Application of Certain Compounds to Manage Postharvest Gray Mold Caused by *Botrytis cinerea* and Enhancing Strawberry Fruits Quality

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Abstract

Effects of pre-and postharvest treatments with potassium phosphite, potassium phosphate, and salicylic acid against the severity of gray mold disease caused by Botrytis cinerea in strawberry fruits were investigated under ambient conditions. Seven B. cinerea isolates were collected from naturally infected strawberry plants. The treatment with potassium phosphite at concentration of 500 mg/L significantly reduced the fungal growth compared to other treatments in vitro. The treatments with potassium phosphite at concentrations of 250 or 500 mg/L resulted in the highest disease reduction, followed by the treatment with salicylic acid. Additionally, effects of postharvest treatments with these compounds on disease reduction and biochemical attributes of strawberry fruits were evaluated during storage for 9 days at ambient conditions. The highest disease reduction percentage was found with potassium phosphite. Besides, the phenolics content and peroxidase activity of potassium phosphate were found to be greater than those of potassium phosphite or salicylic acid. However, a reduction in the total soluble solids, titratable acids, and ascorbic acid contents of fruits was found for all treatment groups at the end of storage period. Based on the obtained results, potassium phosphite and salicylic acid can be recommended as fungicide alternatives for extending postharvest shelf life of strawberry fruits.

Keywords: Strawberry; Botrytis cinerea; Postharvest; Gray mold; Peroxidase; Ascorbic acid.

Introduction

Strawberry fruits (Fragaria ananassa Duch.) are rich in nutrients such as minerals and vitamins, as well as other bioactive substances that may have health advantages (Coles, L. 2013; Giampieri, et al., 2015). Egypt is the world's leading exporter of frozen strawberries, accounting for 20% of global exports in 2019 (Anonymous, 2019). Strawberry fruits are naturally infected with B. cinerea, which is the most common strawberry postharvest disease in Egypt and around the world, known as the gray mold disease (Yang *et al.*, 2010; El-Ghanam, 2015; Petrasch *et al.*, 2019 and El-fawy *et al.*, 2020). Furthermore, the gray disease causes significant economic loss in strawberry production around the world (Hahn, M. 2014).

Because of the favorable conditions that exist throughout the postharvest handling chain, such as injuries, high humidity, sensitive plant tissue, and high sugar content, *B. cinerea* is a significant postharvest pathogen. Mostly, the postharvest infection by *B. cinerea* occurs in differ-

ent types of fruit such as strawberry and grapes can sometimes spoil entire lots in field and during storage. Romanazzi and Feliziani (2014)found that *B. cinerea* influences fruits in the field, during storage, during transportation, and in the market. Also, it was reported that the gray mold disease is the most often cause for rejection of fruit shipping and exportation (Petrasch et al., 2019). During the growing season of the strawberry, the gray mold or B. cinerea prefers moderate temperatures and high humidity, and strawberry fruits may be contaminated with higher amounts in marketplaces and during shipment (Elad and Stewart 2007 and Choquer et al., 2007).

Synthetic fungicides are now employed in combination with natural or chemical treatments to control the gray mold disease because of the significant risk of fungus resistance (Romanazzi et al., 2016). Several compounds and strategies, including promotion of plant defense levels of resistance, can help strawberry fruits fight the gray mold diseases (Fu and Dong, 2013; Walters et al., 2013; Zhou and Wang, 2018). For example, in strawberry tissues, treatments with chitosan and a calcium-organic acid mixture reduced pathogen development and increased expression of enzymes connected to defensive mechanisms (Landi et al., 2014). Salicylic acid (SA) one of the compounds that gave good results to suppress diseases caused by B. cinerea on different hosts (Terry and Joyce, 2004). Plant defense mechanisms were activated by SA in response to a variety of abiotic and biotic physiological, biochemical, and morphological alterations (War et al., 2011). Phosphite also known to has an impact on severity of the plant pathogens (Ribeiro Junior et al., 2006). Effects of tomato-juice and KH2PO4 on the infection of tomato gave noticeable results to control B. cinerea (Hyun, et al., 2011). The gray mold severity can be determined by biochemical changes in infected strawberry plants or fruits. The polyphenol oxidase (PPO) and peroxidase (PO) activity in strawberry fruits infected with B. cinerea were found to be enhanced by a mixture of chemicals (El-Ghanam et al., 2015). Furthermore, treatment with SA enhanced PO and PPO activities as well as phenols induction (War et al., 2011).

