EFFECT OF PRE HARVEST CALCIUM APPLICATIONS ON THE INCIDENCE OF ONION STORAGE ROTS

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(Manuscript received 28 June 2003)

Abstract

Two field experiments were carried out in Giza and Ismailia governorates on onion Giza 20 cv. to determine the efficacy of seedling dip and pre harvest spray applications of two calcium forms *i.e.* calcium nitrate [$Ca\ (No_3)_2$] or calcium chelate on rot occurrence after 2 and 4 months of storage. Correlation with calcium content and Ca accumulation in cell wall of healthy onions was studied. In both experiments, significant reduction in storage rots (neck, black and basal rots) occurred due to the application of each of Ca forms. In Ismailia experiment the incidence of storage rots decreased as the Ca rate increased. However, in Giza experiment, only after 4 month-storage the decrease in storage rot incidence occurred with the medium Ca rates (2% of $Ca\ (No_3)_2$, 0.2% of calcium chelate) in addition to the absence of neck rot. Upon the application of $Ca\ (No_3)_2$ at 2 % or calcium chelate at 0.2 %, the greatest reduction in either basal and black rots was experienced with the highest Ca content. Scanning electron microscope (SEM) observations revealed cell wall structure integrity as a result of application of either calcium forms at the highest rates.

INTRODUCTION

Onion bulbs (*Allium cepa L.*) are highly susceptible to post harvest rots due to pre harvest infections by rot causal pathogens. Black mould, neck rot and basal rot are the major post harvest rots of onions in Egypt. The causal pathogens are *Aspergillus niger, Botrytis allii* and *Fusarium oxysporum* (Abdel - Rahim and Arbab, 1985; Walker, 1926 and Everts *et al.*, 1985). Infection with black rot is thought to take place usually via the neck tissue when the foliage dies down at maturity (Machacek, 1929). Infection with neck rot occurs down the leaves and may be deep within the neck tissues of the onion bulbs at the time of harvesting. An infected crop may be in an apparently good condition when shipped or stored. The percentage of rotted bulbs in store is a direct reflection of the percentage of plants with neck infection in the field

(Kritzman, 1983). Infection with basal rot occurs via the roots and stem base (Shalaby and Struckmeyer, 1966) and old infection is usually detectable at harvest which subsequently leads to bulbs rot in store (Rath and Mohanty, 1986).

Losses caused by post harvest rots in onions are greater than is often realized and the avoidable losses between the farm gate and the consumer are of great concern.

Soils may contain adequate levels of calcium, but most of what is taken up by plants is translocated to the leaf. During fruit ripening, Ca participates in maintaining the integrity of the middle lamella (Clarkson and Hanson, 1980). However, cytoplasmic Ca also participates in a broad range of plant functions that may influence fruit decay. Most Ca entering the tissues accumulates in cell walls and membrane that are thought to be sites of its anti senescence action (Glenn *et al.*, 1988).
An application of lime to the topped bulbs has been found to reduce losses caused by black mold rot in stores (Tanaka and Nonaka, 1981).

Calcium level necessary to reduce decay significantly in some fruits such as apples and potato tubers, is usually higher than can be obtained with standard fertilization practices (Faust, 1989). Therefore, an attempt was proposed to increase onion bulb calcium concentrations through several pre harvest application of calcium nitrate or calcium chelate at certain concentrations to determine their efficacy in reducing post harvest rots of onions and to determine calcium accumulation as well as its content in tissues of outer scales of onion due to calcium treatment.

MATERIALS AND METHODS

Calcium salt preparations:

Two forms of calcium salts *i.e.* pure calcium nitrate [$Ca(NO_3)_2$] and commercial organic calcium chelate prepared by EL- Naser Company for Fertilizers and Biocides, were used at three rates *i..e.* 1, 2, 4 and 0.1, 0.2 and 0.4% (w/v) respectively for either seedling dip or plant spray applications .

Field applications:

Experiments were carried out in two locations *i.e.* Nekla, Giza, and Ismailia, Agricultural Research Station on onion Giza 20 cv..Two experiments were conducted at each site in the growing season of 2001-2002. Onion seedlings were firstly treated by dipping separately in the calcium salt preparations at the suggested rates. Dipping was targeted at seedling heads for 10-15 minutes. Seedlings were then transplanted.

