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SUPPRESSING PATULIN PRODUCTION AND APPLE ROTS BY CERTAIN SALTS

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ABSTRACT

Apple fruits were heavily infected with *Botrytis cinerea* and *Penicillium expansum* during cold storage, causing serious economic losses. Certain salts as alternatives to fungicides, i.e. ammonium sulfate ($(NH_4)_2SO_4$), calcium chloride ($CaCl_2$), magnesium sulfate ($MgSO_4$) and potassium disulfite ($K_2S_2O_5$) at 100, 200 or 300 ppm and sodium hypochlorite (NaOCl) at 50, 100 or 200 ppm were tested *in vitro* against both fungi. All tested salts inhibited mycelial fungal growth, particularly $K_2S_2O_5$and $CaCl_2$, while $(NH_4)_2SO_4$ was the least effective one. Activity of PG, PME and C_x as cell degrading enzymes in treated fruits was investigated. However, it was suggested that PG had a remarkable role in infection of apples with *B. cinerea*, while C_x had an impact in infection of apples with *P. expansum*. Salt treatments that inhibited PG, C_x and PME activity concomitantly controlled decay of apple fruits during cold storage. $K_2S_2O_5$ and $CaCl_2$ at 200 ppm proved to be the most effective postharvest treatments to control apple fruit rots during cold storage at 0-1°C for 2 months. Patulin, a mycotoxin, was produced in Czapek's broth medium with *P. expansum* at concentration of 105.93 µg/litre, when incubated at 25°C for 2 weeks, while tested salts completely suppressed patulin production, except for NaOCl and $(NH_4)_2SO_4$. *P. expansum* produced patulin at 52.180 µg/kg in the control apple fruits. No patulin was detected in apples treated with $K_2S_2O_5$, where low amount of patulin, 3.125 µg/kg fruit, was detected in apples treated with either $CaCl_2$ or $MgSO_4$.

Generally, it could be concluded that postharvest treatment of apple fruits with sodium disulfite and calcium chloride at 200 ppm was very effective in controlling fungal decay incited by *B. cinerea* and *P. expansum* and simultaneously in suppression of patulin production. Salt treatments maintained good fruit quality including firmness, TSS, TA and TSS/TA ratio during the cold storage at 0-1°C for 2 months.

Keywords: apple, patulin, mycotoxin, *Botrytis cinerea*, *Pencillium expansum*, storage, rot, decay, salt, calcium, potassium, sodium, magnesium, ammonium, cell-degrading enzyme, fungus, quality

INTRODUCTION

Apple fruit rots cause great losses during cold storage and marketing. *Penicillium expansum* and *Botrytis cinerea* were the most frequently isolated fungi from decayed apple fruits in storage. Most *P. expansum* and *B. cinerea* isolates showed high virulence at both 22°C and 3°C (Romano *et al.*, 1983, Mir *et al.*, 1986 and Veronica and Ximena, 1998).

The uncertain future of postharvest fungicide treatments has created the need to find alternative control methods for reducing storage rots in apples. Sodium metabisulphite was more effective against *P. expansum* on apple fruits than ammonium carbonate and ammonium chloride + magnesium oxide (Kaul and Munjal, 1983). Calcium salts have been reported to play an important role in the inhibition of postharvest decay of apples. Calcium-induced resistance to postharvest fungal pathogens is broad in spectrum (Conway *et al.*, 1991). Increasing concentration of $CaCl_2$ (25-175 mM)

resulted in decreased spore germination and germ-tube growth of *B. cinerea* and *P. expansum*. (Wisniewski *et al.*, 1995). Chlorine (as calcium hypochlorite) drenching was as effective as metalaxyl + carbendazim in reducing the incidence of *Botrytis* rots in Bramley apples (Colgan and Johnson, 1998). Calcium chloride solutions reduced decay to a greater extent in apple fruit inoculated with *P. expansum* (37%) or *B. cinerea* (50%). Treatment of apples with calcium chloride reduced decay caused by all three *B. cinerea* isolates by 3-5 times (Chardonnet *et al.*, 2000). Preharvest treatment of apple with calcium nitrate, significantly increased fruit firmness and brix, and decreased reducing sugars and malic acid (Tuna, 1999).