As mentioned above, the excessive usage of chemical fungicide to control B. cinerea causal pathogen in strawberry and other fruits develops resistance. environmental fungal problems. and health hazards (Hauschild, 2012; Leroch et al., 2013). Therefore, finding safe and eco-friendly fungicide alternatives is very important (Sylla et al., 2015). The aim of this investigation was to evaluate efficiency of treatment with phosphite. potassium potassium phosphate, and SA as fungicide alternatives to control B. cinerea the causal pathogen of gray mold and preserve postharvest quality of strawberry fruits.

Materials and Methods Materials

Strawberry fruits of Fortuna cultivar were harvested at maturegreen stage in March 2020 from Fortuna cultivar, cultivated in Plant Pathology farm of Faculty of Agriculture, Assiut university, Assiut, Egypt. Fruits were uniform in shape and size, and free of injuries, wounds, scratches, insect infestation, fungal infection, and mechanical harms. Fruits packaged randomly in sterilized polypropylene punnets and stored at 25° C for subsequent analyses at 0, 3, 6 and 9 days of storage periods. Treated fruits surface was disinfected with 70% ethanol. Chemicals and reagents used in this study were of analytical grade and purchased from El Nasr Co. Egypt.

Fungal Isolation and identification

Fungi were harvested from naturally infected fruits and grown on potato dextrose agar (PDA) for 3 to 9 days at 22 to 25°C. Using hyphal tip and single spore isolation techniques, a pure culture was generated. Isolates were identified based on their morphological and cultural characters as described by Khazaeli et al. (2010) and Dowling et al. (2017). Each plate received ten mL of sterilized distilled water, and the colonies were scraped using a sterile needle. The suspension was determined with a hemocytometer and adjusted as required with sterilized distilled water to approximately 2×10^5 CFU/mL the conidial suspension mixed with carborundum as described by Mansfield et al. (1974).

In vitro treatments

Effect of treatments by SA, potassium diphosphate and potassium phosphite at concentrations of 125, 250, 500 ppm on mycelial growth of *B. cinerea* were performed on PDA medium. The plates were inoculated with 5 mm in diameter mycelia discs of 7 days old of *B. cinerea*, four petri dishes were used for each replicate, incubated at 25°C and compared with the control. Mycelial growth diameter length was taken and the reduction in growth diameter was measured as follows:

% Reduction= (A – B/A) 100

Where A is the control radial growth and B is the treatment radial growth

In vivo treatments

For *in vivo* artificial infection, strawberry fruits were sprayed with spore suspension of *B. cinerea* at about $2x10^5$ cfu/mL, packed in polypropylenePunit, and stored at 25 °C and 85-95% relative humidity for 24 hours and tested at 0, 3, 6 and 9 days of storage period. Disease severity measured as Romanazzi *et al.* (2000)

Where d is the category of rot intensity scored on the fruit, f is the frequency, N is the total number of examined fruits (uninfected and infected), and D is the highest category of decay intensity present on the empirical scale, using scale 0-4, where 0 = healthy fruit, 1 = decayed area of the fruit ranging from 1 to 25%, 2 = decayed area of fruit ranging from 26 to 50%, 3 = decayed area of fruit ranging from 51 to 75% and 4 = decayed area of fruit ranging from 76 to 100%. and used the followed equation

Disease Severity (%) = $(\sum (d \times f)/(N \times D))$ 100

The experiment was randomly designed, and each treatment group was sprayed with compounds immediately after harvesting. The control plants were sprayed with distilled water. The fungicide switch was applied at concentration of one mL/L.

Total phenolics content (TPC)

One gram of treated and untreated strawberry fruits was cut into smallpieces and extracted with 95% ethanol alcohol for 10 min. The total phenolic content was measured according to Folin-Ciocalteau method, and the absorbance of measured at 765 nm using a spectrophotometer as described by Ough and Amerine (1988). Measurements were performed in duplicate and the mean values were expressed as g of gallic acid per kg of the sample using a standard gallic acid curve.

Peroxidase (POD) activity assay

The POD activity was assessed according to Vicente *et al.* (2006). In a total volume of 3.0 mL, the reaction mixture contains 100 μ L of extract, 0.024 mol H2O2, 0.1 mol phosphatebuffered saline and 0.008 mol guaiacol was prepared. At wavelength of 460 nm and 30°C, the enzyme activity was measured with guaiacol as substrate, the results were reported as U/g min.

Measurement of total soluble solids

Tissues (50 g) from 4-8 fruits were homogenized and centrifuged for 20 min at $10000 \times g$. The supernatant was collected, and a refractometer was used to determine the total soluble solids concentration.

