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Postharvest Application of Potassium Sorbate and Sodium Carbonate for Controlling Blue and Green Moulds of Navel Orange

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The food preservatives potassium sorbate or sodium carbonate were evaluated to study their effect on green and blue moulds incidence of navel orange fruits. Potassium sorbate and sodium carbonate at concentration of 4.0% completely inhibited the linear growth and spore germination of *Penicillium digitatum* and *P. italicum*. Fresh navel orange fruits were artificially wounded and inoculated with spore suspension (10^6 spores/ml) of *P. digitatum* or *P. italicum* then dipped in potassium sorbate or sodium carbonate at different concentrations then air dried and stored for 15 days. The most effective treatment was potassium sorbate at concentration of 4.0% which reduced the disease incidence and rotted part tissue by 80.0 & 89.0 and 83.3 & 90.0% for blue and green moulds, respectively. Diameter of inhibition growth zone of both fungi gradually increased as salt concentrations increased, individually or combined with water wax. In other experiment, potassium sorbate and sodium carbonate were mixed with carnova wax to study their durable protective effect against green and blue moulds incidence on navel orange fruits during 45 days of storage. All treatments have significantly reduced both diseases incidence and rotted part tissue during 45 days. The highest reduction was obtained with potassium sorbate at concentration 4.0% which reduced the blue & green moulds incidence and rotted part tissue more than 90.0 and 92.0%, respectively. While, potassium sorbate at concentrations of 2.0% and sodium carbonate at 4.0% reduced both diseases incidence and rotted part tissue more than 80.0 and 84.0%, respectively. So, postharvest application of potassium sorbate or sodium carbonate could be considered for controlling postharvest diseases of navel orange fruits.

Keywords: Blue mould, green mould, navel orange, potassium sorbate and sodium carbonate.

Fruit decay caused by *Penicillium digitatum* Pers. Sacc. (green mould), and *P. italicum* Wehmer (blue mould) are the most important diseases affecting harvested citrus fruits during handling, transportation, exportation and storage (Ismail and Zhang, 2004; Abd-El-Kareem and Abd-Alla, 2002 and Abd-El-Kareem, 2002).

Several investigations found that chemical fungicides currently provide the primary mean for controlling postharvest decay in citrus fruit (Hyas *et al.*, 2007). However, the use of chemical fungicides for postharvest disease control in under scrutiny due to growing consumer concern about pesticide residues along with the development of pathogen resistance to approved pesticides (Lanza *et al.*, 2004). The

food preservatives as potassium sorbate, sodium carbonate and bicarbonate had antifungal activities for control postharvest decaying fungi (Palou *et al.*, 2001and Smilanick *et al.*, 2008) Sorbic acid and its salts potassium sorbate are the most widely used antimicrobial agents for food preservation worldwide. Using potassium sorbate against postharvest diseases of tomato, apple, carrots and potato was reported by Ryu and Holt (1993). The food preservative potassium sorbate was applied to citrus fruits inoculated with *Penicillium digitatum* have similar fungicidal activity and are equivalent to the traditional treatment used as a postharvest fungicide for controlling citrus fruit decay (Montesinos-Herrero *et al.*, 2009). Carbonic acid salts, such as sodium carbonate (Na_2CO_3, soda ash) is common food additives allowed with no restrictions for many applications by European and North American regulations (Lindsay, 1985 and Palou *et al.*, 2002). Sodium carbonate has been used to improve cleaning and also to reduce postharvest decay of lemons in California, USA for more than 70 years (Eckert and Brown, 1986). Sodium carbonate solution used correctly, approach the effectiveness of common synthetic fungicides used to control *P. digitatum* on lemons and oranges (Smilanick *et al.*, 1997).

The purpose of the present study is evaluating the effects of potassium sorbate and sodium carbonate on linear growth and spore germination of pathogenic fungi. Moreover, their efficacy on green mould and blue moulds incidence of navel orange fruits. Furthermore, to evaluate their durable protective effect against both diseases during 45 days of storage.

