Alternative Strategy for Controlling the Postharvest Pepper Gray Mould Caused by *Botrytis cinerea* W.I. Shaban

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The gray mould disease caused by *Botrytis cinerea* Pers., is one of the most postharvest economically important diseases of many plants all over the world. Potassium carbonate and potassium bicarbonate were tested to show their antifungal activities against the mycelia growth of the pathogen in vitro and in vivo on the postharvest gray mould on pepper fruits. Tested potassium salts, i.e. K₂CO₃ and KHCO₃, reduced the growth of *B. cinerea* to 38.5 and 49.8%, respectively, after 7 days compared with the check treatment. In addition, among the tested materials for controlling pepper gray mould, carbonate and bicarbonate at concentrations of 1 and 2%, significantly reduced fruit rot severity, compared with Rovral WG 50% at 0.9g/l. Treating the undamaged pepper fruits with bicarbonate significantly reduced the gray mould infection. Also, submerging pepper fruits in the two tested salt solutions was more effective than spraying them. As for the best storage temperature, storing pepper fruits at 3°C followed by 13°C significantly decreased disease severity in all treatments. Also, changes in fruit weights as a result of infection under the influence of different storage temperatures were investigated. Treating pepper fruits with either carbonate or bicarbonate salt at 2%, led to enhance their resistance to gray mould disease and also decreased the loss in fruits weight and increased its shelf-life compared to check treatment.

Keywords: Botrytis cinerea, gray mould, pepper and potassium salts.

Pepper or paprika (Capsicum annuum L.) is a group tracking type pepper variety of annuals Solanaceae and comes on the colour green, yellow, red and orange. Gray mould caused by the fungus Botrytis cinerea Pers. is the most important postharvest disease of fruits and vegetables as pepper. Gray mould appears as tan or brown water soaked lesion that becomes greyish or dried-out on fruits. The fungus produces abundant white surface mycelia, which spread from infected to healthy plant, on pepper, the infection appears during storage but it starts already in the field. The infected fruits are covered with fine white-gray or tan mould (Black, 2003). Postharvest gray mould is usually controlled by initial sulphur dioxide fumigation, followed by weekly fumigations during cold storage (Smilanick et al., 1990). Regarding biocontrol, several fungi, yeasts and bacteria have been found to be effective in controlling Botrytis diseases due to enzymes and antibiotics they produced (Vagelas et al., 2009). Alternative safe, effective and economical strategies to control gray mould are needed. Sodium carbonate and bicarbonate, potassium carbonate and bicarbonate and ammonium bicarbonate are common food additives for taste (Lindsay, 1985), pH control, leavening, texture modification,

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spoilage control, and they inhibit different plant pathogens (Palmer *et al.*, 1997). Carbonates and bicarbonate salts also successfully controlled powdery mildew on cucumbers (Ziv and Zitter, 1992). Also, sodium bicarbonate controlled the postharvest green mould, caused by *Penicillium digitatum* on citrus fruits (Smilanick *et al.*, 1999).

The inhibitory activity of carbonate or bicarbonate solutions against several microorganisms including *P. digitatum* is low and generally fungistatic. Therefore, it is probable that a residue of carbonate or bicarbonate must remain on the fruit, or at least within the wound infection courts occupied by this pathogen, to control green mould (Miyasaki *et al.*, 1987).

Sodium and potassium salt solutions were applied in different concentrations to wounded pepper fruits prior to inoculation with *Colletotrichum capsici*, 39% to 82% of smaller lesions were observed when compared to the check treatment. From the results, it is evident that both sodium and potassium salts have ability to control the anthracnose on pepper fruits (Vagelas *et al.*, 2009). The addition of surfactants improved effectiveness of sodium bicarbonate against green mould on citrus (Homma *et al.*, 1981). Pre-harvest application of 2% potassium bicarbonate on bell peppers significantly reduced postharvest gray mould caused by *B. cinerea*, while concentration of 3% was phytotoxic, causing shrivelling, weight loss and increased gray mould incidence (Fallik *et al.*, 1997).

In some instances, salt (potassium bicarbonate) recommended against diseases of a wide range of fruits and vegetable crops, has commercially been available in Switzerland (Milling *et al.*, 2012). Although several studies have been conducted in which these salts were applied in their purest form, few reports regarding testing of commercial products exist (Youssef *et al.*, 2014).

The present study was designed to: (i) evaluate the *in vitro* ability of potassium bicarbonate and carbonate salts to inhibit the growth of *B. cinerea*, (ii) assess their effectiveness against postharvest pepper decay during cold storage and shelf-life and (iii) to compare the efficacy of these solutions alone with fungicides.