Daniel and Dube (1986) found that *P. expansum* inciting soft rot of stored apple can cause complete cellulolysis, from attacking crystalline cellulose up to the release of monomers. The pectinolytic activity of crude enzyme obtained from the culture medium of *B. cinerea* and *P. expansum* was inhibited by $CaCl_2$ (Wisniewski *et al.*, 1995). Symptoms of water-soaked lesions on apple fruits caused by *B. cinerea* were related to pectolytic activity, including pectin lyase (PNL) and polygalacturonase (PG) activity (Chilosi and Magro, 1997). Calcium ($CaCl_2$) inhibited polygalacturonase activity at 16 g $litre^{-1}$ for *B. cinerea* isolate from decayed apple (Chardonnet *et al.*, 2000).

Patulin was once considered a promising antibiotic, but its toxicity prevented medicinal use. Patulin is of some public health concern, because of its carcinogenic properties, and because it has been found in commercial apple juice (Bullerman, 1986). Patulin as a mycotoxin produced by isolates of *P. expansum* varied from 268 to 2225 µg/ml, depending on the carbon source in the liquid medium and temp., where less patulin was produced on apple tissue than on liquid Czapek (Pytel and Borecka, 1985). Prieta *et al.* (1994) suggested that patulin should be used as a quality indicator of the apples employed in the elaboration of food and drink. The potential capacity for patulin production was detected in 60.7% of *P. expansum* strains isolated from 5 apple packinghouses in Spain (Vinas *et al.*, 1995). On the basis of a PMTDI of 0.4 µg/kg bw, limit values of 50 µg/kg or 50 µg/litre of patulin have been set in apple fruit derivatives (Beretta *et al.*, 2000).

Control of postharvest diseases of apple fruit during cold storage and eliminating patulin contamination was approached in the current study by using certain naturally safe salts. Keeping fruit quality by such treatment was also investigated.

MATERIALS AND METHODS

Suppression of *Botrytis cinerea* and *Penicillium expansum* growth *in vitro* by certain salts:

Pathogenic isolates of *B. cinerea* and *P. expansum* previously isolated from decayed apple fruits during cold storage were used. *P. expansum* isolate proved to be a producer of patulin in preliminary evaluation. Sterilized Czapek's broth medium containing ammonium sulfate ($(NH_4)_2SO_4$), calcium chloride ($CaCl_2$), magnesium sulfate ($MgSO_4$) and potassium disulfite ($K_2S_2O_5$) at 100, 200 or 300 ppm and sodium hypochlorite (NaOCl) at 50, 100 or 200 ppm. Three flasks used for each concentration and inoculated with *B. cinerea* or *P. expansum* were kept at 20°C and 25°C, respectively, for 2

weeks. After incubation period, mycelial mat of fungal cultures was harvested and dried at 70°C overnight. Dry mycelial mat was weighted. Fungal growth inhibition by such treatment was calculated relating to the growth in the control treatment.

Determination of cell wall degrading enzymes activity:

The activity of pectin methylestrase (PME), polygalacturonase (PG) and cellulases (C_x) produced by *B. cinerea* and *P. expansum* in artificially inoculated or naturally infected apple fruits as affected by tested salt treatments were determined. Crude enzymes from apple fruits were extracted according to Nanson (1955). PME activity was assayed according to method modified by Smith (1958) and expressed by milliliters of 0.01 M NaOH required to 5.6 pH supernatant adjustment. PG and C_x activities were determined using Ostwald Viscometer pipette according to the method described by Talboys and Busch (1970). PG and C_x activity was expressed as percentage of loss of viscosity.