Plants were further sprayed with calcium forms each at the same rates. Spray application was repeated at monthly intervals for three months starting one month after transplanting. Spray application were done by double repeated runs using manual back handled application modified with 3 nozzles at medium pressure (2 bar) that delivers approximately 0.4 l/acre /each.

Onion plants were subjected to routine cultural practices without pesticide applications and left for natural infection. At harvest time at the end of May (30-35 °C), when bulbs become visually mature, onions were harvested, topped then packed in plastic net containers and stored in the storage room.

Post harvest storage

Harvested onions collected from Ismailia were stored at room temperature of 23 ± 2 °C for up to 4 month. While onion bulbs collected from Giza were stored at similar room temperature for 2 months and for up to 4 months in storage room (20 ± 2 °C) to evaluate the development of major post harvest rots *i.e.* neck, black and basal rots. Disease assessment was expressed as a percentage of rotted onions at the time the control onions were completely rotted.

Calcium content

Calcium content of bulb tissue was determined by first removing the outer dry scales and cutting a wedge of flesh scales to the entire depth from two sides of the bulb. After removal from the bulb, the flesh was immediately freeze- dried, and ground. The two segments from each of four bulbs made up one sample, and three samples from each treatment were analyzed. From each sample, 0.250 ± 0.001 g was ashed at 500 ° C overnight and the residue was dissolved in 5 ml of 6 N HCl. The samples were then diluted and analyzed with Jarrell-Ash atomic absorption spectrophotometer and Ca values were calculated on a dry- weight basis (Conway, *et al.*, 1987).

Scanning electron microscopy (SEM):

Onions resulting from plants at Ismalia and receiving the application of either [$Ca(N0_3)_2$] or calcium chelate at 4% and 0.4 % respectively, in addition to untreated onions (control) were subjected to SEM observations (Harley and Fergusen, 1990).

Onion samples harvested at maturity stage were randomly chosen and immediately transported to the laboratory and stored in cold storage room at 18 ± 2 °C till use. Several scanning electron microscope (SEM) observations were made through a Jeol Scanning Electron microscope (JSM-T330 A) equipped with image recording and processing system (SEM Afore) to illustrate the supporting action on onion scale-tissue due to calcium treatments.

RESULTS

Efficacy of Pre harvest Calcium treatments on onion rot incidence at Ismailia governorate :

Figures (1a) and (1b) show that the calcium treatments i.e. [Ca $(NO_3)_2$ (N) as well as calcium chelate (H) reduced the onion rots when compared to the untreated control plants and the percentage of disease incidence as illustrated in figures (2a, 2b and 2c). Generally, the incidence of neck rot was the lowest, while it was the highest for black rot. The percentage of neck, basal and black rots was constantly reduced after 2 and 4 months on onions that received calcium compared to the control. As the rate of Ca application increased, the incidence of rot was significantly decreased at both storage periods.

Regarding different calcium forms, the efficacy of calcium chelate in reducing rot incidence was generally better than calcium nitrate. Its effect was better against basal and black rots after 2 or 4 months (5.27, 5.8 and 16, 14.46%) each at 0.4% respectively. Comparable results were obtained by calcium nitrate after 4 month storage at 4% (3.77%) with respect to neck rot.

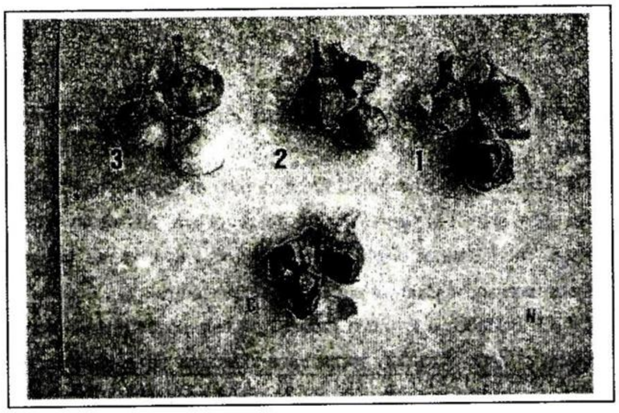


Fig. (1a): Symptoms of storage rots upon the effect of pre harvest application of Ca $(No_2)_3$ -N- at 1, 2 and 4% concentrations (1,2,3 respectively) and control (c).