Materials and Methods

Source of B. cinerea and spore suspension preparation:

The pathogen *B. cinerea* was isolated from diseased pepper fruits and maintained on potato dextrose agar (PDA) under aseptic conditions. Spore suspension of *B. cinerea* was prepared by the placing disk (5-mm-diam.) in the centre of the Petri plates and then incubating at $25\pm0.5^{\circ}$ C for 15 days. Conidia were harvested by adding 10 ml of sterilized distilled water, containing 0.05% (v/v) Triton X-100, to culture plates grown at $25\pm0.5^{\circ}$ C (2-week-old). Then the liquid was vortexed gently to dislodge the spores with a sterile glass rod and then the spore suspension was filtered through four layers of sterile cheese cloth to remove mycelial fragments. The required concentration of 10^{6} spore/ml was prepared by diluting the suspension after counting them with the aid of a Haemocytometer. The suspension was diluted with water to an absorbance of 0.25 at 425nm as determined by a Spectrophotometer. This density contains approximately 1×10^{6} spore/ml (Mlikota and Smilanick, 2001).

In vitro effect of potassium carbonate and bicarbonate on B. cinerea growth:

In this trial, the tested potassium carbonate or bicarbonate (Sigma Chemical Co., St. Louis, MO) solutions were added to PDA medium at rate of 0, 0.2, 0.5, 1 or 2%. Then *B. cinerea* disks (5-mm-diam.) were transferred into the centre of PDA plates. Three replicates were used for each treatment.

In vitro effect of five fungicides on B. cinerea growth:

In this trial, five fungicides, *i.e.* Ipromise 40% SC, Swich 62.5%, Ranger 50% SC, Luna Tranquility 50% SC and Rovral 50% WP, were tested *in vitro* for their efficacy against *B. cinerea*. Tested fungicides were prepared in distilled sterilized water then added to the melted sterilized PDA culture medium at 45°C in order to obtain the desired concentrations, *i.e.* 100, 250, 500 and 1000 ppm, before inoculation as abovementioned with the tested fungus. Fungicide free medium was used as control (check) treatment. Three replicates were used for each treatment. Colony diameter was measured and colony growth inhibition (%) was calculated 7 days after incubation at $25\pm0.5^{\circ}$ C. Mycelial growth inhibition (%) at each concentration was calculated by the following formula:

P= [C-T] /C x 100

Whereas: (P) is mycelial growth inhibition, (C) is the colony diameter in check treatment and (T) is the treated colony diameter (Pârvu *et al.*, 2008).

In vivo effects of potassium carbonate and bicarbonate salts on pepper gray mould infection:

Freshly harvested pepper fruits were transferred to Plant Pathol. Lab., Fac. Agric., Suez Canal Univ. Pepper fruits were surface sterilised by 2% sodium hypochlorite solution for 1 min. Two inoculation methods were employed with *B. cinerea* spore suspension (10^6 spores/ml), (1) Each pepper fruit was artificially wounded by piercing the tested fruits with sharp cork borer (7-mm-diam.) and the epidermal disk was removed with a sterile scalpel then the spore suspension was injected into the fruit; and (2) the prepared spore suspension was applied on the intact fruit surfaces, then the treated fruits were left to dry for 45 min. after inoculation.

Effect of spraying and immersion methods:

The inoculated pepper fruits were treated with potassium carbonate or bicarbonate as: (1) spraying until run-off and (2) immersion the fruits in the solution for 30 sec. Also, the inoculated pepper fruits were treated with potassium carbonate or bicarbonate with addition of Triton X-100 at the rate of 0.1% v/v as surfactant. Pepper fruits were placed post treating inside plastic boxes humidified with paper tissue soaked with sterile water on the bottom of each box.

Later on, the treated fruits with potassium salt solutions with two concentrations, *i.e.* 1% and 2%, as well as Rovral fungicide at 0.9 g/l, in addition to check treatment as dipped fruits in sterile deionised water were incubated at $25\pm0.5^{\circ}$ C for 15 days. After developing the gray mould infection on treated fruits, the infected fruits were counted and the percentage of disease incidence was calculated.

