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Efficacy of alkaline and acidic electrolysed water generated by some salt solutions against gray mold of table grape: pre and postharvest applications

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Abstract

The objectives of the this research were to assess the activity of alkaline (alEW) and acidic electrolysed water (acEW), *in vitro* and *in vivo*, against the development of gray mold caused by *Botrytis cinerea* Pers. on table grape cv. Crimson seedless and to investigate these treatments on grape quality. Thirteen salt solutions (potassium bicarbonate, potassium sorbate, sodium silicate, EDTA-Fe, EDTA-Ca, sodium bicarbonate, sodium carbonate, potassium carbonate, potassium phosphate dibasic, potassium dihydrogen phosphate, ammonium molybdate, sodium metabisulphite, sodium chloride), most of them are generally recognized as safe (GRAS) compounds, were used to generate alEW and acEW. *In vitro* results showed that in alEW, sodium carbonate and potassium sorbate were the most effective salts reducing the percentage of colony forming units (CFUs) by 67.3 and 63.7%, respectively. In acEW, sodium carbonate, potassium bicarbonate, sodium silicate, potassium sorbate and EDTA-Fe were the most effective salts reducing CFUs by 88, 86, 85, 89 and 84%, respectively. Under artificial infection, at the end of cold storage, for alEW, the percentage of reduction ranged from 55-91.7% and potassium carbonate gave the best results against the grapes gray mold (91.7%). For acEW, the percentage of reduction ranged from 77-98% and ammonium molypdate gave the best results against gray mold (98%). Electrolysed water generated by potassium sorbate, sodium carbonate and sodium metabisulphite was effective against development of gray mold disease under natural infection. In most cases, the results of the present research showed that alEW and acEW did not influence the physicochemical properties of berry quality. This study confirms that electrolysed water is an effective treatment and has a good control activity against gray mold of table grape and it demonstrates that certain salts improve the electrolysis potential.

Keywords: grape, electrolysed water, gray mold, *Botrytis cinerea*, salts.

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Introduction

Postharvest losses of table grape could reach 30–40% and more in some developing countries such as Egypt due to pathological and physiological deterioration (Prigojin et al., 2005), due to improper handling and lack of proper methods to prevent decay and senescence (Prusky, 2011). Grape berries are susceptible to severe losses by gray mold caused by *Botrytis cinerea* after harvest. Fungicide resistance has been detected in *B. cinerea* exposed to fungicides applied for controlling gray mold in grape vineyards (Jacometti et al., 2010). Market and regulatory pressure regarding residues and concerns of environmental and human health are increasing, so new management techniques are needed to be adopted. Development of alternative means to control postharvest decays and to sanitize fresh products is derived by economic and consumer demand motivations (Feliziani et al., 2016; Mahajan et al., 2014). Various studies have documented the strong antimicrobial activity of electrolysis of water (Cui et al., 2009; Huang et al., 2008; Guentzel et al., 2008) against postharvest diseases of several commodities such as peaches, pears and citrus (Fallanaj et al., 2011; Guentzel et al., 2010; Al Haq et al., 2002). Most previous studied have assessed electrolysing parameters (flow rate, current intensity and time for electrolysing) effect on free chlorine, electric conductivity and pH of the resulted electrolysed water (Guentzel et al., 2010; Abbasi & Lazarovits 2006; Hsu 2003), while very few account have studied the effect of electrolysing parameters on the ability of electrolysed water to deactivate pathogen unit (Fallanaj et al., 2015). While salt solutions used in electrolysing play a major role in the efficacy of electrolysed water, few number of salts were studied for their effect on electrolysed water efficiency; sodium chloride (Khayankarn et al., 2014; Whangchai et al., 2013; Guentzel et al., 2010; Abbasi & Lazarovits, 2006; Hsu, 2003); potassium chloride (Yaseen et al., 2013) and sodium bicarbonate (Fallanaj et al., 2016). During the last seven years, although our experiments performed on grape clearly demonstrate the advantage of preharvest application of alternative control means such natural salts (Youssef & Roberto, 2014a,b), generally, farmers are not interested in the storage life of products once fruit are sold on the plant. In this regard, an integrated control approach along the whole production chain is necessary to obtain a good quality of grape. The objectives of the present research were: (i) Evaluate the efficacy of electrolysed water generated by different salt solutions *in vitro* against *B. cinerea*; (ii) Assess the efficiency of the treatments against gray mold under artificial infection on table grapes; (iii) Study the treatments as preharvest applications (spray in the field), postharvest applications (spray after harvest) and the combined pre and postharvest applications against natural infection; (iv) Study the effect of treatments on grape quality profile.

Materials and methods

Isolation and inoculum preparation of *B. cinerea*: *B. cinerea* was isolated from red grapes cv. Crimson seedless showing typical symptoms of gray mold disease collected from orchard located at El Hamam city in Matrouh Governorate, Egypt and identified according to the morphological and cultural characteristics (Barnett & Hunter, 1986).

The spore counts were made using Thoma counting chamber (Tiefe 0.100 mm, 1/400 qmm, Lutzellinden, Germany) and the suspension was diluted with sterile distilled water to obtain final concentration of 10^5 conidia ml^{-1}.

***In vitro* assessment of salt solutions alone on viability of *B. cinerea*:** Thirteen different salts (Table 1) were tested at 1% concentration for their efficacy on vitality of *B. cinerea* spores. The conidial suspension (100 µl of *B. cinerea* at initial concentration of 10^5 conidia ml^{-1}) was added to 900 µl of each salt solution and incubated for 2 min at room temperature. In order to keep the number of colonies expected to grow on the 9 cm Petri dishes at a countable limit, a 10-fold dilution was made after contact time for each treatment by mixing 100 µl of the conidial suspension in salt solution with 900 µl of sterilized distilled water. Volumes of 100 µl from the mixture were spread on PDA amended with 250 mg l^{-1} of ampicillin and 250 mg l^{-1} of streptomycin on 9 cm Petri dishes. Distilled water was used as a control. Each treatment contains five PDA plates as replicates and the entire experiment was repeated twice. The plates were incubated at 24±1°C and colony forming units (CFU) were recorded after 3-4 days. The percentage of reduction was determined according to the following formula: Percentage of colony reduction = (CFU in salt treatment – CFU in distilled water) / CFU in distilled water x 100.

Effect of water flow rate and electric current intensity on electrolysis efficiency against *B. cinerea*: To evaluate the effect of different current intensities on electrolysed water parameters, the water flow rate was fixed whereas the current intensities were changed. While for testing the effect of water flow rates, the current intensities were fixed and water flow rates were manipulated. Water supply pressure was maintained within 0.1-0.75 Mpa by using the pressure regulation valve. Three levels of water flow rate (low = 3 l/min, medium = 4 l/min and high = 6 l/min) and three different amperes (5, 9 and 13A) were investigated. The pathogen suspensions were freshly prepared as described before and added to the electrolysed water at final concentration of 10^5 CFU ml^{-1} and then incubated for 2 min at room temperature. The *in vitro* tests were carried out as describe above. The results were expressed as percentage of colony reduction.

