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The Efficacy of Preharvest Salts Treatment on Incidence of Snap Bean Pod Rots during Storage

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Seven salts, *i.e.* potassium metabisulfite, calcium nitrate, potassium carbonate, potassium sulfate, calcium sulfate, calcium chloride and potassium thiosulfate, were tested at concentrations of 0.2% and 0.4% as a preharvest spray on snap bean to protect pods during cold storage, transportation and marketing to control *Botrytis cinerea* and *Sclerotinia sclerotiorum* during cold storage at 10°C and 90-95% RH for 30 days in seasons 2003 and 2004. Calcium nitrate was the most effective treatment to control pod rots on naturally infected snap bean pods at concentrations of 0.2% and 0.4%, the efficacy of high concentrations of calcium nitrate exceeded 70%. Potassium carbonate and calcium chloride at high concentrations achieved efficacy more than 50% to control decay on naturally infected pods, which is also considered very applicable as effective and safe treatments. Also, calcium nitrate and potassium carbonate were the most effective treatments in decreasing snap bean pod rots caused by *Sclerotinia sclerotiorum* at concentrations of 0.2%, the efficacy of low concentrations of calcium nitrate and potassium carbonate exceeded 90%, 60%, respectively, during the two successive seasons. On the other hand, calcium nitrate and calcium chloride were the best effective treatments in controlling snap bean pod rots caused by *Botrytis cinerea* at concentrations of 0.2%, the efficacy of low concentrations of calcium nitrate and calcium chloride exceeded 90%, 77% respectively during the two successive seasons. Generally, spraying snap bean plants (30, 20, 10 days before harvesting) with calcium nitrate, calcium chloride and potassium carbonate at a concentration of 0.4% effectively controlled snap bean pod rots (gray and white molds) caused by *Botrytis cinerea* and *Sclerotinia sclerotiorum*, respectively during cold storage at 10°C and 90-95% RH for 30 days.

Key words: *Botrytis cinerea*, cold storage control, gray mold, salts, snap bean, white mold and *Sclerotinia sclerotiorum*.

Snap bean (*Phaseolus vulgaris* L.) is one of the most popular vegetable crops in Egypt grown for local human consumption and exportation. Postharvest diseases of Snap bean pods caused by fungi constitute a serious problem. Such pathogenic fungi in the field attack the fruits, during harvest, storage, and marketing. The most serious postharvest diseases were gray mould caused by *Botrytis cinerea* and white mould caused by *Sclerotinia sclerotiorum* as reported by (Ragab, 1980; Wong *et al.*, 1980 and Soltan, 1993). Many attempts were made to protect snap bean pods against certain post-harvest pathogens during marketing and storage. Salts were used to suppress fungal decay occurrence and development in fruits and vegetables. Chlorine solutions, calcium hypochlorite and sodium hypochlorite, were used at appropriate concentrations to control postharvest diseases of fruits and vegetables (Pordesimo, 1984, Bartz, 1988 and Boyette *et al.*, 1991). Sodium metabisulphite and salicylic acid were the most effective treatments against *Cladosporium sphaerospermum* and *Fusarium moniliforme* [*Gibberella fujikuroi*] on orange; *Pestalotia* [*Pestalotiopsis*] *versicolor* and *Rhizoctonia solani* on potato; and *Rhizopus stolonifer* on apple fruits (Gaur and Chenulu, 1982). Among tested fungicides, sodium metabisulphite gave equal control to benzimidazole fungicides on *Aspergillus niger* in inoculated banana fruits (Ram and Vir, 1984). Calcium chloride was the most effective salt to control of *Botrytis cinerea* on Golden delicious apple (McLaughlin *et al.*, 1984). Rushid (2001) found that potassium chloride revealed highest effectiveness against *B. cinerea* infection on artificially infected Flame Seedless and Thompson Seedless grapes. Conway *et al.* (1994) found that the natural resistance of plant tissues was enhanced by application of calcium. On the other hand, Cheour *et al.* (1991) stated that calcium content of the fruit appeared to depend mainly on the ability of the plant to accumulate and distribute Ca^{++}. This finding was realized in current work by increasing salt effectiveness by increasing salt concentration during preharvest application. Kaile *et al.* (1991) suggested that *B. cinerea* had the potential to release a high level of Ca^{2+} normally bound to pectic substances in the middle lamella during host tissue maceration by secreted pectolytic enzymes and that these ions may be implicated in host cytotoxicity during necrotrophic pathogenesis. Generally, potassium chloride proved to be the most effective salt to control *B. cinerea* infection. Sugar *et al.* (2003) found that calcium chloride was effective against gray mold on pear fruits.

