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Use of Food Additives to Control Postharvest Citrus Blue Mold Disease

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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 diseases. The antifungal activity of 10 salt compounds, considered as common food additives was assayed in *in vitro* and *in vivo* trials against *Penicillium italicum*, causal agent of citrus blue mold. Among the 10 tested salt compounds, sodium carbonate, ammonium carbonate, copper sulfate, sodium EDTA and sodium metabisulfite completely inhibited mycelial growth of Penicillium italicum at 20 mM. Colony growth of *P. italicum* on pH adjusted medium was evaluated. Results indicate that *P. italicum* can grow on both acidic and alkaline pH, with the optimum growth occurred in the range of 4.0 and 8.0. Results of the *in vivo* trials with tested salt compounds indicate that sodium metabisulfite (100 and 200 mM), boric acid (400 mM), sodium salicylate and sodium sulfite (200, 300 and 400 mM) completely inhibited blue mold development on citrus fruit. Boric acid (400 mM) and sodium metabisulfite (100 mM) gave the best results as they completely inhibited the fungus development without damaging fruit rind. Such healthy products therefore may represent a sustainable alternative to the use of chemical fungicides for controlling postharvest diseases of citrus fruit.

Key words: Citrus, food additives, blue mold, Penicillium italicum.

Introduction

Citrus fruit cultivation is very important in Morocco, being the first exporting agricultural sector and playing a major role in the national economic development. The largest volume of citrus fruit for fresh fruit consumption and export is grown and shipped from packing houses in Souss-Massa-Draa (SMD) Valley (Boubaker et al., 2009).

Postharvest green mold, caused by *Penicillium digitatum* (pers.:Fr.) Sacc. and blue mold, caused by *P. italicum* wehmer are the most important postharvest diseases that cause commercially significant losses, in Morocco (Elkhamass et al., 1994) and worldwide (Eckert JW and IL Ears 1989, Holmes GJ and JW Eckert, 1999; Zhu et al., 2006). These Penicillium species are strict wound pathogens, they are ubiquitous and produce profuse amount of asexual conidia that are readily disseminated by air current (Boubaker et al., 2009; Holmes and Eckert, 1995; Holmes and Eckert, 1999). Therefore they can infect the fruit in the grove, the packinghouses and marketing, through wounds occurred during harvest and subsequent handling (Boubaker, et al., 2009; Brown and Miller, 1999). 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, such fungal diseases are commonly controlled worldwide by applying chemical fungicides that are usually incorporated into waxes before fruit storage (Boubaker et al., 2009; Smilanick and Sorenson, 2001). However, the use of fungicides is becoming increasingly restricted due to stringent regulation, pathogen resistance development and growing public concern about chemical residues

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in fruit (Palou et al., 2008; Zhang and Swingle, 2003). These issues have resulted in an intensive search for non polluting control methods. Various alternative measures such as the application of microorganisms (El-Ghaouth et al., 2000; Lahlali et al., 2011; Taqarort et al., 2008), plant extracts (Ameziane et al., 2007; Askarne et al., 2013) or the use of effective natural substances like food additives that have minimal adverse effect on the environment and health (Arslan et al., 2009) and which exhibit a broad-spectrum antifungal activity (Corral et al., 1988), in combination or in a replacement for fungicide have been developed. 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; Arslan et al., 2009; Nigro et al., 2006). 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* (Smilanick et al., 2006; Smilanick et al., 2008; Zhang and Swingle, 2003). 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 *Helminthosporium solani* (Hervieux et al., 2002; Mills et al., 2006; Olivier et al., 1998) and potato dry rot caused by *Fusarium sambucinum* (Mecteau et al., 2002). Sodium 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 10 salt compounds, considered as common food additives, for *in vitro* and *in vivo* control of *Penicillium italicum* the causal agent of blue mold of citrus fruit.