***In vitro* assessment of the effect of alEW and acEW generated by certain salt solutions on *B. cinerea*:** Electrolysed water was generated by ROX-10 WBE (Hoshizaki Electric Co., LTD.) machine which produces both acidic electrolysed water (acEW) and alkaline electrolysed water (alEW) separately, and salt solutions were used to improve the electrolysing process (10% NaCl salt solution stock as recommended by the manufacturer). The concentrated salt solution stock is directly injected into the water flow in 1:10 (salt solution: water) dilution rate. Built-in flow rate adjusting valve automatically controls the flow rate according to the selection (three levels are available: low, medium and high). The separation membrane in this electrolysis machine is bipolar membrane composed of vinylidinepolyflouride. The acEW or alEW were produced using water flow rate (high =6 l/min) and current intensity (13A). Salt solutions (Table 1) and tap water from the laboratory supply line were simultaneously introduced into the equipment to generate electrolysed water as recommended by the manufacturer. The acEW and alEW were collected from their respective outlets in separate flasks just before the experiment. Volumes of 100 µl of *B. cinerea* spores were suspended in sterilised water to prepare suspension with concentration of 10^5 conidia ml^{-1}. The conidial suspension was added to the acEW or alEW for each salt and incubated for 2 min at room temperature and then diluted and spread on PDA plates. Each treatment contains five PDA plates as replicates. The plates were incubated at 24±1°C and then CFU was determined after 3-4 days and percentage of colony reduction was determined according to the above formula.

Assess the efficiency of the treatments against gray mold under artificial infection on grapes: The effect of electrolysed water generated by thirteen salt solutions on gray mold was tested *in vivo* under artificial inoculation. Healthy table grape cv. Crimson seedless bunches were harvested at commercial maturity from orchard located at El Hamam city in Matrouh Governorate, Egypt. Bunches were selected and immersed in 1% sodium hypochlorite for 2 min to sterilize the surface and rinsed with sterile distilled water and air-dried. Grape bunches were sprayed with acEW or alEW generated by each salt or sterile distilled water, as a control, until dripping and left to dry. After 1 h, bunches were sprayed with a conidial suspension of the pathogen (10^6 conidia ml^{-1}) (50ml/10kg of grapes). Treated bunches were arranged in plastic boxes, covered with plastic bag and stored at 2±1°C for one month followed by one week of shelf-life at 22±2°C and high relative humidity (Youssef & Roberto, 2014a). A completely randomized design, including three boxes as replicates (each contain five small bunches) for each treatment, was used. The percentage of gray mold incidence (number of rotted berries/total number of berries x 100) was recorded at the end of cold storage and after one week of shelf-life.

Study the efficacy of certain treatments individually and in combination as pre and postharvest against natural infection of grape gray mold disease: Electrolysed water generated by different salt solutions was examined against naturally occurring infection of gray mold. The application methods were pre-harvest treatment, pre and postharvest treatments and postharvest application after harvest. For preharvest treatments, the experiments were carried out on 6-year-old table grapes cv. Crimson seedless in a commercial vineyard located in El Hamam city in Matrouh Governorate, Egypt. Experiments were arranged in a completely randomized design and then grape bunches were treated. Electrolysed salt solutions were sprayed one week before harvest using a compressed plastic air sprayer. Grapes were harvested and placed into covered carton boxes (3 boxes per plant, nine boxes per treatment). Grapes were stored for 6 weeks at 2±1°C and high relative humidity, followed by one week of shelf-life at 22 ±2°C. For pre and postharvest treatment, grapes from vines treated in the field were placed in nine carton boxes for each treatment and were transferred to the laboratory. Then, grapes from each group were sprayed by the same electrolysed salt solutions used in the field. After treatment, grapes were stored as described for field treatments. For postharvest trials, grapes harvested from untreated vines were pooled and arranged in nine boxes per treatment in a completely randomized experimental design. The procedure was the same described for combined treatments. The percentage of gray mold incidence (number of rotted berries/total number of berries x 100) was recorded at the end of cold storage and after one week of shelf life.

Determinate the effect of certain treatments on grape quality measurement parameters: Weight loss during postharvest storage was calculated by dividing the weight change during storage by the original weight.

$$\text{Weight loss (\%)} = Wi - Ws/ Wi \quad \times 100$$

Where Wi= initial weight and Ws= weight at examined time.

To assess grape color, total soluble solids (TSS) and titratable acidity (TA), 30 grape berries were collected from each treatment at the end of cold storage. Grape color was measured by a model colorimeter (color reader CR-10, Konica Minolta Inc.), obtaining the following variables from the equatorial portion of the berries: L^* (luminosity); C^* (saturation) h° (hue angle). The color index for red grapes (CIRG) was determined using the formula: CIRG = $(180 - h^\circ)/(L^* + C^*)$ (Carre˜no & Martinez, 1995). TSS was determined using a digital refractometer (Atago PAL-S). The pH of grape juice was recorded using Adwa Professional pH-ORP-Conductivity TDS temp Bench Meter with GLP model AD8000 and then TA was determined by potentiometric titration with 0.1 N NaOH up to pH 8.2, using 10 ml of diluted juice in 40 ml distilled water.

Statistical Analysis: All results were introduced to analysis of variance using Statistica 6.0 software. Mean values of treatments were compared by using Fisher's protected LSD test and judged at $P \leq 0.05$ level. Percentage data were arcsine transformed to normalize variance.

Table 1: List of different salts used to generate alkaline and acidic electrolysed water.

Salts	Chemical formula	Manufacturer
Potassium bicarbonate	$KHCO_3$	Certis
Potassium sorbate	$C_6H_7KO_2$	Vetec
Sodium silicate	Na_2SiO_3	Vetec
EDTA-Fe	$C_{10}H_{12}N_2O_8FeNa.3H_2O$	Synth
EDTA-Ca	$C_6H_{12}N_2O_8CaNa.2H_2O$	Vetec
Sodium bicarbonate	$NaHCO_3$	El Gomhouria
Sodium carbonate	Na_2CO_3	Adwic
Potassium carbonate	K_2CO_3	Adwic
Potassium phosphate dibasic	K_2HPO_4	Adwic
Potassium dihydrogen phosphate	KH_2PO_4	Adwic
Ammonium molybdate	$(NH_4)Mo_7O_{24}.4H_2O$	Roth
Sodium metabisulphite	$Na_2S_2O_5$	Roth
Sodium chloride	NaCl	Adwic

Results

***In vitro* assessment of certain salt solutions on viability of *B. cinerea*:** The percentage of reduction of germinated Botrytis spores when immersed in different salts was variable and ranged from 12.37-49.69%. Sodium carbonate, potassium sorbate, sodium metabisulphite, ammonium molybdate and sodium bicarbonate reduced the percentage of colony forming units by 49.7, 47.4, 35.4, 35.4 and 34.4%, respectively (Table 2).

Effect of water flow rate and electric current intensity on electrolysis efficiency against *B. cinerea*: All types of parameters used in the electrolysis machine to produced electrolysed alkaline water have shown significant effect on *B. cinerea* compared to distilled water and salt solution (10% NaCl). Electrolysing at Hi flow rate and current intensity 13 A has shown the most significant effect in reducing pathogen population count. The percentage of reduction was 86.8% as compared to distilled water (Table 3). Acidic electrolysed water has shown significant reduction in *B. cinerea* count. As was highlighted in alkaline water, electrolysed water at Hi flow rate and current intensity 13 A has shown the most significant reduction in pathogen population taking them down to zero. The percentage of reduction was 100% as compared to control (Table 3).

Table 2: The reduction percentages of germinated *B. cinerea* spores determined after treatment with different salts alone (1%), alkaline EW and acidic EW.

