International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN: 0974-4290
Vol.8, No.9, pp 237-244 2015

# Evaluation of safe postharvest treatments for controlling Valencia orange green and blue moulds

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**Abstract:** Postharvest treatments of salt preservatives viz., sodium benzoate (SB) and potassium sorbate (PS) against *Penicillium digitatum* and *P. italicum*, the cause of Valencia orange fruits green and blue mould, respectively, were evaluated. *In vitro* studies, complete inhibition of both linear mycelial growth and conidial germination of both *P. digitatum* and *P. italicum* was obtained with (SB) at 20 g/ L and (PS) at 15 g/ L. Also coated peel disks of Valencia orange with (SB) or (PS) showed antifungal activity against *P. digitatum* and *P. italicum* by formation inhibition zone in agar assays. With increase in salt concentrations, the radius of the inhibition zone was increased. Our experiments were performed on a medium at pH of 4.5. *In vivo* studies, single treatment of (SB) or (PS) were applied to Valencia orange fruits at different concentrations, *i.e* 0.0, 5.0, 10.0,15.0 and 20.0 g/ L water wax by immersing the fruit for 30 seconds, and then artificially inoculated individually with *P. digitatum* or *P. italicum*. Treated fruits were stored at 20±2$^{o}$C and 90-95% relative humidity for 20 days. Results revealed that all treatments reduced significantly the incidence (%) and severity (%) of Valencia orange green and blue mould compared to the control. With increase in concentrations, the efficacy of salts was increased. The highest reduction in postharvest diseases of Valencia orange fruits was obtained with (SB) or (PS) at 20.0 g / L water wax, which reduced the disease incidence and disease severity by (83.0 & 80.0 and 86.0 & 84.0%) and (88.0 & 85.3 and 90.0 & 88.0%) for green and blue moulds, respectively. The moderate effect was obtained with (SB) and (PS) at 15.0 g /L water wax. It could be suggested from the present study that immersing Valencia orange fruits in water wax amended with sodium benzoate (SB) or potassium sorbate (PS) considered as one of the applicable safely treatments for controlling green and blue moulds.

**Key word :** Valencia orange fruits, green and blue moulds, salts preservatives, postharvest treatments.

## Introduction

Postharvest green mould, caused by *Penicillium digitatum* (Pers.:Fr.) Sacc., and postharvest blue mould, caused by *P. italicum* Wehmer, are the most economically important postharvest diseases of Valencia orange fruits in Egypt, and all citrus production areas characterized by low summer rainfall [1, 2, 3, 4].

Currently, these diseases are primarily controlled by application of synthetic fungicides such as imazalil or thiabendazole [5, 6]. However, resistance development to fungicides by plant pathogens is a factor limiting fruit production worldwide due to the decrease in efficacy of fungicides [7]. Furthermore, in the development and use of chemical fungicides for postharvest disease control, considerable attention must be given to the preservation of the global environment.

Alternative methods that have been pursued for the control of postharvest diseases include biological control, physical methods such as heat or radiations, and the use of safe low-toxicity chemicals such as food additives [8, 3, 9, 10, 11, 12]. Food additives are widely utilized for controlling food pH, taste and texture. Some of them such as potassium sorbate (PS) and sodium benzoate (SB) also have a broad spectrum antimicrobial activity and are commonly used as food preservatives [13]. The main advantages of using salt compounds as fungicides include their relatively low mammalian toxicity, a broad spectrum of modes of action, and relatively low cost [14].

Sorbic acid and its water-soluble salts, especially potassium sorbate (PS), are common food preservatives. Sorbates are the best characterized of all food antimicrobials as to their spectrum of action. They inhibit certain bacteria and food-related yeasts and mould species [15]. Sodium benzoate (SB) is the sodium salt of benzoic acid. It is used as an antifungal agent [16]. However, inhibition of microorganisms by (PS) and (SB) varies, depending on species and strain differences, extent of contamination, type and composition of the substrate, concentration and pH of sorbate, water activity, presence of other additives, temperature of storage, and type of packaging [17]. Using (PS) or (SB) against postharvest diseases of tomato, apple, carrots, potato and citrus fruits was reported by several workers [18, 19, 20, 21, 9, 16, 22, 8, 12]. The objectives of the present work were to evaluate the efficiency of potassium sorbate (PS) and sodium benzoate (SB) against postharvest diseases of Valencia orange fruits.

