

Evaluation of Safe Postharvest Treatments for Controlling Grey Mould and Soft Rot diseases of Strawberry Fruits

Farid Abd-El-Kareem

Plant Pathology Department, National Research Centre, Giza, Egypt.

Abstract : The strawberry fruit rot (grey mould and soft rot) caused by *Botrytis cinerea* and *Rhizopus stolonifer* respectively are the most important diseases attach strawberry fruits. Evaluate the efficiency of potassium sorbate and sodium benzoate against postharvest diseases of strawberry fruits were tested In vitro trails, results revealed that compete inhibition of linear growth was obtained with potassium sorbate and sodium benzoate at concentrations of 20.0 and 25.0 g / L for *B. cinerea* and *R. stolonifer* respectively. The highest reduction was obtained potassium sorbate and sodium benzoate at concentrations of 15.0 g / L which reduced linear growth of both tested fungi more than 63.3 %. As for spore germination results revealed that compete inhibition of linear growth was obtained with potassium sorbate and sodium benzoate at concentrations of 20.0 g / L for both *B. cinerea* and *R. stolonifer*. The highest reduction was obtained potassium sorbate and sodium benzoate at concentrations of 15.0 g / L which reduced spore germination of both tested fungi more than 85.1 %.

Moreover, in vivo trails results indicated that all tested concentrations of potassium sorbate and sodium benzoate significantly reduced the grey mould and soft rot (incidence and severity) of strawberry fruits . The highest reduction was obtained with potassium sorbate and sodium benzoate at concentrations of 20.0 and 25.0 g / L which reduced the grey mould and soft rot incidence of strawberry fruits more than 88.0 and 86.0 % respectively. Treated strawberry fruits with potassium sorbate and sodium benzoate at concentrations of 15.0 g / L resulted in reducing grey mould and soft rot incidence more than 65.5 % . As for disease severity the highest reduction was obtained with potassium sorbate and sodium benzoate at concentrations of 20.0 and 25.0 g / L which reduced the grey mould and soft rot severity of strawberry fruits more than 90.0 and 88.0 % respectively. It could be suggested that potassium sorbate and sodium benzoate are excellent treatments for controlling postharvest diseases of strawberry fruits.

Key words : Strawberry fruit- grey mould- Soft rot - Potassium sorbate - Sodium benzoate- Postharvest diseases.

Introduction

Botrytis cinerea, and *Rhizopus stolonifer* are the microorganisms that most commonly attack strawberries¹. The strawberry fruit rot (grey mould) caused by *Botrytis cinerea* develops in any part of the fruit, but is mostly found on the calyx end or on the sides of fruit in contact with other rotten fruit. *Rhizopus stolonifer* fruit rot is associated with the presence of wounds in the fruit, but may also occur in intact fruit². 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^{3,4,5,6,7,8}. Food additives such as potassium sorbate (PS) and sodium benzoate (SB) have a broad spectrum antimicrobial activity and are commonly used as food preservatives^{9,10}.

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^{11,12,13}. Sodium benzoate (SB) is the sodium salt of benzoic acid. It is used as an antifungal agent⁽¹⁴⁾. 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⁽¹⁵⁾. Using (PS) or (SB) against postharvest diseases of tomato, apple, carrots, potato and citrus fruits was reported by^{8,16,17,18,19,20,21}. The objectives of the present work were to evaluate the efficiency of potassium sorbate (PS) and sodium benzoate (SB) against postharvest diseases of strawberry orange fruits.

Materials and Methods

Fungal isolates and strawberry fruits

Botrytis cinerea, and *Rhizopus stolonifer*, the causal organisms of grey and soft moulds respectively were obtained from Plant Pathol. Dept., (NRC) Egypt and were maintained on potato dextrose agar PDA for further study. While, strawberry fruits were obtained from the Department, of Vegetable Crop Research, Agricultural Research Centre, Giza, Egypt.