Treatment	Percentage of colony reduction (%)		
	Salt only	Alkaline	Acidic EW
Sodium metabisulphite	35.43	43.80	58.12
Ca chelate	30.40	48.29	55.13
Sodium carbonate	49.69	67.30	88.89
Potassium dihydrogen phosphate	24.74	24.78	27.56
Potassium bicarbonate	31.24	34.82	86.32
Sodium silicate	26.21	28.20	85.25
Potassium sorbate	47.38	63.67	89.10
EDTA-Fe	13.63	24.14	84.40
Sodium bicarbonate	34.38	40.17	52.99
Potassium carbonate	32.29	48.93	58.97
Potassium phosphate dibasic	12.37	18.16	29.70
Ammonium molybdate	35.43	35.47	46.79
Sodium chloride	16.56	73.50	87.60

***In vitro* assessment of the effect of acEW or alEW generated by different salt solutions on the viability of *B. cinerea* spores:** The evaluation of the effect of 13 salt solutions combined with alkaline and acidic electrolysed water on spore suspension of *B. cinerea* has revealed general reduction in viability of the pathogens spore. In alEW, the percentage of colony reduction ranged from 18-73.5%. In particular, sodium carbonate and potassium sorbate were the most effective salts reducing the percentage of CFUs by 67.3 and 63.7%, respectively (Table 2). In acEW, the percentage of colony reduction ranged from 27-89%. Sodium carbonate, potassium bicarbonate, sodium silicate, potassium sorbate and EDTA-Fe were the most effective salts reducing Botrytis colonies by 88, 86, 85, 89 and 84%, respectively (Table 2).

Table 3: Effect of alkaline and acidic electrolysed water produced by electrolysis machine different parameters (electric current intensity and water flow rate) on *B. cinerea in vitro*.

Treatment		Percentage of colonies	
Electric current intensity	Water flow rate	Alkaline EW	Acidic EW
5	Low	25.85	93.43
9	Low	28.39	93.85
13	Low	42.37	94.27
5	Mid	41.10	93.85
9	Mid	32.20	95.34
13	Mid	34.53	95.33
5	Hi	24.15	96.39
9	Hi	46.61	97.03
13	Hi	86.86	100
NaCl without electrolysis		16.10	

Assess the efficiency of the treatments against gray mold under artificial infection on grapes: At the end of cold storage, for alEW, the percentage of reduction ranged from 55-91.7% and potassium carbonate gave the best results against the grapes gray mold (91.7%). For acEW, the percentage of reduction ranged from 77-98% and ammonium molypdate gave the best results against the gray mold rot (98%) (Fig. 1). After one week of shelf life, for alEW, the percentage of reduction ranged from 69-88%. Sodium metabisulphite, potassium dihydrogen phosphate, potassium bicarbonate, sodium carbonate and potassium phosphate dibasic showed the best activity against grapes gray mold (88, 88.3, 87.3, 86.8 and 85.9%). For acEW, the percentage of reduction ranged from 79-96%. Sodium carbonate, potassium sorbate, sodium metabisulphite and potassium phosphate dibasic were the most effective treatment reducing the percentage grapes gray mold (96, 91.6, 90.6 and 90.6%) (Fig. 2). In all cases, no surface injury was observed on the berries after the treatment.

Efficacy of the treatments as preharvest and postharvest applications and the combined pre and postharvest applications against natural infection: Under natural infection, electrolysed water showed a variable activity against the decay. At the end of cold storage, for alEW, as a preharvest treatment, the percentage of reduction ranged from 15-76% and sodium carbonate gave the best results against the rot (76%). For pre and postharvest treatment, the percentage of reduction ranged from 49-87% and potassium sorbate gave the best results against the disease (87%). When the treatments applied only after harvest, the percentage of reduction ranged from 44-71% and potassium sorbate gave the best result against the rot (71%) (Fig. 3). For acEW, as a preharvest treatment, the percentage of reduction ranged from 39-95% being sodium metabisulphite the best treatment against the rot (95%). For pre and postharvest treatment, the percentage of reduction ranged from 55-97% and sodium metabisulphite and potassium sorbate gave the best results against the gray mold (97% each). When the treatments applied only after harvest, the percentage of reduction ranged from 40-91% and potassium sorbate gave the best results against the rot (91%) (Fig. 4).

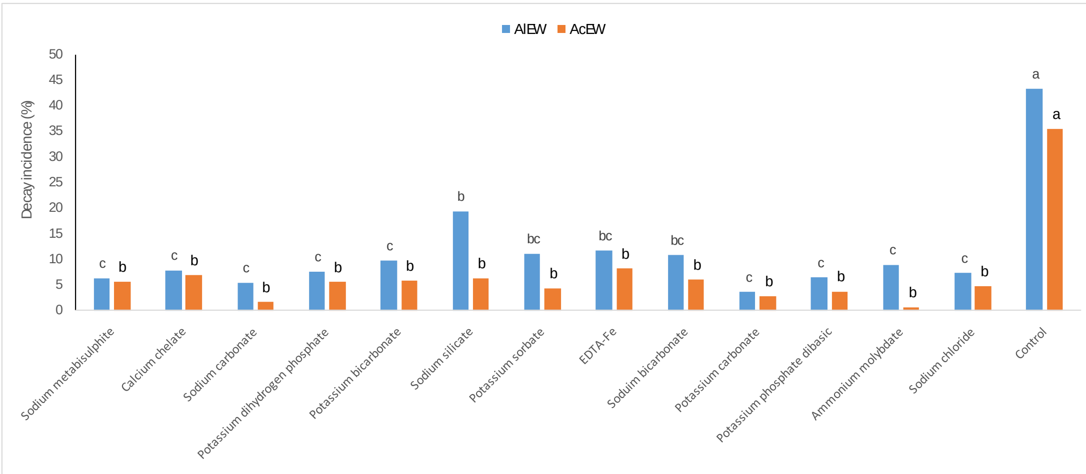

Figure 1: Effect of alkaline and acidic electrolysed water generated by different salt solutions on the development of gray mold on table grape cv. Crimson seedless bunches artificially inoculated with *B. cinerea* (10^6 conidia ml^{-1}) assessed at the end of cold storage at 2±1°C. Columns followed by different letters are statistically different according to Fisher's protected least significant difference (LSD) ($P \leq 0.05$).

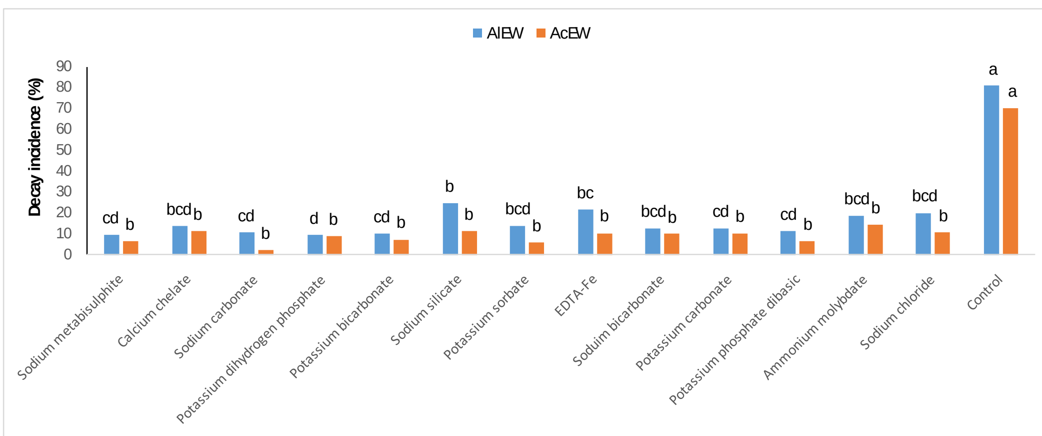

Figure 2: Effect of alkaline and acidic electrolysed water generated by different salt solutions on the development of gray mold on table grape cv. Crimson seedless bunches artificially inoculated with *B. cinerea* (10^6 conidia ml^{-1}). Grape bunches were cold stored one month at 2±1 ◦C followed by one week of shelf life at 22±2 ◦C. Columns followed by different letters are statistically different according to Fisher's protected least significant difference (LSD) ($P \leq 0.05$).