Determination of ascorbic acid content

The content of ascorbic acid (AA) in strawberry was determined according to 2, 6- dichlorophenolindophenol titration method. Briefly, tissue from fruits was homogenized in 50 mL of 0.02 g/mL oxalic acid solution. The mixture was centrifuged for 15 min at 15000 \times g and 4 °C. A total of 10 mL supernatant was titrated with 0.1 percent 2.6dichlorophenolindophenol to get a persistent pink color. The AA concentration was estimated using the titration volume and the results were reported as mg per 100 g of fresh weight.

Measurement of titratable acidity (TA)

Using phenolphthalein indicator, the TA % of the treated and untreated strawberry fruits was measured by titrating 10 mL of clear strawberry juice 0.1 N NaOH. The TA% was calculated as malic acid according to the following equation of AOAC (1980)

Statistical analysis

The obtained data were statistically analyzed using MSTAT-C version 2.10 (1991) software. The least significant difference (L.S.D.) was used for the comparison between means as described by Gomez and Gomez (1984).

Results and Discussion

Fungal isolates sources

Data in Table 1 shown that isolates were collected from Naturally infected strawberry, Fortuna strawberry cultivar grown in El Behira governorate (El Nubaria and Badr territories) with symptoms of gray mold. B. cinerea 1, 2, and 3 were collected from El Behira-El Nubaria, while B. cinerea 4, 5, 6, and 7 were collected from El Behira-Badr. These results are consistent with those reported by Wagih et al. (2019), who investigated the pathological diversity related to 51 B. cinerea isolates those infected grapes and strawberry in Egypt. In another study, fifteen B. cinerea isolates were obtained from different vegetables cultivated in various Egyptian locations and identified by Gaber et al. (2020). Furthermore, four isolates of B. cinerea were collected and tested for fenhexamid- regardless of host plant, location, and plant organ by Wahab (2015).

Pathogenicity

The isolated and identified fungi isolates used to conduct pathogenicity test and the results are shown in Table 1. The pathogenicity test was conducted using 7 isolates of B. cinerea on strawberry plants of the Fortuna cultivar, eighty days after planting in the first stage of maturity and the start of red discoloration on the fruits next to the uninfected control. The infection with B. cinerea 1 isolate resulted in incidence of 75% and disease severity of 58.40%, which were the highest percentages among the seven isolates, followed by B. cinerea 2, while the lowest percentages of disease severity and incidence were found for B. cinerea 6. On the other hand, no disease symptoms were found are the infection with the isolate *B. cinerea* 7. The *B. cinerea* infection was found to take place very rapidly on strawberry fruits, the germination starts within 90 min of infection (Hennebert and Gilles, 1958) and the penetration occurs after 20 h of inoculation. Additionally, it was reported that the first symptom can appear in two days of strawberry fruit ripening (Guillon, 1906). Furthermore, Jarvis (1968) found that only about 1% of infections of ripened intact strawberry with B. cinerea occurred conidia germinated in a persistent drop of water. Valiuskaite et al. (2010) investigated pathogenicity traits of B. cinerea isolates on strawberry fruits and found different degrees of virulence on strawberry by the three isolates of B. cinerea. Reddy et. al. (2000) tested the pre-harvest and post-harvest infection by B. cinerea decay incidence under storage period and temperature that recorded increasing in disease incidence by time over 10 days.

Igolatog numbor	Source of isolate	Gray	mold
isolates number	Source of isolate	Incidence %	Severity %
B. cinerea 1	El Behira-El Nubaria	75.00	58.40
B. cinerea 2	El Behira-El Nubaria	43.75	36.50
B. cinerea 3	El Behira-El Nubaria	37.5	12.25
B. cinerea 4	El Behira-Badr	25.00	10.40
B. cinerea 5	El Behira-Badr	22.60	10.85
B. cinerea 6	El Behira-Badr	18.75	8.25
B. cinerea 7	El Behira-Badr	0.0	0.0
Control	-	0.0	0.0
L.S.D. 0.05		8.85	8

Table 1. Isolates of strawberry gray mold, sources and their pathogenic capability.