Control of apple decay during cold storage by postharvest dip in certain salt solutions:

Freshly harvested apple fruits were obtained from Ismailia governorate during seasons 1999 and 2000. Apparently healthy apples free from mechanical injury, bruising and wounds were selected. Apples were thoroughly washed with water and surface sterilized with 70% ethyl alcohol for 2 min. The fruits were punctured and sprayed with *B. cinerea* or *P. expansum* at $4x10^5$ conidia/ml or left without inoculation for natural infection. Artificially inoculated fruits with tested fungi were kept overnight at 20-25°C. Three replicates each of 24 fruits were used for each postharvest dip treatment in salt solutions or in water as a control. The used salts were $(NH_4)_2SO_4$, $CaCl_2$, $MgSO_4$, $K_2S_2O_5$ and NaOCl all at a concentration of 200 ppm, except for the last salt which was used at 100 ppm. The apple fruits of each replicate were air dried and then packed in a carton box for storage at 0-1°C for 2 months. After cold storage, fruits were evaluated for decay incidence, where decay was determined as disease severity, which was rated according to the following scale (Kremer and Unterstenhofer, 1967):

0 = Healthy apple fruit, 1 = decayed tissues of the fruit up to 25%, 2 = 26 - 50% decayed tissues, 3 = 51 - 75% decayed tissues, and 4 = decayed tissues more than 75% of the fruit.

Disease severity (%) =

$$\Sigma(\text{score} * \text{number of fruits}) * 100 / 4 * \text{total number of fruits.}$$

Efficacy of tested salts to control decay of apple fruits was calculated.

The data were statically analyzed using the Fisher's LSD at 5% according to Gomez and Gomez (1983).

Quality of treated apples with salts:

After cold storage, naturally infected apples and those treated with salts or untreated were tested for the internal quality described by AOAC (1980). Total soluble solids (TSS, %) in apple juice was determined using a hand refractometer. Titratable acidity (TA, %) was determined in filtrated juice of apple fruits through Whattman paper No. 1. Fruit juice was titrated against 0.1 N NaOH. TA (%) was expressed as matic acid. TSS/TA ratio was obtained by relating TSS to TA value. Fruit firmness was determined using

Ametic Firmness Tester mounted on a drill press and fitted with an 8-mm diameter probe. Skin of apple fruits vras removed from an approximately 12-mm diameter area from both sides of each fruit. Resistance to compression was determined and expressed in Newton (N).

Patulin production in response to certain salts:

Czapek's broth medium containing salts or not as a control was inoculated with 4 mm-disc of 7-day-old culture of *P. expansum* and kept at 25°C for 2 weeks. After incubation period, patulin was extracted and cleaned-up according to AOAC (1980). Patulin was visualized on TLC plate by spraying 2% phenyl hydrazine chloride in distilled water, then heated at 130°C for 15 min according to Subramanian (1982). Desaga densitometer was used for detection and measurement of patulin at 540 ηm.

Patulin was determined in artificially inoculated apple fruits as affected by such salty treatment or kept untreated as a control after 2 months of cold storage at 0-1°C according to AOAC (1980) and Subramanian (1982).

RESULTS AND DISCUSSION

Tested salts, i.e. $(NH_4)_2SO_4$, $CaCl_2$, $MgSO_4$, $K_2S_2O_5$ and NaOCl, inhibited growth of *B. cinerea* and *P. expansum in vitrto* (Table 1). *B. cinerea* growth in Czapek's broth medium was more affected by most tested salts than *P. expansum*. All tested concentrations of calcium chloride and potassium disulfite as well as 200 ppm of sodium hypochlorite reduced *B. cinerea* growth by more than 90%. Calcium chloride and potassium disulfite at 200 ppm and 300 ppm gave similar trend against *P. expansum*. Wisniewski *et al.* (1995) reported that calcium cation rather than the chloride anion was responsible for the inhibition of *P. expansum*. Ammonium sulfate was non efficient against *B. cinerea* and *P. expansum*. Magnesium sulfate and sodium hypochlorite were not efficient against *P. expansum* only.