## Materials and Methods

### Valencia orange fruits

The mature Valencia orange (*Citrus sinensis* L., osbeck) fruits used in the experiment were grown in National Research center NRC orchards, and brought to the laboratory immediately after harvest. They were selected for their uniformity, size, color and shape, and for being free of damage and fungal infection.

### Fungal isolates

High virulent isolates of *Penicillium digitatum* and *P. italicum*, the cause of citrus green and blue mould, respectively were obtained from Plant Pathol. Dept., (NRC). The pathogenic isolates were isolated and selected based on their pathogenic ability to Valencia orange fruits in previous study [23].

*Penicillium digitatum* and *P. italicum*, were maintained on potato dextrose agar PDA (potato 20 g ; dextrose 20 g ; streptomycine 0.03 g and agar 20g in 1 liter of distilled water) at 25 ± 2°C for two weeks. A spore suspension was obtained by flooding 2-week-old cultures of *P. digitatum* and *P. italicum* with sterile distilled water that contained 0.1% (v/v) Tween 80. Spores were counted using a hemacytometer slid, and the spore concentrations from the pathogens were adjusted with sterile distilled water to obtain $1 \times 10^6$ spores per ml.

### Salt preservatives

Salt preservatives viz., sodium benzoate (SB) and potassium sorbate (PS) were purchased from Sigma chemical Co. and used in the present study. The media of potato dextrose agar PDA and potato dextrose broth PDA was used. The maximum pH for antimicrobial activity of sodium benzoate (SB) and potassium sorbate (PS) is 4.5 [24, 25, 26]. Hence for both preservatives to be effective, the pH of the medium was adjusted to 4.5 with 1m HCl.

### Water wax

Water wax used for soluble the salts preservatives, were purchased from citrus exportation company located at Qalubia governorate and used in the present study.

### Controlling fungal growth with salts preservatives *in vitro*

### Linear mycelial growth method

The inhibition by salts preservatives toward the linear mycelial growth of *P. digitatum* and *P. italicum* were performed according to the method described by [26, 27]. The prepared PDA medium was dispersed into 250 ml Erlenmeyer flasks and sterilized by autoclaving at 121°C for 15 min. Both preservatives were then added to

PDA medium individually before its solidification to obtain the final concentrations of 0.0, 5.0, 10.0, 15.0 and 20.0 g/ L (w/v) and mixed gently with 0.1% Tween 80 (Sigma) to enhance solubility. Each flask was then disbanded in sterilized Petri- plates (9- cm diameter) before its solidification. Plates were individually inoculated with equal disks (6- mm diameter) taken from 7-days old cultures of each *P. digitatum* and *P. italicum*, then incubated at 20 ± 2°C. Linear mycelial growth of fungus was measured, when the control plates reached full growth and the average growth diameter was calculated. Each treatment was represented by 5 plates as replicates.

**Spore germination method**

The inhibition by salts preservatives toward the spores germination of both *P. digitatum* and *P. italicum* were performed according to the method described by [26, 27]. The prepared potato dextrose broth (PDB) was dispersed into 10 ml test tube and sterilized by autoclaving at 121°C for 15 min. Both preservatives were then added to PDB to obtain the final concentrations of 0.0, 5.0, 10.0, 15.0 and 20.0 g/ L (w/v) and mixed gently with 0.1% Tween 80 (Sigma) to enhance solubility. Each tube was then inoculated with 1.0 ml of the spore suspension at a concentration of $10^3$/ml. Inoculated test tubes were incubated at 20 ± 2°C for 20 hours on rotary shaker (200 rpm). Germinated spores were examined microscopically to determine the germination rate**.** Experiment was represented by one hundred spores per replicate and five replicates per treatment were used.