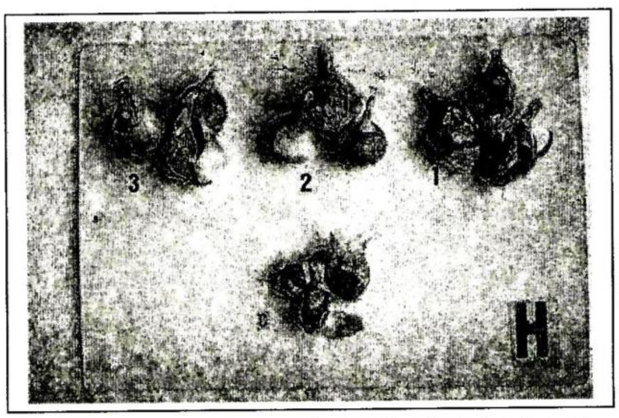


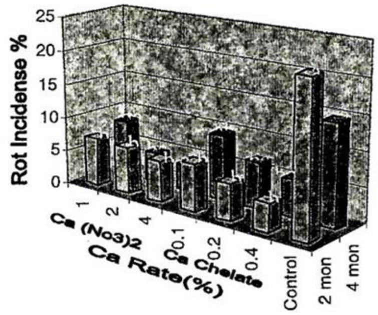
Fig. (1b): Symptoms of storage rots upon the effect of pre harvest application of Ca chelate -H- at 0.1, 0.2 and 0.4% concentrations

Efficacy of Pre harvest calcium treatments on onion rot incidence at Giza governorate:

Rot incidence on onions stored for 2 months at room temperature or for 4 months at 20 °C is shown in figures 3a, 3b. Neck rot was absent on onions at both storage periods. The incidence of basal and black rots was evident; however, it was lower on onions receiving calcium treatments than the control.

Basal rot incidence was much reduced by calcium treatment than the black rot. Tested rates of each of calcium forms have differently affected the percentage of rot incidence with no constant trend associated with increasing calcium rates after two month storage period. However, after four month storage, the efficacy of Calcium nitrate or calcium chelate at the rates of 2 % and 0.2% respectively was remarkable on basal and black rots as it gave the best results (5, 8.67 or 11.67, 18.33% after 2 and 4 months, respectively, for basal rot and 15.33, 26 and 23,26 after 2 and 4 months respectively, for black rot) in reducing rot incidence compared to the other tested rates.

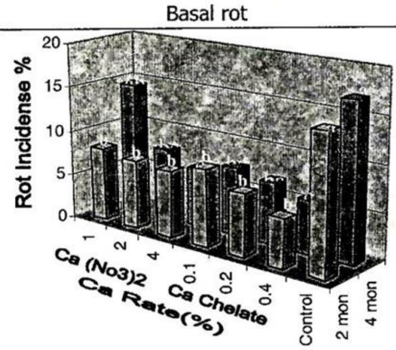
Neck rot



4 mon Rot(%)	2 mon Rot(%)	Ca Rate(%)
8.3	7.02	1
4.5	6.87	2
3.77	6	4
9.5	6.9	0.1
6.7	5.33	0.2
5.5	4	0.4
14.6	21.8	Control

2 a

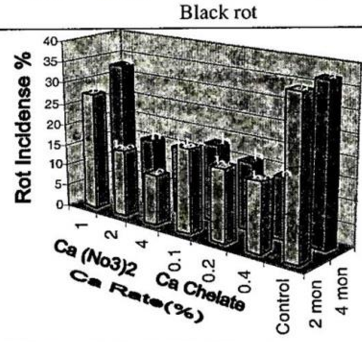
Basal rot



4 mon Rot (%)	2 mon Rot(%)	Ca Rate(%)
14.53	8.2	1
7.76	7.7	2
6.46	7.4	4
7.5	8.5	0.1
6.8	6.9	0.2
5.8	5.27	0.4
17	14.9	Control