Materials and Methods

In vitro inhibition of linear growth of pathogenic fungi by different concentrations of potassium sorbate and sodium carbonate:

Potassium sorbate and sodium carbonate (Sigma Co.) at different concentrations, *i.e.* 0.0, 0.5, 1.0, 2.0 and 4.0% (w/v) were prepared. Salt solutions were added to conical flasks containing sterilized PDA medium to obtain the proposed concentrations, then mixed gently and dispensed in sterilized Petri plates (9-cm-diam.). Plates were individually inoculated at the centre with equal disks (5-mm-diam.) of 10-day-old culture of *P. digitatum* or *P. italicum* (isolated from citrus fruits in the laboratory, Plant Pathol. Dept., Fac. Agric., Moshtohor). Five plates were used as replicates for each particular treatments. Inoculated plates were incubated at 20±2°C. The average linear growth of fungi tested was calculated after 10 days.

In vitro inhibition of spore germination of pathogenic fungi by different concentrations of potassium sorbate and sodium carbonate:

Preparation of spore suspension:

Spores suspension were prepared by inoculated sterilized PDA medium with disk (6-mm-diam.) taken from 10-day-old cultures of *P. digitatum* and *P italicum*. Plates were incubated at 20±2°C for 10 days. Spores of *P. digitatum* and *P. italicum* were harvested and transferred to sterilized water (containing 0.01% Tween 80) to obtain spore suspension which was adjusted to 10^6 spores/ml using hemocytometer slide (Palou *et al.*, 2001).

Potassium sorbate and sodium carbonate at different concentrations, *i.e.* 0.0, 0.5, 1.0, 2.0 and 4.0% (w/v) were prepared as mentioned before. One ml of each prepared spore suspension was placed in Petri plates. PDA media containing different salt concentrations were poured before solidifying into the previous inoculated plates and rotated gently to ensure even distribution of fungal spores. Inoculated plates were incubated at 20°C for 24 h. Germinated spores were counted microscopically and percentage of spore germination was calculated.

Testing of different concentrations of potassium sorbate and sodium carbonate on green and blue mould incidence on navel orange fruits:

Different concentrations of potassium sorbate and sodium carbonate were tested to study their effect against green and blue moulds incidence on navel orange fruits. Fresh navel orange fruits apparently free from physical damage and diseases were artificially wounded using sterilized scalpel. Inoculation of wounded fruits was carried out by spraying fruits with a spore suspension (10^6 spores/ml) of *P. digitatum* or *P. italicum* then air dried. Inoculated fruits were dipped in potassium sorbate or sodium carbonate at concentrations of 0.0, 2.0 and 4.0% (w/v) containing 0.01% Tween 80 for 3 min, then air dried. All treated or un-treated fruits were placed into carton boxes at the rate of 10 fruits/box. Each particular concentration as well as control treatment was represented by three carton box. All boxes were stored at 20±2°C for 15 days. Percentage of infected fruits (disease incidence) and rotted parts of fruits (disease severity) were recorded after 15 days.

Testing of long-term protective effect of potassium sorbate and sodium carbonate mixed with carnova wax against postharvest diseases of navel orange fruits:

a- Testing the compatibility effect between potassium sorbate or sodium carbonate and water wax on zone inhibition area:

Potassium sorbate and sodium carbonate at three concentrations, *i.e* 0.0, 2.0 and 4.0% were tested to study their compatible effect with water wax (carnova wax) as a commercial coating material used for prolonging the shelf life of citrus fruits (Brown, 1984). Peel disks of navel orange were used as carrier material for testing chemicals. Peel disks (10-mm-diam.) of navel orange were dipped in tested salts, or water wax containing salt to obtain final concentration, then air dried and transferred to Petri plates containing PDA medium inoculated with spore suspension (10^6 spore/ml) of *P. digitatum* or *P. italicum.*

Inoculated plates were incubated at 20°C for 5 days and diameter of inhibition zone (mm) was measured.

b- Long-term protective effect of potassium sorbate and sodium carbonate mixed with carnova wax against green and blue moulds of navel orange fruits:

Potassium sorbate and sodium carbonate at three concentrations, *i.e.* 0.0, 2.0 and 4.0% were evaluated for their durable protective effect against green and blue moulds incidence on navel orange fruits during 45 days of storage. Navel orange fruits were dipped in water wax containing 0.0, 2.0 or 4.0% of tested treatments then air dried for 2h and incubated at 20±2°C for 40 days. To test the long acting protecting effect was carried out by inoculated treated fruits with spore suspension (10^6spores/ml) of *P digitatum* or *P italicum* every 10 days. Percentage of infected fruits and rotted parts were recorded after 15, 30 and 45 days of incubation. All

treated or un-treated fruits were placed into carton boxes at the rate of 10 fruits/box. Each particular concentration as well as control treatment was represented by three carton boxes.