**Inhibition zone method**

The inhibition zone resulting from salts preservatives against the growth of *P. digitatum* and *P. italicum* were performed according to the method described by [28] with some modification. Peel disks (10- mm diameter) of Valencia orange were soaked with potassium sorbate or sodium benzoate diluted in deionized water at the concentrations of 0.0, 5.0, 10.0, 15.0 and 20.0 g/ L for 30 seconds. After soaking, the peel disks were air dried under sterilized conditions. The developed peel disks activated with salts films were assayed for antifungal activity against *P. digitatum* and *P. italicum*. Individual samples (10- mm diameter) of the treated peel disks were located on the surface of Potato dextrose agar medium. Agar plates were seeded with spore suspension ($10^6$ spore / ml ) of *P.digitatum* or *P.italicum*. The treated peel disks were in connection with agar. The plates were incubated at 20±2°C for 5 days. We estimated the activity of potassium sorbate and sodium benzoate treated peel disks by observing the growth inhibition as a clear zone around the peel.

**Controlling green and blue moulds with salt preservatives *in vivo***

Valencia orange fruits were surface-sterilized with 2% sodium hypochlorite for 2 min at room temperature, rinsed with tap water in order to remove the heavy dirt, pesticides and fungal spores that are covering the fresh harvested produce and allowed to dry at room temperature. Fruits were immersing in water wax amended with potassium sorbate and sodium benzoate at different concentrations , *i.e* 0.0, 5.0, 10.0, 15.0 and 20.0 g/ L for 30 seconds and then air dried for two hours in laminar flow. Inoculation of fruits was carried out by spraying fruits with spore suspension ($10^6$ spores/ml) of *P. digitatum* or *P. italicum*, individually then air dried. A set of inoculated fruits with *P. digitatum* or *P. italicum*, individually only were left as control. Each treatment as well as the control was performed in triplicate. All treated or un-treated (control) fruits were placed into carton boxes (46 × 23 × 30 cm) at the rate of 20 fruits/box and stored for 20 days at 20±2°C and 90-95% relative humidity for assessment. The fruits were examined regularly to detect mould and regarded as infected if a visible lesion was observed. Results were expressed as percentage of fruit infected. Disease incidence (%) were expressed as percentage of fruit infected, while disease severity (%) were expressed as percentage of rotted part of fruit which was calculated from the following formula:

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

**Statistical analysis**

Tukey test for multiple comparison among means was utilized [29].

## Results

### Suppressive effect of salts preservatives against *P. digitatum* and *P. italicum in vitro*

### Linear mycelial growth method

The *in vitro* suppressive effect of salts preservatives against the linear mycelial growth of *P. digitatum* and *P. italicum* are shown in Table 1. Results indicate that all the tested concentrations of both sodium benzoate (SB) and potassium sorbate (PS) significantly reduced the linear mycelial growth of both *P. digitatum* and *P. italicum*. It is clear that the suppressive effect increased with the increase in concentrations of salt. The highest reduction was obtained with (SB) at 15 g/ L and (PS) at 10 g/ L which reduced the linear mycelial growth by 78.9 & 74.4 and 77.0 & 70.0 % for *P. digitatum* and *P. italicum*, respectively. Complete inhibition of linear mycelial growth of both *P. digitatum* and *P. italicum* was obtained with (SB) at 20 g/ L and (PS) at 15 g/ L, while salts preservatives at the concentration of 5.0 g / L, showed less suppressive effect.

**Table 1. Suppressive effect of salt preservatives against the linear mycelial growth of *P. digitatum* and *P. italicum in vitro*.**

| Salt | Conc. (g/L) | Linear mycelial growth (mm) and reduction (%) | | | |
|---|---|---|---|---|---|
| | | *P . digitatum* | | *P. italicum* | |
| | | Growth | Reduction | Growth | Reduction |
| Sodium benzoate (SB) | **00.0** | 90.0 a | - | 90.0 a | - |
| | **05.0** | 71.0 b | 21.1 | 74.2 b | 17.6 |
| | **10.0** | 42.0 d | 53.3 | 45.0 d | 50.0 |
| | **15.0** | 19.0 e | 78.9 | 23.0 e | 74.4 |
| | **20.0** | 00.0 f | 100 | 00.0 f | 100 |
| Potassium sorbate (PS) | **00.0** | 90.0 a | - | 90.0 a | - |
| | **05.0** | 52.4 c | 41.8 | 56.0 c | 37.8 |
| | **10.0** | 20.7 e | 77.0 | 27.0 e | 70.0 |
| | **15.0** | 00.0 f | 100 | 00.0 f | 100 |
| | **20.0** | 00.0 f | 100 | 00.0 f | 100 |