The objective of this study was evaluating the use of salts to suppress gray and white molds on snap bean pods during cold storage.

Materials and Methods

Source of fungal pathogens:

Pathogenic isolates of *Botrytis cinerea* and *Sclerotinia sclerotiorum* isolated from decayed snap bean pods were used for all control experiments.

Effect of salts:

A- In vitro experiments:

The effect of certain salts at different concentrations on mycelial growth of *B. cinerea* and *S. sclerotiorum* was *in vitro* tested. The tested salts were potassium metabisulfite, calcium nitrate, potassium carbonate, potassium sulfate, calcium sulfate, calcium chloride and potassium thiosulfate. Each salt was amended to PDA medium at a concentration of 0.05, 0.1 and 0.15%. Treated or untreated medium with salts was poured into three plates per each treatment. After medium solidification, plates were inoculated with 3-mm discs of 7-day-old culture of each fungus and incubated at 25± 2°C for 7 days where the linear growth was measured. The percentage of reduction in colony diameter was calculated as follows:

$$\text{Reduction (\%) of colony diameter} = (d_c - d_t) \times 100 / d_c$$

Whereas: d_c = average diameter of linear growth in control set, and
d_t = average diameter of linear growth in treatment set.

B- Field experiments:

Under field conditions for the two successive seasons 2003 and 2004, the effect of salts spraying on snap bean (pre harvest) to protect snap bean pods during harvesting and storage was studied. Seven salts, *i.e.* potassium metabisulfite, calcium nitrate, potassium carbonate, potassium sulfate, calcium sulfate, calcium chloride and potassium thiosulfate at rate the of 200 and 400g/100 l. were tested for their ability to control snap bean pod rots under field conditions. This experiment was carried out at El-Kassasin in Ismailiya governorate. Plots, each consisting of three rows were used as an experimental unit. Snap bean plants (cv. Paulista) were sprayed with the three sprays, the first at bloom stage, the second after ten days from the first spray and third after ten days from the second spray. Plots, containing snap bean plants, receiving no salts were used as control. After harvest, the pods were sprayed separately with spore or hyphal suspension of *B. cinerea* or *S. sclerotiorum* at concentration of 4×10^6/ml. Each treatment contained three replicates (one kilogram pods for each replicate). Then, packed in polyethylene bags, inoculated and non-inoculated (natural infection) snap bean pods incubated at $10^{O}C$ and 90-95% RH for 30 days. Severity of infection was recorded as loss of weight according to the equation suggested by Spalding and Reeder (1974):

$$\text{Disease severity (\%)} = \frac{\text{Weight of diseased pods}}{\text{Total weight of treatment}} \times 100$$

Statistical design and analysis:

A complete randomized design was used for control of snap bean pod rots during cold storage. Each treatment contained three replicates, each one kilogram pods. Obtained data were analyzed according to Gomez and Gomez (1983). 'F' test and Fisher's L.S.D. using Excel Microsoft Software obtained the differences among the treatments.

Results and Discussion

A- In vitro experiments:

Effect of several salts used at different concentrations on reduction of colony diameter of *B. cinerea* and *S. sclerotiorum.* Data in Table (1) indicate that all treatments were significantly effective against *B. cinerea* and *S. sclerotiorum.* Potassium metabisulfite and potassium carbonate were the best effective on reduction of colony diameter of *B. cinerea* and *S. sclerotiorum* at concentration 0.15% followed by calcium chloride. On the other hand, calcium nitrate, potassium sulfate and calcium sulfate were the less effective in reduction of colony diameter of *B. cinerea* and *S. sclerotiorum.* But potassium thiosulfate was the less effective compared with calcium nitrate.

