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# Influence of preharvest spraying with the chemical salts on grey mold disease and keeping quality of table grapes during storage

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**Abstract**

**The efficacy of calcium chloride, calcium nitrate, potassium metabisulfate, calcium carbonate and sodium bicarbonate to protect grape bunches during harvesting was studied under storage conditions. This experiment was carried out in a private vineyard, El-Khatatba region, Alexandria desert road, Egypt during 2014 and 2015 growing seasons on Thompson seedless variety. The chemical used at 0.1 and 0.2% g /liter water. The healthy grape bunches of all treatments were harvested at proper ripeness, packed in perforated plastic bags, put inside carton boxes and stored at 0°C under 90-95% R.H. for 36 days. Other grape bunches either treated or untreated with the salts were artificially inoculated with spore suspension of *Botrytis cinerea* at a concentration of $3x10^6$ spores/ml before packing and cold storage. Disease severity of grape bunches with *B. cinerea* was estimated after 36 days of cold storage. All treatments exhibited disease reduction under field conditions of Thompson seedless grape variety. The field applications of salts gave a significant reduction of botrytis rot during storage. In this respect, the grape rots were significantly reduced when sprayed by the salts three times.**

**Key words:** grey mold, *Botrytis cinerea*, salts, table grapes.

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## 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 following citrus. Concerning, the cultivated areas reached 171973 acres (153269 acres as table grapefruits) with a production of 1320801 tons (According to annual statistical of the Egyptian Ministry of Agricultural, 2014). Grape orchards are increased rapidly as more desert areas are being planted every year for local market consumption and exportation. Grapes are subjected to the infection with several pre and postharvest diseases. The bunch rot disease caused by *Botrytis cinerea* is serious fungus on grape bunches all over the world where it cause great losses and also affect grape quality, especially during picking, packing, marking, exporting and storage. The fungus *B. 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$^{o}$C by the sea for exportation or during cold storage for local market. Use of synthetic fungicides has been the traditional option to control plant diseases, but the extensive and prolonged use of synthetic fungicides has resulted in the resistance development of the fungis. Furthermore, the residual effect on the crop and environmental pollution are other problems associated with the use of these chemicals. Therefore, the use of pre- and postharvest chemical treatments is increasingly limited due to consumer concerns. Present focuses on the development of alternative means of controlling fungal in the field and during storage for local market. This alternative means, are safe to human and environment, have been initiated (Soltan et al., 2008; Abd Elghany et al., 2007; Abo rehab et al., 2007; Rushed, 2001). In the search for biocompatible products, which could be defined as chemicals exhibiting low mammalian and environmental toxicity, many salts have been recently tested as alternative control means (Soltan et al., 2008; Abd Elghany et al., 2007; Abo rehab et al., 2007; Ippolito et al., 2005; Karabulut et al., 2005; Rushed, 2001; Smilanick et al., 1999). 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 as growth inhibitors of *B. cinerea* (Soltan et al., 2006; Ricker & Mills et al., 2004; Gabler & Smilanick, 2001; Karabulat et al., 2001; Rushed, 2001; Fallik et al., 1997; Palmar et al., 1997; Punja, 1991). Pre- and postharvest application of different kinds of potassium, Sodium and calcium salts on table grapes reduced the incidence of grey mold disease (Soltan et al., 2008; Ippolito et al., 2005; Rushed, 2001). Different kinds of potassium and calcium salts sprayed three weeks before harvest on Thompson seedless grapevines showed highly reduction of disease severity with *B. cinerea* comparing with the control grapes under field and storage condition at 0-1$^{o}$C (Rushed, 2001). The purpose of this study was to find out the best antifungal activity of tested salts against *B. cinerea* the causal organism of grey mold disease of table grapes, Thompson seedless variety under cold storage conditions.

## Materials and methods

**Fungal isolates:** Phytopathogenic cultures of *Botrytis cinerea* from grape berries were isolated and purified following single spore method. The agar slant of purified fungus was stored at 4°C and served for all experiments.