After 6 weeks of cold storage followed by one week of shelf life, for alEW, as a preharvest treatment, the percentage of reduction ranged from 7-71% and sodium carbonate gave the best results against the rot (71%). For pre and postharvest treatment, the percentage of reduction ranged from 40-78% and potassium sorbate gave the best results against the disease (78%). When the treatments applied only after harvest, the percentage of reduction ranged from 39-68% and potassium sorbate gave the best results against the rot (68%) (Fig. 5). For acEW, as a preharvest treatment, the percentage of reduction ranged from 32-83% being potassium sorbate the best treatment against the rot (83%) while potassium phosphate dibasic showed the lowest activity (32%). For pre and postharvest treatment, the percentage of reduction ranged from 61-91% and potassium sorbate gave the best results against the gray mold (91%). When the treatments applied only after harvest, the percentage of reduction ranged from 50-75% and potassium sorbate gave the best results against the rot (75%) (Fig. 6). In most cases, EDTA-Fe showed the lowest activity against gray mold while potassium sorbate was the best treatment against the decay.

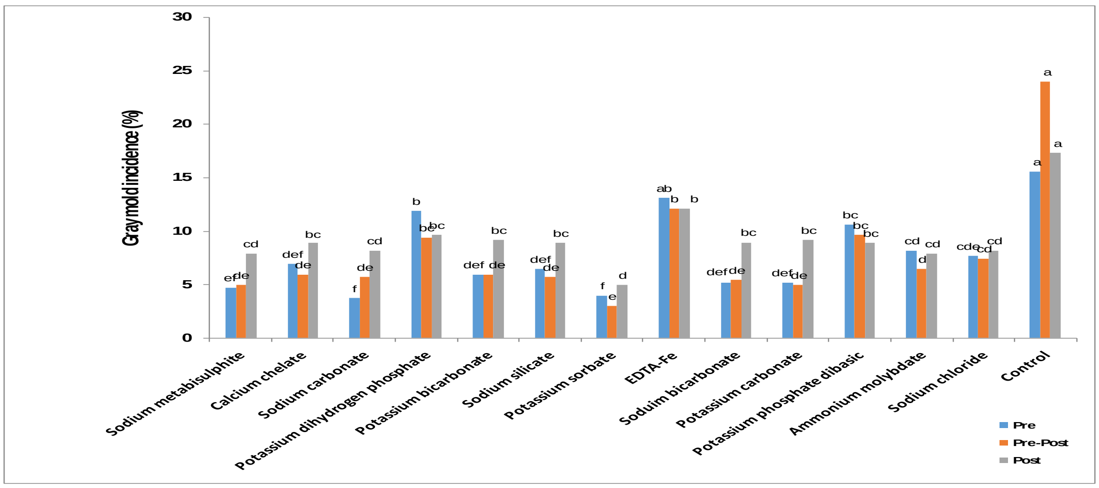

Figure 3: Effect of alkaline electrolysed water generated by different salt solutions on the development of gray mold on table grape cv. Crimson seedless bunches under natural infection assessed at the end of cold storage at 2±1°C. Treatments were applied before harvest, after harvest and double treatments. Columns followed by different letters are statistically different according to Fisher's protected least significant difference (LSD) ($P \leq 0.05$).

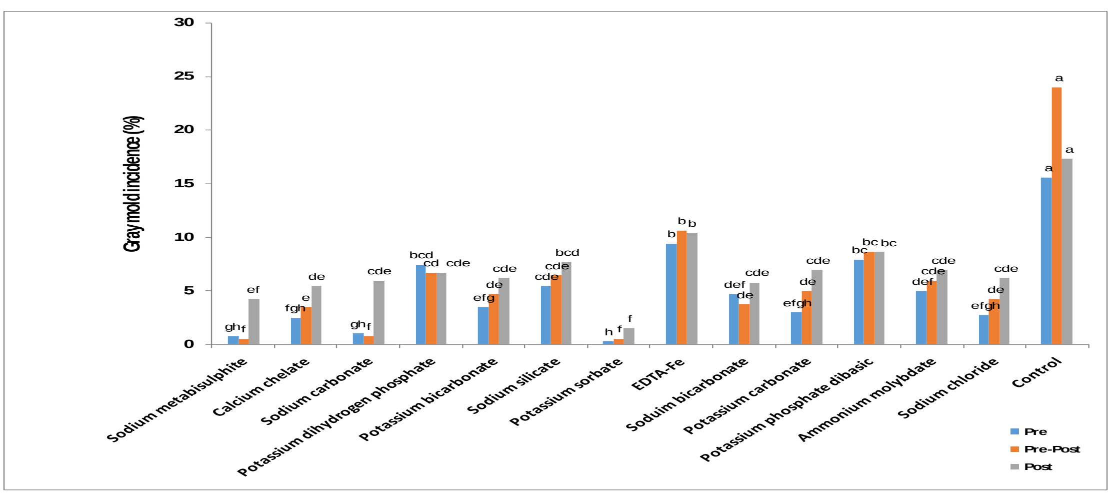

Figure 4: Effect of acidic electrolysed water generated by different salt solutions on the development of gray mold on table grape cv. Crimson seedless bunches under natural infection assessed at the end of cold storage at 2±1°C. Treatments were applied before harvest, after harvest and double treatments. Columns followed by different letters are statistically different according to Fisher's protected least significant difference (LSD) ($P \leq 0.05$).

