

EFFICIENCY OF SOME SALTS ON THE INCIDENCE OF GRAPE BUNCH ROT CAUSED BY *Botrytis cinerea* IN THE FIELD AND DURING STORAGE AND FRUIT CHARACTERISTICS OF FLAME SEEDLESS GRAPEVINES.

Soltan, H.H.M.¹ ; A.H. Abd Elaal² and M.E.A. Abo Rehab¹

1- Institute of Plant Pathology, Agriculture Research Center, Giza, Egypt
aborehab666@yahoo.com.

2- Hort. Dept., Fac. Agric., El-Azhar Univ., Assiut, Egypt.

ABSTRACT

The efficiency of calcium chloride, calcium nitrate, potassium metabisulfite, calcium carbonate and sodium bicarbonate was assessed by *in vivo* and *in vitro* tests against grape bunch rot in field and storage conditions by spraying salts three times, the first at bloom stage, the second after two weeks from the first spray and third after two weeks from the second spray. The investigation was carried out in a private vineyard El-Khatatba region, Cairo –Alexandria desert road during two growing seasons, 2006/2007 on Flame seedless grapevines. All tested salts were found to reduce the growth of *Botrytis cinerea* Pers. through the use of three concentrations 0.05%, 0.1% and 0.2% a complete inhibition was achieved by Potassium metabisulfite at the rate of 0.2% and by Sodium bicarbonate at the rate of 0.2%. All tested salts significantly reduced disease severity of bunch rot in field conditions, the highest percentage of reductions was shown when vines were treated by Potassium metabisulfite during seasons 2006 at the rate of 2g/L. Field applications of salts resulted in a significant reduction of botrytis storage rots. Potassium metabisulfite was the most effective treatment to control rots on naturally and artificially infected at the rate of 2g/L. in both seasons during storage at 0°C under 90-95% R.H. for 50 days. Fruit physical and chemical were positively affected by different treatment. All applied salts increased cluster weight and yield / vine except sodium bicarbonate and calcium chloride were a decrease in these parameters. Weight and berry diameters were positively affected by all applied salts except sodium bicarbonate. A similar positive effect was also observed concerning TSS, total acidity and TSS/TA ratio for grapes stored for 50 days at 0°C and 90-95% R.H. with the exception of sodium bicarbonate which reduced juice acidity and TSS /TA ratio. Loss of weight after cold storage of grapes was substantially decreased in all salts treatments. From the economic point of view, the cost of using salts is very cheap compared to use classic fungicide and also increased yield to 1404.8 and 2205.6 kg/ fed in season 2006 and 2007 respectively; in addition the fruit quality was improved.

INTRODUCTION

Grapevine (*Vitis vinifera* L.) is considered one of the most important fruit crops in Egypt as well as all over the world. In Egypt, grape is the second fruit crop preceded citrus. The cultivated areas reached 159929 feddans (138499 feddan of fruiting vines) with a production of 1275288 tons (According to the annual statistics of the Ministry of Agricultural in 2005). This number is increasing rapidly as more desert areas are being planted every year either for local market or exportation. Grapes are subjected to the infection with several pre and post harvest diseases. The Bunch rot disease is serious on grape bunches all over the world where it cause, great losses and also affects grape quality, especially during harvesting, packing, storage,

exporting and marketing. The fungus *Botrytis cinerea* is considered one of the most serious fungi that attack grape berries during their progressive stages of development especially when shipped at 0-1°C by sea for exportation or during cold storage for local market.

Use of synthetic fungicides has been the traditional option of control plant diseases but, the extensive and prolonged use of these fungicides has resulted in the development of resistance in the fungus. Furthermore the residual effects on the crop and environmental pollution are other problems associated with the use of these chemicals. Therefore, the use of pre- and post harvest chemical treatments are increasingly limited due to consumer concerns. Present focuses on the development of alternative means for controlling fungal disease in the field and during cold storage for local market that are safe to human and environment have been initiated (Rushed, 2001 and Nigro, *et al* 2006, Abo Rehab *et al* 2007, Abd Elghany *et al* 2007).

In the search for biocompatible products, which could be defined as chemicals exhibiting low mammalian and environmental toxicity, many salts have recently been tested as alternative control means (Smilanick *et al.*, 1999; Rushed, 2001; Ippolito *et al.*, 2005, Karabulut *et al.*,2005, Abd-El-Karem 2007, Nigro *et al* 2006, Abo Rehab *et al* 2007 and Abd Elghany *et al* 2007) These salts are inexpensive, easily accepted by consumers, non-toxic, with minor environmental impact at the effective concentrations, and usually used in the food industry. Potassium, Sodium and calcium salts have been shown to be effective growth inhibitors of *Botrytis cinerea* (Ricker and Punja,1991, Palmar *et al.*, 1997, Fallik *et al.*, 1997; Gabler and Smilanick,2001, Karabulat *et al.*,2001, Rushed 2001, Mills *et al* 2004, Soitan *et al* 2006 and Nigro *et al* 2006).

Pre- and post harvest application of different kinds of potassium, Sodium and calcium salts on table grapes reduced the incidence of grey mould storage rot (Ippolito *et al.*, 1997 ; Miceli *et al.*, 1999;Rushed 2001and Nigro *et al* 2006)

Different kinds of potassium and calcium salts sprayed 3 weeks before harvest on Flame Seedless and Thompson seedless grapevines showed a highly reduced infection percentage of grapes with *B. cinerea* compared to control grapes in the open field. Spraying of these salts also showed strong varied effects to control grey mold during cold storage at 0-1°C. Rushed (2001).Many researchers pointed out that using of these compounds to control diseases in vineyards was followed by improving productivity (Abo Rehab *et al* 2007; Abd Elghany *et al* 2007; Kilany *et al* 2002; shoaieb *et al* 2002; Abd El-hady 2002).

The objective of this work is to study the effect of preharvest treatment of grapevines with salts on protecting plants from bunch rot disease caused by *Botrytis cinerea* in the field conditions and during storage at 0°C for 50days and to study their influence on some bunch and berry characteristics in Flame seedless cv..