Materials and Methods

Food Additives and Fungal Species

The food additives tested for their antifungal activity were listed in Table 1. The *P. italicum* isolate used in this study was obtained from naturally decayed orange fruit. Small pieces of fruit tissue, previously surface-disinfected with 90% ethanol, were aseptically excised from the advancing edge of the rot and transferred to Petri plates containing potato dextrose agar (PDA) acidified with 1 ml of lactic acid (80%) per litre. After a 4-day incubation period at 25°C, plates were examined under a stereomicroscope to determine colony identities. The isolate used in this work was the most aggressive one in our collection and produced the largest lesions on inoculated fruit. This fungus was purified and maintained on PDA and stored at 4°C, with periodic transfers through citrus fruit to maintain its aggressiveness (Taqarort et al., 2008).

Fruit

Fruit of mandarin (*Citrus reticulata* Blanco) cv. Clementine were used. Fruit were harvested from orchards of the M'brouka cooperative, in the Souss-Massa Valley, Morocco. Only healthy and commercially mature fruit were used in the experiments. Freshly harvested or briefly stored (no longer than 2 days) fruit were used in the screening tests.

In Vitro Antifungal Tests

The inhibitory effects of 10 food additives 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 autoclaved and cooled PDA medium at 50°C to achieve final concentrations of 2, 5, 10, 15, 20, 50,75, 100, 150 and 200 mM. The food additive-amended medium was dispensed (15ml/plate) aseptically into 9-cm-diameter Petri plates. Chemical 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 reached the edge of the Petri plates. The antifungal activity was expressed in terms of percentage of reduction of mycelial growth calculated according to the following formula: Reduction (%) = [(Diameter in control– Diameter in treatment) / Diameter in control] × 100.

The concentrations of food additives that caused 50% reduction (EC50) of mycelial growth were calculated using probit

Table 1. Chemicals used in this study.

Food Additive	Chemical Formula	Molecular Weight
Boric acide	H_3BO_3	61.83
Ammonium carbonate	$(NH_4)_2CO_3$	96.09
Copper sulfate	$CuSO_4$, $5H_2O$	249.68
Sodium EDTA	$C_{10}H_{14}N_2Na_2O_8$, $2H_2O$	372.24
Potassium carbonate	K_2CO_3	138.21
Sodium bicarbonate	$NaHCO_3$	84.01
Sodium carbonate	Na_2CO_3	105.99
Sodium metabisulfite	$Na_2S_2O_5$	190.1
Sodium salicylate	$C_7H_5NaO_3$	160.11
Sodium Sulfite	Na_2SO_3	126.04

analysis (POLO software). The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were also determined in parallel experiment. The nature of toxicity (fungistatic/fungicidal) of the food additives was determined by following the method of Tripathi et al. (2004). The inhibited fungal discs showing no growth were taken from the food additive treated Petri plates, and then re-inoculated separately into the fresh medium and revival of their growth was observed for the next 9 days at 25°C.

Effect of pH on Mycelial Growth of P. italicum

Since some food additives could affect the pH of PDA medium, the effects of pH on *P. italicum* colony growth was examined on adjusted PDA at pH 2, 4, 6, 8, 10 and 12 with 1N HCl or NaOH. Hyphal plugs (5 mm diameter) cut from the periphery of actively growing colonies (7 day-old) were transferred aseptically, mycelium down, to three replicate Petri plates containing PDA at different pH. Radial growth was determined daily, by measuring colony size along two perpendicular axes. Percentage of colony growth which is the ratio of colony growth at various food additive concentrations compared with that of control was determined.

Effects of Food Additives on Blue Mould Development in Artificially Wounded and Inoculated Fruit

The *in vivo* test was conducted as previously described by (Askarne L, et al. 2012). Briefly, fruit were wounded (2 mm deep and 3 mm wide) using a sterile needle at the equatorial side. The wounds were treated with 40 µl of food additive solutions at concentrations of 50, 100, 200, 300 and 400 mM. Controls were treated with the same volume of sterile distilled water under the same conditions. After 2-h incubation at room temperature, each wound was inoculated with 20 µl of an aqueous suspension of conidia of *P. italicum* adjusted to 106 spores ml^{-1} (Palou et al., 2002). Treated fruit were placed on plastic tray in cardboard boxes and stored at 20°C and ~95% relative humidity (RH) for 5 days. The number of infected wounds and lesion diameters of the overall treated fruit were determined daily. All treatments were arranged in a complete randomized block design. Sixteen oranges constituted a single replicate, and each treatment was replicated three times. The experiment was conducted twice, obtaining consistent results. The reported values are the average of the measurements. The incidence and severity of disease were calculated as follows:

Disease incidence (%) = [(number of rotten wounds/ number of total wounds)] x 100.