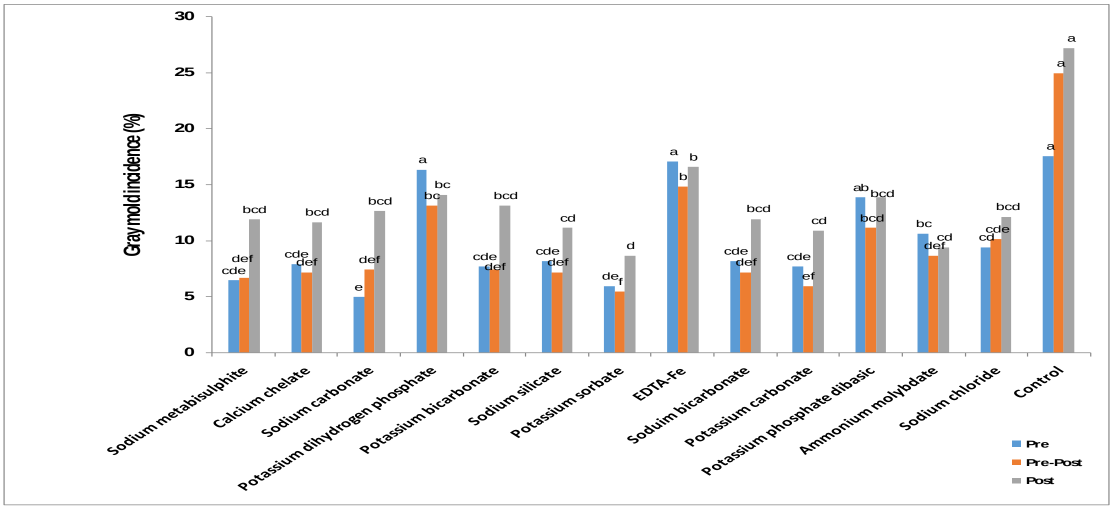

Figure 5: Effect of alkaline electrolysed water generated by different salt solutions on the development of gray mold on table grape cv. Crimson seedless bunches under natural infection assessed at the end of cold storage at 2±1°C followed by one week of shelf life at 22±2 ◦C. Treatments were applied before harvest, after harvest and double treatments. Columns followed by different letters are statistically different according to Fisher's protected least significant difference (LSD) ($P \leq 0.05$).

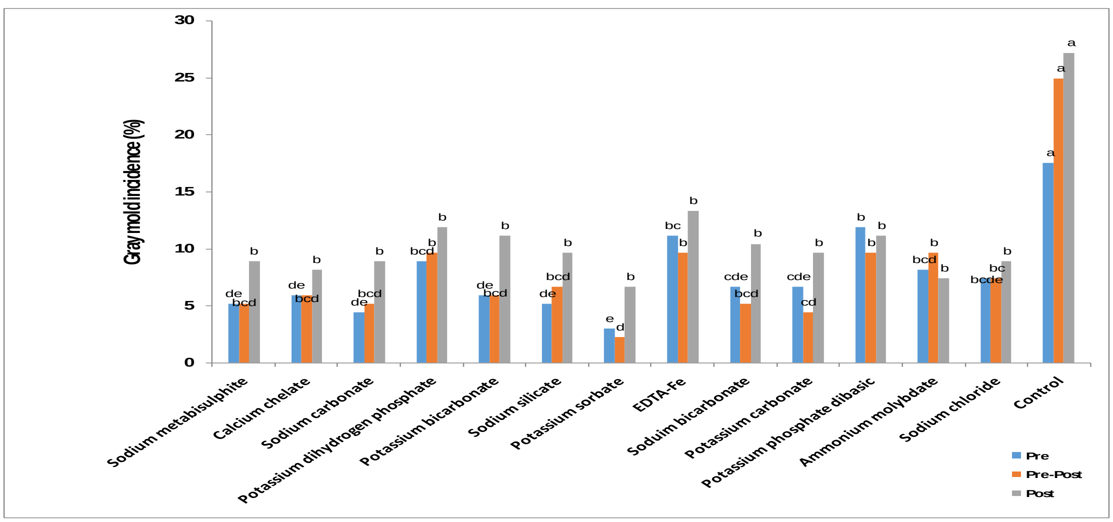

Figure 6: Effect of acidic electrolysed water generated by different salt solutions on the development of gray mold on table grape cv. Crimson seedless bunches under natural infection assessed at the end of cold storage at 2±1°C followed by one week of shelf life at 22±2 ◦C. Treatments were applied before harvest, after harvest and double treatments. Columns followed by different letters are statistically different according to Fisher's protected least significant difference (LSD) ($P \leq 0.05$).

Study the effect of these treatments on physical and chemical properties for grape quality measurement parameters: The percentage of weight reduction is shown in (Fig. 7-8). alEW and acEW did not influence weight reduction and no statistical differences were found among all treatments as compared to the control. Regarding TSS, no statistical differences were found among all treatments as compared to the control (Fig. 9-10). For TA, no statistical differences were found also among all treatments as compared to the control except for alEW generated by EDTA-Fe when applied as preharvest treatment (Fig. 11-12). Concerning grape color, in all cases no significant differences were observed among the treatments as compared to the control except for acEW generated by ammonium molybdate when applied before harvest and the color index was decreased by 18.3% (Fig. 13-14).

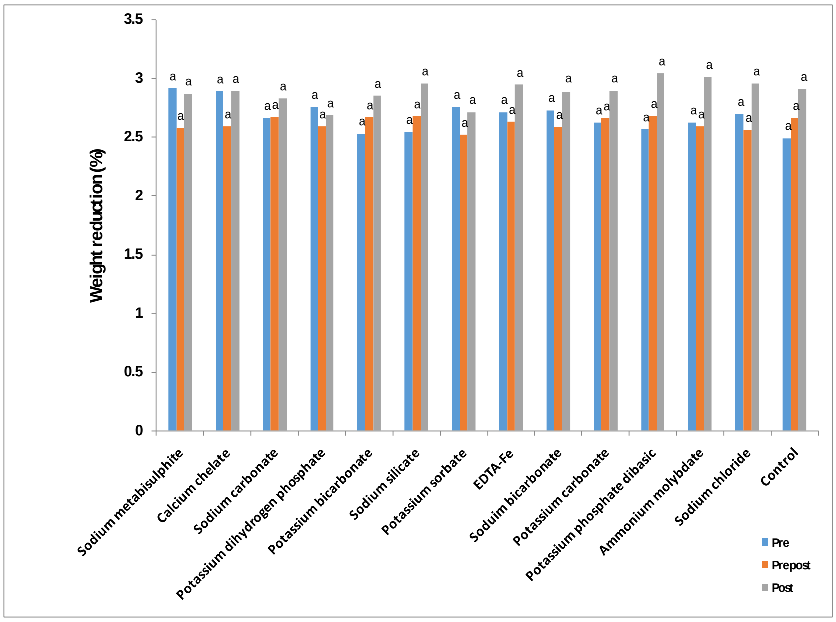

Figure 7: Weight reduction percentages of table grape cv. Crimson seedless treated with alkaline electrolysed water at the end of cold storage at 2±1◦C. Columns followed by different letters are statistically different according to Fisher's protected least significant difference (LSD) (P ≤ 0.05).

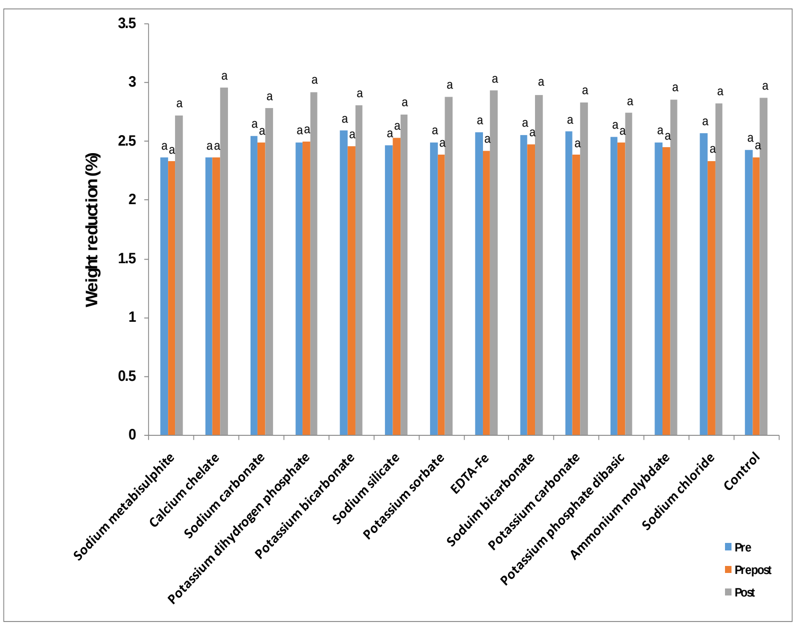

Figure 8: Weight reduction percentages of table grape cv. Crimson seedless treated with acidic electrolysed water at the end of cold storage at 2±1◦C. Columns followed by different letters are statistically different according to Fisher's protected least significant difference (LSD) (P ≤ 0.05).