MATERIALS AND METHODS

Source of fungus pathogen:

Pathogenic isolate of *Botrytis cinerea* previously isolated from diseased grape berries with gray mold was used for all control experiments.

Effect of salts:

In vitro experiments:

The effect of certain salts at different concentrations on mycelial growth of *B. cinerea* was tested *in vitro*. The tested salts were; calcium chloride, calcium nitrate, potassium metabisulfite, calcium carbonate and sodium bicarbonate. Each salt was amended in PDA medium at a concentration of 0.05, 0.1 or 0.2%. Treated or untreated medium with salts was poured into five Petri dishes per each treatment. After medium solidification, Petri dishes were inoculated with 3-mm discs of 7-day-old culture of *B. cinerea* and incubated at $20 \pm 2^\circ\text{C}$ for seven days. Effect of salt treatments on linear growth (mm) of *B. cinerea* was measured after incubation period. The percentage of reduction in colony diameter was calculated as follows:

Reduction (%) of colony diameter = $(d_e - d_t) \times 100/d_e$

Whereas: d_e = average diameter of linear growth in control set. And

d_t = average diameter of linear growth in treatment set.

Filed experiments:

For two successive seasons 2006 / 2007, five salts, i.e. calcium chloride, calcium nitrate, potassium metabisulfite, calcium carbonate and sodium bicarbonate at rate of 1g/L. and 2g/L were tested for their ability to control grape bunch rots under field conditions. This experiment was carried out in private vineyard in El-Khatatba region, Cairo –Alexandria desert road. The double- cordon system was used in training the vines which were supported by telefon trellis system. Vines were pruned during the last week on December of 2006 / 2007 seasons leaving 60-65 buds per vine. The vines were grown at 1.5×3 meters in a sandy soil where drip irrigation system was used. Ninety nine uniform Flame seedless grapevines were chosen and devoted for achieving this experiment. All vines were adjusted to bear a cluster number of (i.e.30 clusters) perior to anthesis. The above mentioned salts were sprayed on the grapevines three times the first spray at bloom stage, the second after two weeks from the first spray and third after two weeks from the second spray. Salts were used at a concentration of 1g/L or 2g/L. five grapevines were used for each treatment. Five grapevines were left between each two treatments as a barrier. Untreated grapevines kept as a control were sprayed with water.

During the two tested seasons, the following parameters were determined.

- 1- Yield/Vine, average yield Kg/ vine were recorded at harvest time.
- 2- Physical and chemical properties of the berries:

Representative random samples of 12 clusters per each treatment (four clusters for each replicate) were picked at the harvesting time (middle of

June in both seasons) and transferred to the laboratory for the determination of following parameters:

- Average weight of cluster (g)
- Berry weight (g) as an average of 50 berries /replicate.
- Berry dimensions (length and diameter, cm) the berry shape index was calculated by dividing berry length by diameter.
- Total soluble solids % using a hand refractometer.
- Total acidity % (As gram tartaric acid /100g juice) according to the A.O.A.C. (1990).
- TSS/ Acidity ratio was calculated.

Grape bunches of all treatments were harvested at the proper degree of ripeness, packed in plastic bags inside carton boxes and stored at 0°C under 90-95% R.H. for 50 days. Other grape bunches either treated or untreated were artificially inoculated with a spore suspension of *B. cinerea* at a concentration of 4×10^6 spores/ml before packing and cold storage. Each treatment contained three replicates (one kilogram of bunches for each replicate). Infection percentage of grape bunches with *B. cinerea* was estimated at the end of cold storage.

Bunch rot disease assessment:

The bunch rot disease were evaluated on the bunch in the following scale on 15 bunches for each replication, three replicates of each treatment.

- No symptoms.
- 1-10 % infection on the bunch.
- 11-20% infection on the bunch
- Up to 20 % infection on the bunch.

The disease severity (DS) was calculated according to the following formula Disease severity (%) = $(\sum (n \times v) / N \times V) \times 100$, where, n = No. of bunches at rate, v (disease score), N = total no. of bunches investigated and V = highest disease severity.

Experimental design and statistical analysis:

From the statistical point of view the complete randomized block design was chosen and the data were statically analyzed using the Fisher's LSD according to Gomez and Gomez (1983).

RESULTS

As shown in table (1) the *in vitro* tests showed a variable effect of salts on *B. cinerea* growth. After 7 days incubation at $20 \pm 2^\circ\text{C}$ most salts reduced the colony diameter of the pathogen. A complete inhibition was achieved by Potassium metabisulfite at 0.2% and by Sodium bicarbonate at 0.2% while the lowest percentage of reduction of colony diameter was observed in treatments of calcium nitrate (35.2%) at the rate of 0.05% and Calcium chloride (38.8%) at the rate of 0.05%

From the results illustrated in (Fig.1) can be noticed that. All tested salts of calcium, sodium and potassium reduced disease severity of bunch rot disease in open field at the two rates 1g/L. and 2g/L. in both seasons. The highest percentage of reductions occurred when grapes were treated with Potassium metabisulfite during the first season (87.5%), meanwhile Calcium carbonate during 2007 seasons resulted in the highest percentage of reductions (85.5%) the lowest percentage of reductions (28.4%) was observed in Calcium chloride treatment during season 2007.

Table (1) Effect of different concentrations of certain salts on the reduction of colony diameter of *Botrytis cinerea* on PDA medium after incubation at 20+2°C for 7 days.