Salts preservatives

Salts preservatives, *i.e.* sodium benzoate (SB) and potassium sorbate (PS) were purchased from Sigma chemical Co. and used in the present study.

In vitro trails

Testing of potassium sorbate and sodium benzoate on linear growth of pathogenic fungi

Potassium sorbate and sodium benzoate at different concentrations *i.e.* 0.0, 5.0, 10.0, 15.0, 20.0 and 25.0 g/L were tested to study their inhibitory effect on linear growth of *B. cinerea* and *R. stolonifer*. Salts 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-diameter). Plates were individually inoculated at the center with equal disks (6-mm-diameter) of 10-days old culture of *B. cinerea* and *R. stolonifer*. Five plates were used as replicates for each particular treatments. Inoculated plates were incubated at $20 \pm 2^\circ\text{C}$. The average linear growth of tested fungi was calculated after 10 days of incubation.

Testing of potassium sorbate and sodium benzoate on spore germination of pathogenic fungi

Potassium sorbate and sodium benzoate at different concentrations *i.e.* 0.0, 5.0, 10.0, 15.0, 20.0 and 25.0 g/L were tested to study their inhibitory effect on spore germination of *B. cinerea* and *R. stolonifer*. Spores of 10-days- old cultures of each fungus were harvested in sterilized water (containing 0.01% Tween 80) then adjusted to reach concentration of 10^6 spore/ml. 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.

Controlling grey mould and soft rot of strawberry fruits in vivo

Testing of potassium sorbate and sodium benzoate on spore germination of pathogenic fungi

Healthy and fresh strawberry fruits cv. Camarosa apparently free of physical damages and diseases were used in this experiment.

Fruits were immersing in water solutions containing potassium sorbate or sodium benzoate at different concentrations, *i.* 0.0, 5.0, 10.0, 15.0, 20.0 and 25.0 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

B. cinerea and *R. stolonifer* individually then air dried. A set of inoculated fruits with *B. cinerea* and *R. stolonifer*, 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 50 fruits/box and stored for 10 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:

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

Statistical analysis

Tukey test for multiple comparison among means was utilized ⁽²²⁾.

Results

Effect of potassium sorbate and sodium benzoate on linear growth of pathogenic fungi

Potassium sorbate and sodium benzoate at different concentrations *i.e* 0.0, 5.0, 10.0, 15.0, 20.0 and 25.0 g /L were tested to study their inhibitory effect on linear growth of *B. cinerea* and *R. stolonifer*. Results in Table (1) reveal that all tested concentrations of potassium sorbate and sodium benzoate significantly reduced the linear growth of *B. cinerea* and *R. stolonifer*. Complete inhibition of linear growth was obtained with potassium sorbate and sodium benzoate at concentrations of 20.0 and 25.0 g / L for *B. cinerea* and *R. stolonifer* respectively. The highest reduction was obtained potassium sorbate and sodium benzoate at concentrations of 15.0 g/L which reduced linear growth of both tested fungi more than 63.3 %. Other concentrations were less effective .

Table 1. Linear growth of *Botrytis cinerea*, and *Rhizopus stolonifer* as affected with potassium sorbate and sodium benzoate

Salt	Conc. (g/L)	Linear growth (mm)			
		<i>Botrytis cinerea</i>		<i>Rhizopus stolonifer</i>	
		Linear growth	Reduction	Linear growth	Reduction
Sodium benzoate	05.0	65.0 b	27.8	74.0 b	17.8
	10.0	32.0 d	64.4	62.4 c	30.7
	15.0	18.4 e	79.7	33.0 e	63.3
	20.0	00.0 f	100.0	12.0 g	86.7
	25.0	00.0 f	100.0	00.0 h	100.0
Potassium sorbate	05.0	59.0 b	34.4	63.0 c	30.0
	10.0	44.0 c	51.1	49.0 d	45.6
	15.0	18.2 e	79.8	32.1 e	64.3
	20.0	00.0 f	100.0	21.4 f	76.2
	25.0	00.0 f	100.0	00.0 h	100
Control	00.0	90.0 a	00.0	90.0 a	00.0