Disease severity (%) = [(average lesion diameter of treatment/ average lesion diameter of control)] x 100. In all experiments, the possible phytotoxic effect on orange fruit was examined.

Statistical Data Analysis

All data were subjected to statistical analysis of variance (ANOVA) using STATISTICA software, version 6, Stat- Soft, 2001, France. Percentage values 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 EC50 values were calculated for each compound by probit analysis using POLO software.

Results

In vitro Antifungal Tests

The *in vitro* experiments (Table 2) show that among the ten food additives tested, only ammonium carbonate, copper sulfate, sodium EDTA, sodium carbonate and sodium metabisulfite completely inhibited the growth of *P. italicum*. Among these salt compounds only ammonium carbonate that showed a fungicidal effect at 20 mM, the remaining salts were fungistatic. The lowest EC50 values against tested fungus were recorded in sodium

Table 2. Reduction (%) at 20mM, EC50, minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values of various compounds tested against *P. italicum*.

Food Additive	Reduction (%)	EC_{50} (mM)	MIC (mM)	MFC (mM)
Ammonium carbonate	100 ± 0 [a]	3.43	10	20
Boric acid	68.78 ± 2.67 [b]	16.14	50	> 200
Copper sulfate	100 ± 0 [a]	3.69	10	20
Sodium EDTA	100 ± 0 [a]	2.67	10	150
Potassium carbonate	55.66 ± 5.22 [c]	25.96	150	> 200
Sodium bicarbonate	48.88 ± 3.75 [d]	21.63	75	> 200
Sodium carbonate	100 ± 0 [a]	12.07	20	> 200
Sodium metabisulfite	100 ± 0 [a]	1.69	5	5
Sodium salicylate	44.47 ±3.48 [e]	27.29	150	200
Sodium Sulfite	42.41 ± 2.01 [e]	24.52	150	200

Values expressed are mean of three replicates; Means followed by the same letter do not differ significantly according to Duncan multiple range tests at $P<0.05$.

metabisulfite (1.69 mM), sodium EDTA (2.67 mM), ammonium carbonate (3.43 mM) and copper sulfate (3.68 mM) (Table 2). Similar results were recorded in MIC values. The MIC value of sodium metabisulfite was lower than that of copper sulfate, sodium EDTA, and ammonium carbonate. The lowest MFC value was recorded in sodium metabisulfite too. The MFC values of ammonium carbonate and copper sulfate were lower than that of sodium EDTA. Boric acid and potassium carbonate reduced the mycelial growth by more than 50 %. The remaining compounds inhibited mycelial growth by less than 50%.

pH Tests

The obtained results demonstrate that *P. italicum* grew on both acidic and alkaline pH (Fig.1). The data indicate that the optimum growth of tested fungus was obtained between pH 4 and pH 8 as the colony diameter is not significantly affected after 7 days of incubation at 25°C. bellow pH 4 and above pH 8, *P. italicum* grew at a reduced rate.