L.S.D. 0.05

Antifungal effect of chemical concentration *in vitro*

In vitro, the effect of chemical compounds on *B. cinerea* growth was performed with three concentrations of each chemical compound and the results are shown in Table 2. Three compounds were used including po-

tassium phosphite and potassium phosphate at concentrations of 125, 250 and 500 μ g/L as well as SA at concentrations of 250, 500, and 1000 μ g/L, compared with fungicide (Switch) and control infected with the fungal strain *B. cinerea 1*. The treatment with potassium phosphate did not reduce the mycelium growth of the fungus as well as the treatment with SA at concentration of 250 µg/L. However, a reduction in the growth of the mycelium was found after treatment with SA at 500 and 1000 ug/L. Growth reductions were also found after the treatment with potassium phosphite at concentrations of 125, 250 and 500 mg/L. Estrada-Ortiz, et al. (2013) found that treatment with phosphite enhanced quality of strawberry fruits and plant resistance. Also, phosphite was found to have effect against oomycetes (Orbovic et al., 2008). Volatile substances and plant extracts have been shown to have antifungal effects. For benzaldehyde, example. acetaldehyde, ethanol, benzyl alcohol, ethyl benzoate, methyl salicylate, and isothiocyanates inhibited the B. cinerea infection on a laboratory scale (Tripathi and Dubey, 2004).

L	8	
Treatments	B. cinerea G	rowth
Ireatments	Diameter mm	eduction
K_2 HPO _{4 500 mg/L}	70.0	0.0
K_2 HPO _{4 250 mg/L}	70.0	0.0
K ₂ HPO _{4 125 mg/L}	70.0	0.0
KPhi 500 mg/L	22.0	68.57
KPhi 250 mg/L	46.0	34.28
KPhi 125 mg/L	60.0	14.28
SA 1000 mg/L	64.0	8.57
SA 500 mg/L	65.0	7.14
SA 250 mg/L	70.0	0.0
Switch 100 mg/L	0.0	100
Control (untreated)	70.0	0.0
L.S.D. 5	8.2	6.4

Table 2. Effect of chemical compounds on *in vitro radial growth*.

L.S.D. 5

Preharvest treatments on strawberry plants

Antifungal of effect chemical treatments

Treatments in vivo were carried out on strawberry plants of Fortuna cultivar to investigate the effect of chemical treatments against the fungal strain B. cinerea 1 under greenhouse conditions, the results are presented in Table 3. A reduction in severity of the disease and significant differences were found after treatments with potassium phosphite, potassium phosphate, and SA. The lowest reduction percentage in the infection with the causal pathogen were

found after treatment with SA at 250 mg/L, potassium di phosphate at 125 and 250 mg/L, and SA at 500 and 1000 mg/L, and potassium di phosphate 500 mg/L. However, the treatment with potassium phosphite at concentrations of 500, 250 and 125 mg/L resulted in the higher diseases reductions than other treatment, but lower that of treatment with fungicide. Estrada-Ortiz et al. (2013) reported that adding 20% phosphite to the nutrient solution improved the quality of strawberry fruits. They also found that the addition of 30% phosphite to the nutrient solution triggered defense systems in plants and improved the quality of fruits and anthocyanins accumulation. In another study, Kamal *et al.* (2008) found that treatment with di-potassium phosphate can reduce the infection of onion plants by *Stemphylium vesicarium*.

Table 3.	Effect of treatments	with chemical	compounds at	different conce	ntrations
aga	ainst the infection of	strawberry wit	h fungal strain	B. cinerea.	

Compounds	Strawberry gray mold				
Compaunds	Disease severity (%)	Reduction (%)			
K ₂ HPO _{4 500 mg/L}	39.2	28.7			
K ₂ HPO _{4 250 mg/L}	42.4	22.9			
K ₂ HPO _{4 125 mg/L}	46.8	14.9			
KPhi 500 mg/L	10.6	80.7			
KPhi 250 mg/L	14.6	73.5			
KPhi 125 mg/L	18.8	65.8			
SA 1000 mg/L	40.6	26.2			
SA 500 mg/L	40.8	25.8			
SA 250 mg/L	48.6	11.6			
Switch 100 mg/L	2.5	95.5			
Control1 (infected)	55.0	0.0			
Control 2 (uninfected)	0.0	100.0			
L.S.D. 0.05	10.4	9.6			

Biochemical changes as response to infection and treatments

Changes in the phenolics content and peroxidase activity of strawberry after 6 days of treatments with different concentrations of chemical compounds are presented in Table 4. Generally, the total phenolics content and peroxidase activity significantly increased in the infected control with chemical treatments as compared to the uninfected control. On the other hand, the phenolics content and peroxidase activity of the uninfected and chemically treated strawberry are lower than those of the infected and chemically treated strawberry. Also, the total phenolics content and peroxidase activity of the infected and chemically treated strawberry are lower than those of the infected control without chemical treatments. These results indicate that the chemical treatments have inhibition effect against *B. cinerea 1*.

Table 4	Effect of different compounds on total phenolic contents and peroxidase
ac	tivity of the untreated and treated strawberry plants after 6 days of treat-
m	ents.