Figures with the same letter are not significantly different (p=0.05)

Effect of potassium sorbate and sodium benzoate on spore germination of pathogenic fungi

Potassium sorbate and sodium benzoate at different concentrations *i.e* 0.0, 5.0, 10.0, 15.0, 20.0 and 25.0 g /L were tested to study their inhibitory effect on spore germination of *B. cinerea* and *R. stolonifer*. Results in Table (2) reveal that all tested concentrations of potassium sorbate and sodium benzoate significantly reduced the spore germination of *B. cinerea* and *R. stolonifer*. Complete inhibition of linear growth was obtained with potassium sorbate and sodium benzoate at concentrations of 20.0 g / L for both *B. cinerea* and *R. stolonifer*. The highest reduction was obtained potassium sorbate and sodium benzoate at concentrations of 15.0

g / L which reduced spore germination of both tested fungi more than 85.1 %. Other concentrations showed moderate effect .

Table 2. Spore germination of *Botrytis cinerea*, and *Rhizopus stolonifer* as affected with potassium sorbate and sodium benzoate

Salt	Conc. (g/L)	Spore germination			
		<i>Botrytis cinerea</i>		<i>Rhizopus stolonifer</i>	
		Spore germination	Reduction	Spore germination	Reduction
Sodium benzoate	05.0	61.0 b	35.1	70.0 b	24.7
	10.0	28.0 d	70.2	57.0 c	38.7
	15.0	14.0 e	85.1	29.0 e	86.8
	20.0	00.0 f	100	00.0 g	100.0
	25.0	00.0 f	00.0	00.0 g	100.0
Potassium sorbate	05.0	55.0 b	41.5	59.0 c	36.6
	10.0	39.0 c	58.5	42.0 d	54.8
	15.0	12.0 e	87.2	14.0 f	84.9
	20.0	00.0 f	100.0	00.0 g	100.0
	25.0	00.0 f	100.0	00.0 g	100
Control	00.0	94.0 a	00.0	93.0 a	00.0

Figures with the same letter are not significantly different (p=0.05)

Controlling grey mould and soft rot of strawberry fruits in vivo

Effect of potassium sorbate and sodium benzoate on grey mould and soft rot of strawberry fruits in vivo

Potassium sorbate or sodium benzoate at different concentrations , i. 0.0 , 5.0, 10.0, 15.0 ,20.0 and 25.0 g /L were tested to study their effect against grey mould and soft rot of strawberry fruits in vivo.

Effect on disease incidence

Results in Table (3) indicate that all tested concentrations of potassium sorbate and sodium benzoate significantly reduced the grey mould and soft rot of strawberry fruits . The highest reduction was obtained with potassium sorbate and sodium benzoate at concentrations of 20.0 and 25.0 g / L which reduced the grey mould and soft rot incidence of strawberry fruits more than 88.0 and 86.0 % respectively. Treated strawberry fruits with potassium sorbate and sodium benzoate at concentrations of 15.0 g / L resulted in reducing grey mould and soft rot incidence more than 65.5 % .Meanwhile, other concentrations were less effective .

Effect on disease severity

Results in Table (4) indicate that all tested concentrations of potassium sorbate and sodium benzoate significantly reduced the grey mould and soft rot severity of strawberry fruits. The highest reduction was obtained with potassium sorbate and sodium benzoate at concentrations of 20.0 and 25.0 g / L which reduced the grey mould and soft rot severity of strawberry fruits more than 90.0 and 88.0 % respectively. Treated strawberry fruits with potassium sorbate and sodium benzoate at concentrations of 15.0 g / L resulted in reducing grey mould and soft rot severity more than 76.5 % .Meanwhile, other concentrations were less effective .