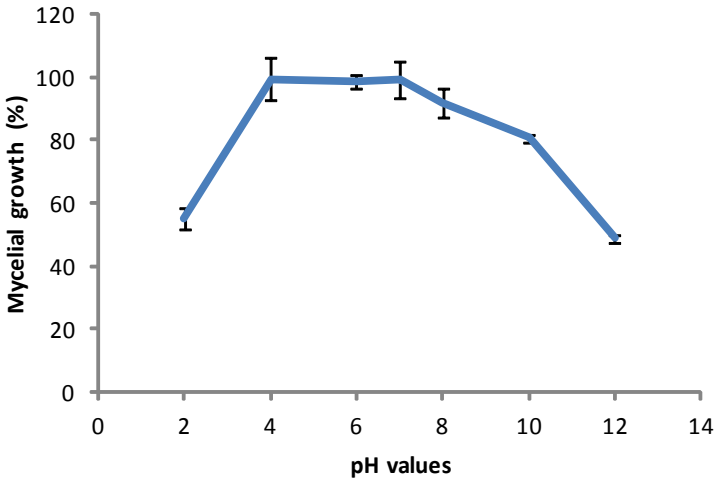

Fig. 1. Effect of pH on in vitro mycelial growth of P. italicum. Medium pH was adjusted with NaOH or HCl. Bars represent standard deviations of means.

Table 3. Blue mold incidence on oranges fruit 'cv. Clementine' treated with various concentrations of salts and stored at 20°C and ~95% relative humidity for 5 days .

Salts	Disease Incidence (%)				
	Concentration (mM)				
	50	100	200	300	400
Control	100,00 ^{c}	100,00 ^{c}	100,00 ^{d}	100,00 ^{d}	100,00 ^{d}
Sodium carbonate	100,00 ^{c}	100,00 ^{c}	100,00 ^{d}	NA*	31,25 ^{b}
Sodium metabisulfite	27,08 ^{a}	0,00 ^{a}	0,00 ^{a}	NA	NA
Boric acid	NA	NA	33,33 ^{b}	NA	0,00 ^{a}
Sodium Sulfite	83,33 ^{b}	35,42 ^{b}	0,00 ^{a}	0,00 ^{a}	0,00 ^{a}
Sodium salicylate	97,92 ^{bc}	39,58 ^{b}	0,00 ^{a}	0,00 ^{a}	0,00 ^{a}
Potassium carbonate	NA	NA	83,33 ^{c}	66,67 ^{b}	58,33 ^{b}
Ammonium carbonate	NA	NA	100,00 ^{d}	91,67 ^{cd}	91,67 ^{cd}
Sodium bicarbonate	NA	NA	91,67 ^{cd}	83,33 ^{bc}	83,33 ^{c}
Copper sulfate	91,67 ^{bc}	83,33 ^{c}	41,67 ^{b}	NA	NA
Sodium EDTA	100,00 ^{c}	100,00 ^{c}	91,67 ^{cd}	NA	NA

Values were the mean of three replicates. Means followed with different letters in each column are statistically different according to Duncan multiple range tests ($P<0.05$) applied after an analysis of variance of the arcsine of the square root of the proportion of decayed fruit. Non transformed data are shown. *: not applied.

Table 4. Blue mold severity on oranges fruit 'cv. Clementine' treated with various concentrations of salts, and stored at 20°C and ~95% relative humidity for 5 days .

Salts	Disease severity (%)				
	Concentration (mM)				
	50	100	200	300	400
Control	100,00 ^{d}	100,00 ^{d}	100,00 ^{g}	100,00 ^{d}	100,00 ^{d}
Sodium carbonate	94,88 ^{cd}	96,18 ^{d}	88,60 ^{ef}	NA*	23,81 ^{b}
Sodium metabisulfite	28,20 ^{a}	0,00 ^{a}	0,00 ^{a}	NA	NA
Boric acid	NA	NA	19,90 ^{b}	NA	0,00 ^{a}
Sodium Sulfite	71,99 ^{b}	33,58 ^{b}	0,00 ^{a}	0,00 ^{a}	0,00 ^{a}
Sodium salicylate	86,12 ^{bc}	27,35 ^{b}	0,00 ^{a}	0,00 ^{a}	0,00 ^{a}
Potassium carbonate	NA	NA	75,50 ^{de}	52,98 ^{b}	44,70 ^{b}
Ammonium carbonate	NA	NA	98,35 ^{fg}	80,54 ^{c}	83,92 ^{c}
Sodium bicarbonate	NA	NA	94,02 ^{fg}	64,42 ^{b}	76,72 ^{c}
Copper sulfate	73,$^{50\,b}$	72,45 ^{c}	36,42 ^{bc}	NA	NA
Sodium EDTA	92,80 ^{cd}	60,14 ^{c}	55,22 ^{cd}	NA	NA

Values were the mean of three replicates. Means followed with different letters in each column are statistically different according to Duncan multiple range tests ($P<0.05$) applied after an analysis of variance of the arcsine of the square root of the percentage of lesion diameters. Non transformed data are shown. *: not applied.