**Means designated with the same letter in the same column are not significantly different at 0.05 level of probability.**

### Spore germination method

The effect of PDB amended with different concentrations of each of sodium benzoate (SB) and potassium sorbate (PS) on conidial germination (%) of *P. digitatum* and *P. italicum* are shown in Table 2. Results indicate that all the tested concentrations of (SB) and (PS) significantly reduced the germinated spores of both *P. digitatum* and *P. italicum*. With increase in concentration, the efficacy of salts preservatives was increased. The highest reduction in spore germination was obtained with (SB) at 15 g/ L and (PS) at 10 g/ L , which reduced the conidial germination by 94.6 & 90.0 and 93.0 & 87.0 % for *P. digitatum* and *P. italicum*, respectively. Complete inhibition of the conidial germination of both pathogens was obtained with (SB) at 20.0 g/ L and (PS) at 15.0 g / L. Meanwhile, other concentrations of both salts showed moderate effect.

**Table 2. Effect of salt preservatives on conidial germination (%) of *P. digitatum* and *P. italicum in vitro*.**

| Salt | Conc. (g/L) | Conidial germination (%) and reduction (%) | | | |
|---|---|---|---|---|---|
| | | *P . digitatum* | | *P. italicum* | |
| | | Germination | Reduction | Germination | Reduction |
| Sodium benzoate (SB) | **00.0** | 93.0 a | - | 92.0 a | - |
| | **05.0** | 47.0 b | 49.5 | 54.0 b | 41.3 |
| | **10.0** | 23.0 d | 75.3 | 30.4 d | 67.0 |
| | **15.0** | 05.0 e | 94.6 | 09.2 e | 90.0 |
| | **20.0** | 00.0 e | 100 | 00.0 f | 100 |
| Potassium sorbate (PS) | **00.0** | 93.0 a | - | 92.0 a | - |
| | **05.0** | 34.5 c | 62.9 | 38.0 c | 58.7 |
| | **10.0** | 06.5 e | 93.0 | 12.0 e | 87.0 |
| | **15.0** | 00.0 e | 100 | 00.0 f | 100 |
| | **20.0** | 00.0 e | 100 | 00.0 f | 100 |

**Means designated with the same letter in the same column are not significantly different at 0.05 level of probability.**

**Inhibition zone method**

Antifungal activity of sodium benzoate (SB) and potassium sorbate (PS) coated peel disks of Valencia orange against *P. digitatum* and *P. italicum* are shown in Table 3. The study showed interesting results; indeed, 30 seconds after soaking the peel disks into sodium benzoate (SB) or potassium sorbate (PS) solution at different concentrations, *i.e* 5.0, 10.0, 15.0 and 20.0 g/ L, the peel disks always showed activity against *P. digitatum* and *P. italicum* in agar inhibition assays. In all the cases untreated peel disks did not show any antifungal activity. Increasing the sodium benzoate (SB) or potassium sorbate (PS) concentration by more than 10.0 g/ L, resulted a large increase of the radius of the inhibition zone. The highest inhibition zone radius was obtained with (SB) and (PS) at 20.0 g/ L, being 46.2 & 32.0 and 60.5 & 51.0 mm for *P. digitatum* and *P. italicum*, respectively. Meanwhile, both salts at the concentration of 5.0 g/L were less effective.