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Effect of storage temperature degrees:

In separate trial, the effect of different temperature degrees on gray mould development was studied. In this respect, wounded pepper fruits were immersed in the previously prepared solutions of potassium salts and Rovral fungicides in addition to check treatment before storage at three different temperature degrees, *i.e.* 3, 13 and $25\pm0.5^{\circ}$ C for 15 days. Then the fruits that developed gray mould were counted and the disease incidence percentages were calculated. This experiment was conducted twice with three replicates of each treatment and twelve fruits per replicate were used (Ajith and Lakshmidevi, 2011).

Disease severity of gray mould was scored in four classes, *i.e.* 1 = no rind blemishes; 2 = slight blemishes present; 3 = moderate blemishes present and 4 = severe rind injury. Fruit with rind injuries associated with classes 3 and 4 were of sufficient significance that the fruit could not normally be sold commercially without discount. The experiment was done twice with each treatment. According to Reddy *et al.* (1997), disease control with different tested materials was estimated and measured. All fruits were kept in closed plastic boxes. Equal numbers of healthy fruits, treated with either sterilized distilled water or fungus spore suspension were kept as check.

Effect of tested treatments on fruits weight losses:

Five infected and/or healthy fruits, chosen from each potassium carbonate or bicarbonate treatment were examined for their weight loss after storage at 3, 13 and $25\pm0.5^{\circ}$ C for 15 days when the percentages were calculated using the following formula:

Fruit weight loss (%) = [(Initial weight - final weight) / Initial weight] \times 100

Statistical analysis:

All experiments were set up in a complete randomized block design. Analysis of variance (ANOVA) for unequal sample sizes and means was separated by least significant differences (LSD) at p 0.05 as described by Song and Keane (2006).

Results

Effect of potassium bicarbonate and carbonate on the in vitro growth of B. cinerea:

This study was carried out to evaluate the *in vitro* inhibitory action of potassium carbonate (K_2CO_3) and potassium bicarbonate (KHCO₃) salts against *B. cinerea* at 0, 0.5, 1 and 2% concentrations. Potassium carbonate and bicarbonate strongly reduced mycelial growth of *B. cinerea* at the higher concentration. After seven days, moderate inhibitory effect was observed with the higher concentrations of tested salts. KHCO₃ at 1% and 2% significantly inhibited the growth of *B. cinerea* by 16.6% and 38.5%, respectively, compared with the check treatment. Also, K₂CO₃ at 1% and 2% concentrations inhibited the growth of *B. cinerea* with 13.7% and 49.8%, respectively. On the other hand, no inhibition was observed at the lower concentrations (0.2% and 0.5%) with both tested salts (Table 1).

	Tested salt concentration							
Treatment	0.2%		0.5%		1%		2%	
	Lg*	R**	Lg	R	Lg	R	Lg	R
Potassium carbonate	90.0	0.0	90.0	0.0	77.6	13.7	45.1	49.8
Potassium bicarbonate	90.0	0.0	90.0	0.0	75.0	16.6	55.3	38.5
Check	90.0	0.0	90.0	0.0	90.0	0.0	90.0	0.0
L.S.D at 5%	0.0		0.0		4.2		2.9	

Table 1. Effect of potassium carbonate and bicarbonate salts on the *in vitro* growth of *B. cinerea*

* Lg = Linear growth (mm).

**R = Growth reduction (%).

Effect of different fungicide concentrations on the in vitro growth of B. cinerea:

Data presented in Table (2) show that all tested fungicides had the ability to inhibit, with different degrees, the growth of *B. cinerea*. Also, among the tested fungicides, Rovral, Luna Tranquility and Ranger at low concentration (100 ppm) completely inhibited the growth of *B. cinerea* with superiority of them in this respect. Meanwhile, Swich exhibited moderate effect at 100 and 250 ppm. At the same time, they inhibited completely the growth of *B. cinerea* at 500 and 1000 ppm. In the contrary, Ipromise fungicide couldn't be able to completely inhibit the growth of *B. cinerea* at all tested concentrations.

Table 2. Radial growth of B. cinerea at different fungicide concentrations

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	Fungicide concentration (ppm)							
Tested fungicide	100		2	50	500		1000	
_	Lg*	R	Lg	R	Lg	R	Lg	R
Ipromise	40.6	54.8	30.5	66.1	11.3	87.4	7.8	91.3
Swich	40.5	55.0	26.3	70.7	0.0	100.0	0.0	100.0
Ranger	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
Luna Tranquility	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
Rovral	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
Check	90.0		90.0		90.0		90.0	
L.S.D. at 5%	1.36		1.85		2.6		1.2	

* As described in footnote of Table (1).