Table 1: Effect of different concentrations of certain salts on reduction of colony diameter of *B. cinerea* and *S. sclerotiorum* on PDA medium after incubation at 25°C for 7 days

Treatment	Reduction (%) in colony diameter					
	B. cinerea			*S. sclerotiorum*		
	Concentration (%)					
	0.05	0.1	0.15	0.05	0.1	0.15
Potassium metabisulfite	83	100	100	77.8	100	100
Calcium nitrate	21	27	33	0.0	0.0	0.0
Potassium carbonate	20	41	100	20	100	100
Potassium sulfate	11	22	33	0.0	33	44
Calcium sulfate	44	44	44	0.0	0.0	44
Calcium chloride	24	35.5	55.5	0.0	0.0	38.9
Potassium thiosulfate	22	25.5	33	22	33	33
LSD at 0.05	3.3	2.8	1.5	2.5	1.03	1.5

B- Field experiments:

Data presented in Table (2) show that all salt treatments significantly decreased decay development on naturally infected snap bean pods during cold storage (seasons 2003 and 2004). It was observed that the efficacy of most treatments was almost constant along the two season results in-spite of different infection percentages were obtained. This finding is attributed to the different percentages of infection of control pods, which were the reference to calculate the efficacy of each treatment. Calcium nitrate was the most effective treatment to control pod rots on naturally infected snap bean pods at two tested concentrations. The efficacy of high concentrations (400g/100L) of calcium nitrate exceeded 70%. Potassium carbonate and calcium sulfate at high concentrations (400g/100L) achieved efficacy more than 50% to control decay on naturally infected pods, which is also considered very applicable as effective and safe treatments. These results are similar with those obtained by El-Sheikh *et al.*(1998), Catherino *et al.* (2000) and Saber *et al.* (2003). Potassium metabisulfite showed the least effectiveness treatment against postharvest decay of snap bean pods. High efficacy of salts resulted to control fungi causing postharvest decay of snap bean pods was also recorded by many investigators on different fruits, where potassium sorbate was found to be effective against many fungi such as *Penicillium* spp. and *Geotrichum candidum* (Azzouz and Bullerman, 1982, Nelson *et al.*, 1982 and Kitagawa and Kawada, 1985).

Effect of certain salts at two concentrations on snap bean pod rots caused by *S. sclerotiorum* stored at 10°C and 90-95% R.H. for 30 days, seasons 2003 and 2004 is demonstrated in Table (3). While all tested preservatives at application rates significantly reduced snap bean pod rots caused by *S. sclerotiorum*, only some treatments showed proper control. The higher concentration of most tested salts the higher efficacy against *S. sclerotiorum* infection was obtained. The infection of untreated snap bean pods after cold storage was 90% and 100% during seasons 2003 and 2004, respectively. Calcium nitrate was the most effective treatment in

Table 2: Effect of preharvest spraying of snap bean plants with certain salts on severity of infection of natural infection snap bean pods during storage at 10°C and 90-95% RH for 30 days during seasons 2003 and 2004

Treatment	Conc. (%)	Natural infection			
		Season 2003		Season 2004	
		DS *	E	DS	E
Potassium metabisulfite	0.2	33	5.7	50	16.7
	0.4	30	14.3	46.7	20.5
Calcium nitrate	0.2	14	60	33.3	44.5
	0.4	10	71.4	16.7	72.2
Potassium carbonate	0.2	30	14.3	46.7	22.2
	0.4	12	65.7	30	50
Potassium sulfate	0.2	25	28.6	43.3	27.8
	0.4	30	14.3	50	16.7
Calcium sulfate	0.2	25	28.6	36.7	38.8
	0.4	33	5.7	53.3	11.2
Calcium chloride	0.2	17	51.4	40	33.3
	0.4	13	62.8	33.3	44.5
Potassium thiosulfate	0.2	16	54.3	40	33.3
	0.4	25	28.6	53.3	11.2
Control	-	35	-	60	-
LSD at 0.05		1.84		2.34	