**Table 3. Antifungal activity of sodium benzoate (SB) and potassium sorbate (PS) coated peel disks at different concentrations against *P. digitatum* and *P. italicum*.**

| Salt | Conc. (g/L) | Inhibition zone area (mm) around peel disk | |
|---|---|---|---|
| | | ***P . digitatum*** | ***P. italicum*** |
| **Sodium benzoate (SB)** | **00.0** | 00.0 h | 00.0 g |
| | **05.0** | 04.0 g | 03.2 g |
| | **10.0** | 18.0 e | 15.4 e |
| | **15.0** | 31.4 d | 21.0 d |
| | **20.0** | 46.2 c | 32.0 c |
| **Potassium sorbate (PS)** | **00.0** | 00.0 h | 00.0 g |
| | **05.0** | 10.4 f | 08.4 f |
| | **10.0** | 31.0 d | 24.0 d |
| | **15.0** | 52.0 b | 42.0 b |
| | **20.0** | 60.5 a | 51.0 a |

**Means designated with the same letter in the same column are not significantly different at 0.05 level of probability.**

**Suppressive effect of salts against postharvest diseases of Valencia orange fruits**

Table 4 shows the *in vivo* protective effect of water wax amended with salts against postharvest diseases of Valencia orange fruits caused by *P. digitatum* and *P. italicum* as disease incidence (%) and disease severity (%) after 20 d. of treatment and inoculation, when fruit were stored at 20±2$^{o}$C. Results indicate that all treatments reduced significantly postharvest diseases incidence (%) and severity (%) of Valencia orange fruits compared to the control. With increase in concentration, the efficacy of salts preservatives was increased. After storage period, 100% of control fruit developed green and blue moulds in Valencia orange fruits. It was noticed that, the highest reduction in postharvest diseases of Valencia orange fruits was obtained with sodium benzoate (SB) or potassium sorbate (PS) at 20.0 g / L water wax, which reduced the disease incidence and disease severity by (83.0 & 80.0 and 86.0 & 84.0%) and (88.0 & 85.3 and 90.0 & 88.0%) for green and blue moulds, respectively. The moderate effect was obtained with sodium benzoate and potassium sorbate at 15.0 g /L which reduced the disease incidence more than 77.0 and 70.0% and reduced the disease severity more than 82.0 and77.9% for green and blue moulds respectively. While, other salts concentration showed moderate effect.

**Table 4. Protective effect of water wax amended with salts against postharvest diseases of Valencia orange fruits.**

| Salt | Conc. (g/L) | Postharvest disease of Valencia orange fruits* | | | |
|---|---|---|---|---|---|
| | | Incidence (%) | | Severity (%) | |
| | | Green mould | Blue mould | Green mould | Blue mould |
| **Sodium benzoate (Sb)** | **00.0** | 100.0 a | 100.0 a | 100.0 a | 100.0 a |
| | **05.0** | 60.00 b | 65.00 b | 51.00 b | 54.00 b |
| | **10.0** | 42.00 c | 47.00 c | 38.40 d | 41.20 c |
| | **15.0** | 23.00 e | 30.00 d | 18.00 e | 22.10 e |
| | **20.0** | 17.00 f | 20.00 f | 12.00 f | 14.70 f |
| **Potassium sorbate (Ps)** | **00.0** | 100.0 a | 100.0 a | 100.0 a | 100.0 a |
| | **05.0** | 42.00 c | 45.00 c | 45.00 c | 48.00 c |
| | **10.0** | 31.00 d | 34.00 d | 33.00 d | 33.00 d |
| | **15.0** | 22.00 e | 25.00 e | 17.00 e | 21.00 e |
| | **20.0** | 14.00 f | 16.00 f | 10.00 f | 12.00 f |

**Means designated with the same letter in the same column are not significantly different at 0.05 level of probability.**

Disease incidence (%) were expressed as percentage of fruit infected, while disease severity (%) were expressed as percentage of rotted part of fruit which was calculated from the following formula:

$$\textbf{Percentage of rotted part (\%)} = \frac{\textbf{Rotted part weight of fruit}}{\textbf{Fruit weight}} \textbf{ X 100}$$

