Ait Benaoumar, 2009. Sensitivity of *Penicillium digitatum* and *P. italicum* to imazalil and thianbendazole in morocco. Plant Pathol. J., 8: 152-158.

Brown, G.E. and W.R. Miller, 1999. Maintaining Fruit Health after Harvest. In: Citrus Health Management, Timmer, L.W. and L. W. Duncan (Eds.). APS Press, St. Paul, MN., pp: 175-188.

Corral, L.G., L.S. Post and T.J. Montville, 1988. Antimicrobial activity of sodium bicarbonate. J. Food Sci., 53: 981-982.

Droby, S., M. Wisniewski, A. El Ghaouth and C. Wilson, 2003. Influence of food additives on the control of postharvest rots of apple and peach and efficacy of the yeast-based biocontrol product Aspire. Postharvest Biol. Technol., 27: 127-135.

Eckert, J.W. and I.L. Eaks, 1989. Postharvest Disorders and Diseases of Citrus Fruits. In: The Citrus Industry: Crop protection, Postharvest Technology and Early History of Citrus Research in California, Calavan, E.C. and G.E. Carman (Eds.). Vol. 5, University of California Press, Berkeley, pp: 179-269.

El-Ghaouth, A., J.L. Smilanick and C.L. Wilson, 2000. Enhancement of the performance of *Candida saitoana* by the addition of glycolchitosan for the control of postharvest decay of apple and citrus fruit. Postharvest Biol. Technol., 19: 103-110.

Elkhamass, M., B. Oulahcen, A. Lekchiri, A. Sebbata and Y. Charhabaili, 1994. Strategies used to Control Postharvest Diseases of Citrus Fruit. In: Postharvest Pathology and Technology for Horticultural Commodities: Recent Advances, Ait Oubahou, A. and M. El-otmani (Eds.). Institut Agronomique et Vétérinaire Hassan II, Agadir, Maroc, pp: 388-398.

Errampalli, D., 2004. Effect of fludioxonil on germination and growth of *Penicillium expansum* and decay in apple cvs. Empire and Gala. Crop Prot., 23: 811-817.

Fallik, E., O. Ziv, S. Grinberg, S. Alkalai and J.D. Klein, 1997. Bicarbonate solutions control powdery mildew (*Leveillula taurica*) on sweet red pepper and reduce the development of postharvest fruit rotting. Phytoparasitica, 25: 41-43.

Gabler, F.M. and J.L. Smilanick, 2001. Postharvest control of table grape gray mold on detached berries with carbonate and bicarbonate salts and disinfectants. Am. J. Enol. Viticulture, 52: 12-20.

Hervieux, V., E.S. Yaganza, J. Arul and R.J. Tweddell, 2002. Effect of organic and inorganic salts on the development of *Helminthosporium solani*, the causal agent of potato silver scurf. Plant Dis., 86: 1014-1018.

Holmes, G.J. and J.W. Eckert, 1995. Relative fitness of imazalil-resistant and sensitive biotypes of *Penicillium digitatum*. Plant Dis., 79: 1068-1073.

Holmes, G.J. and J.W. Eckert, 1999. Sensitivity of *Penicillium digitatum* and *P. italicum* to postharvest citrus fungicides in Californa. Phytopathol., 89: 716-721.

Hwang, L. and L.J. Klotz, 1938. The toxic effect of certain chemical solutions on spores of *Penicillium digitatum* and *P. italicum*. Hilgardia, 12: 1-38.

Kazemi, M., M. Aran and S. Zamani, 2011. Effect of calcium chloride and salicylic acid treatments on quality characteristics of kiwifruit (*Actinidia deliciosa* cv. Hayward) during storage. Am. J. Plant Physiol., 6: 183-189.

Mecteau, M.R., J. Arul and R.J. Tweddell, 2002. Effect of organic and inorganic salts on the growth and development of *Fusarium sambucinum*, a causal agent of potato dry rot. Mycol. Res., 106: 688-696.

Mills, A.A.S., H.W. Platt and R.A.R. Hurta, 2004. Effect of salt compounds on mycelial growth, sporulation and spore germination of various potato pathogens. Postharvest Biol. Technol., 34: 341-350.

Mills, A.A.S., H.W. Plat and R.A.R. Hurta, 2006. Sensitivity of *Erwinia* spp. to salt compounds *in vitro* and their effect on the development of soft rot in potato tubers in storage. Post Harvest Biol. Technol., 41: 208-214.

Montesinos-Herrero, C., J.L. Smilanick, J.S. Tebbets, S. Walse and L. Palou, 2011. Control of citrus postharvest decay by ammonia gas fumigation and its influence on the efficacy of the fungicide imazalil. Posth. Biol. Technol., 59: 85-93.