Treatments (salts)	Reduction of colony diameter %		
	Concentrations%		
	0.05	0.1	0.2
Calcium chloride	38.8	59.2	77.8
Calcium nitrate	35.2	42.5	53.7
Potassium metabisulfate	72.2	74.1	81.1
Calcium carbonat	77.7	96.3	100
Sodium bicarbonate	74.1	88.9	100
L.S.D at 0.05	6.70	5.10	4.52

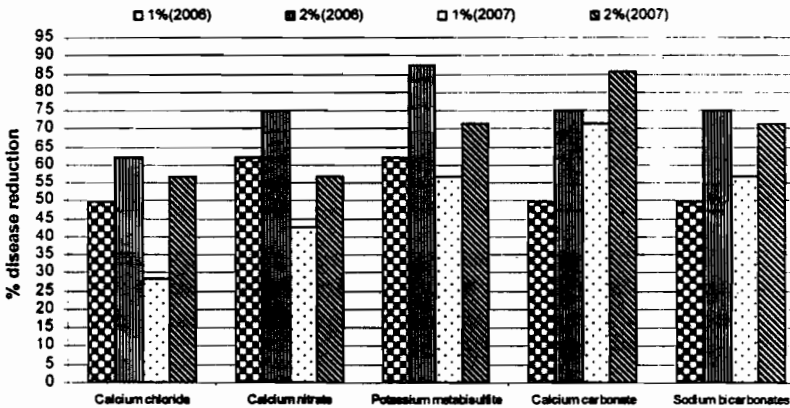


Fig (1): Effect of different salts on bunch rot disease severity caused by *Botrytis Cinerea* under natural infections in open field on Flame seedless grapevines 2006/2007 (scale % disease reduction)

Data presented in Table (2) show that all salt treatments significantly decreased decay development on naturally infected Flame seedless grapevines during cold storage (seasons 2006 and 2007). It can be noticed that Potassium metabisulfite was the most effective treatment to control berries rots on naturally infected vines at 2g/L. concentrations in both seasons (82.4% in 2006 and 83.3% in 2007) followed by calcium carbonate (80.0% in 2006 and 66.7% in 2007), Calcium chloride (76.5% in 2006 and 77.8% in 2007), Sodium bicarbonate (58.8% in 2006 and 22.2% in 2007) and Calcium nitrate (41.2% in 2006 and 44.4% in 2007) respectively.

Data presented in (Table 3) reveal that all tested salts reduced storage rots as compared to the untreated (control). The highest effective salt in reducing rot incidence in artificial infection by *Botrytis cinerea* during storage at 0°C under 90-95% R.H. for 50 days of Flame seedless grapes was calcium carbonate (63.7%) followed by calcium nitrate, potassium metabisulfite, calcium chloride and sodium bicarbonate 60.75, 41.13, 35.6 and 17.8 respectively during the first season. It is obvious that the highest effective salt in season 2007, was calcium nitrate (57.9%) followed in a

descending order by potassium metabisulfite, sodium bicarbonate, calcium carbonate, and calcium chloride 48.7, 47.2, 37.5 and 30.9 respectively

Table (2): Effect of pre harvest spraying of Flame seedless grapevines with certain salts on infection percentage of naturally infected vines during storage at 0°C and 90-95% RH for 50 days.

Treatments	Concen. g./L	Natural infection			
		Season 2006		Season 2007	
		Rot incidence %	Efficacy %	Rot incidence %	Efficacy %
Calcium chloride	1	50	41.2	60	33.3
	2	20	76.5	20	77.8
Calcium nitrate	1	60	29.4	70	22.2
	2	50	41.2	50	44.4
Potassium metabisulfite	1	20	76.5	17	81.1
	2	15	82.4	15	83.3
Calcium carbonate	1	20	76.5	50	44.4
	2	17	80.0	30	66.7
Sodium bicarbonate	1	30	64.7	50	44.4
	2	35	58.8	70	22.2
Control	-	85	-	90	-
L.S.D. at 0.05		9.5		8.4	

Table (3): Effect of pre harvest spraying of Flame seedless grapevines with certain salts on infection percentage of artificially inoculated grapes with *Botrytis cinerea* during storage at 0°C and 90-95% RH for 50 days.

Treatments	Concen. g/l	Artificial infection by <i>Botrytis cinerea</i>			
		Season 2006		Season 2007	
		Rot incidence %	Efficacy %	Rot incidence %	Efficacy %
Calcium chloride	1	50.1	35.0	80.9	19.1
	2	50.2	35.6	69.1	30.9
Calcium nitrate	1	46.6	40.23	43.7	54.3
	2	30.6	60.75	42.1	57.9
Potassium metabisulfite	1	45.9	41.13	73.9	26.1
	2	58.9	24.2	51.6	48.4
Calcium carbonate	1	35.5	54.5	68.1	31.9
	2	28.3	63.7	63.5	37.5
Sodium bicarbonate	1	64.1	17.8	93.0	7.0
	2	66.9	14.2	52.8	47.2
Control	-	77.97		100	
L.S.D at 0.05		10.4		11.2	

As shown in table (4) it is obvious that all salts in season 2006 remarkably increase of cluster weight from 419 to 452 at the concentration of 1g/L. and from 516 to 535 at 2g/L except sodium bicarbonate at 1g/L., 2g/L. and calcium chloride at 2g/L which decrease cluster weight as compared to control. The same results were obtained concerning the yield / vine (g). All application salts remarkably increased yield /vine (g) which ranged from 12.9 to 13.5 at 1g/L. and 15.48 to 16.5 at 2g/L. except sodium bicarbonate at 1 g/L., 2 g/L. and calcium chloride at 2 g/L. which decreased the yield/ vine (g) compared to control. Furthermore, all salts had a positive effect on physical

characteristics (i.e. weight (g), Length (L.cm), width (w.cm) and berry shape index (L/W)) as compared with control treatment.

Table (4): Effect of pre harvest spraying with certain salts on the yield/vine and some fruit physical characteristics of Flame seedless grapevines season 2006.