Discussion

The objectives of the this research were to assess the activity of both alkaline and acidic electrolysed water, *in vitro* and *in vivo*, against the development of gray mold caused by *B. cinerea* on table grape cv. Crimson seedless and to investigate these treatment on the grape quality. The *in vitro* tests showed that the percentage of reduction of germinated spores when immersed in different tested salt solutions without electrolysing was variable and ranged from 12.4-49.7%.

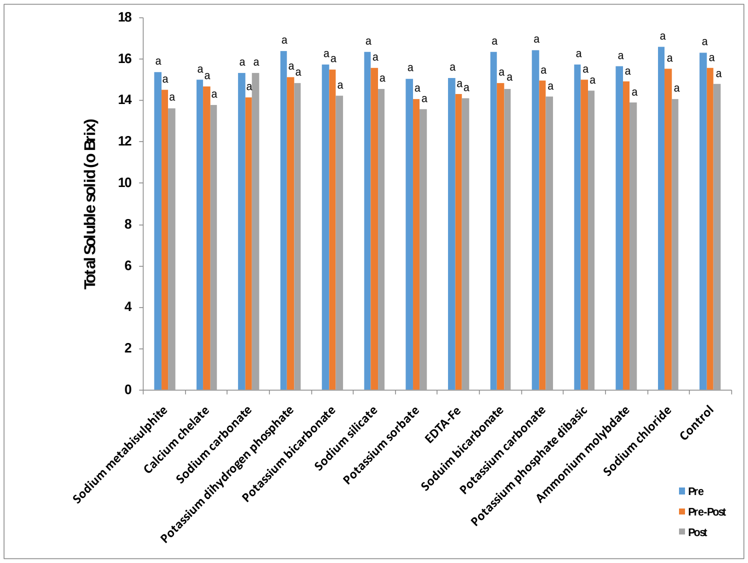

Figure 9: Total soluble solids of table grape cv. Crimson seedless treated with alkaline electrolysed water at the end of cold storage at 2±1◦C. Columns followed by different letters are statistically different according to Fisher's protected least significant difference (LSD) ($P \leq 0.05$).

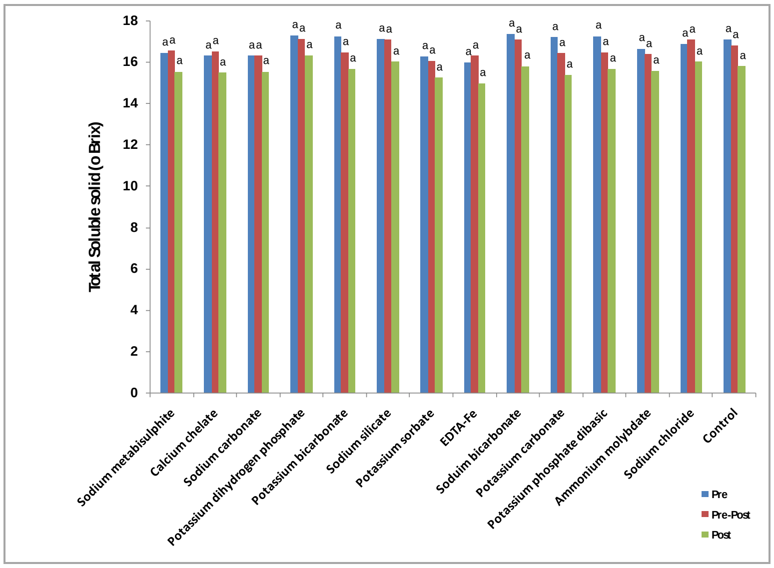

Figure 10: Total soluble solids of table grape cv. Crimson seedless treated with acidic electrolysed water at the end of cold storage at 2±1◦C. Columns followed by different letters are statistically different according to Fisher's protected least significant difference (LSD) ($P \leq 0.05$).

Several studies have dealt with the use of different salt solutions to control gray mold of table grape with satisfactory results (Youssef & Roberto, 2014a,b; Romanazzi et al., 2012; Nigro et al., 2006). Regarding the different machine parameters, the results showed that electrolysing at Hi flow rate and current intensity 13 A has shown the most significant effect in reducing *B. cinerea* population count. The percentage of reduction was 86.8% and 100% for alkaline and acidic electrolysed water, respectively as compared to control. Those parameters were used for all the experiments carried out in this research. Physiochemical characteristics of electrolysed water have been shown to greatly influence its antimicrobial activity (Fallanaj et al., 2015; Forghani et al., 2015; Whangchai et al., 2013; Rahman et al., 2012; Hsu, 2005). After setting the electrolysis parameters, 13 salt solutions were used to generate alkaline and acidic electrolysed water and investigated against spore suspension of *B. cinerea* and have revealed general reduction in viability of the pathogen spores. In alkaline water, the percentage of colony reduction ranged from 18-73.5% as compared to control. In acidic electrolysis water, the percentage of colony reduction ranged from 27-89%. Nevertheless, the use of sodium chloride as electrolyte may pose problems for processing equipments, and the efficacy of chlorine has been questioned after the emergence of tolerant pathogens in fresh-cut vegetable industry (Singh et al., 2002).

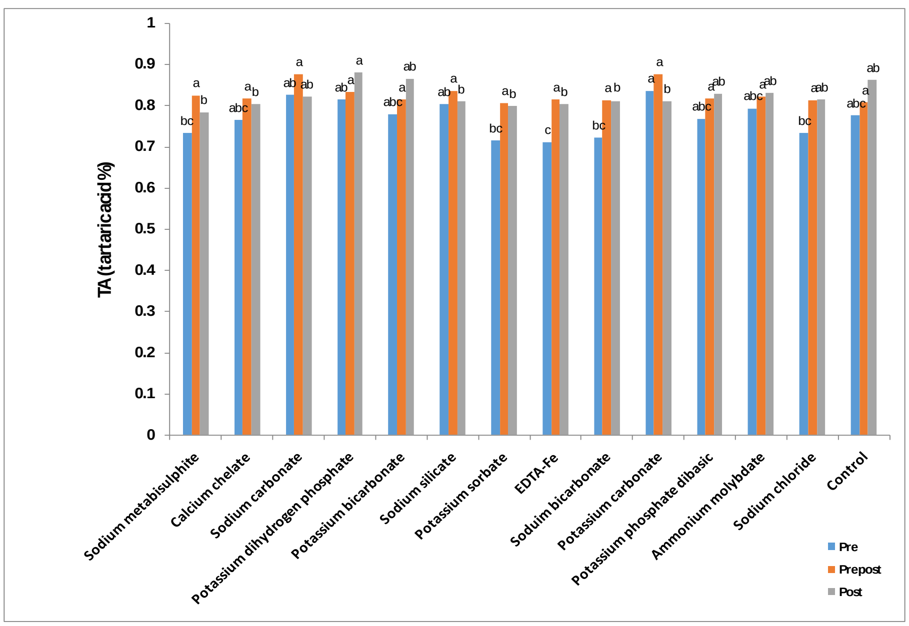

Figure 11: Titratable acidity of table grape cv. Crimson seedless treated with alkaline electrolysed water at the end of cold storage at 2±1◦C. Columns followed by different letters are statistically different according to Fisher's protected least significant difference (LSD) ($P \leq 0.05$).