Nigro, F., L. Schena, A. Ligorio, I. Pentimone, A. Ippolito and M.G. Salerno, 2006. Control of table grape storage rots by pre-harvest applications of salts. Postharvest Biol. Technol., 42: 142-149.

Olivier, C., D.E. Halseth, E.S.G. Mizubuti and R. Loria, 1998. Postharvest application of organic and inorganic salts for suppression of silver scurf on potato tubers. Plant Dis., 82: 213-217.

Palmer, C.L., R.K. Horst and R.W. Langhans, 1997. Use of bicarbonates to inhibit *in vitro* colony growth of *Botrytis cinerea*. Plant Dis., 81: 1432-1438.

Palou, L., J.L. Smilanick, J. Usall and I. Vinas, 2001. Control of postharvest blue and green molds of oranges by hot water, sodium carbonate and sodium bicarbonate. Plant Dis., 85: 371-376.

Palou, L., J.L. Smilanick and S. Droby, 2008. Alternatives to conventional fungicides for the control of citrus postharvest green and blue moulds. Stewart Postharvest Rev., 4: 1-16.

Palou, L., J.L. Smilanick and C.H. Crisosto, 2009. Evaluation of food additives as alternative or complementary chemicals to conventional fungicides for the control of major postharvest diseases of stone fruit. J. Food Protection, 174: 1037-1046.

Punja, Z.K. and M.M. Gaye, 1993. Influence of postharvest handling practices and dip treatments on development of black root rot on fresh market carrots. Plant Dis., 77: 989-995.

Rani, P.U., and P. Devanand, 2011. Efficiency of Different Plant Foliar Extracts on Grain Protection and Seed Germination in Maize Res. J. Seed Sci., 4: 1-14.

Shirzadeh, E. and M. Kazemi, 2011. Effect of malic acid and calcium treatments on quality characteristics of apple fruits during storage. Am. J. Plant Physiol., 6: 176-182.

Smilanick, J.L., D.A. Margosan, F. Mlikota, J. Usall and I.F. Michael, 1999. Control of citrus green mold by carbonate and bicarbonate salts and the influence of commercial postharvest practices on their efficacy. Plant Dis., 83: 139-145.

Smilanick, J.L. and D. Sorenson, 2001. Control of postharvest decay of citrus fruit with calcium polysulfide. Postharvest Biol. Technol., 21: 157-168.

Smilanick, J.L., M.F. Mansour, D.A. Margosan, F. Mlikota-Gabler and W.R. Goodwine, 2005. Influence of pH and $NaHCO_3$ on the effectiveness of imazalil to inhibit germination of spores of *Penicillium digitatum* and to control green mold on citrus fruit. Plant Dis., 89: 640-648.

Smilanick, J.L., M. Mansour, G.G. Mlikota and W.R. Goowdwine, 2006. The effectiveness of pyrimethanil to inhibit germination of spores of *Penicillium digitatum* and to control citrus green mold after harvest. Postharv. Biol. Technol., 42: 75-85.

Smilanick, J.L., M.F. Mansour, F.M. Gabler and D. Sorenson, 2008. Control of citrus postharvest green mold and sour rot by potassium sorbate combined with heat and fungicides. Postharvest Biol. Technol., 47: 226-238.

Taqarort, N., A. Echairi, R. Chaussod, R. Nouaim and H. Boubaker *et al*., 2008. Screening and identification of epiphytic yeasts with potential for biological control of green mold of citrus fruits. World J. Microbiol. Biotechnol., 24: 3031-3038.

Tripathi, P. and N.K. Dubey, 2004. Review exploitation of natural products as an alternative strategy to control postharvest fungal rotting of fruit and vegetables. Postharvest Biol. Technol., 32: 235-245.

Valencia-Chamorro, S.A., L. Palou, M.A. del Rio and M.B. Pérez-Gago, 2008. Inhibition of *Penicillium digitatum* and *Penicillium italicum* by hydroxypropyl methylcellulose- lipid edible composite films containing food additives with antifungal properties. J. Agric. Food Chem., 56: 11270-11278.

Zhang, J. and P. Swingle, 2003. Control of green mold on Florida citrus fruit using bicarbonate salts. Conf. Proc., 116: 375-378.

Zhu, J.W., Q.Y. Xie and H. Li, 2006. Occurrence of imazalil-resistant biotype of *Penicillium digitatum* in China and the resistant molecular mechanism. J. Zhejiang Univ. Sci., 7: 362-365.