Cell wall degrading enzymes activity including pectin methylestrase (PME), polygalacturonase (PG) and cellulases (C_x) in naturally infected or artificially inoculated apple fruits with *B. cinerea* or *P. expansum* and treated with salt solutions was demonstrated after 2 months of cold storage in Table (2). All salt treatments reduced the activity of PG, PME and C_x enzymes. PG, PME and C_x activity in apples artificially inoculated with *B. cinerea* was greatly decreased in apples treated with $K_2S_2O_5$ or $CaCl_2$ than in the control treatment. PG activity in artificially inoculated apples with *B. cinerea* in all treatments was higher than that in naturally infected apple fruits. This suggested that PG had a remarkable role in infection of apples with *B. cinerea*. On the other hand, effect of tested salts to reduce incidence of *B. cinerea* could be attributed to the suppression of PG enzyme secretion or activity. This finding was supported by Chilosi and Magro (1997) who found that symptoms on apple fruits caused by *B. cinerea* were related to pectolytic activity, including polygalacturonase (PG) activity. High activity of C_x enzymes was observed in apples artificially inoculated with *P. expansum* comparing with naturally infected apples or artificially inoculated ones with *B. cinerea*. $K_2S_2O_5$, $CaCl_2$ and $MgSO_4$ strongly suppressed PME and C_x activity.

Table (1): Inhibition (%) of *Botrytis cinerea* and *Penicillium expansum* growth on Czapek's broth medium amended with certain salts and kept at 20°C and 25°C, respectively, for 2 weeks

Salt	Conc. (pm)	Fungal growth inhibition (%)	
		Botrytis cinerea	*Penicillium expansum*
Ammonium sulfate	100	14.3	15.2
	200	22.5	19.2
	300	32.0	20.3
Calcium chloride	100	94.1	51.3
	200	95.9	92.4
	300	100	99.4
Magnesium sulfate	100	23.0	12.3
	200	40.1	18.4
	300	59.5	24.7
Potassium disulfite	100	93.5	71.5
	200	99.0	100.0
	300	99.7	100.0
Sodium hypochlorite	50	41.8	6.3
	100	75.9	18.0
	200	96.2	25.9
LSD at 0.05		5.8	7.5

On the other hand, PME activity in apples infected with *P. expansum* was higher than that of naturally infected apples and lower than that apples infected with *B. cinerea*. So, treatments affected PG and C_x activities were expected to be effective against *P. expansum* infection of apple fruits. This finding was supported by Kim *et al.* (1990) who found that the strongly pathogenic isolate of *P. expansum* (S1) and weakly pathogenic (R2) produced considerable amounts of endo-polymethylgalacturonase, endo-polygalacturonase, pectin methyl-trans-eliminase and polygalacturonate-trans-eliminase in an artificial medium. In apple medium, the activities of endo-polymethylgalacturonase and endo-polygalacturonase of S1 were >6 times higher than those of R2. The addition of these enzymes increased the pathogenicity of weak pathogenic isolates R1-4.

On naturally infected fruits, all salt treatments strongly suppressed decay of apples (Table 3). $K_2S_2O_5$, $CaCl_2$ and $MgSO_4$ almost completely suppressed the fungal decay in season 1999. While decay of apples was higher during season 2000, these salts achieved good control with average ranged from 1.38% to 5.10% comparing to 26.7% decay of the control treatment. Artificial inoculation of apple fruits with *B. cinerea* and *P. expansum* increased the decay incidence during the cold storage. However, all salt treatments suppressed infection of apples with *B. cinerea* by more than 50% of the control treatment. $K_2S_2O_5$, $CaCl_2$ and NaOCl reduced this infection to 15.34-18.49% and 12.87-22.10% comparing with 77.62% and 81.54% of the control fruits during seasons 1999 and 2000, respectively.

Table (2): Determination of pectinolytic and cellulytic enzymes activity in naturally infected or artificially inoculated Anna apple fruits with *Botrytis cinerea* or *Penicillium expansum* after dipping in certain salt solutions at 200 ppm and storage at 0-1°C for 2 months