Effects of tested potassium salts on pepper gray mould infection:

Effect of potassium salts with different inoculation methods of pepper fruits:

Data illustrated in Fig. (1) indicate that treating the inoculated unwounded pepper fruits with potassium carbonate or bicarbonate at 2% followed by 1% significantly reduced the gray mould infection caused by *B. cinerea*. Meanwhile, treating the inoculated wounded pepper fruits with bicarbonate or carbonate exhibited moderate fruits protection against *B. cinerea* infection at both tested concentrations compared with the tested fungicide and the check treatment.

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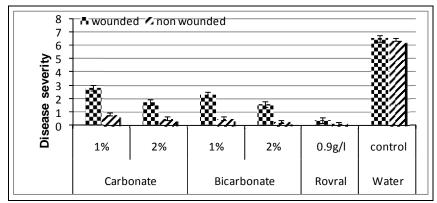


Fig. 1. Disease severity of gray mould on inoculated wounded or unwounded pepper fruits after application with tested materials followed by storage for 15 days at 25°C.

Effect of spraying or immersion methods of potassium salts:

Submerging the pepper fruits in the selected solutions of potassium salts was effective more than spraying the pepper fruits with the same solutions (Fig. 2). The addition of the surfactant Triton X-100 (0.1% v/v) significantly (p=0.05) improved the efficacy of tested materials (bicarbonate and carbonate) when the fruits were immersed or sprayed with the tested solutions. Potassium bicarbonate at 2% with addition of the surfactant was superior to potassium carbonate with the same addition and both were superior to the check treatment. On the other hand, using the tested salts as spraying or sub application without any surfactant to the wounds was less effective than those applied by immersing of the fruits.

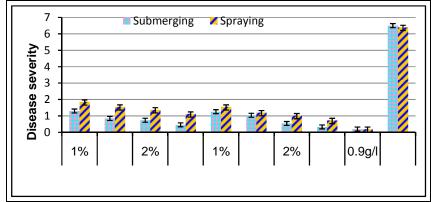


Fig. 2. Disease severity of gray mould on inoculated pepper fruits after spraying or submerging application with tested materials followed by storage for 15 days at 25°C.

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Data presented in Table (3) show that reduction in gray mould incidence was observed with lower disease severity (0.13%) when pepper fruits treated with potassium carbonate at 2% and kept at 3°C. In addition, no significant differences were recorded between carbonate at 2% concentration and Rovral treatment. Potassium bicarbonate at 1% was not effective in controlling the gray mould infection comparing with check treatment. At the same time, keeping the treated pepper fruits at 3°C and 13°C was showed significant results of disease reduction percentage with all treatments compared with the check treatment. No significant differences were detected between carbonate and bicarbonate salts at 2% on disease reduction at all tested temperatures storage degree. Furthermore, treating pepper fruits with the tested salts or Rovral and keeping them at 25°C exhibited that bicarbonate carbonate salts and Rovral at tested concentrations were effective in reducing disease severity on treated fruits more than the check treatment.

Treatment	Disease severity (%)					
Treatment	25°C	13°C	3°C			
Carbonate 1%	0.83	0.56	0.33			
Carbonate 2%	0.56	0.36	0.13			
Bicarbonate 1%	0.70	0.36	0.66			
Bicarbonate 2%	0.43	0.33	0.26			
Rovral at 0.9 g/l	0.16	0.20	0.10			
Check	6.00	1.40	0.40			
L.S.D at 5%	0.18	0.13	0.13			

Table 3. Effect of potassium carbonate and bicarbonate salts in controlling gray mould compared with Rovral fungicide at 3, 13 and 25°C storage temperature.

Effect of tested potassium salts on weight loss of stored pepper fruits for 15 days at different temperature degrees:

Data illustrated in Figs. (3 and 4) indicate that treating pepper fruits with potassium carbonate or bicarbonate in addition to Rovral on healthy or infected pepper fruits before storing at three different temperature degrees (3, 13 and 25°C) for 15 days affected clearly the fruit weight loss in addition mould control. In this respect, stored healthy pepper fruits, treated with each of tested potassium salts with both concentrations as well as Rovral fungicide at 3°C exhibited low weight losses followed by fruits stored at 13°C. Meanwhile, fruits stored at 25°C with the same treatments exhibited higher weight losses comparing with check treatment.