Parameter 2006	Physical characteristics											
	Yield/vine (kg)		Bunch weight (g)		Average Berry						Berry shape index (L/W)	
					Weight (g)		Length (cm)		Width (cm)			
	1g/L.	2g/L.	1g/L.	2g/L.	1g/L.	2g/L.	1g/L.	2g/L.	1g/L.	2g/L.	1g/L.	2g/L.
Concentrations	13.08	11.45	419	392	3.00	2.40	1.65	1.53	1.60	1.40	1.031	1.093
Calcium chloride	13.05	16.05	435	535	2.80	3.13	1.60	1.70	1.55	1.65	1.032	1.030
Calcium nitrate	13.50	16.5	452	550	2.90	3.40	1.70	1.80	1.65	1.75	1.030	1.029
Potassium metabisulfite	12.90	15.48	425	516	2.70	3.10	1.50	1.55	1.45	1.50	1.034	1.033
Calcium carbonate	9.00	9.30	300	310	2.15	2.20	1.45	1.45	1.35	1.38	1.074	1.051
Sodium bicarbonate	12.00	12.00	400	400	2.70	2.70	1.55	1.55	1.50	1.50	1.030	1.030
control	1.01	2.3	12.1	15.2	.038	0.21	0.15	0.12	0.09	0.10	0.012	0.010
L.S.D at 0.05												

Data in table (5) reveal that all application salts resulted in a significant increase of TSS %, total acidity (TA) % and TSS/TA ratio as compared with control except sodium bicarbonate which reduce TSS/TA ratio. In contrast, all application salts lead to a significant decrease the loss of weight %, the biggest reduction was observed when vines were treated by calcium chloride in the concentration of 1g/L. (3.2% loss of weight)

Table (5): Effect of pre harvest spraying of Flame seedless grapevines with certain salts on some chemical characteristics of berries at cold storage (0°C and 90-95% RH for50) days season 2006.

Treatments 2006	Chemical berry characteristics							
	TSS %		Acidity (TA) %		TSS/TA ratio		Loss of weight %	
	1g/L.	2g/L.	1g/L.	2g/L.	1g/L.	2g/L.	1g/L.	2g/L.
Calcium chloride	20.17	18.40	0.680	0.652	29.66	29.44	3.2	8.00
Calcium nitrate	21.80	22.50	0.675	0.675	32.29	33.3	6.40	9.00
Potassium metabisulfite	22.00	24.00	0.629	0.622	35.20	38.58	7.40	9.30
Calcium carbonate	21.00	22.00	0.629	0.650	33.60	33.85	9.20	6.00
Sodium biocarbonate	18.80	18.70	0.850	0.800	21.38	23.37	8.00	6.40
Control	18.20	18.20	0.628	0.628	28.98	28.98	9.40	9.40
L.S.D. at 0.05	2.6	2.9	0.011	0.015	2.8	3.1	1.6	2.1

Data in table (6) showed that all application salts in season 2007 remarkably increased cluster weight ranged from 435 to 460 at a concentration. 1 g/L. and from 530 to 565 at 2 g/L. except sodium bicarbonate at 1 g/L., 2 g/L. and calcium chloride at 2 g/L. Which decrease cluster weight 310,308 and 403 respectively compared to control 410 The same result was obtained in the yield / vine (g) where all application salts remarkably increased yield / vine (g) which ranged from 13.05 to 13.80 at 1 g/L. and from 15.90 to 16.95 at.2 g/L. except sodium bicarbonate at 1 g/L.,2 g/L. and calcium chloride at 2 g/L.which decrease yield/ vine (g) 9.30,9.25

and 12.09 respectively compared to control 12.30. Furthermore, all salts were found to have a positive effect on the physical characteristics (*i.e.* weight (g), Length (L.cm), width (w.cm) and berry shape index (L/W)) as compared with control.

Table (6): Effect of pre harvest spraying with certain salts on fruit physical characteristics of Flame seedless grapevines (season 2007).

Parameter 2006	Physical characteristics											
	Yield/vine (kg)		Bunch weight (g)		Average Berry						Berry shape index (L/W)	
					Weight (g)		Length (cm)		Width (cm)			
Concentrations	1g/L.	2g/L.	1g/L.	2g/L.	1g/L.	2g/L.	1g/L.	2g/L.	1g/L.	2g/L.	1g/L.	2g/L.
Calcium chloride	13.25	12.09	440	403	3.10	2.50	1.68	1.53	1.63	1.43	1.031	1.073
Calcium nitrate	13.35	16.50	445	550	2.90	3.30	1.63	1.73	1.58	1.68	1.031	1.030
Potassium metabisulfite	13.80	16.95	460	565	3.00	3.50	1.73	1.73	1.68	1.77	1.030	1.034
Calcium carbonate	13.05	15.90	435	530	2.80	3.20	1.53	1.57	1.48	1.52	1.036	1.033
Sodium bicarbonate	9.30	9.25	310	308	2.25	2.30	1.48	1.47	1.37	1.40	1.080	1.050
control	1230	12.30	410	410	2.75	2.75	1.58	1.58	1.53	1.53	1.032	1.023
L.S.D at 0.05	1.05	1.09	11.8	12.08	0.24	0.29	0.05	0.04	0.10	0.09	0.002	0.002

Data in table(7) revealed that all application salts lead to a significant increase in TSS %, total acidity (TA) % and TSS/TA ratio as compared with control except sodium bicarbonate which reduced TSS/TA ratio. In contrast, all application salts resulted in significant decrease loss of weight %, the biggest reduction observed when treated by calcium nitrate at concentration 1 g/L. (7.2% loss of weight)

Table (7): Effect of pre harvest spraying of grapes of Flame seedless grapevines with certain salts on chemical characteristics of berries at cold storage (0°C and 90-95% RH for50) days season 2007.

Treatments 2007	Chemical berry characteristics							
	TSS %		Acidity (TA) %		TSS/TA ratio		Loss of weight %	
	1g/L.	2g/L.	1g/L.	2g/L.	1g/L.	2g/L.	1g/L.	2g/L.
Calcium chloride	21.93	18.00	0.720	0.626	30.46	28.75	7.6	8.8
Calcium nitrate	22.00	23.00	0.670	0.672	32.3	34.22	6.4	9.6
Potassium metabisulfite	23.2	24.60	0.620	0.618	37.42	39.80	7.2	12.2
Calcium carbonate	22.20	23.00	0.622	0.625	35.69	36.80	8.9	9.6
Sodium bicarbonate	19.00	19.00	0.40	0.610	22.62	31.15	7.6	10.2
Control	19.00	19.00	0.623	0.623	30.49	30.49	10.4	10.4
L.S.D. at 0.05	1.9	2.10	0.008	0.006	2.18	2.24	1.65	1.92