Effect of Salts on Blue Mold Development

The data presented in Table 3 shows that tested food additives reduced the incidence of blue mold on citrus in a dose dependent manner, with the exception of sodium metabisulfite (100 and 200 mM), boric acid (400 mM), sodium salicylate and sodium sulfite (200, 300 and 400 mM) which completely inhibited rot development on fruit.

Sodium metabisulfite (50 mM), sodium carbonate (400 mM), boric acid (200 mM), sodium sulfite (100 mM), sodium salicylate (100 mM), copper sulphate (200 mM) reduced significantly the incidence of the decay compared with the control. The percentage of rot incidence ranged between 27.08 and 41.67%.

Regarding severity of the disease, which is the ratio of lesion diameter at various food additive concentrations compared with that of control, we found that boric acid (400 mM) and sodium metabisulfite (100 mM) that gave the best results as they completely inhibited the development of the fungus without damaging fruit (Table 4). The other treatments that completely inhibited the fungus in *in vivo* experiments, lead to a drying of the rind of the fruit around the wounds which could be accompanied by browning that increases with increasing concentration.

Treatment with sodium carbonate (400 mM), sodium metabisulfite (50 mM), boric acid (200 mM), sodium sulfite (100 mM), sodium salicylate (100 mM), potassium carbonate (300 and 400 mM) and sodium EDTA (200 mM) significantly reduced the severity of the decay without damaging the fruit. The percentages of severity varied between 19.9 and 55.22% (Table 4).

Discussion

The obtained results demonstrate that several food additives can inhibit significantly the growth of *P. italicum*. In our study, we found that sodium metabisulfite completely inhibited mycelial growth of *P. italicum*. Several previous studies demonstrate that sodium metabisulfite has been shown to completely inhibit *in vitro* mycelial growth of H. solani (Hervieux et al., 2002), *Fusarium sambucinum* (Mecteau et al., 2002), *Geotrichum candidum* (Talibi I, et al. 2011) and a wide range of potato postharvest pathogens (Mills et al., 2004). The present study shows that copper sulfate completely inhibited the mycelial growth of *P. italicum*. However, Mills et al. (2004) reported that copper sulfate had only a reduced effect on mycelial growth of *Phytophthora erythroseptica*. Sodium carbonate completely inhibited the mycelial growth of *P. italicum*. It has also a strong effect on *Geotrichum candidum* as reported by Talibi et al. (2011). Palou et al. (2001), demonstrated that sodium carbonate had fungistatic rather than fungicidal activity against *P. italicum* which is consistent with our data.

Considering that several salt compounds could influence medium pH, the effect of pH on *P. italicum* growth was determined. The results showed that *P. italicum* grew at acidic pH as well as at alkaline pH, which agree with the results of Talibi et al. (2011) concerning *Geotrichum candidum*.

The optimum growth of *P. italicum* was obtained in the range of 4.0 and 8.0 as colony growth was not significantly affected by pH modifications. Panasenko (1967) reported that most of *Penicillium* species could develop even at pH 2.0 and they are generally fruit contaminants. Byrde and Willets (1977) stud-

ied the effect of pH on *Monilinia* sp. growth and found that the specie can grow at varied pH (from 1.5 to 9.00) with optimum growth occurred under acidic pH.

This ability of pathogens to grow over a wide range of pH, shows that differences in mycelial growth in salts amended medium (Table 2) cannot be only due to the effect of pH. In addition, we have got a total inhibition in the radial growth of *P. italicum* both in the case of treatment with salt solutions at acidic or alkaline pH. Hervieux et al. (2002) reported also that differences in the behavior of fungi toward salts could not be only due to the effect of pH.