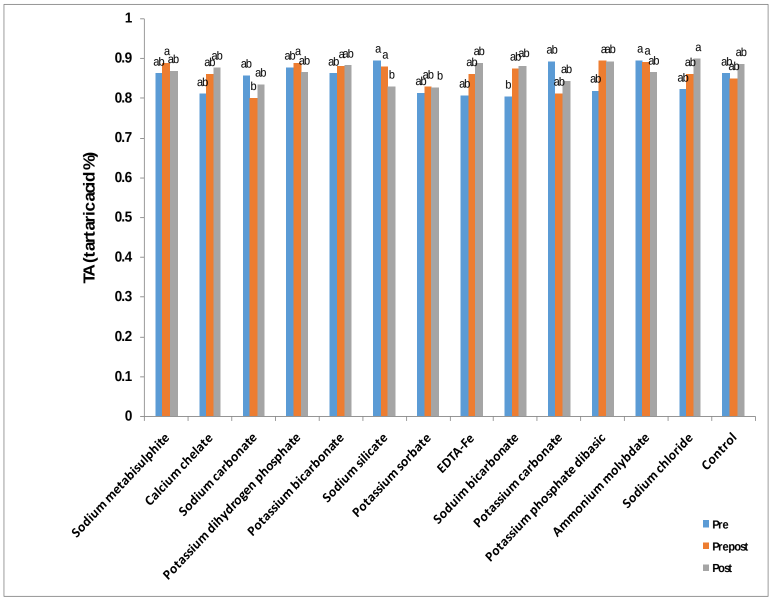

Figure 12: Titratable acidity of table grape cv. Crimson seedless treated with acidic electrolysed water at the end of cold storage at 2±1◦C. Columns followed by different letters are statistically different according to Fisher's protected least significant difference (LSD) (P ≤ 0.05).

In addition, in most of the previous studies, electrolysis was performed by adding sodium chloride as electrolyte, with the consequent formation of free chlorine and chlorinated organic compounds including chloramines (NH2Cl), dichloramines (NHCl2), and trichloromethanes (NCl3), which are respiratory irritants suspected to be carcinogenic (Fallanaj et al., 2013).

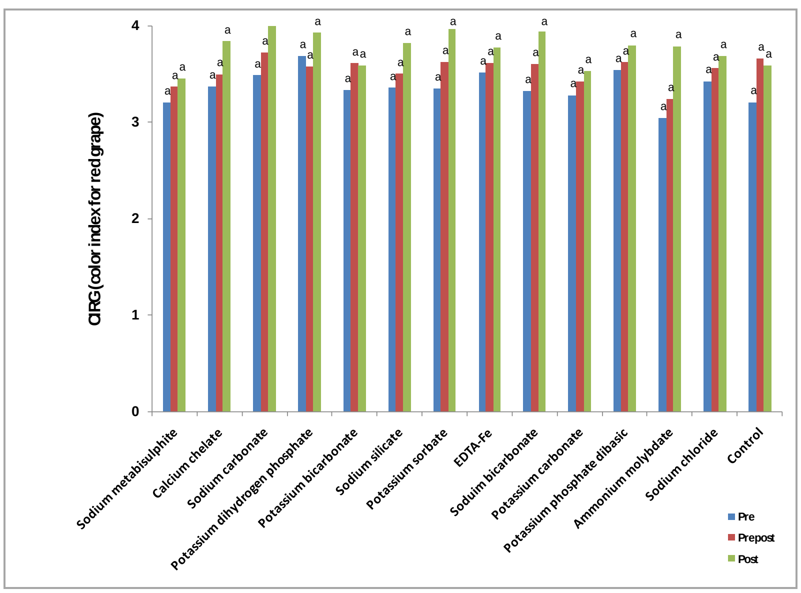

Figure 13: Color index of red table grape cv. Crimson seedless treated with alkaline electrolysed water at the end of cold storage at 2±1◦C. Columns followed by different letters are statistically different according to Fisher's protected least significant difference (LSD) (P ≤ 0.05).

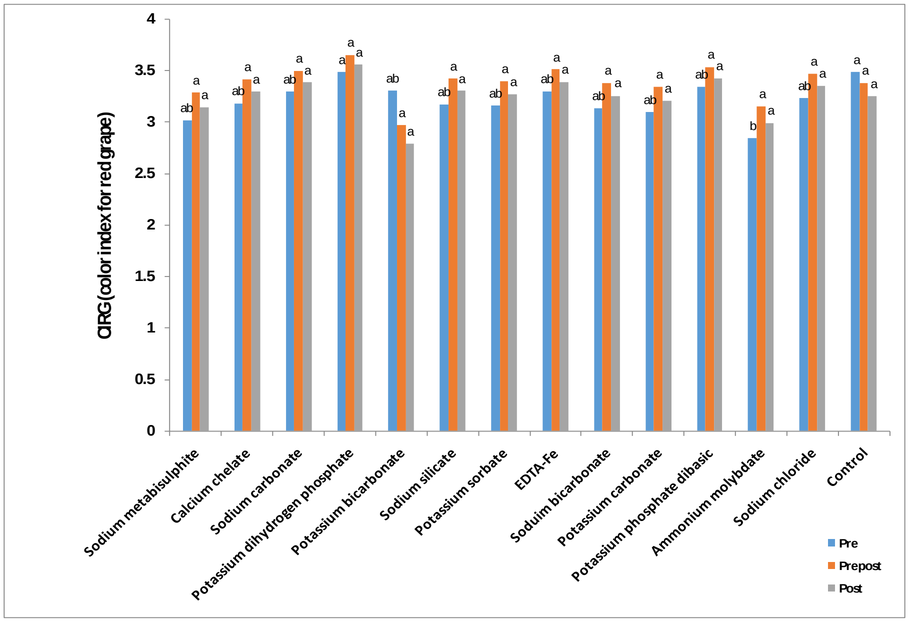

Figure 14: Color index of red table grape cv. Crimson seedless treated with acidic electrolysed water at the end of cold storage at 2±1◦C. Columns followed by different letters are statistically different according to Fisher's protected least significant difference (LSD) ($P \leq 0.05$).