Treatment	Natural infection					*Botrytis cinerea*					*Penicillium expansum*				
	% reduction in viscosity				PME 0.01 N NaOH	% reduction in viscosity				PME 0.01 N NaOH	% reduction in viscosity				PME 0.01 N NaOH
	PG		C_x			PG		C_x			PG		C_x		
	15 min	30 min	15 min	30 min		15 min	30 min	15 min	30 min		15 min	30 min	15 min	30 min	
Ammonium sulfate	13.58	23.76	6.07	15.58	2.2	33.66	43.56	10.98	20.24	7.7	24.29	35.72	49.09	65.45	6.0
Calcium chloride	10.47	19.38	2.74	9.21	1.8	27.91	38.36	8.54	17.05	6.2	21.63	29.10	32.26	50.00	3.4
Magnesium sulfate	13.04	22.79	3.90	9.41	1.7	28.13	39.53	10.39	17.07	4.9	23.24	31.51	35.94	50.00	3.6
Potassium disulfite	9.77	15.96	2.38	4.11	1.6	25.76	34.85	4.94	14.82	4.7	20.64	28.05	25.42	38.98	3.0
Sodium hypochlorite	13.48	23.60	6.58	11.90	2.0	28.77	42.19	10.71	19.48	6.9	23.66	34.35	36.21	56.90	5.5
Control	14.45	24.11	11.76	18.82	3.4	43.20	52.80	14.77	30.49	9.0	29.80	37.17	51.92	67.31	7.9

Table (3): Decay (%) of naturally infected and artificially inoculated apple fruits with *Botrytis cinerea* or *Penicillium expansum* as affected by postharvest dipping in solutions of certain salts at 200 ppm for 2 min then storage at 0-1°C for 2 months during seasons 1999 and 2000

Treatment	Season 1999						Season 2000					
	Natural infection		*Botrytis cinerea*		*Penicillium expansum*		Natural infection		*Botrytis cinerea*		*Penicillium expansum*	
	Decay (%)	Efficacy (%)	Decay (%)	Efficacy (%)	Decay (%)	Efficacy (%)	Decay (%)	Efficacy (%)	Decay (%)	Efficacy (%)	Decay (%)	Efficacy (%)
Ammonium sulfate	8.13	56.96	34.25	55.87	35.00	32.42	11.68	56.21	39.20	51.93	34.25	43.20
Calcium chloride	0.22	98.84	17.57	77.36	14.12	72.74	2.41	90.96	15.90	80.50	15.00	75.12
Magnesium sulfate	0.30	98.41	22.06	71.58	24.58	52.54	5.10	80.88	26.70	67.26	20.63	65.79
Potassium disulfite	0.00	100.00	15.34	80.24	13.43	74.07	1.38	94.83	12.87	84.22	13.43	77.73
Sodium hypochlorite	5.38	71.52	18.49	76.18	24.90	51.92	9.90	62.88	22.10	72.90	31.72	47.40
Control	18.89	—	77.62	—	51.79	—	26.67	—	81.54	—	60.30	—
LSD at 0.05	3.83		4.93		5.41		4.63		4.62		5.17	

This result revealed that these salts were very effective against *B. cinerea*. While infection of the control apples with *P. expansum* was lower than infection with *B. cinerea*, the efficacy of tested salts to control *P. expansum* was relatively lower. However, $K_2S_2O_5$ and $CaCl_2$ were significantly very effective against *P. expansum* during both seasons. Conway *et al.* (1988) concluded that since Ca is known to stabilize the cell wall, decay in apples with high levels of Ca may be decreased, because maceration by polygalacturonase is reduced. CaCl2 was also expected to be effective against *P. expansum* as mentioned by Popushoi *et al.* (1993). They found that decay intensity of Golden Delicious apples, caused by inoculation with *P. expansum*, decreased with increasing Ca concentration. It was shown that polygalacturonase produced by *P. expansum* did not readily hydrolyse Ca-pectate and it was concluded that Ca ions affect the structure of plant cell walls making them less accessible to cell wall degrading enzymes produced by fungal pathogens.

Firmness of treated apple fruits with tested salts was enhanced and was higher than the control treatment (Table 4). Enhancement of firmnes could be attributed to calcium where it affects fruit softening, because it is an essential part of the cell wall structure and it also influences cell membrane integrity (Fallahi *et al.*, 1997) or to the suppression of fungal development and so delaying fruit respiration. All other internal quality parameters, i.e. TSS, TA and TSS/TA ratio were better in the control treatment than in salt treatments, which could be attributed to the decay development promoting the fruit toward the senescence. So, the quality was developed by such salt treatments in spite of lower TSS and TSS/TA ratio and higher TA than the control apples.