Yaganza (2005) explained the adaptation of pathogens to a wide range of pH by several mechanisms known as "pH homeostasis" (White, 2000). These mechanisms exist in the cell membranes of pathogens. They maintain the stability of the macromolecules such as enzymes and therefore the growth and metabolism of these microorganisms. Their mode of action is based on the regulation of ion transport across membranes, even when the extracellular pH varies significantly and this by means of the selectivity and energy coupling to the translocation of solutes (Booth, 1988).

Sofos et al. (1986) reported that the inhibitory effect of salts to microorganisms could be due to the altered function of the transport cells and enzymes involved in the glycolytic pathway.

For the *in vivo* tests, the results showed that sodium metabisulfite (100 and 200 mM), boric acid (400 mM), sodium salicylate and sodium sulfite (200, 300 and 400 mM) completely inhibited the development of blue mold on treated citrus fruit (Table 3). Talibi et al. (2011) also reported that boric acid and sodium salicylate applied at a concentration of 3% significantly reduced the incidence and severity of sour rot caused by *G. candidum*. Although sodium salicylate was effective against both citrus blue mold and citrus sour rot, it was phytotoxic to fruit rind at all most tested concentrations.

Talibi et al. (2011) reported also that sodium EDTA significantly reduced the incidence and severity of sour rot. This is consistent with previous studies that have shown that sodium EDTA is effective against citrus green mold, caused by *P. digitatum* (Valencia-Chamorro et al., 2008), and against gray mold of apples caused by *B. cinerea* (Droby et al., 2003). The present study showed that sodium EDTA significantly reduced the severity of postharvest citrus blue mold, but he had only a limited effect in reducing the incidence of the disease. In the current study, we found that sodium carbonate and potassium carbonate (400 mM) reduced significantly the incidence of citrus blue mold compared with the control. Palou et al. (2009), reported that sodium carbonate and potassium carbonate reduced also the incidence of the decay caused by *Monilinia fructicola*, *Botrytis cinerea*, *Geotrichum candidum*, and *Penicillium expansum* in many stone fruit. However, treatment of citrus fruit with ammonium carbonate showed a high decay incidence of blue mold (current data), while Talibi et al. (2011) reported that ammonium carbonate at 3% (312,2 mM) reduced the incidence of sour rot caused by *G. candidum* in postharvest citrus fruit by more than 51% and the severity by more than 74% .

These behavioral differences of ammonium carbonate toward post-harvest fungi could be explained by changes in the pH of the environment of the wounds. Indeed, *G. candidum* is a post-harvest fungus that leads to an increase in the pH of the medium. Furthermore, Palmer et al. (1997) reported that ammonium salts are effective under alkaline rather than acidic conditions, where the production of ammonia gas (NH_3) is favored over the form (NH^{4+}) which is inefficient. Montesinos-Herrero et al. (2011) reported that they effectively controlled post-harvest green and blue molds on lemons and oranges by applying ammonium in its active form, which is fumigation of fruit with a dose of ammonia gas not exceeding 6000 µl/l for 6 h at 22°C. These authors found also that germination of *P. italicum* conidia was more sensitive to the treatment compared with those of *P. digitatum*, and fumigation with ammonia gas could even control an isolate of *P. digitatum* resistant to the treatment with imazalil.

Conclusion

The result of this study showed that among tested salt compounds boric acid, sodium metabisulfite, sodium sulfite, sodium salicylate, sodium carbonate and copper sulfate showed high antifungal activity against citrus blue mold in both *in vitro* and *in vivo* tests. The use of these compounds can be considered a useful strategy to be included in an integrated approach for controlling postharvest diseases of citrus fruit. However, the potential use of salt compounds to control postharvest diseases requires a detailed examination of their biological activity *in vivo* and the development of formulation which inhibits the growth of the pathogens at non-phytotoxic concentrations.

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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. Agronomy for Sustainable Development 27(3): 273-277.

Arslan U, K Ilhan and OA Karabulut (2006) Evaluation of food additives and low-toxicity compounds for the control of bean rust and wheat leaf rust. Journal of Phytopathology 154(9): 534-541.

Arslan U, K Ilhan, C Vardar and OA Karabulut (2009) Evaluation of antifungal activity of food additives against soilborne phytopathogenic fungi. World Journal of Microbiology and Biotechnology 25(3): 537-543.