**Filed experiments:** Under filed conditions for the two successive seasons 2014 and 2015, the effect of salts spraying on grapevines as preharvest treatment to protect grape bunches during harvesting and storage was studied. Five salts, i.e. calcium chloride, calcium nitrate, potassium metabisulfate, calcium carbonate and sodium bicarbonate at rate of 100 and 200g/100L,were tested for their ability to control grape bunches rots under field conditions. This experiment was carried out in private vineyard, El-Khatatba region,–Alexandria desert road. Ninety nine uniform Thompson seedless grapevines were chosen and devoted for achieving this experiment. All vines were adjusted to the same cluster number (i.e.30 clusters) prior to an thesis. 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 the third after two weeks from the second spray. Salts were used at a concentration of 1 and 2g/L. water. Five grapevines were used for each treatment. Five grapevines were left between each two treatments as a barrier. Five grapevines were sprayed with the water and served as a control. Grape bunches of all treatments were harvested at proper ripeness, packed in plastic bags inside carton boxes and stored at 0°C under 90-95% R.H. for 36 days of Thompson seedless variety. Other grape bunches either treated or untreated were artificially inoculated with spore suspension of *B. cinerea* at a concentration of $3x10^6$ spores/ml before packing and cold storage. Each treatment contained three replicates (one kilogram grape bunches for each replicate). Disease severity of grape bunches with *B. cinerea* was estimated at the end of cold storage.

The bunch rot disease was evaluated on the bunch using the following scale on 15 bunches for each replicate, three replicates of each treatment. The disease severity (DS) was calculated according to the following formula:

$$\text{Disease severity (\%)} = \frac{\sum (ni \times v_i)}{(V \times N)} \times 100$$

Where: ($n_i$) = the number of bunches with disease rating, ($v_i$) = disease score (0= no symptoms, 1= 1-10% infection on the bunch, 2= 11-20% infection on the bunch, 3= up to 20 % infection on the bunch), (N) = the total number of bunches investigated, (V) = the highest disease score (3).

Titratable acidity in untreated and treated Thompson Seedless grape berries was determined by titrating 10 ml of grape juice against 0.1N NaOH using phenolphthalein indicator. The percentage of acidity was calculated as malic acid according to the following equation of AOAC (1980):

$$\text{Acidity \%} = \frac{\text{ml of used NaOH} \times \text{N of NaOH} \times 0.064}{\text{sample volume of grape sample (ml)}}$$

Loss of grape bunches, weight was estimated in naturally infected grape bunches treated with certain salt.

**Experimental design and statistical analysis:** The experiments were adopted according to the complete randomized block design and the data were statically analyzed using the Fisher's LSD according to Gomez and Gomez (1983).

## Results and Discussion

**Comparative effectiveness of preharvest salt treatments on grape major fruit rot caused by *B. cinerea*:** Data in Table (1) mentioned that all tested salts significantly reduced disease severity of bunch rot disease caused by *Botrytis cinerea* during 2014 and 2015 growing seasons. In this respect, the highest percentage of reduction happened, when grape clusters sprayed with Potassium metabisulphate at 1 and 2 g/l. water, as reached 76.7 and 83.5%, respectively during 2014 season. Also, calcium carbonate and sodium bicarbonate gave moderate disease reduction when used at 2 g/l. water, wherever the percentage of disease reduction reached 66.9%, respectively during 2014 season. On the contrary, calcium carbonate with 1 and 2 g/l. water gave the highest disease reduction during 2015 growing season. The percentage of disease reduction reached it 72.1 and 80.1 %, relatively. While, potassium metabisulphate and sodium bicarbonate exhibited the moderate result in decreasing the disease reduction caused by *B. cinerea* during 2015 growing season. The lowest disease reduction was obtained by calcium chloride and calcium nitrite. It was clear from data in Table (2) that disease severity caused with *B. cinerea* significantly reduced on Thompson seedless grape during 2014 and 2015 growing seasons. Data exhibited that calcium carbonate at all concentrations tested was the most effective salt in reducing rot caused with *B. cinerea* compared with control, when applied as preharvest treatment during 2014 growing season. Also, calcium nitrite and calcium chloride were very effective at 1 and 2 g/l. water in reducing the disease severity .While potassium metabisulphate and sodium bicarbonate showed moderate effect during the same season. Also, data in Table (2) indicated that all tested salts significantly decreased the fungal infection of grapes rot during 2015 season if compared with control. However , calcium carbonate, calcium chloride and calcium nitrite at 1 or 2 g/l. water were the most effective salts, which decreased the naturally infection on Thompson seedless grapes. Potassium metabisulphate and sodium bicarbonate exhibited clear effect in reducing the disease severity with two tested concentrations as compared with the control during 2015 growing season. Different concentrations of five salts were evaluated for their effectiveness to control grape fruit rot caused *B. cinerea* during storage. Data presented in Table (3) indicated that all the tested salts reduced storage rot as compared with the control during 2014 and 2015 growing seasons. The highest effective salt in reducing disease incidence caused by *B. cinerea* during storage at $0^{o}$C and 90-95% RH for 36 days was calcium carbonate at 1 and 2 g/l. water as reached 77.3 and 82.7%, respectively, followed by sodium bicarbonate (80.0%) during 2014 season. Calcium chloride showed clear effect in controlling grape fruit rot if compared with the control.