Table (4): Quality of naturally infected apple fruits as affected by postharvest dipped in solutions of certain salts at 200 ppm for 2 min then stored at 0-1°C for 2 months

Treatment	Firmness (N)	TSS (%)	Acidity (TA), (%)	TSS/TA ratio
Ammonium sulfate	48.74	10.6	0.3518	30.13
Calcium chloride	58.15	9.2	0.3082	29.85
Magnesium sulfate	50.41	10.0	0.2546	39.28
Potassium disulfite	51.78	10.0	0.3183	31.42
Sodium hypochlorite	49.82	10.8	0.2161	49.98
Control	47.56	11.2	0.1976	56.68
LSD at 0.05	5.46	ns	ns	6.77

ns: non significant differences were obtained among all treatments.

P. expansum produced patulin at 105.93 µg/litre in Czapek's broth medium (Table 5). No patulin was detected in inoculated medium with *P. expansum* containing tested salts, except for NaOCl and $(NH_4)_2SO_4$. This finding could be occurred due to suppression of fungal growth by the tested salts or due to the effect of the tested salts on toxin production and excretion in the broth medium. While $MgSO_4$ did not inhibit fungal growth, it prevented

toxin production. This finding revealed that $MgSO_4$ may affect toxin metabolism and excrition in the liquid medium. Taniwaki *et al.* (1989) found that patulin was produced at 933-10 000 µg/litre, 472-144 444 µg/litre and 53.3-400 µg/litre in culture medium at 7°C, 25°C and 35°C, respectively. Patulin was produced at 150-311 µg/litre and 100-300 µg/litre in the apples stored at 25°C or at 4°C, respectively.Patulin production in artificially inoculated apples with *P. expansum* was reduced by postharvest treatment with salt solutions (Table 6). Concentration of patulin in the control apples was 52.180 µg/kg. Leggott *et al.* (2000) suggested that higher temperature and total solids concentration (°Brix) caused a significant increase in patulin concentration. So, detected patulin in the current investigation in the control apples could be attributed to direct growth of the fungus as a secondary metabolite or to the promotion of toxin production resulted by increasing TSS as a response to the fungal growth. On the other hand, no patulin was detected in apples treated with $K_2S_2O_5$ as a result of suppression of fungal growth as well as toxin production. However, $K_2S_2O_5$ produces high amount of SO_2 in the medium which was found to react rapidly with patulin and break it down offerring a suitable method for decontamination (CAST, 1979 and Altmayer *et al.*, 1985). Low amount of patulin, 3.125 µg/kg, was detected in apples treated with either $CaCl_2$ or $MgSO_4$.

Table (5): Patulin production by *Penicillium expansum* on Czapeck's medium containing different concentrations of certain salts after incubation at 25°C for 2 weeks

Salt	Conc. (ppm)	Patulin concentration (µg toxin/L medium)
Ammonium sulfate	100	83.84
	200	75.06
	300	68.12
Calcium chloride	100	0.0
	200	0.0
	300	0.0
Magnesium sulfate	100	0.0
	200	0.0
	300	0.0
Potassium disulfite	100	0.0
	200	0.0
	300	0.0
Sodium hypochlorite	50	11.66
	100	96.15
	200	99.21
Control		105.93

Table (6): Patulin production in artificially inoculated apple fruits with *Penicillium expansum* as affected by postharvest dipped in solutions of certain salts at 200 ppm for 2 min then stored at 0-1°C for 2 months

Treatment	Patulin (µg/kg)
Ammonium sulfate	25.000
Calcium chloride	3.125
Magnesium sulfate	3.125
Potassium disulfite	0.000
Sodium hypochlorite	45.206
Control	52.180

Generally, it could be concluded that postharvest treatment of apple fruits with sodium disulfite and calcium chloride at 200 ppm was very effective in controlling fungal decay incited by *B. cinerea* and *P. expansum* and simultaneously in suppression of patulin production. Both treatments maintained good fruit quality including firmness, TSS, TA and TSS/TA ratio during the cold storage at 0-1°C for 2 months.