* DS = Disease severity (%).
E = Treatment efficacy (%)= 1-(DS in treatment / DS in control) × 100

decreasing snap bean pod rots with efficacy ranged from 93.3% to 95.6% and from 93.3% to 95% during the two successive seasons, respectively. The most effective treatment following calcium nitrate was potassium carbonate at low concentrations with efficacy 66.7% for both during the two successive seasons, respectively. While potassium metabisulfite and potassium carbonate has completely inhibited mycelia growth of *S. sclerotiorum in vitro*, it showed the least effectiveness *in vivo*. This finding demonstrates that potassium metabisulfite can not control well established infection with *S. sclerotiorum*, but show better effectiveness when snap bean pod are subjected to the infection after treatment with salt. On the other hand, calcium nitrate was almost the least effective salt treatment *in vitro*, but showed the highest effectiveness *in vivo*. This finding indicates that calcium nitrate may play a significant role in enhancing fruit resistance to infection with *S. sclerotiorum*.

Artificial inoculation of snap bean pods with *B. cinerea* resulted in 85% and 90% disease severity during seasons 2003 and 2004, respectively, as shown in Table (4). While all tested salts at the applied rates have significantly reduced snap bean pod rots caused by *B. cinerea*, only some treatments showed proper control. Calcium nitrate and Calcium chloride were the most effective treatment in decreasing snap

Table 3: Effect of preharvest spraying of snap bean plants with certain salts on severity of infection of snap bean pods artificially infected by *S. sclerotiorum* during storage at 10°C and 90-95% RH for 30 days during seasons 2003 and 2004

Treatment	Conc. (%)	*S. sclerotiorum*			
		Season 2003		Season 2004	
		DS *	E **	DS	E
Potassium metabisulfite	0.2	78.3	13	83.3	16.7
	0.4	63.3	29.7	66.7	33.3
Calcium nitrate	0.2	6	93.3	6.7	93.3
	0.4	4	95.6	5	95
Potassium carbonate	0.2	30	66.7	33.3	66.7
	0.4	36.7	59.2	43.3	56.7
Potassium sulfate	0.2	80	11.1	86.7	13.3
	0.4	50	44.4	53.3	46.7
Calcium sulfate	0.2	70	22.2	73.3	26.7
	0.4	33.3	63	43.3	56.7
Calcium chloride	0.2	75	16.7	83.3	16.7
	0.4	81.7	9.2	86.7	13.3
Potassium thiosulfate	0.2	33.3	63	36.7	63.3
	0.4	65	27.8	70	30
Control	-	90	-	100	-
LSD at 0.05		2.41		2.48	

* DS = Disease severity (%) and E = Treatment efficacy (%).
** E = Efficacy (%). For explanation refer to footnote of Table (2).

bean pod rots with efficacy ranged from 92.1 to 88.2, 91.4 to 85.2 and 80.4 to 86.2, 77.8 to 85.2 during the two successive seasons, respectively. On the other hand, potassium carbonate, calcium sulfate, potassium thiosulfate had moderate effectiveness. Almost similar finding was obtained by Azzouz and Bullerman (1982) who mentioned that potassium sorbate at 0.3% was highly effective against four *Penicillium* species. High effectiveness of potassium sorbate against *Penicillium* was also realized by Nelson *et al.* (1982) and Kitagawa and Tani (1984). Gabler and Smilanick (2004) tested carbonate and bicarbonate salts and disinfectants to control gray mold of table grape. They found that among the bicarbonates, each applied at 500 mM, ammonium bicarbonate (ABC) was significantly more effective than sodium bicarbonate (SBC) and potassium bicarbonate (PBC). It was also superior to potassium carbonate (PC) (100 mM) and chlorine (200 µg/ml) and equal in effectiveness to sodium carbonate (SC) (100 mM) and ethanol (70% w/v).