The results obtained herein from artificial inoculation showed a variable activity of electrolysed water against the pathogen. At the end of cold storage, for alkaline electrolysed water, the percentage of reduction ranged from 55-91.7% and potassium carbonate gave the best results against the gray mold. For acidic electrolysed water, the percentage of reduction ranged from 77-98% and ammonium molypdate gave the best results against the rot. After one week of shelf life, for alkaline electrolysed water, the percentage of reduction ranged from 69-88%. For acidic electrolysed water, the percentage of reduction ranged from 79-96%. Several studies have dealt with the use of different salt solutions to control gray mold of table grape with satisfactory results (Youssef & Roberto, 2014a,b; Romanazzi et al., 2012; Nigro et al., 2006). Regarding the different machine parameters, the results showed that electrolysing at Hi flow rate and current intensity 13 A has shown the most significant effect in reducing *B. cinerea* population count. The percentage of reduction was 86.8% and 100% for alkaline and acidic electrolysed water, respectively as compared to control. Those parameters were used for all the experiments carried out in this research. Physiochemical characteristics of electrolysed water have been shown to greatly influence its antimicrobial activity (Fallanaj et al., 2015; Forghani et al., 2015; Whangchai et al., 2013; Rahman et al., 2012; Hsu, 2005). After setting the electrolysis parameters, 13 salt solutions were used to generate alkaline and acidic electrolysed water and investigated against spore suspension of *B. cinerea* and have revealed general reduction in viability of the pathogen spores. In alkaline water, the percentage of colony reduction ranged from 18-73.5% as compared to control. In acidic electrolysis water, the percentage of colony reduction ranged from 27-89%. Nevertheless, the use of sodium chloride as electrolyte may pose problems for processing equipments, and the efficacy of chlorine has been questioned after the emergence of tolerant pathogens in fresh-cut vegetable industry (Singh et al., 2002). In addition, in most of the previous studies, electrolysis was performed by adding sodium chloride as electrolyte, with the consequent formation of free chlorine and chlorinated organic compounds including chloramines (NH_2Cl), dichloramines ($NHCl_2$), and trichloromethanes (NCl_3), which are respiratory irritants suspected to be carcinogenic (Fallanaj et al., 2013). The results obtained herein from artificial inoculation showed a variable activity of electrolysed water against the pathogen. At the end of cold storage, for alkaline electrolysed water, the percentage of reduction ranged from 55-91.7% and potassium carbonate gave the best results against the gray mold. For acidic electrolysed water, the percentage of reduction ranged from 77-98% and ammonium molypdate gave the best results against the rot. After one week of shelf life, for alkaline electrolysed water, the percentage of reduction ranged from 69-88%. For acidic electrolysed water, the percentage of reduction ranged from 79-96%. Its well known that electrolysed water shows antimicrobial effect against a variety of microorganisms and eliminates most common types of viruses, bacteria, fungi, and spores in a relatively short amount of time in food products, food processing surfaces, and nonfood surfaces (Ding et al., 2015; Hao et al., 2015; Huang et al., 2008). Also, the acceptance of electrolysed water as a sanitizer is obvious from its use in a number of applications in various fields including agriculture, medical sterilization, food sanitation, livestock management, and other fields that employ antimicrobial techniques (Rahman et al., 2016; Huang et al., 2008; Kim et al., 2000). Depending on the results obtained *in vitro* and under artificial inoculation, certain salt solutions were deeply studied to examine their efficacy against gray mold on naturally occurring infection, using different strategies of application. Generally, potassium sorbate, sodium carbonate and sodium metabisulphite were the most effective salts to generate the electrolysed water under natural infection of gray mold of table grape. At the end of cold storage, for alkaline electrolysed water, as a preharvest treatment, the percentage of reduction ranged from 15-76% and sodium carbonate gave the best results against the rot. For pre and postharvest treatment, the percentage of reduction ranged from 49-87% and potassium sorbate gave the best results against the rot. When the treatments applied only after harvest, the percentage of reduction ranged from 44-71% and potassium sorbate gave the best results against the rot. Our results were in agreement with Askarne et al. (2013) who summarized that sodium carbonate and sodium metabisulfite completely inhibited mycelia growth of *Penicillium italicum* at 20 mM and his results of the *in vivo* trials indicate that sodium metabisulfite (100 and 200 mM) completely inhibited blue mold on citrus fruit. Moreover, numerous studies showed that sodium metabisulfite has been shown to completely inhibit mycelial growth of *H. solani*, *F. sambucinum*, *G. candidum* and a large range of potato postharvest diseases (Talibi et al., 2011; Mills et al., 2004; Hervieux et al., 2002; Mecteau et al., 2002). At the end of cold storage, for acidic electrolysed water, as a preharvest treatment, the percentage of reduction ranged from 39-95% being sodium metabisulphite the best treatment against the rot. For pre and postharvest treatment, the percentage of reduction ranged from 55-97% and sodium metabisulphite and potassium sorbate gave the best results against the rot. When the treatments applied only after harvest, the percentage of reduction ranged from 40-91% and potassium sorbate gave the best results against the rot. Sulfur dioxide is well known to be used on table grapes to avoid decay during storage, by either initial fumigation of fruit from the field followed by weekly fumigation of storage rooms or slow release from in-package pads containing sodium metabisulphite (Palou et al., 2010). Youssef et al. (2014a) summarized that of potassium sorbate reduced gray mold of 'Italia' table grape by 86, 97 and 100% when applied as preharvest application, pre- and postharvest application and postharvest treatment, respectively. In addition, Youssef et al. (2014b) showed that a significant difference was observed for sodium carbonate after one week of shelf-life and reduced the percentage of rotted 'Benitaka' table grapes by 74% when the fruit were treated in its solution at 1%. Moreover, application of potassium salts enhanced maturity of 'Thompson Seedless' grapes (Obenland et al., 2015; Feliziani et al., 2013). Previous studies confirmed that acidic electrolysed water helps control foliar diseases of many plants (Jia et al., 2015; Kusakari et al., 2013; Hou et al., 2012; Guentzel et al., 2011). Electrolysed water has been utilized to disinfect kitchen cutting boards, and other surfaces, fresh cut vegetables, alfalfa seeds and sprouts, broccoli, straw- berry, lettuce, tomatoes, apple and poultry (Bari et al., 2003; Buck et al., 2002) and it can also be used in agriculture for sterilization of fruits and vegetables (Fallanaj et al., 2013; Al-Haq et al., 2001), food materials (Rahman et al., 2016; Al-Haq et al., 2002). Guentzel et al. (2010) showed that treatment of *B. cinerea* with near-neutral electrolysed water (10, 25, 50, 75, 100 ppm TRC; pH=6.3–6.5; 10 minute contact time) in pure culture resulted in a 6 $\log_{10}$ sporesml^{-1} reduction and 100% inactivation. Acidic electrolysed water (TRC=54–56 ppm; pH=2.8) prevented growth of *B. cinerea* and *M. fructicola* in laboratory culture studies (Buck et al., 2002). Recently, Hirayama et al. (2016) applied neutral electrolysed water throughout an overhead irrigation system for controlling of strawberry anthracnose. The results of the present research showed that alkaline and acidic electrolysed water did not influence weight reduction**.** It should be noted that the effect of treatments on fruit quality is frequently ignored since laboratory scale experiments tend to focus on the effectiveness of a treatment to control decay and do not sufficiently take into account the final quality of the produce, which is necessary for a potential commercial application. Regarding TSS and TA, no statistical differences were noted among all treatments as compared to the control with minor exception. For grape color, in all cases no significant differences were observed between treatments as compared to the water control except for acidic electrolysed water generated by ammonium molybdate when applied before harvest. This study confirms that electrolysed water is an effective treatment and has a good control activity against gray mold of table grape caused by *B. cinerea* and it also demonstrates that some salts improve the electrolysis potential. Potassium sorbate, sodium carbonate and sodium metabisulphite were the most effective salts to generate the electrolysed water under natural infection of gray mold of table grape as they showed higher activity against the development of disease. Finally, because postharvest treatments can be too late with infection having already started, only one treatment in the field before harvest with electrolysed water might be of essential importance especially on table grapes.

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