Compounds	Phenolics	Peroxidase
Compaunus	(mg galic acid equivalent/g)	(U/g. min)
K ₂ HPO ₄ 500 mg/L+B	6.55	1.40
K ₂ HPO ₄ 500 mg/L	2.45	0.84
K ₂ HPO ₄ 250 mg/L+B	7.35	1.48
K ₂ HPO _{4 250} mg/L	2.24	0.80
K ₂ HPO ₄ 125 mg/L+B	8.24	1.64
K ₂ HPO ₄ 125 mg/L	2.24	0.80
KPhi 500 mg/L+B	4.35	0.62
KPhi 500 mg/L	2.34	0.32
KPhi 250 mg/L+B	4.68	0.75
KPhi 250 mg/L	2.46	0.58
KPhi 125 mg/L+B	4.98	0.88
KPhi 125 mg/L	2.65	0.68
SA 1000 mg/L+B	6.98	0.90
SA 1000 mg/L	3.48	0.72
SA 500 mg/L+B	5.95	0.88
SA 500 mg/L	3.22	0.70
SA 250 mg/L+B	5.65	0.95
SA 250 mg/L	2.98	0.70
Switch 100 mg/L+B	3.46	0.68
Switch 100 mg/L	3.20	0.42
Control1 (infected)	8.94	1.84
Control 2 (uninfected)	2.31	0.16
L.S.D. 0.05	0.32	0.18

Postharvest treatments and changes in biochemical attributes of strawberry fruits

Antifungal effect of treatments

The antifungal effect of treatments of harvested strawberry fruits with different concentrations of potassium phosphite, potassium phosphate, and SA was investigated, and the results are presented in Table 6. The untreated and treated strawberry fruits were stored at room temperature for 9 days, and samples were taken for analysis every 3 days of storage period, It can be seen that the treatment of strawberry fruits with potassium phosphite at concentration of 500 mg/L resulted in the highest disease reduction, followed by treatment with SA at concentration of 1000 mg/L, while the treatment with potassium phosphate resulted in the lowest disease reduction percentage after storage to up to 9 days compared with infected control. Additionally, the obtained results indicate that the treatment with potassium phosphite is the best and comparable to the treatment with the recommended fungicide. These results match previous studies for other researchers, Rebollar-Alviter and Ellis (2005) mentioned that potassium phosphite reduced the leather rot in strawberry fruits. In another study, to minimize botrytis and anthracnose rot, potassium phosphite was recommended as a supplemental ingredient to strawberry in the field during prolonged wetness conditions (Rebollar-Alviter et al., 2010).

		Storage periods						
Cl.	Concentration	3 days		6 days		9 days		
Compounds	Concentration	DS	reduction	DS	reduction	DS	reduction	
		(%)	(%)	(%)	(%)	(%)	(%)	
K ₂ HPO ₄	500 mg/L	6.8	59.0	22.2	36.2	52.4	22.9	
KPhi	500 mg/L	4.2	74.7	12.0	65.5	18.6	72.6	
SA	1000 mg/L	10.2	38.6	19.4	44.3	26.8	60.6	
Switch	100 mg/L	2.6	84.3	8.2	76.4	10.0	85.3	
Control1 (infected)	-	16.6	0.0	34.8	0.0	68.0	0.0	
Control 2 (uninfected)	-	0.0	100.0	0.0	100.0	0.0	100.0	
L.S.D. 0.05		2.24	6.41	2.61	4.78	7.20	6.62	

 Table 5. Effect of different compounds and storage periods on strawberry gray mold disease severity caused by B. cinerea under ambient conditions.

Total phenolic content

Changes in the content of total phenolics in the untreated and treated strawberry fruits during storage at room temperature for 9 days are presented in Table 7. The total phenolic content of the untreated strawberry fruits significantly decreased as the storage time extended to up to 9 days. However, the total phenolic content of the postharvest B. cinerea 1 infected and chemical compounds or fungicide treated strawberry fruits significantly increased as storage prolonged, especially during the first 3 days of storage. Also, a significant increase was found in the total phenolic content of B. cinerea 1 infected strawberry fruits without potassium phosphate, potassium phosphite, and SA treatments. The increase in the total phenolic content of the infected strawberry fruit during storage may be attributed to stress effects caused infection (Coltro et al., 2014). The pathogen infection stress may lead to physiological disturbances and activation of certain phenylpropanoid metabolism proteins, increasing the phenolic compounds accumulation. Additionally, the damage of cells by