Diseases assessment:

Disease incidence was expressed as percentage of infected fruits. The percentage of rotted part of fruit was calculated from the following formula:-

$$\text{Rotted part of fruit (\%)} = \frac{\text{Rotted part weight}}{\text{Fruit weight}} \times 100$$

Statistical analysis:

Tukey test for multiple comparisons among means was utilized (Neler *et al.*, 1985).

Results

Effect of different concentrations of potassium sorbate and sodium carbonate on linear growth of pathogenic fungi:

Potassium sorbate and sodium carbonate at different concentrations, *i.e.* 0.0, 0.5, 1.0, 2.0 and 4.0% (w/v) were tested to study their inhibitory effect on linear growth and spore germination of *P. digitatum* and *P. italicum*. Data in Table (1 and 2) indicate that all treatments significantly reduced the linear growth and spore germination of both tested fungi. Potassium sorbate and sodium carbonate at concentration of 4.0% completely inhibited the linear growth, while, either 2.0 or 4.0% completely inhibited the spore germination of *P. digitatum* and *P. italicum*. The highest reduction was obtained with potassium sorbate and sodium carbonate at concentration of 1.0% which reduced the linear growth and spore germination more than 74.4 and 72.2% for *P. digitatum* and *P. italicum*, respectively. Other treatments were less effective.

Table 1. Linear growth of *P. digitatum* and *P. italicum* as affected by different concentrations of sodium carbonate or potassium sorbate

Tested salt	Conc. (%)	*P. digitatum*		*P. italicum*	
		Linear growth (mm)	Reduction (%)	Linear growth (mm)	Reduction (%)
Sodium carbonate	0.5	65.0 b *	27.8	70.0 b	22.2
	1.0	23.0 d	74.4	25.0 d	72.2
	2.0	00.0 e	100.0	6.0 e	93.3
	4.0	0.00 e	100.0	0.00 e	100.0
Potassium sorbate	0.5	40.5 c	55.0	52.0 c	42.2
	1.0	18.0 d	80.0	22.5 d	75.0
	2.0	00.0 e	100	4.0 e	95.6
	4.0	00.0 e	100	00.0 e	100
Control	00	90.0 a	—	90.0 a	—

* Figures with the same letter are not significantly different (P= 0.05).

Table 2. Average percent of spore germination of pathogenic fungi as affected with different concentrations of sodium carbonate or potassium sorbate

Tested salt	Conc. (%)	*P. digitatum*		*P. italicum*	
		Spore germination (%)	Reduction (%)	Spore germination (%)	Reduction (%)
Sodium carbonate	0.5	50.0 b *	46.2	55.0 b	39.6
	1.0	18.0 d	80.6	23.0 d	74.7
	2.0	00.0 e	100.0	00.0 e	100.0
	4.0	00.0 e	100.0	00.0 e	100.0
Potassium sorbate	0.5	42.0 c	54.8	50.0 b	45.1
	1.0	23.0 d	74.4	32.0 c	64.8
	2.0	00.0 e	100.0	00.0 e	100.0
	4.0	00.0 e	100.0	00.0 e	100.0
Control	00	93.0 a	—	91.0 a	—

* Figures with the same letter are not significantly different (P= 0.05).