Table 1: Effect of different salts on bunches rot disease caused by *Botrytis cinerea* under natural infection during 2014 and 2015 growing seasons.

| Treatments | Conc. g/l | Severity of infection (%) | | | |
|---|---|---|---|---|---|
| | | Season 2014 | | Season 2015 | |
| | | D.S.* | R.D.S.** | D.S. | R.D.S. |
| Calcium chloride | 1 | 7.1 | 46.6 | 7.3 | 34.2 |
| | 2 | 6.7 | 49.6 | 6.7 | 39.6 |
| Calcium nitrite | 1 | 5.3 | 60.2 | 7.1 | 360 |
| | 2 | 4.4 | 66.9 | 6.7 | 39.6 |
| Potassium metabisulfate | 1 | 3.1 | 76.7 | 4.9 | 55.9 |
| | 2 | 2.2 | 83.5 | 4.4 | 60.0 |
| Calcium carbonate | 1 | 4.9 | 63.2 | 3.1 | 72.1 |
| | 2 | 4.4 | 66.9 | 2.2 | 80.1 |
| Sodium bicarbonates | 1 | 4.7 | 64.7 | 5.1 | 54.1 |
| | 2 | 4.4 | 66.9 | 4.4 | 60.0 |
| Control | | 13.3 | -- | 11.1 | -- |
| L.S.D (0.05) | | | | | |
| Conc. | | 1.39 | | 1.19 | |
| Salt. | | 2.22 | | 1.89 | |
| C&S | | 2.89 | | 2.69 | |

* D.S. = Disease severity, ** R.D.S. = Reduction of Disease severity

Table 2: Effect of pre harvest spraying of Thompson seedless grapevines with certain salts on infection percentage of naturally infected during storage at 0°C and 90-95% RH for 36 days.

| Treatments | Conc. g/l | Severity of natural infection (%) | | | |
|---|---|---|---|---|---|
| | | Season 2014 | | Season 2015 | |
| | | Rot incidence | Efficacy | Rot incidence | Efficacy |
| Calcium chloride | 1 | 5 | 75 | 5 | 80 |
| | 2 | 3 | 85 | 2 | 92 |
| Calcium nitrite | 1 | 2 | 90 | 2 | 92 |
| | 2 | 4 | 80 | 3 | 88 |
| Potassium metabisulfate | 1 | 7 | 65 | 6 | 76 |
| | 2 | 5 | 75 | 4 | 84 |
| Calcium carbonate | 1 | 0.0 | 100 | 0.0 | 100 |
| | 2 | 2 | 90 | 3 | 88 |
| Sodium bicarbonates | 1 | 5 | 75 | 5 | 80 |
| | 2 | 7 | 65 | 6 | 76 |
| Control | | 20 | -- | 25 | -- |
| L.S.D (0.05) | | | | | |
| Conc. | | 1.3 | | 1.1 | |
| Salt. | | 2.0 | | 1.8 | |
| C&S | | 2.8 | | 2.6 | |

Table 3: Effect of pre harvest spraying of Thompson seedless grapevines with certain salts on infection percentage of artificially inculcated grapes with *Botrytis cinerea* during storage at 0°C and 90-95% RH for 36 days.

| Treatments | Conc. g/l | Severity of natural infection (%) | | | |
|---|---|---|---|---|---|
| | | Season 2014 | | Season 2015 | |
| | | Rot incidence | Efficacy | Rot incidence | Efficacy |
| Calcium chloride | 1 | 22 | 70.6 | 15 | 81.3 |
| | 2 | 20 | 73.3 | 10 | 87.5 |
| Calcium nitrite | 1 | 40 | 46.7 | 40 | 50.0 |
| | 2 | 50 | 33.3 | 50 | 37.5 |
| Potassium metabisulfate | 1 | 40 | 46.7 | 10 | 87.5 |
| | 2 | 30 | 60.0 | 7 | 91.3 |
| Calcium carbonate | 1 | 17 | 77.3 | 10 | 87.5 |
| | 2 | 13 | 82.7 | 15 | 81.3 |
| Sodium bicarbonates | 1 | 15 | 80.0 | 20 | 75.0 |
| | 2 | 25 | 66.7 | 30 | 62.5 |
| Control | | 75 | -- | 80 | -- |
| L.S.D (0.05) | | | | | |
| Conc. | | 3.2 | | 2.3 | |
| Salt. | | 4.0 | | 3.6 | |
| C&S | | 4.7 | | 4.1 | |