Askarne L, I Talibi, H Boubaker, E Boudyach, F Msanda, B Saadi and A Ait Ben Aoumar (2013) Use of Moroccan medicinal plant extracts as botanical fungicide against citrus blue mould. Letters in Applied Microbiology 56(1): 37-43.

Askarne L, I Talibi, H Boubaker, EH Boudyach, F Msanda, B Saadi, MA Serghini and A Ait Ben Aoumar (2012) *In vitro* and *in vivo* antifungal activity of several Moroccan plants against *Penicillium italicum*, the causal agent of citrus blue mold. Crop Protection 40(0): 53-58.

Booth I (1988) Control of proton permeability: its implications for energy transduction and pH homeostasis. In: Whittenburry R., Banks J. G., Gould G. W. and B. R. G. (eds.) Homeostatic mechanisms in micro-organisms. FEMS symposium. Bath University Press, pp. 1-12.

Boubaker H, B Saadi, EH Boudyach and A Ait Benaoumar (2009) Sensitivity of Penicillium digitatum and *P. italicum* to Imazalil and Thianbendazole in Morocco. Plant Pathology Journal 8(4): 152-158.

Brown GE and WR Miller (1999) Maintaining fruit health after harvest. In: L. Timmer and L. Duncan (eds.) Citrus Health Management. The American Phytopathological Society Press. St. Paul, MN, pp. 175-188.

Byrde RJW and HJ Willets (1977) The brown rot fungi of fruit. Oxford, Pergamon Press, p. 58.

Corral LG, LS Post and TJ Montville (1988) Antimicrobial Activity of Sodium Bicarbonate. Journal of Food Science 53(3): 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 Biology and Technology 27(2): 127-135.

Eckert JW and IL Ears (1989) Postharvest Disorders and Diseases of Citrus Fruits. The Citrus Industry: Crop protection, postharvest technology, and early history of citrus research in California 5: 179.

El-Ghaouth A, JL Smilanick and CL 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 Biology and Technology 19(1): 103-110.

Elkhamass M, B Oulahcen, A Lekchiri, A Sebbata and Y Charhabaili (1994) Stratégie de lutte contre les maladies de post-récolte des fruits d'agrumes. In: A. Ait Oubahou and M. Elotmani (eds.) Postharvest Pathology and technology for Horticultural Commodities: Recent advances. Institut Agronomique et Vétérinaire Hassan II, Agadir, Maroc, pp. 388–398.

Hervieux V, ES Yaganza, J Arul and RJ Tweddell (2002) Effect of organic and inorganic salts on the development of *Helminthosporium solani*, the causal agent of potato silver scurf. Plant disease 86(9): 1014-1018.

Holmes GJ and JW Eckert (1995) Relative fitness of imazalil-resistant and-sensitive biotypes of *Penicillium digitatum*. Plant disease 79(10): 1068-1073.

Holmes GJ and JW Eckert (1999) Sensitivity of *Penicillium digitatum* and *P. italicum* to Postharvest Citrus Fungicides in California. Phytopathology 89(9): 716-721.

Lahlali R, Y Hamadi, ME guilli and MH Jijakli (2011) Efficacy assessment of *Pichia guilliermondii* strain Z1, a new biocontrol agent, against citrus blue mould in Morocco under the influence of temperature and relative humidity. Biological Control 56(3): 217-224.

Mecteau MR, J Arul and RJ Tweddell (2002) Effect of organic and inorganic salts on the growth and development of *Fusarium sambucinum*, a causal agent of potato dry rot. Mycological Research 106(6): 688-696.

Mills AAS, HW Platt and RAR Hurta (2004) Effect of salt compounds on mycelial growth, sporulation and spore germination of various potato pathogens. Postharvest Biology and Technology 34(3): 341-350.

Mills AAS, HW Platt and RAR 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. Postharvest Biology and Technology 41(2): 208-214.

Montesinos-Herrero C, JL Smilanick, JS 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. Postharvest Biology and Technology 59(1): 85-93.