Effect of different concentrations of potassium sorbate and sodium carbonate on green and blue moulds incidence of navel orange fruits:

Potassium sorbate and sodium carbonate at three concentrations, *i.e.* 0.0, 2.0 and 4.0% were tested to study their effect against green and blue moulds incidence on navel orange fruits. Results in (Table 3 and 4) indicate that all treatments significantly reduced the diseases incidence and rotted part tissue (Disease severity). The most effective treatment was potassium sorbate at concentration 4.0% which reduced the disease incidence and rotted part tissue by 80.0 & 89.0 and 83.3 & 90.0% for blue and green moulds, respectively. The highest reduction was obtained with potassium sorbate at concentrations of 2.0% and sodium carbonate at 4.0% with reduced both diseases incidence and rotted part tissue more than 80.0 and 86.0%, respectively. Meanwhile, sodium carbonate at concentration 2.0% showed moderate effect.

Table 3. Percent of green and blue moulds incidence on navel orange fruits affected with different concentrations of sodium carbonate or potassium sorbate

Tested salt	Conc. (%)	Disease incidence (%)			
		Blue mould	Reduction (%)	Green mould	Reduction (%)
Sodium carbonate	2.0	30.0 b	70.0	25.0 b	75.0
	4.0	20.0 c	80.0	16.7 bc	83.3
Potassium sorbate	2.0	16.7 c	83.3	13.3 c	86.7
	4.0	6.7 d	93.3	6.7 d	93.3
Control	0.0	100.0 a	—	100.0 a	—

* Figures with the same letter are not significantly different (P= 0.05).

Table 4. Percent of rotted part tissue caused by green and blue moulds diseases on navel orange fruits as affected with different concentrations of sodium carbonate or potassium sorbate

Tested salt	Conc. (%)	Rotted part tissues (%)			
		Blue mould	Reduction (%)	Green mould	Reduction (%)
Sodium carbonate	2.0	26.0 b	74.0	22.0 b	78.0
	4.0	11.0 c	89.0	10.0 c	90.0
Potassium sorbate	2.0	14.0 c	86.0	11.0 c	89.0
	4.0	6.0 d	94.0	4.0 d	96.0
Control	0.0	100.0 a	—	100.0 a	—

* Figures with the same letter are not significantly different (P= 0.05).

Long-term protective effect of potassium sorbate and sodium carbonate mixed with carnova wax against postharvest diseases of navel orange fruits

a- Compatibility effect between potassium sorbate or sodium carbonate and carnova wax on zone inhibition area:

Potassium sorbate and sodium carbonate at three concentrations, *i.e.* 0.0, 2.0 and 4.0% were tested to study their compatible effect with water wax (carnova wax) on zone inhibition area of *P. digitatum* and *P. italicum* growth. Data in Table (5) indicate that all salts have inhibitory effect on the growth of pathogenic fungi appeared as a clear zone of fungal growth. Diameter of inhibited growth zone of both fungi gradually increased as salts concentrations increased as individually or combined with water wax. The highest increase in inhibition zone diameter was obtained with potassium sorbate at 4.0% as water solution for tested fungi. All salts at 2.0% as single treatments had moderate effect. Statistical analysis indicates that the efficacy of all tested salts reduced when mixed with water wax.

Table 5. Compatibility effect between sodium carbonate or potassium sorbate and carnova wax on zone inhibition area

Treatment	Zone of inhibition (mm)			
	P. digitatum		*P. italicum*	
	Salt	Salt + wax	Salt	Salt + wax
Sodium carbonate 2.0%	A* 8.0 b	B 3.0 b	A 6.0 c	B 2.0 c
Sodium carbonate 4.0%	A 12.0 b	B 5.0 b	A 10.5 b	B 3.0 bc
Potassium sorbate 2.0%	A 12.0 b	B 4.0 b	A 9.5 b	B 4.0 b
Potassium sorbate 4.0%	A 18.0 a	B 8.5 a	A 14.0 a	B 6.5 a
Control	A 00.0 d	A 00.0 c	A 00.0 d	A 00.0 d

* Figures with the same letter are not significantly different, small letters between treatments and capital letters between salt and salt + wax. (P = 0.05).

b- Long-term protective effect of potassium sorbate and sodium carbonate mixed with carnova wax against green and blue moulds on navel orange fruits:

Potassium sorbate and sodium carbonate at three concentrations, *i.e.* 0.0, 2.0 and 4.0% were evaluated for their durable protective effect against green and blue moulds incidence on navel orange fruits during 45 days of storage.