## Discussion

The efficiency of sodium benzoate (SB) and potassium sorbate (PS) treatments has been investigated on a wide range of postharvest diseases and horticultural crops [30, 31, 16]. On navel orange fruits, the effectiveness of (SB) and (PS) treatments against green and blue moulds has already been demonstrated by *in vitro* and *in vivo* studies but a little were conducted on Valencia orange fruits [9, 10, 32, 12]. The present results showed that sodium benzoate (SB) and potassium sorbate (PS), have a significant effect on the growth (*in vitro* and *in vivo*) of both *P. digitatum* and *P. italicum*. *In vitro* studies, complete inhibition of both linear mycelial growth and conidial germination of *P. digitatum* and *P. italicum* was obtained with (SB) at 20 g/ L and (PS) at 15 g/ L. Also coated peel disks of Valencia orange with (SB) or (PS) showed antifungal activity against *P. digitatum* and *P. italicum* by formation inhibition zone in agar assays. Our experiments were performed on a medium at pH of 4.5. *In vivo* studies, under artificial inoculation with *P. digitatum* or *P. italicum*, immersing Valencia orange fruits for 30 second in water wax amended with (SB) or (PS) at 20.0 g / L, reduced the disease incidence and disease severity by (83.0 & 80.0 and 86.0 & 84.0%) and (88.0 & 85.3 and 90.0 & 88.0%) for green and blue moulds, respectively. The antifungal properties of (PS) and (SB) against *P. digitatum* and *P. italicum* have already been reported by other authors [33, 9, 34, 12] who linked the antimicrobial activity of (PS) and (SB) to the pH of the substrate, reporting an enhancement of the activity in the pH 3.0-6.5 range. [33] demonstrated a complete inhibition of the growth of *P. digitatum* seeded on PDA amended with (PS) at 1000 mg/L and adjusted to pH 4.5. Similarly, [9] showed an increase of (PS) toxicity with decreasing pH, and pointed out that the concentration of (PS) able to inhibit the germination of conidia of *P. digitatum* was similar from pH 4 to 6, and it was about 3- and 10-fold less toxic at pH 7 or pH 8, respectively. The antimicrobial activity of (PS) and (SB) is dependent on the presence of sorbic and benzoic acid in the solution, where the dissociated ionic form and the undissociated one (sorbic acid and benzoic acid) are in equilibrium. At pH below 4.76 the undissociated form prevails. According to [35] the effectiveness of organic acids is pH-dependent and the undissociated form of the acid is primarily responsible for antimicrobial activity. The mode of action of sorbate could be through the alteration of the morphological structure of the cell, genetic changes, cell membrane alterations, inhibition of cell transport processes, and inhibition of enzymes involved in metabolism of transport functions [17]. One of the primary targets of sorbic acid in vegetative cells appears to be the cytoplasmic membrane. It reduces the cytoplasmic membrane electrochemical gradient and consequently active transport, which in turn inhibits amino acid transport and could eventually result in the inhibition of many cellular enzyme systems [36]. [37] showed that sorbate reacts with the thiol group of cysteine and suggested that this is a mechanism of inactivation of sulfhydryl enzymes. [38] reported that a decrease in adenosine triphosphate (ATP) level in conidia of *Aspergillus parasiticus* was related to decreased viability after exposure to sorbic acid. Sorbate treatment may also induce defensive responses in citrus fruit to pathogens, although nothing is known about this. [39] reported potassium sorbate (PS) treatment induced scoparone, caused structural changes, and increased the pH of rind tissue, all of which, in addition to the fungitoxicity of this compound, contributed to control of green and blue moulds by this treatment. Sodium benzoate (SB) has activity against yeast, mold, and bacteria. The effectiveness of sodium benzoate (SB) as a preservative and antifungal increases with decreasing pH (increasing acidity). This is because the ratio of undissociated (*i.e.*, free) benzoic acid to ionized benzoic acid increases as the pH decreases. It is generally accepted that the undissociated benzoic acid is the active antimicrobial agent. Although no definite theory has been yet proposed to explain this antimicrobial effect, it is believed to be related to the high lipoid solubility of the undissociated benzoic acid which allows it to accumulate on the cell membranes or on various structures and surfaces of the microbial cell, effectively inhibiting its cellular activity [34]. It could be suggested from the present study that immersing Valencia orange fruits in water wax amended with sodium benzoate (SB) or potassium sorbate (PS) considered as one of the applicable safely treatments for controlling green and blue moulds.

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