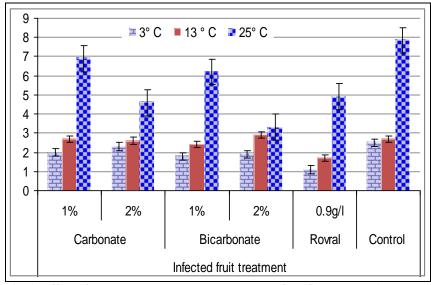


Fig. 3. Effect of three storage temperature degrees for 15 days on weight losses of infected pepper fruits.

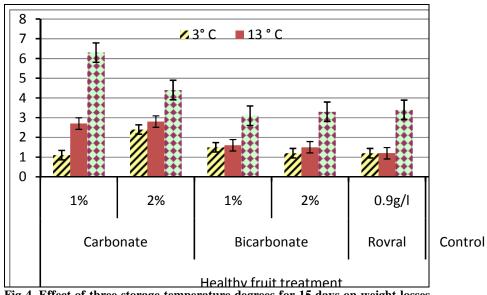


Fig 4. Effect of three storage temperature degrees for 15 days on weight losses of healthy pepper fruits.

Potassium carbonate and bicarbonate are widely used in the food industry and have antifungal activity in controlling several plant fungal diseases (Ziv and Zitter, 1992). In the present study, results indicate that potassium carbonate and bicarbonate at concentrations 1% and 2% had inhibitory effect on *Botrytis cinerea* growth. Obtained data showed that, tested concentrations of KHCO₃ or K₂CO₃ produced gradual increases of cultured medium pH (from 7.0 to 8.0) compared to the check (pH=6.5). Potassium salts were more effective at concentration of 2% than that of 1% in reducing the *in vitro* growth of *Botrytis cinerea*.

In practical tests to control gray mould on pepper fruits, bicarbonate was superior and consistently effective than carbonate. The observed antifungal effect of KHCO₃ on the tested fungus is in accordance with Bombelli and Wright (2006) who found that bicarbonate inhibited the *in vitro* growth of *B. cinerea*. In this regard, Palmer *et al.* (1997) found that carbonates and bicarbonates inhibited the formation and germination of sclerotia formed by *Sclerotium rolfsii* and *B. cinerea*. Also, Wilson (1999) stated that carbonates and bicarbonates of sodium and potassium are effective chemical compounds against *B. cinerea* the causal agent of postharvest gray mould disease on grapes. The inhibitory effects of bicarbonates on the formation and/or germination of spores have been demonstrated for fungal plant pathogens such as *Colletotrichum gloeosporioides* and *Penicillium* spp. (Korsten *et al.*, 2000 and Gamagae *et al.*, 2003).

In the present study, potassium bicarbonate had the most toxic affect on the mycelial growth of *B. cinerea*, followed by potassium carbonate. These results are in harmony with those reported by Mills *et al.* (2004) who mentioned that mycelial growth and spore germination of *Alternaria alternata*, *B. cinerea* and *Verticillium dahlia* strongly inhibited by applying sodium metabisulfite and propylparaben. Moreover, in a previous study, Mecteau *et al.* (2002) stated that the pH values of organic and inorganic salts have a minor role in their toxic effects. On the contrary, Arslan *et al.* (2009) found that potassium carbonate recorded greater fungicidal activity against *Fusarium oxysporum* f.sp. *melonis* than potassium bicarbonate. They also noted that mycelial growth of the tested fungus was totally inhibited by ammonium bicarbonate, but not by sodium carbonate or sodium bicarbonate.

Treating the wounded inoculated pepper fruits with bicarbonate or carbonate at both tested concentrations caused moderate fruit protection against *B. cinerea* infection when compared to either tested fungicide or check treatment. These results indicated also that effect of bicarbonate and carbonate was weak in controlling gray mould infection when placing the tested spore suspension inside wounds. Although, in case of uninoculated wounded pepper fruits, the wounds could become contaminated during subsequent handling. In this regard, De Kock and Holz (1994) stated that because berries become infected primarily during harvest, packing operations and storage, the necessity for reducing *B. cinerea* inoculum on harvested grapes should be emphasized. Good sanitation during the harvest and packing operation to minimize contamination of these wounds is necessary. Consequently, applying these treatments before fruit storing or marketing will be more effective in

minimizing the fungal inoculum on the fruits that might be wounded during harvesting and packing. In the same trend, Smilanick et al. (1999) reported that treating the wounded citrus fruits with sodium bicarbonate or carbonate solutions, after inoculating with spores of P. digitatum, was very effective in preventing postharvest green mould. In the same trend, Atia and Esh (2005) found that wounded tomato and pepper fruits were most susceptible to A. alternata fruit rots than unwounded fruits. According to presented results in this research, applying bicarbonate salt on wounded pepper inoculated with the tested pathogen was more effective in controlling gray mould infection than that of carbonate salt. In addition, spraying the salt to the wounds, without any surfactant, was less effective than that of fruits immersing treatment. These findings are in harmony with those reported by Ogawa et al. (1990) who found that B. cinerea spores placed into surface injuries of tomato fruits were not inactivated by chlorine or ozone at doses that killed free spores rapidly. Similarly, Spotts and Peters (1980) reported that decay of inoculated wounded pears by P. expansum, B. cinerea or Mucor piriformis was not affected by chlorine.