Data in Table (6 and 7) indicate that all treatments significantly reduced the both diseases incidence and rotted part tissue (Disease severity) during 45 days. The highest reduction was potassium sorbate at concentration 4.0% which reduced the blue & green moulds incidence and rotted part tissue more than 90.0 and 92.0%, respectively. Followed by potassium sorbate at concentrations of 2.0% and sodium carbonate at 4.0% with reduced both diseases incidence and rotted part tissue more than 80.0 and 84.0%, respectively. Meanwhile, sodium carbonate at concentration 2.0% showed moderate effect.

Table 6. Effect of long-term protective effect (1) of sodium carbonate or potassium sorbate mixed with carnova wax on postharvest diseases of navel orange during 45 days

Tested salt	Conc. (%)	Disease incidence (%)			
		Blue mould	Reduction (%)	Green mould	Reduction (%)
Sodium carbonate	2.0	33.3 b*	66.7	26.7 b	73.3
	4.0	23.3 c	76.7	20.0 bc	80.0
Potassium sorbate	2.0	20.0 c	80.0	16.7 cd	83.3
	4.0	10.0 d	90.0	10.0 d	90.0
Control	0.0	100.0 a	—	100.0 a	—

* Figures with the same letter are not significantly different (P =0.05). (1) Testing of protective effect was carried out by inoculating treated fruits with spore suspension (10^6 spores/ml) of *P. digitatum* or *P. italicum* every 10 days.

Table 7. Effect of long-term protective effect (1) of sodium carbonate or potassium sorbate mixed with carnova wax on rotted part tissue of navel orange fruits during 45 days

Tested salt	Conc. (%)	Rotted tissues (%)			
		Blue mould	Reduction (%)	Green mould	Reduction (%)
Sodium carbonate	2.0	25.0 b *	75.0	23.0 b	77.0
	4.0	15.0 c	85.0	14.0 c	86.0
Potassium sorbate	2.0	16.0 c	84.0	13.0 cd	87.0
	4.0	8.0 d	92.0	7.5 d	92.5
Control	0.0	100.0 a	—	100.0 a	—

* Refer to footnote of Table (6).

Discussion

Navel orange is the most important exportation fruits. Postharvest diseases caused by *Penicillium digitatum* (green mould), and *P. italicum* (blue mould) are the most important diseases affecting harvested citrus fruits (Ismail and Zhang, 2004; Abd-El-Kareem and Abd-Alla, 2002 and Abd-El-Kareem, 2002).The food preservatives potassium sorbate or sodium carbonate, had antifungal activities against postharvest decaying fungi (Palou *et al.*, 2001and Smilanick *et al.*, 2008). In present study, potassium sorbate and sodium carbonate at 4.0% completely inhibited the linear growth and spore germination of both pathogenic fungi. Inhibition of microorganisms by sorbic acid and its salts may be caused by alternation of cell transport function, inhibition of enzymes involved in the glycolytic pathway or tricarboxylic acid cycle by inhibition of RNA, DNA, and protein synthesis, and by uncoupling of the oxidative phosphorylation in mitochondria (Sofos, 1992). The depletion of ATP was reported in conidia of various moulds fungi after exposure to sorbic acid (Cheng and Piper, 1994). The very low levels of mammalian toxicity of potassium sorbate (LD 50 in rate of 4-7 g/kg body weight, equals 500g for an adult human) and its wide application as a food preservative would make it an excellent candidate for postharvest treatment (Sofos, 1992). Using potassium sorbate or sodium carbonate for controlling postharvest diseases of tomato, apple, carrots and potato was reported by Palou *et al.* (2001). In present study, potassium sorbate and sodium carbonate at 2.0 and 4.0% significantly reduced the blue and green moulds of navel orange fruits when applied as water solution. In addition to prolonged their protective effect for 45 days of storage against both diseases when mixed with carnova wax (as a commercial wax using for fruit coating). Several reports used potassium sorbate for controlling postharvest diseases on different fruits (Ryu & Hold, 1993). Sorbic acid and its salts derivatives are the most widely used antimicrobial agents for food preservation worldwide, they are also used as preservation in animal feeds, pharmaceuticals and cosmetics (Sofos, 1992). Carbonic acid salts, such as sodium carbonate (Na_2CO_3, soda ash) is a common food additive allowed with no restrictions for many applications by European and North American regulations (Lindsay, 1985). Sodium carbonate was listed as approved ingredients on products labelled "organic" as proposed by the United States Department of Agriculture. The antimicrobial activity of these compounds has been described *in vitro* (Corral *et al.*, 1988) and on leaves and fruit (Smilanick *et al.*, 1997). Sodium carbonate has been used to improve cleaning and also to reduce postharvest decay of lemons in California for more than 70 years (Eckert and Eaks, 1989). Both salts can be useful tools to manage postharvest decay because they are inexpensive, readily available, and can be used with a minimal risk of injury to the fruit. Recent work shows that sodium carbonate solution, approach the effectiveness of common synthetic fungicides used to control *P. digitatum* on oranges (Smilanick *et al.*, 1997). Recently, the influence of commercial postharvest practices on the control of green mould by sodium carbonate and sodium bicarbonate was published to facilitate their commercial adoption (Smilanick *et al.*, 1999).