pathogen may be contributed to the increased phenolics, which released from vacuoles and oxidized to quinones (Thipyapong et al., 2004). Kamal et al. (2008) found that treatment with di-potassium phosphate enhances peroxidase and total phenol contents when fungal infection occurs on onion plants by fungal strain of Stemphylium vesicarium. On the other hand, slight changes were found in the total phenolics content of uninfected and potassium phosphate, potassium phosphite, and SA treated strawberry fruits. Recently, it was found that calcium chloride and SA treated strawberry fruits showed an increased phenolic content during cold storage, while the phenolic content of the untreated fruits decreased as storage prolonged (Shahzad et al., 2020). Nguyen & Nguyen (2021) found a reduction in the total content of phenolics in strawberry fruits stored at different temperatures (2, 5, 10, and 25C). The decrease in the total phenolic content can be attributed to oxidation of polyphenols by polyphenoloxidase and partial degradation of anthocyanins during storage.

	Concentation	Storage periods					
Compaunds	(mg/I)	Total phenolics contents (mg galic acid equivalent/g)					
	(mg/L)	0 days	3 days	6 days	9 days		
K_2HPO_4+B	500	1.25	4.26	5.84	5.82		
K ₂ HPO ₄	500	1.23	1.32	1.30	1.40		
KPhi +B	500	1.24	3.14	2.08	1.42		
KPhi	500	1.22	1.25	1.25	1.39		
SA +B	1000	1.20	4.26	3.44	2.64		
SA	1000	1.14	1.85	2.18	2.24		
Switch +B	100	1.26	2.42	1.90	1.22		
Switch	100	1.23	1.34	1.34	1.22		
Control1 (infect- ed)		1.24	4.62	6.20	5.06		
Control 2 (unin- fected)	-	1.22	1.02	0.81	0.42		
LSD 0.05	-	_	0.48	0.62	0.31		

 Table 6. Effect of different compounds and storage periods on total phenolic contents of strawberry fruits infected with *B. cinerea* under ambient conditions.

Peroxidase activity

Peroxidase activity was measured for the untreated and treated strawberry fruits and the results are presented in Table 8. Peroxidase activity of the uninfected and untreated strawberry fruits significantly decreased as the storage time prolonged. However, the postharvest B. cinerea 1 infected strawberry fruits without chemical compounds treatment showed increased peroxidase activity as storage time extended to up to 6 days. A significant increase was also found in peroxidase activity of B. cinerea 1 infected and chemical compounds fungicide or treated strawberry fruits during 6 days of storage. On the other hand, slight changes were found in the peroxidase activity of chemical compounds and fungicide treated strawberry fruits without B. cinerea 1 postharvest in-Asghari and Hasanlooe fection. (2016) found that treatment by methyl jasmonate enhanced peroxidase and some other defense enzymes activities as well as the total antioxidant content of harvested strawberry fruits during storage. In another study, Shahzad et al. (2020) found an increase in the catalase and peroxidase activities of calcium chloride and SA treated strawberry fruits during the cold storage compared to the untreated ones, especially during the first days of storage.

Table 7. Effect of different compounds and storage periods on peroxidase activity (U/g. min) of the untreated and treated strawberry fruits at ambient conditions.

Tuestanouta	Concentration	Storage periods				
1 reatments	(mg/L)	0 days	3 days	6 days	9 days	
K_2HPO_4+B	500	0.32	1.47	1.22	0.80	
K ₂ HPO ₄	500	0.30	0.38	0.42	0.42	
KPhi +B	500	0.36	0.46	0.58	0.50	
KPhi	500	0.30	0.32	0.48	0.46	
SA +B	1000	0.32	1.25	1.56	1.42	
SA	1000	0.32	0.42	0.70	0.62	
Switch +B	100	0.36	0.56	0.62	0.60	
Switch	100	0.40	0.42	0.44	0.42	
Control 1 (infected)	-	0.38	1.82	1.86	1.42	
Control 2 (uninfected)	-	0.35	0.30	0.23	0.15	
LSD 0.05	-	-	0.11	0.09	0.12	

Total soluble solids

Total soluble solids contents of the untreated and treated strawberry fruits during storage at room temperature for 9 days are shown in Table 9. Generally, a reduction in the total soluble solids content of the postharvest B. cinerea 1 infected and chemical compounds or fungicide treated strawberry fruits was found as the storage time extended to up to 9 days. The highest reduction rates in the total soluble solids were found for the B. cinerea 1 infected straw berry fruits without chemical compounds treatments. Also, the decrease rate in total soluble solids content was greater for the potassium phosphate treated strawberry fruits than other treatments. Mandour et al. (2019) found a reduction in the total soluble solids contents untreated and chemically treated strawberry fruits during cold storage for 15 days. However, in another study, it was found that treatment with different concentrations of calcium chloride and SA effectively maintained the total soluble solids contents of strawberry during cold storage (Shahzad et al., 2020). Recently, a reduction in the total soluble solids content of strawberry fruits during storage at 25°C was reported by Nguyen and Nguyen (2021). The decrease in the total soluble solids content can be attributed the higher respiration rate and the metabolic utilization of sugars during storage at room temperature (Yang et al., 2010; and Nguyen and Nguyen, 2021).