Table 3. Grey mould and soft rot incidence of strawberry fruits as affected with potassium sorbate and sodium benzoate

Salt	Conc. (g/L)	Strawberry fruit rots			
		Grey mould		Soft rot	
		Disease incidence	Reduction	Disease incidence	Reduction
Sodium benzoate	05.0	52.0 b	48.0	62.0 b	38.0
	10.0	41.4 c	58.6	52.0 c	48.0
	15.0	24.0 d	76.0	31.4 e	68.6
	20.0	10.0 e	90.0	14.0 g	86.0
	25.0	10.0 e	90.0	14.0 g	86.0
Potassium sorbate	05.0	50.0 b	50.0	56.0 bc	44.0
	10.0	39.4 c	60.6	41.0 d	59.0
	15.0	21.0 d	79.0	34.5 e	65.5
	20.0	12.0 e	88.0	14.0 g	86.0
	25.0	12.0 e	88.0	13.0 g	87.0
Control	00.0	100.0 a	0.0	100.0 a	0.0

Figures with the same letter are not significantly different (p=0.05)

Table 4. Grey mould and soft rot severity of strawberry fruits as affected with potassium sorbate and sodium benzoate

Salt	Conc. (g/L)	Strawberry fruit rots			
		Grey mould		Soft rot	
		Disease severity	Reduction	Disease severity	Reduction
Sodium benzoate	05.0	49.0 b	51.0	58.0 b	42.0
	10.0	34.0 c	66.0	41.2 d	58.8
	15.0	18.0 d	82.0	23.4 e	76.6
	20.0	8.0 e	92.0	12.0 g	88.0
	25.0	7.0 e	93.0	11.0 g	89.0
Potassium sorbate	05.0	43.2 b	56.8	48.2 c	51.8
	10.0	33.2 c	66.8	35.4 d	64.6
	15.0	19.0 d	81.0	22.1 e	78.9
	20.0	10.0 e	90.0	12.0 g	88.0
	25.0	9.0 e	91.0	12.0 g	88.0
Control	00.0	100.0 a	0.0	100.0 a	0.0

Figures with the same letter are not significantly different (p=0.05)

Discussion

The strawberry fruit rot (grey mould and soft rot) caused by *Botrytis cinerea* and *Rhizopus stolonifer* respectively are the most important diseases attach strawberry fruits^{1,2}.

Sorbic acid and its water-soluble salts inhibit ed certain bacteria and food-related yeasts and mould species^{11,12,13}. Sodium benzoate is used as an antifungal agent^{9,10,14}.

In the present study results indicate that in vitro trails, results revealed that compete inhibition of linear growth and spore germination was obtained with potassium sorbate and sodium benzoate at concentrations of 25.0 g / L for *B. cinerea* and *R. stolonifer*. Moreover, in vivo trails results indicated that all tested concentrations of potassium sorbate and sodium benzoate significantly reduced the grey mould and soft rot (incidence and severity) of strawberry fruits. The highest reduction was obtained with potassium sorbate

and sodium benzoate at concentrations of 20.0 and 25.0 g / L which reduced the grey mould and soft rot incidence or severity of strawberry fruits more than 88.0 and 86.0 % respectively. In this respect, the efficiency of sodium benzoate (SB) and potassium sorbate (PS) treatments has been investigated on a wide range of postharvest diseases and horticultural crops^(14,23,24). The antifungal properties of (PS) and (SB) against several pathogenic fungi was reported several investigators^{8,25,26}. Smilanick *et al.*⁸ 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 acid 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 to²⁷ 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 functions¹⁵. 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 systems²⁹. It was 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 pathogens²⁹. 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 decreases³⁰. 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 lipid 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 activity³¹. It could be suggested that sodium benzoate (SB) or potassium sorbate (PS) considered as one of the applicable safely treatments for controlling postharvest diseases of strawberry fruits

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