It can be shown from data presented in table (8) that the cost of used salts very cheap as compared to used classic fungicides. Furthermore the use of salts as control of bunch rot disease exhibited low mammalian and environmental toxicity also increased yield / fedaan 1404.8 Kg in season 2006 and 2205.6 Kg in seasons 2007 in addition the fruit quality was

improved .The net profit of increase in yield was reach 1826.24 LE in season 2006 and 2205.6 LE season 2007

Table (8) some economical data concerning the use of some salts to control bunch rot and improving fruit quality.

season	Price of the salts (LE)	Price of the fungicide (LE)	Yield /feddan (Kg) treated vine	Yield /feddan (Kg) untreated vine	Increase in yield over control (Kg)	Price of one Kg grapes (LE)	Price of Increase in yield over control (LE)
2006	39	250	11004.8	9600	1404.8	1.30	1826.24
2007	39	300	11310.4	9840	1470.4	1.50	2205.60

DISCUSSION

The objective of the present study is to determine the ability of some salts to control bunch rot in open field, botrytis storage rot and the effect of these salts on some physical and chemical characteristics of flame seedless grapes by means of pre-harvest applications. In the present study the *in vitro* tests showed a variable effect of salts on *B. cinerea* growth. After 7 days incubation at 20±2°C most of salts were found to reduced the colony diameter of the pathogen. A complete inhibition was achieved by Potassium metabisulfite at 0.2% and by Sodium bicarbonate at 0.2% while the lowest percentage of colony diameter reduction was found when calcium nitrate applied at the 0.05% 35.2% Calcium chloride at the rate 0.05% (38.8%).

In preliminary papers, several salts showed in vitro and/or in vivo inhibitory effect against *B. cinerea*, although no correlation was found between the two activities. Ammonium bicarbonate, ammonium phosphate, sodium acetate, and sodium sulfate strongly reduced *B. cinerea* mycelial growth but displayed an inconsistent activity against grey mould on small bunches. In particular, sodium phosphate reduced the growth of the pathogen in vitro, but increased rot incidence on small table grape bunches (Nigro *et al* 2006). As reported for other phosphates (Palou *et al.*, 2002), it is assumed that this salt provided additional nutrients and/or enhanced environmental conditions for the development of the pathogen. In addition, Calcium carbonate showed almost no in vitro inhibitory activity but strongly reduced grey mould incidence on table grapes. Other salts such as Potassium carbonate, Sodium bicarbonate, and Sodium carbonate showed the same behavior, with both in vitro and in vivo assays. The difference in vitro and in vivo behavior of salts suggests that a specific salt–host tissue interaction may involve biochemical reactions, such as defense mechanisms contributing to the control of grey mould (Hervieux *et al.*, 2002). Furthermore, the interaction between the salt and agar medium (Biggs *et al.*, 1997) as well as the interaction between the salt and environment (Punja and Grogan, 1982) may play an important role in this respect. In the present work, the results of filed experiment demonstrated that pre-harvest applications of salts are an effective strategy to reduce the incidence of bunch rot and botrytis storage rot of table grapes. Calcium chloride, calcium nitrate, potassium metabisulfite, calcium carbonate and sodium bicarbonate provided higher

level of protection compared to control the highest percentage of reduction of bunch rot in open felid happened when vines were treated by Potassium metabisulfite during seasons 2006 (87.5%), the highest percentage of reduction (85.5%) resulted from Calcium carbonate during 2007 season, the lowest percentage of reduction (28.4%) when Calcium chloride was applied during season 2007. Furthermore, early applications of calcium chloride provided better control of field rots. It is presumed that an early application of calcium chloride favored the penetration of calcium through the skin and that a second application increased the calcium level inside the fruit, resulting in higher levels of protection compared to control. Data obtained are in accordance with some previous reports on pre-harvest application of calcium chloride. Pre-harvest applications of calcium chloride increased the Ca^{2+} content of sweet cherries and apples (Brown et al., 1996), and table grape berries (Miceli et al., 1999). As demonstrated by the penetration of calcium chloride via aqueous pores, exogenous Ca^{2+} easily penetrates the fruit epidermis (Sch" nherr, 2000). The mechanism by which calcium improves the resistance of plant tissues to pathogens is not completely understood. Most of the calcium, which penetrates into the fruit tissue, seems to accumulate in the middle lamella region of the cell wall where it exerts a stabilizing effect forming ionic bridges between and within pectic polysaccharides, thereby conferring rigidity to the cell wall. The formation of calcium cross-linkage between pectin polymers could make the cell wall more resistant to hydrolytic enzymes produced by decay causing organisms (Tobias et al., 1993). Furthermore, calcium treatments affect pathogens directly, inhibiting the activity of polygalacturonase enzyme. A reduced virulence or a fungistatic activity of calcium has been already reported for *Penicillium expansum* and *B. cinerea* (Conway et al., 1999; Droby et al., 1997). Other studies have shown that calcium treatments preserve the membrane integrity of carrot shreds by delaying senescence-related membrane lipid changes and enhancing membrane-restructuring processes (Picchioni et al., 1996). Calcium also seems to increase the synthesis of phytoalexins and/or phenolic substances (Kohle et al., 1985).. The activity of carbonates and bicarbonates in inhibiting spore germination, germ tube elongation, and production of pectinolytic enzymes in several pathogens is well recognized (Punja and Grogan, 1982; Hervieux et al., 2002; Mills et al., 2004; Smilanick et al., 2005). Sodium and potassium salts strongly inhibited mycelial growth and spore germination of *B. cinerea* as well as polygalacturonase activity. Considering that the proportion of CO_3^{2-} ion is high at pH 11.3 (Potassium carbonate and Sodium carbonate) and that HCO_3^- is the predominant ion at pH 8.4 (Sodium bicarbonate), the results suggest that both HCO_3^- and CO_3^{2-} forms display an inhibitory activity on mycelial growth and spore germination (Palmer et al., 1997). Like calcium chloride. Carbonates and bicarbonate salts were more effective when applied during the early phase of fruit development and when applied twice. Since the inhibitory action of carbonates and bicarbonates depends on the presence of salt residues within the wound infection site occupied by fungi, it is possible that earlier applications, especially when followed by a later one, provide a more effective action against pathogens and prevent the