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استخدام بعض الأملاح فى تثبيط إنتاج الباتيولين وأعفان ثمار التفاح

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تصاب ثمار التفاح بشدة بفطرى بوتريتس سينماريا وبنيسيليوم اكسبانسم أثناء التخزين المبرد مسببة خسائر اقتصادية كبيرة.

وقد تم اختبار فعالية عدد من الأملاح مثل كبريتات أمونيوم وكلوريد كالسيوم وكبريتات ماغنيسيوم وثانى كبريتيت بوتاسيوم بتركيز ١٠٠، ٢٠٠، ٣٠٠ جزء فى المليون وهيبوكلوريت صوديوم بتركيز ٥٠، ١٠٠، ٢٠٠ جزء فى المليون على الفطرين المذكورين كبدائل للمبيدات فى المعمل، وأوضحت النتائج أن كل الأملاح المختبرة لديها القدرة على تثبيط الفطرين وخاصة ثانى كبريتيت بوتاسيوم وكلوريد كالسيوم بينما كان أقل الأملاح فعالية هو كبريتات أمونيوم.

وقد تم تقدير نشاط الإنزيمات المحللة لجدر الخلايا (بولى جالاكتوروتيز PG، بكتين ميثيل استريز PME والإنزيمات المحللة للسليلوز C_x حيث وجد أن انزيم PG له دور واضح فى إصابة ثمار التفاح بفطر بوتريتس سينماريا، بينما كان الإنزيمات C_x لها تأثير فى إصابة ثمار التفاح بفطر بنيسيليوم اكسبانسم، وقد أدت الأملاح المستخدمة فى مقاومة أعفان ثمار التفاح أثناء التخزين المبرد إلى تثبيط نشاط الإنزيمات المحللة لجدر الخلايا (PG, PME, C_x)، وأثبت ملحى ثانى كبريتيت بوتاسيوم وكلوريد كالسيوم بتركيز ٢٠٠ جزء فى المليون أنهما أكثر الأملاح فعالية فى مقاومة أعفان ثمار التفاح أثناء التخزين المبرد على درجات حرارة صفر – ١°م لمدة شهرين.

ووجد أن فطر بنيسيليوم اكسبانسم ينتج باتيولين (سم فطرى) فى بيئة تشابكس السائلة بتركيز ١٠٥,٩٣ ميكروجرام سم فطرى/لتر بيئة عند التحضين على درجة حرارة ٢٥°م لمدة أسبوعين، وقد أدى استخدام الأملاح إلى تثبيط إنتاج الباتيولين، فيما عدا ملحى هيبوكلوريت صوديوم وكبريتات أمونيوم، وثبط ملح ثانى كبريتيت بوتاسيوم إنتاج الباتيولين كلية بينما أنتج فطر بنيسيليوم اكسبانسم كمية قليلة من الباتيولين (٣,١٢٥ ميكروجرام سم فطرى/كيلو جرام) فى ثمار التفاح المعاملة بملحى كلوريد كالسيوم وكبريتات ماغنيسيوم.

وبصفة عامة فإنه يمكن استنتاج أن معاملة ثمار التفاح بعد الحصاد بمحلول ملحى ثانى كبريتيت بوتاسيوم أو كلوريد كالسيوم بتركيز ٢٠٠ جزء فى المليون كانت فعالة فى مقاومة أعفان الثمار المتسببة عن فطرى بوتريتس سينماريا وبنيسيليوم اكسبانسم، كما أدت إلى تثبيط إنتاج السم الفطرى باتيولين وفى نفس الوقت حافظت على جودة ثمار التفاح شاملة الصلابة والمواد الصلبة الذائبة الكلية والحموضة ونسبة المواد الصلبة الذائبة الكلية إلى الحموضة أثناء التخزين المبرد على صفر – ١°م لمدة شهرين.