It could be suggested the use of potassium sorbate or sodium carbonate for wide application as a food preservative and also for controlling postharvest diseases of navel orange fruits.

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مقاومة العفن الاخضر والعفن الازرق في البرتقال (أبو سرة) باستخدام سوربات البوتاسيوم وكربونات الصوديوم

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تم اختبار تأثير بعض المواد الحافظة الآمنة التى تستخدم فى حفظ الأغذية وهى سوربات البوتاسيوم وكربونات الصوديوم فى مقاومة مرضي العفن الاخضر والازرق فى ثمار البرتقال (أبو سرة). وتتلخص النتائج فيما يلى :

أدت سوربات البوتاسيوم وكربونات الصوديوم عند تركيز ٤,٠% الى التثبيط الكامل للنمو الطولى وانبات الجراثيم لكلا لفطرين المختبرين .تم تجريح وعدوي ثمار البرتقال بمعلق جراثيم بتركيز (10^6 spores/ml) من الفطرين *P. digitatum* و *P. italicum* وتم غمرها لمدة ٣ دقائق في محلول مائي من سوربات البوتاسيوم أوكربونات الصوديوم بتركيزات مختلفة وجففت وتم تخزينها لمدة ١٥ يوم علي (٢٠±٢°م) وأوضحت النتائج أن المعاملة بسوربات البوتاسيوم 4.0% أدت الي انخفاض نسبة حدوث المرض و النسبة المئوية للجزء المتحلل بمقدار ٨٠ & ٨٩% و ٨٣,٣ و ٩٠,٠% بالنسبة لمرضي العفن الازرق والاخضر علي الترتيب.

حدوث زيادة في قطر منطقة التثبيط لنمو الفطرين المختبرين بازدياد تركيز المادتين سواء منفردة أو مخلوطة مع الشمع. في تجربة أخري تم تحميل سوربات البوتاسيوم وكربونات الصوديوم بتركيزات ٢% و ٤% مع الشمع الذي يستخدم تجاريا في تغليف ثمار البرتقال المعدة للتصدير ودراسة الحماية ممتدة المفعول ضد مرضي العفن الازرق والاخضر حيث خزنت علي (٢٠±٢°م) لمدة ٤٥ يوم و اجراء العدوي الصناعية كل ١٠ أيام وأوضحت النتائج أن المعاملة بسوربات البوتاسيوم بتركيز ٤% أدت الي انخفاض نسبة حدوث المرض و النسبة المئوية للجزء المتحلل بمقدارة ٩٠ و٩٢% علي الاقل علي الترتيب .بينما أدت المعاملات سوربات البوتاسيوم بتركيز ٢% وكربونات الصوديوم بتركيز ٤% الي انخفاض نسبة حدوث المرض والنسبة المئوية للجزء المتحلل بمقدارة ٨٠ و ٨٤% علي الاقل علي الترتيب.

وتوضح النتائج امكانية استخدام سوربات البوتاسيوم وكربونات الصوديوم وهى مواد آمنة فى مقاومة أمراض ما بعد الحصاد فى ثمار الموالح على نطاق تجارى.