Calcium nitrite and potassium metabisulphate were less effective during 2014 season. On the converse, potassium metabisulphate followed by calcium chloride gave the most effective in reducing disease severity during 2015 season. The disease severity decreased with the increasing of salt concentrations. Calcium carbonate at 1 g/l. water was very effective salt against rot development during storage at 0°C and 90-95% RH for 36 days. Also, calcium carbonate at 2 g/l. water gave moderate effect, while, calcium nitrite was the less effective in reducing the disease incidence. Gray mold caused by *B. cinerea* is the main postharvest decay of table grapes. Some inorganic salts used in the food industry as preservartives and antimicrobial agents have been found suitable alternatives for synthetic fungicide in plant disease control. These salts have shown broad spectrum antimicrobial activity and are generally recognized as safe. Karabulut et al. (2003) found that baking sodium bicarbonate significantly reduced the total number of decayed grape berries caused by *B. cinerea*. Nigero et al. (2006) reported that several salts could reduce the growth of *B. cinerea* under lab. conditions but under greenhouse conditions, calcium chloride, sodium bicarbonate and sodium carbonate significantly reduced the incidence of grey mold on small table grape bunches. Their result related to effectiveness of sodium bicarbonate against *B. cinerea* is inagreement with our results, while their findings in regard of calcium chloride effectiveness against this fungus is not in favour of our results. However, there are some reports in contrary to our results, but more documents are available in favour of proving the antifungal efficacy of potassium and sodium salts.

Table 4: Effect of pre harvest spraying of Thompson seedless grapevines with certain salts on biochemical quality parameters stored 0°C and 90-95% RH for 36 days.

| Treatments | Conc. g/l | Quality characters of treated and untreated bunches (%) | | | |
|---|---|---|---|---|---|
| | | TSS | Acidity (TA) | TSS/TA ratio | Loss of weight |
| Calcium chloride | 1 | 25.0 | 0.645 | 38.75 | 3.2 |
| | 2 | 27.0 | 0.694 | 38.90 | 8 |
| Calcium nitrite | 1 | 26.0 | 0.686 | 37.90 | 6.4 |
| | 2 | 25.6 | 0.678 | 37.75 | 10 |
| Potassium metabisulfate | 1 | 26.8 | 0.690 | 38.84 | 7.4 |
| | 2 | 23.8 | 0.692 | 34.39 | 10 |
| Calcium carbonate | 1 | 27.2 | 0.688 | 39.53 | 9.8 |
| | 2 | 26.0 | 0.660 | 39.39 | 6 |
| Sodium bicarbonates | 1 | 27.4 | 0.680 | 40.29 | 18 |
| | 2 | 25.4 | 0.675 | 37.62 | 6.4 |
| Control | | 22.8 | 0.662 | 34.44 | 12.4 |

**Determination of total soluble solids, acidity and loss of weight in grape fruits treated with the chemical salts:** Total soluble solids, acidity and loss of weight of grape clusters treated and untreated Thompson seedless grape cultivar were estimated. Data in Table (4) indicated that there was an increase in total soluble solids, acidity and TSS/TA ratio contents in grape fruits treated with different salts, when compared with untreated fruits. However, fruits treated with calcium chloride at 1 g and calcium carbonate at 2 g /l. water gave the lowest level if compared with the control. On the converse, all the tested salts lead to decrease of fruit weight (%). The biggest decrease was obtained, when grape fruits were sprayed with calcium chloride at 1 g/l. water as reached 3.2% loss of weight compared with the control. Concerning the effect of spraying grape bunches with different chemical salts on biochemical changes with grape grey mold was included. Our results indicated that there was an increase in total soluble solids contents in grape fruits (Thompson seedless cv.) sprayed with different concentrations of chemical salts. Also, these results showed higher acid contents in grape fruits treated with the chemical salts and infected with *B. cinerea*. Also, spraying with the salts demonstrated higher levels in TSS/TA ratio than unspraying with the chemical salts. The increase in TSS, acidity and TSS/TA ratio contents in grape fruits treated with different concentrations of the tested salts could be attributed to their fungistatic effect on the tested pathogen and consequently accumulation of TSS, TA and TSS/TA ratio which were not consumed by the pathogen.

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