Nigro F, L Schena, A Ligorio, I Pentimone, A Ippolito and MG Salerno (2006) Control of table grape storage rots by pre-harvest applications of salts. Postharvest Biology and Technology 42(2): 142-149.

Olivier C, DE Halseth, ESG Mizubuti and R Loria (1998) Postharvest application of organic and inorganic salts for suppression of silver scurf on potato tubers. Plant disease 82(2): 213-217.

Palmer CL, RK Horst and RW Langhans (1997) Use of bicarbonates to inhibit *in vitro* colony growth of *Botrytis cinerea*. Plant disease 81(12): 1432-1438.

Palou L, JL Smilanick and CH Crisosto (2009) Evaluation of food additives as alternative or complementary chemicals to conventional fungicides for the control of major postharvest diseases of stone fruit. Journal of Food Protection 72(5): 1037-1046.

Palou L, JL Smilanick and S Droby (2008) Alternatives to conventional fungicides for the control of citrus postharvest green and blue moulds. Stewart Postharvest Review 4(2): 1-16.

Palou L, JL Smilanick, J Usall and I Viñas (2001) Control of postharvest blue and green molds of oranges by hot water, sodium carbonate, and sodium bicarbonate. Plant Disease 85(4): 371-376.

Palou L, J Usall, JL Smilanick, MJ Aguilar and I Viñas (2002) Evaluation of food additives and low toxicity compounds as alternative chemicals for the control of *Penicillium digitatum* and *Penicillium italicum* on citrus fruit. Pest Management Science 58(5): 459-466.

Panasenko VT (1967) Ecology of microfungi. The Botanical Review 33(3): 189-215.

Smilanick JL, MF Mansour, FM Gabler and WR Goodwine (2006) The effectiveness of pyrimethanil to inhibit germination of *Penicillium digitatum* and to control citrus green mold after harvest. Postharvest Biology and Technology 42(1): 75-85.

Smilanick JL, MF Mansour, FM Gabler and D Sorenson (2008) Control of citrus postharvest green mold and sour rot by potassium sorbate combined with heat and fungicides. Postharvest Biology and Technology 47(2): 226-238.

Smilanick JL and D Sorenson (2001) Control of postharvest decay of citrus fruit with calcium polysulfide. Postharvest Biology and Technology 21(2): 157-168.

Sofos J, M Pierson, J Blocher and F Busta (1986) Mode of action of sorbic acid on bacterial cells and spores. International Journal of Food Microbiology 3(1): 1-17.

Talibi I, L Askarne, H Boubaker, EH Boudyach and A Ait Ben Aoumar (2011) *In vitro* and *In vivo* Antifungal Activities of Organic and Inorganic Salts Against Citrus Sour Rot Agent *Geotrichum candidum*. Plant Pathology Journal 10(4): 138-145.

Taqarort N, A Echairi, R Chaussod, R Nouaim, H Boubaker, A Ait Benaoumar and EH Boudyach (2008) Screening and identification of epiphytic yeasts with potential for biological control of green mold of citrus fruits. World Journal of Microbiology and Biotechnology 24(12): 3031-3038.

Tripathi P, NK Dubey, R Banerji and JPN Chansouria (2004) Evaluation of some essential oils as botanical fungitoxicants in management of post-harvest rotting of citrus fruits. World Journal of Microbiology and Biotechnology 20(3): 317-321.

Valencia-Chamorro SA, L Palou, MA del Rio and MB Pérez-Gago (2008) Inhibition of Penicillium digitatum and *Penicillium italicum* by Hydroxypropyl Methylcellulose- Lipid Edible Composite Films Containing Food Additives with Antifungal Properties. Journal of agricultural and food chemistry 56(23): 11270-11278.

Zhang J and P Swingle (2003) Control of green mold on Florida citrus fruit using bicarbonate salts. pp. 375-378.

Zhu J-w, Q-y Xie and H-y Li (2006) Occurrence of imazalil-resistant biotype of Penicillium digitatum in China and the resistant molecular mechanism. Journal of Zhejiang University - Science A 7(0): 362-365.