establishment of latent infections (Smilanick et al., 1999). The present study show that all salts treatments significantly decreased decay development on naturally infected Flame seedless grapes during cold storage in the two seasons 2006/ 2007. It was observed that Potassium metabisulfite was the most effective treatment to control berries rots on naturally infected grapes at 2g/L. concentration in both seasons (82.4% in 2006 and 83.3% in 2007) followed by calcium carbonate (80.0% in 2006 and 66.7% in 2007), Calcium chloride (76.5% in 2006 and 77.8% in 2007), Sodium bicarbonate (58.8% in 2006 and 22.2% in 2007), Calcium nitrate (41.2% in 2006 and 44.4% in 2007) respectively, Furthermore, The present study declare that all tested salts reduced storage rots as compared to the untreated (control). The highest effective salt to reduce rot incidence in artificial infection by *Botrytis cinerea* during storage at 0°C under 90-95% R.H. for 50 days of Flame seedless grapes was calcium carbonate (63.7%) followed by calcium nitrate, potassium metabisulfite, calcium chloride and sodium bicarbonate 60.75, 41.13, 35.6 and 17.8 respectively during 2006. On the other hand the highest effective salt in season 2007 was calcium nitrate 57.9% followed by potassium metabisulfite, sodium bicarbonate, calcium carbonate, and calcium chloride 48.7, 47.2, 37.5 and 30.9 respectively. (Ippolito and Nigro, 2000). Mlikota Gabler and Smilanick (2001) reported that dip treatments with sodium carbonate and potassium carbonate was highly effective from the control of grey mold on grapes but they darkened the pedicels and caused dark brown spots on the berries. Such problems were completely avoided in the present work by applying salts before harvest. Other advantages of salts are the very low cost and the lack of legislative restrictions to their utilization. However, several additional factors should be explored to optimize table grape disease control with salts.(Nigro et al., 2006) reported that 1% solution of salts was utilized since they were effective and non-phytotoxic to the berries, though higher concentrations have been considered necessary by other authors to achieve high levels of protection (Smilanick et al., 1999; Tian et al., 2002). Furthermore, although our data seem to indicate that salts are more effective when applied during the early phase of berry development and that more salt applications increase their efficacy, additional research is still required to optimize time and number of applications.

The present study revealed also that all application salts resulted in a significant increase of TSS %, total acidity (TA) % and TSS/TA ratio as compared with control except sodium bicarbonate which reduce TSS/TA ratio during the two seasons 2006/2007.

All applied salts lead to a significant decrease in loss of weight % the remarkable reduction was observed when vines were treated by calcium chloride in a concentration 1g/L. (3.2% loss of weight) in season 2006 and a significant decrease in loss of weight % the biggest reduction was noticed when vine were treated by calcium nitrate at 1 g/L. (7.2% loss of weight) in season 2007.

The investigation also demonstrated that all application salts in season 2006 remarkably increased cluster weight which ranged from 419 to 452 at. 1 g/L. and 516 to 535 at.2 g/L. except sodium bicarbonate at 1 g/L., 2 g/L. and calcium chloride at 2 g/L. were cluster weight was decreased

300,310 and 392 respectively compared to control 400 while in season 2007 the increase in cluster weight ranged from 435 to 460 at 1 g/L. and 530 to 565 at 2 g/L. except sodium bicarbonate at 1 g/L., 2 g/L. and calcium chloride at 2 g/L. which decreased cluster weight 310,308 and 403 respectively compared to control 410.

The same result was obtained on yield / vine (g) were all application salts remarkably increased yield / vine (g) which ranged from 12.9 to 13.5 at 1 g/L. and 15.48 to 16.5 at 2 g/L. except sodium bicarbonate at g/L., 2 g/L. and calcium chloride at 2 g/L. which decreased yield/ vine (g) 9.0,9.3 and 11.45 respectively compared to control 12 during season 2006. while in 2007 the increase of yield / vine (g) ranged from 13.05 to 13.80 at 1 g/L. and 15.90 to 16.95 at 2 g/L. except sodium bicarbonate at 1 g/L., 2 g/L. and calcium chloride at 2 g/L. decrease yield/ vine (g) 9.30, 9.25 and 12.09 respectively compared to control 12.30. Furthermore, all application salts showed a positive effect on the physical characteristics (i.e. weight (g), Length (L.cm), width (w.cm) and berry shape index (L/W)) as compared with control during both seasons of the study. These results agreement with (Abd-Elghany et al 2007; Abo Rehab et al 2007 ; Abd El-Hady 2002 ; Shoaieb et al 2002 ; Kilany et al 2002)

REFERENCES

- Abd Elghany A.A. and Abo Rehab, M.E.A. (2007) Effect of calcium, lime, wettable sulfur and copper oxoclride on vine growth and control of Dead-arm disease and bunch rot of Ruby seedless grapevines. *J.Agric. Sci. Mansoura Univ.*, **32**(4):2817-2824.
- Abd El-Hady A.M. (2002) Using sodium azide and some nutrients to promote uniform bud break and improve productivity of Flame seedless grapevines. *Proc.Minia 1st conf.for agric.& Environ.sci.,Minia,Egypt,March 25-28,2002.*
- Abo Rehab, M.E.A.; Ammar, M.I. and Abd Elaal, A.H., (2007) Evaluation of the Effectiveness of some Natural compounds on Powdery Mildew [*Uncinula necator* (Schw.)] and Grape characteristics. *Minufiya j.Agric.Res.Vol.32* No: 5: 1225-1237.
- Anonymous (2005) Annual report of Agric. Statistical Dept. Egyptian Min. of Agric. A.R. E. (in Arabic).
- AOAC. (1990). Official Methods of Analysis of the Association of Official Agriculture Chemists. 15th ed., Washington, D.C.
- Biggs, A.R., El-Kholi, M.M., El-Neshawy, S., Nickerson, R., (1997). Effects of calcium salts on growth, polygalacturonase activity and infection of peach fruit by *Monilinia fructicola*. *Plant Dis.* 81, 399-403.
- Brown, G.S., Kitchener, A.E., McGlasson, W.B., Barnes, S., (1996). The effect of copper and calcium foliar sprays on cherry and apple fruit quality. *Sci.Hortic.* 67, 219-227.
- Conway, W.S., Janisiewicz, W.J., Klein, J.D., Sams, C.E., (1999). Strategy for combining heat treatment, calcium imporation, and biological control to reduce postharvest decay of 'Gala' apples. *HortScience* 34, 700-704.