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Tuestments	Concentration	Storage periods			
Ireatments	(mg/L)	0 days	3 days	6 days	9 days
K ₂ HPO ₄	500	9.2	7.6	7.0	6.4
KPhi	500	9.2	8.8	8.4	8.0
SA	1000	9.1	8.8	7.8	7.4
Switch	100	9.2	9.1	7.9	7.2
Control1 (infected)	-	9.5	8.0	6.2	6.0
Control 2 (uninfected)	-	9.4	9.2	7.4	7.2
LSD 0.05	-	-	0.92	0.55	0.61

Table 8. Effect of different compounds and storage periods on total soluble solids content (%) of the untreated and treated strawberry fruit at ambient conditions.

Titratable acids

The titratable acids contents of the untreated and treated strawberry fruits are presented in Table 10. The titratable acids significantly decreased as the storage time extended to up to 9 days for all samples. The uninfected and chemical compounds untreated strawberry fruits (Control) showed the greatest titratable acids decrease rate, followed by the infected and SA treated strawberry fruits. Shahzad et al. (2020) found a reduction in titratable acids percentage in the untreated and calcium chloride and SA treated fruits stored for 15 days at 4°C, which was attributed to conversion of acids into sugars. Additionally, they found that treatment with 5 mM SA was effective in maintaining strawberry content of acids compared with other treatments. Nunes et al. (2002) found a decrease in the content of titratable acids in strawberry fruits stored at 20°C. Also, Rahman et al. (2016) found a decrease in the content of titratable acids in strawberry fruits stored at ambient temperature (25 °C). The reduction in the acidity of stored strawberry may be due to oxidative metabolism of organic acids.

Table 9. Effect of different compounds and storage periods on titratable acids content (%) of the untreated and treated strawberry fruits at ambient conditions.

Treatmonte	Concentration	Storage periods				
Treatments	(mg/L)	0 days	3 days	6 days	9 days	
K ₂ HPO ₄	500	0.83	0.81	0.54	0.46	
KPhi	500	0.82	0.73	0.68	0.62	
SA	1000	0.81	0.62	0.52	0.49	
Switch	100	0.81	0.80	0.62	0.58	
Control1 (infected)	-	0.83	0.80	0.63	0.42	
Control 2 (uninfected)	-	0.82	0.76	0.58	0.40	
LSD 0.05	-	-	0.19	0.06	0.05	

LSD 0.03

Ascorbic acid content

The Ascorbic Acid content AA content is important factor for determining the quality of stored strawberry fruits (Cordenunsi et al., 2003). Changes in the content of AA in strawberry fruits during storage at room temperature for 9 days were measured, and the results are shown in Table 11. The AA content significantly decreased as the storage time extended to up to 9 days for all samples. The lowest AA content was found for the postharvest infected strawberry fruit without chemical compounds treatment, followed by fungicide treated sample. However, the potassium phosphite and SA treated strawberry fruits showed higher AA content at 9 days of storage than that of other samples. Moor et al. (2009) found an enhancement in synthesis of anthocyanins and AA after fertilizing with phosphite. However, the reduction in the AA content of strawberry fruits is mainly attributed to the increase in level of the oxygen or storage temperature (Sogvar et al., 2016). Additionally, Pavlovska et al. (2015) found a major reduction in the AA content during storage of strawberry fruits for 16 days at room temperature compared to cold and freezing storages. In another study, Rahman et al. (2016) found that the ascorbic content of strawberry fruits

decreased as storage at room temperature prolonged. Also, Mandour *et al.* (2019) found a reduction in the AA content of the untreated and chemically treated strawberry fruits during cold storage for 15 days. Also, Khodaei *et al.* (2021) found a reduction in the AA content during cold storage of strawberry fruits for 16 days. They also found that edible coatings provided protective effect against degradation of AA by reduction of oxygen diffusion and respiration rate. The decrease in the content of AA content can be attributed to oxidation reaction and degradation during storage (Zeb *et al.*, 2015).

 Table 10. Effect of different compounds and storage periods on ascorbic acid (mg/100 g fresh weight) of the untreated and treated strawberry fruits at ambient conditions.