Since alternatives to chemical control do not possess generally a broad spectrum of activity and are not effective as fungicides, a combination of alternative methods could be more effective and consistent than one alternative alone. In this regard, three storage temperatures, *i.e.* 3, 13 and 25°C, were tested. Room temperature (25°C) normally favoured the growth of the pathogens (Snowdon, 1990), whereas 3°C and 13°C are of the temperatures for commercial fruit storage (Spadaro *et al.*, 2002).

Also, in this concern, obtained results showed that applying potassium carbonate and bicarbonate gave a positive effect on shelf-life and also reduced fruit decay. Moreover, it could be observed that coating of stored fruits, which treated with potassium salts, reduced the loss in their weights and keeping them health for long time. Previous reports indicated that postharvest treatments reduced fruit decay. In the present study, the reduction in gray mould incidence was observed with lower disease severity when pepper fruits treated with potassium carbonate at 2% and kept at 3°C. Bicarbonate salt is inexpensive, readily available and can be used with a minimal risk of injured fruits. The inhibitory activity of sodium bicarbonate depends on the presence of salt residues within the wound infection sites occupied by the fungus and on interactions between this residue and constituents of the peel. In this regard, oranges dipped for 3 min. at room temperature in water with 2-4% sodium bicarbonate reduced decay caused by P. italicum by more than 50% (Palou et al., 2001). In the contrary, sodium bicarbonate was applied for controlling B. cinerea on apple at 1%, but it was ineffective (Spadaro et al., 2004). In the same trend, Smilanick et al. (1999) stated that a concentration of 3% sodium bicarbonate was chosen. A disadvantage of sodium bicarbonate is that heating the solution will cause carbon dioxide evolution with a concomitant increase in solution pH, but the addition of hypochlorite should permit the heating of the salt solution.

In the present study, low weight losses were observed when keeping the pepper fruits at 3°C for 15 days either healthy or infected fruits. The maximum weight loss was recorded in infected pepper fruit treated with 1% potassium carbonate, followed

by infected pepper fruit treated with 1% potassium bicarbonate, so the difference between tested salts concentrations was unsignificant. The minimum weight loss was recorded in healthy treated fruits stored at 3°C. The percent weight loss increased significantly with incremental increase in storage duration. These results are in accordance with those of Ghafir *et al.* (2009) stated that the moisture and subsequent weight loss in fruits increased linearly with increase in storage duration due to water loss and respiration.

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استيراتيجية بديلة لمكافحة العفن الرمادى على الفلفل Botrytis cinerea

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ڍُ Botrytis cinerea Pers. هم مراض ما بعد الحصاد التي تصيب ثمار الخضر والفاكهة على . تم اختبار النشاط المثبط لكل من كربونات وبيكربونات البوتاسيوم كمضادات للفطريات ضد نمو الفطر الممرض في المعمل وعلى الذ . ظهرت النتائج ن لكربونات وبيكربونات البوتاسيوم قدرة على تثبيط يام بنسبة . . % . كما خفضت الكربونات والبيكربونات بشكل معنوى عند تركي % % بجانب مبيد الروفرال عند تركيز . / غير ظهر ملح كربونات البوتاسيوم انخفاضاً معنويا في شدة حدوث العفن الرمادي عند المعاملة على الثمار السليمة مٰة . ن جهة أخرى فإ ات وبيكربونات البوتاسيوم مضافا إليها مركب الترايتون X ملاح كربونات وبيكربونات البوتاسيوم تحت تاثير درجة حرارة التخزين المختلفة (, , °). ن التخزين عند °م يليها . من ناحية أ يضاً ثبر المعاملات المختلفة على وزن الثمار التخزين. وقد أظهرت النتائج أن معاملة ثمار الفلفل بكربونات وبيكربونات ، أيضا أدت المعاملات وتاسيوم عند تركيز % غير لي تقليل انخ حرارة تخزين الثمار.