- Droby, S., Wisniewski, M.E., Cohen, L., Weiss, B., Touitou, D., Eilam, Y., Chalutz, E., (1997). Influence of CaCl₂ on *Penicillium digitatum*, grapefruit peel tissue, and biocontrol activity of *Pichia guilliermondii*. *Phytopathology* 87, 310–315.
- Fallik, E., Grinberg, S. and Ziv, O., (1997). Potassium bicarbonate reduces post-harvest decay development on belle pepper fruits. *J. Hort. Sci.* 72, pp. 35–41.
- Galber, F.M and Smilanick, J.L. (2001) Postharvest control of table grape gray mold on detached berries with carbonate and bicarbonate salts and disinfectants. *Am.J.Enol. Viticult.* 52, pp.12-20.
- Gomez, K.A. and A.A.Gomze, (1983) Statistical procedures for agricultural research. John Wiley and Sons, New York.
- Hervieux, V., Yaganza, E.S., Arul, J., Tweddell, R.J., (2002). Effect of organic and inorganic salts on the development of *Helminthosporium solani*, the causal agent of potato silver scurf. *Plant Dis.* 86, 1014–1018
- Ippolito, L. Schena, I. Pentimone and F. Nigro, (2005). Control of postharvest rots of sweet cherries by pre- and postharvest applications of *Aureobasidium pullulans* combination with calcium chloride or sodium bicarbonate. *Postharvest Biol. Technol.* (2005) 36, pp. 245–252.
- Ippolito, A., Nigro, F., 2000. Impact of preharvest application of biocontrol agents on postharvest diseases of fresh fruits and vegetables. *Crop Prot.* 19, 723–725.
- Karabulut, O.A., Lurie, S. and Droby, S., (2001). Evaluation of the use of sodium bicarbonate, potassium sorbate and yeast antagonists for decreasing postharvest decay of sweet cherries. *Postharvest Biol. Technol.* 23, pp. 233–236.
- Karabulut, O.A., U. Arslan, K. Ilhan and G. Kuruoglu, (2005). Integrated control of postharvest diseases of sweet cherry with yeast antagonists and sodium bicarbonate applications within a hydrocooler. *Postharvest Biol. Technol.* 37 (2005), pp. 135–141.
- Kilany, A.E., El-Morsi, F.M. And Ahmed ola, A. (2002). Effect of mineral or chelated calcium and magnesium on growth and fruiting of Flame seedless grapevines on yield and berries quality. *J. Agric. Sci. Mansoura Univ.*, 25(11):7029-7055.
- Kohle, H., Jeblick, W., Poten, F., Blaschek, W., Kauss, H., (1985). Chitosan-elicited callose synthesis in soy bean cells as a Ca²⁺ - dependent process. *Plant Physiol.* 77, 544–551.
- Miceli, A., Ippolito, A., Linsalata, V., Nigro, F., (1999). Effect of preharvest calcium treatment on decay and biochemical changes of table grapes during storage. *Phytopat. Medit.* 38, 47–53.
- Mills, A.A.S., Platt, H.W., Hurta, R.A.R., (2004). Effect of salt compounds on mycelial growth, sporulation and spore germination of various potato pathogens. *Postharvest Biol. Technol.* 34, 341–350.
- Mlikota Gabler, F., Smilanick, J.L., (2001). Postharvest control of table grape gray mold on detached berries with carbonate and bicarbonate salts and disinfectants. *Am. J. Enol. Vitic.* 52, 12–20.

- Nigro, F., Schena L., Ligorio A., Pentimone I., Ippolito A. and Mario G. Salerno. (2006) Control of table grape storage rots by pre-harvest applications of salts. *Postharvest Biol. Technol.* (2006) **42**, pp. 142–149.
- Palmar, C.L., Horst, R.K. and Langhans, R.W., (1997). Use of bicarbonates to inhibit in vitro colony growth of *Botrytis cinerea*. *Plant Dis.* **81**, pp. 1432–1438.
- Palou, L., Usall, J., Munoz, J.A., Smilanick, J.L., Vinas, I., 2002. Hot water, sodium carbonate, and sodium bicarbonate for the control of postharvest green and blue molds of Clementine mandarins. *Postharvest Biol. Technol.* **24**, 93–96.
- Picchioni, G.A., Watada, A.E., Whitaker, B.D., Reyes, A., (1996). Calcium delays senescence related lipid changes and increases net synthesis of membrane lipid components in shredded carrots. *Postharvest Biol. Technol.* **9**, 235–245.
- Punja, Z.K., Grogan, R.G., (1982). Effects of inorganic salts, carbonate and bicarbonate anions, ammonia, and the modifying influence of pH on sclerotial germination of *Sclerotium rolfsii*. *Phytopathology* **72**, 635–639.
- Ricker, M.D. and Punja, Z.K., (1991). Influence of fungicide and chemical salt dip treatments on crater rot caused by *Rhizoctonia carotae* in long-term storage. *Plant Dis.* **75**, pp470-474.
- Rushed, I.A.S. (2001). Pathological studies on grapes prepared for exportation. *M.Sc.Fac.Agric. AL-Azhar University, Egypt.*
- Sch"nherr, J., (2000). Calcium chloride penetrates plant cuticles via aqueous pores. *Planta* **212**, 112–118.
- Shoieb M.M. (2002) A comparative study on the effect of soil or foliar application of potassium to Flame vines. Proc.Minia1st conf. for agric. & Environ. sci. Minia, Egypt, March 25-28, 2002 .
- Smilanick, J.L., Margosan, D.A., Mlikota Gabler, F., Usall, J., Michael, I.F., (1999). Control of citrus green mold by carbonate and bicarbonate salts and the influence of commercial postharvest practices on their efficacy. *Plant Dis.* **83**, 139–145.
- Smilanick, J.L., Mansour, M.F., Margosan, D.A., Mlikota Gabler, F., Goodwine, W.R., (2005). Influence of pH and NaHCO₃ on effectiveness of imazalil to inhibit germination of *Penicillium digitatum* and to control postharvest green mold on citrus fruit. *Plant Dis.* **89**, 640–648.
- Soltan, H.H.M., Tomader J. Abd-Elrahman and Azza M.A.Naffa (2006) The Efficacy of Pre harvest Salts treatment on Incidence of Snap Bean Pod Rots during Storage. *Egypt. J. Phytopathol.*, Vol. **34**, No.1, pp. 31-40.
- Tian, S.P., Fan, Q., Xu, Y., Jiang, A.L., (2002). Effects of calcium on biocontrol activity of yeasts antagonists against the postharvest fungal pathogen *Rhizopus stolonifer*. *Plant Pathol.* **51**, 352–358.
- Tobias, R.B., Conway, W.S., Sams, C.F., Gross, K.C., Whitaker, B.E., (1993) Cell wall composition of calcium-treated apples inoculated with *Botrytis cinerea*. *Phytochemistry* **32**, 35–39.