Tuestments	Concentration	Storage periods			
1 reatments	(mg/L)	0 days	3 days	6 days	9 days
K ₂ HPO ₄	500	70	62	42	34
KPhi	500	76	72	55	46
SA	1000	74	70	45	39
Switch	100	69	65	40	25
Control1 (infected)	-	70	50	35	22
Control 2 (uninfected)	-	68	61	53	35
LSD 0.05	-	2.76	1.25	3.94	2.84

Conclusion

Under green house and ambient conditions, the protective effects of pre- and postharvest treatments with potassium phosphite, potassium phosphate, and SA on strawberry fruits inoculated with B. cinerea were examined. The treatment with potassium phosphite at concentration of 500 mg/L significantly reduced the fungal growth compared with other treatments in vitro. In addition, treating strawberry plants with potassium phosphite at concentrations of 250 or 500 mg/L led to the greatest disease reduction, followed by SA treatment. On the other hand, strawberry fruits were treated with these chemical compounds immediately after harvesting and effects treatments on disease reduction and biochemical characteristics of strawberry fruits were evaluated during storage at room temperature for 9 days. The gray mold disease was significantly reduced in strawberry fruits during storage for 9 days. Furthermore, at the end storage period, the infected and potassium phosphate treated strawberry fruits had higher phenolic content and peroxidase activity than the infected and potassium phosphite or SA treated ones. However, the total soluble solids, titratable acids, and AA contents of strawberry fruits significantly reduced at the end of storage periods for all treatment groups. The obtained results indicate that potassium phosphite and SA can be used as fungicide alternatives for preserving quality of strawberry fruits. Further studies are needed to comprehensively characterize the chemically treated strawberry fruits and its postharvest microbial and chemical safety.

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استخدام بعض المركبات لمكافحة مرض العفن الرمادي المتسبب عن الفطر بوتريتس و تحسين جودة ثمار الفراولة

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الملخص

أجريت هذه الدراسة بهدف التعرف على تأثير المعاملة بفوسفيت البوتاسيوم، وفوسفات البوتاسيوم، وحامض الساليسيليك على مرض العفن الرمادي في الفراولة، والذي يسببه فطر Botrytis cinerea، في مرحلتي ما قبل وبعد الحصاد، وذلك تحت الظروف المناخية الطبيعية. تم جمع سبعة عز لات من الفطر المسبب من نباتات الفر إولة المصابة طبيعيًّا، وإستُخدمت العزلة الأكبر قدرة على إحداث المرض لإجراء الدراسات المختبرية والحيوية. أظهرت النتائج أن المعاملة بفوسفيت البوتاسيوم بتركيز. • • ٥ ملجم/ لتر. أدت إلى خفض نمو. الفطريات بشكل معنوي مقارنة بالمعاملات الأخري في المختبر ، كما أدت معاملة نباتات الفر اولة بفوسفيت البوتاسيوم بتَّر كيز ات ٢٥٠ أو ٠٠٥ ملجم / لتر إلى الحد من المرض بدرجة كبيرة، تلتها المعاملة بحمض الساليسيليك. إضافة إلى ذلك، تم تقييم تأثير المعاملة بتلك المركبات للحد من شدة المرض بعد الحصاد، وذلك عن طريق تقييم التغير في الخصائص الكيموحيوية لثمار الفراولة أثناء التخزين لمدة ٩ أيام على درجة حرارة الغرفة، وقد أوضحت النتائج أن أكبر نسبة خفض في إصابة ثمار الفروالة بالمرض كانت بالمعاملة بفوسفيت البوتاسيوم، وذلك عند نهاية فترة التخزين. إلى جانب ذلك، وجد أن محتوى الفينو لات ونشاط البير وكسيداز في نهاية فترة التخزين لثمار الفر اولة المصابة والمعاملة بغو سفات البوتاسيوم أعلى من تلك المصابة والمعاملة بفوسفيت البوتاسيوم أو بحامض الساليسيليك. وفي نهاية فترة التخرين، وجد انخفاض في المحتوى الإجمالي للمواد الصلبة الذائبة، والأحماض القابلة للمعايرة، ومحتوى حامض الأسكوربيك في ثمار الفراولة لجميع المعاملات. بناءً على النتائج المتحصل عليها، يمكن التوصية باستخدام فوسفيت البوتاسيوم وحمض الساليسيليك كبدائل للمبيدات الاكثر ضررًا لإطالة عمر ثمار الفراولة بعد الحصاد والحفاظ على جودتها.

الكلمات الدالة: الفراولة؛ بوتريتيس سينيريا، ،العفن الرمادي، البيروكسيداز، حامض الأسكوربيك.