Table 4: Effect of pre harvest spraying of snap bean plants with certain salts on severity of infection of snap bean pods artificially infected by *B. cinerea* during storage at 10°C and 90-95% RH for 30 days during seasons 2003 and 2004

Treatment	Conc. (%)	*B. cinerea*			
		Season 2003		Season 2004	
		DS *	E	DS	E
Potassium metabisulfite	0.2	66.7	21.5	71.6	20.4
	0.4	43.3	49.1	46.7	48.1
Calcium nitrate	0.2	6.7	92.1	7.7	91.4
	0.4	10	88.2	13.3	85.2
Potassium carbonate	0.2	48.3	43.2	50	44.4
	0.4	40	52.9	40	55.6
Potassium sulfate	0.2	73.3	13.8	80	11.1
	0.4	50	41.2	56.7	37
Calcium sulfate	0.2	40	52.9	46.7	48.1
	0.4	50	41.2	50	44.4
Calcium chloride	0.2	16.7	80.4	20	77.8
	0.4	11.7	86.2	13.3	85.2
Potassium thiosulfate	0.2	30	64.7	40	55.6
	0.4	50	41.2	70	22.2
Control	-	85	-	90	-
LSD at 0.05		2.16		8.1	

* DS = Disease severity (%) and E = Treatment efficacy (%). For explanation refer to footnote of Table (2).

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فعالية المعاملة بالأملاح قبل الحصاد على أعفان قرون الفاصوليا أثناء التخزين

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تصاب ثمار الفاصوليا الخضراء بالعديد من مسببات الأمراض النباتية أثناء الحصاد و التخزين والنقل والتسويق، حيث يعتبر العفن الرمادى والعفن الأبيض المتسبب عن الفطريات بوتريتس سيناريا، وسكليروتنيا سكليروتيورم من المسببات الرئيسية لاعفان الثمار بعد الحصاد ويهدف هذا البحث الى اختبار كفاءة الأملاح (بوتاسيوم ميتابيسلفيت، نترات كالسيوم، كربونات بوتاسيوم، كبريتات بوتاسيوم، كبريتات كالسيوم، كلوريد كالسيوم و ثيوكبريتات البوتاسيوم) بتركيز ٠,٢، ٠,٤% رشا قبل الحصاد على نباتات الفاصوليا لمقاومة الفطريات المتسببة فى أعفان قرون الفاصوليا بعد الحصاد وأثناء التخزين على درجة ١٠ درجة مئوية ورطوبة نسبية ٩٠-٩٥ % لمدة ٣٠ يوم فى موسمى ٢٠٠٣-٢٠٠٤ ويمكن تلخيص النتائج المتحصل عليها كما يلى:

١- وجد أن نترات كالسيوم كانت الأكثر تأثيرا فى مقاومة أعفان قرون الفاصوليا تحت ظروف العدوى الطبيعية عند تركيز ٠,٢، ٠,٤% وكانت كفاءة التركيز الأعلى أكثر من ٧٠%.

٢- التركيزات الأعلى من كربونات بوتاسيوم و كلوريد كالسيوم أدت إلى كفاءة تزيد عن ٥٠% لمقاومة أعفان قرون الفاصوليا تحت ظروف العدوى الطبيعية التى تعتبر قابلة للتطبيق كمعاملة فعالة وآمنة.

٣- أيضا نترات كالسيوم و كربونات بوتاسيوم كانتا الأكثر فعالية فى مقاومة أعفان قرون الفاصوليا المتسببة عن الحقن الصناعى بالفطر سكليروتنيا سكليرتيورم عند تركيز ٠,٢% حيث أدت إلى كفاءة زادت عن ٩٠%، ٦٠% على التوالى أثناء موسمى ٢٠٠٣، ٢٠٠٤ .

٤- نترات كالسيوم و كلوريد كالسيوم كانتا الأفضل فى مقاومة أعفان قرون الفاصوليا المتسببة عن الحقن الصناعى بالفطر بوتريتس سيناريا بتركيز ٠,٢% حيث أدت الى كفاءة تزيد عن ٩٠%، ٧٧% على التوالى أثناء موسمى ٢٠٠٣، ٢٠٠٤.

٥- عموما رش نباتات الفاصوليا قبل الحصاد بـــ ٣٠، ٢٠، ١٠ أيام بنترات الكالسيوم وكلوريد الكالسيوم بتركيز ٤ جم /لتر لمقاومة أعفان قرون الفاصوليا المتسببة عن الفطر سكليروتنيا سكليرتيورم و بوتريتس سيناريا أثناء التخزين على ١٠ °م ورطوبة نسبية ٩٠-٩٥% لمدة ٣٠ يوم، حيث كانت أفضل المعاملات فى تقليل نسبة الاصابة.