تقيم فعالية بعض املاح الكالسيوم ،الصوديوم والبوتاسيوم علي الاصابة بمرض اعفان ثمار العنب المتسبب عن الفطر *Botrytis cinerea* في الحقل واثناء التخزين وذلك عن طريق الرش اثناء مرحلة ما قبل الحصاد وكذلك تأثيرها علي كمية وجودة المحصول والخصائص الطبيعية والكيميائية لثمار العنب الفليم سيدلس.

حمادة حماد محمد سلطان* , أحمد حسن عبدالعال** , محسن السيد علي أبو رحاب*
* معهد بحوث أمراض النباتات - مركز البحوث الزراعية - جيزة - مصر
** قسم البساتين - كلية الزراعة - جامعة الأزهر - أسيوط - مصر.

أجريت هذه الدراسة بغرض تقييم فعالية كلا من كلوريد الكالسيوم ،نترات الكالسيوم ، ميتا بيسلفات البوتاسيوم ،كاربونات الكالسيوم وبيكربونات الصوديوم في مقاومة مرض اعفان ثمار العنب في الحقل وكذلك قدرتها علي حماية حبات العنب من الاصابة بالمرض اثناء مراحل التخزين تحت درجات الحرارة المنخفضة (صفر درجة مئوية) وذلك برش كرمات العنب بالاملاح السابقة قبل الحصاد باستخدام تركيزين من هذه الاملاح اجم/لتر ، ٢جم/لتر وتم رش هذه الاملاح في ثلاث مواعيد ، الرشة الاولى خلال مرحلة الازهار الكامل والرشة الثانية بعد اسبوعين من الاولى والثالثة بعد اسبوعين من الثانية علي اشجار العنب الفليم سيدلس خلال موسمين متتاليين ٢٠٠٦-٢٠٠٧. حيث قيمت فعالية هذه الاملاح علي حدوث الاصابة في الحقل تحت ظروف العدوي الطبيعية اما في مرحلة التخزين فتم تقييمها تحت ظروف العدوي الطبيعية وكذلك تحت ظروف العدوي الصناعية عن طريق تلقيحها بالفطر بوتريتس سينيريا. كذلك قيمت قدرة هذه الاملاح علي اختزال نمو الفطر بوتريتس سينيريا في المعمل وذلك باستخدام ثلاثة تركيزات مختلفة ٠,٥ جم /لتر ، اجم /لتر و ٢جم /لتر وكذلك تم دراسة تأثير هذه الاملاح علي كمية المحصول والخواص الطبيعية والكيميائية للثمار. أشارت النتائج التي تم الحصول عليها الي ان جميع الاملاح المستخدمة ادت الي نقص نمو الفطر بوتريتس سينيريا في المعمل باستخدام التركيزات ٠,٥ جم /لتر ، اجم /لتر و ٢جم /لتر ولوحظ التنشيط الكامل لنمو الفطر عند استخدام املاح ميتا بيسلفات البوتاسيوم بمعدل ٢جم /لتر وبيكربونات الصوديوم بمعدل ٢جم /لتر كما انخفضت شدة الاصابة بهذا المرض تحت ظروف الحقل وكذلك عند التخزين البارد للثمار سواء تحت ظروف العدوي الطبيعية او الصناعية عند استخدام جميع الاملاح كلا علي حدة وكانت المعاملة باستخدام ميتا بيسلفات البوتاسيوم هي الافضل حيث انخفضت درجة الاصابة بشكل واضح سواء بالنسبة للاصابة الطبيعية او العدوي الصناعية وفيما يتعلق بتأثير هذه الاملاح علي الخواص الطبيعية والكيميائية للثمار فقد اثبتت النتائج انها قد ادت اي زيادة جوهريه في متوسط وزن العنقود وكمية المحصول للاشجار المعاملة باستثناء املاح بيكربونات الصوديوم وكلوريد الكالسيوم تسببت في خفض هاتين الصفتين كما ادي استخدام الاملاح باستثناء بيكربونات الصوديوم الي زيادة وزن الحبة وابعادها كما كان تأثير الاملاح ايجابيا علي الثمار المخزنة بالتبريد حيث تمثل ذلك في زيادة المواد الصلبة الذائبة الكلية والحموضة بعصير الحبات وكذا النسبة بينهما باستثناء بيكربونات الصوديوم التي تسببت في نقص الحموضة وكذلك ادي استخدام الاملاح كل علي حدة الي خفض واضح في النسبة المئوية للفقء في وزن الثمار. ومن الناحية الاقتصادية وجد أن تكاليف استخدام الأملاح كان أقل بصورة معنوية من استخدام المبيدات الفطرية كما أنه أدى الي زيادة محصول اللقدان حيث كانت في سنة ٢٠٠٦ ١٤٠٤,٨ كجم للقدان وسنة ٢٠٠٧ كانت ٢٢٠٥,٦ كجم للقدان بالاضافة الي تحسين جودة الثمار.