2 b

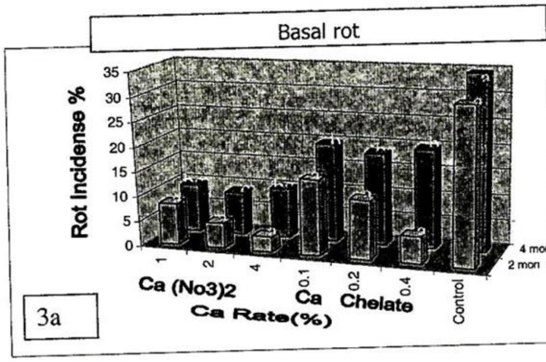
Black rot



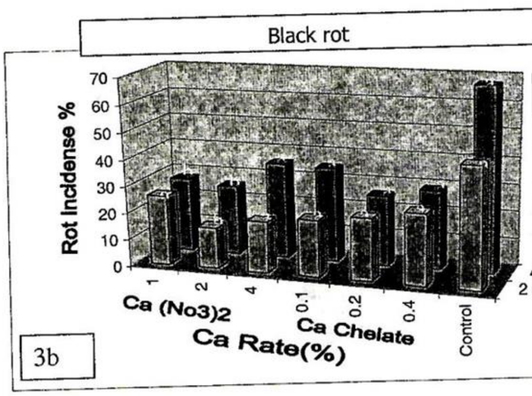
4 mon Rot(%)	2 mon Rot (%)	CaRate (%)
32.73	27.6	1
16	15.53	2
15.66	11.6	4
18.2	19.6	0.1
15.9	17	0.2
14.46	16	0.4
37.2	36.6	Control

2 c

Figures 2a, 2b, 2c. Effect of pre harvest application of two calcium forms i.e. [Ca $(No_3)_2$] or C $^{2+}$ chelate on storage rot incidence after 2 and 4 months at 20 °C (Ismailia).



4 mon Rot(%)	2 mon Rot(%)	Ca Rate(%)
9.67	8.33	1
8.67	5	2
10	3	4
19.67	15	0.1
18.33	11.67	0.2
19.67	5.33	0.4
34.6	30.67	Control



4 mon Rot(%)	2 mon Rot(%)	Ca Rate(%)
27.67	26	1
26	15.33	2
35.6	19	4
35	21	0.1
26	23	0.2
29	26	0.4
67.33	44	Control

Figure (3a, 3b): Effect of Pre harvest application of two calcium forms i.e. Ca $(No_3)_2$ or calcium chelate on storage rot incidence after 4 months. Different letters on the top of the columns indicate significant differences of rot incidences (Giza).

Calcium content determination in bulb tissues of treated onions:

Data presented in Tables (1a, 1b) show that pre harvest application of either Ca $(No_3)_2$ or calcium chelate at the tested rates resulted in an increase in calcium content in tissues of onions compared to the untreated control. The increase was, however, higher in bulbs collected from Giza than those from Ismailia. The differences between the two governorates may be due to soil contents of Ca and its pH.

In onions collected from Giza, the applications of both Ca $(No_3)_2$ or calcium chelate at 2 % or 0.2 %, respectively resulted in the highest Ca content in tissues (2.96, 1.95 µg Ca/ g) compared to those obtained by the two other rates (Table 1a). However the higher rates (0.4%,4%) had a reverse effect compared with the medium rate (2% , 0.2%). On the other hand, in onion collected from Ismailia the application of both Ca $(No_3)_2$ or calcium chelate at 4% or 0.4% respectively, has caused a similar effect in onions (0.813, 1.188 µg Ca/ g) compared to that caused by the lower rates (Table 1b).

Table (1). Calcium content (% dry matter) in outer scales of healthy onions (Giza 20 cv.) upon pre harvest application of Ca $(No_3)_2$ or calcium chelate each at three rates in two governorates {1a (Giza), 1b (Ismailia)}.

1a	Calcium content (% dry weight)		
	Ca Content (µg Ca/g dry wt)		
$Ca(No_3)_2$		Ca Chelate	
Rate %	Ca Content	Rate %	Ca Content
1	1.64	0.1	0.7
2	2.96	0.2	1.95
4	0.85	0.4	0.85
Untreated Control		0.30	

1b	Calcium content (% dry weight)		
	Ca Content (µg Ca/g dry wt)		
$Ca(No_3)_2$		Ca Chelate	
Rate %	Ca Content	Rate %	Ca Content
1	0.625	0.1	0.688
2	0.563	0.2	0.438
4	0.813	0.4	1.188
Untreated Control		0.688	

Onions resulted from plants treated with Ca^{2+} forms at both governorates were harvested, sampled and dried for determination of calcium content.

Scanning electron microscopy of outer flesh scales of healthy onions treated with either* Ca $(NO_3)_2$ *and calcium chelate at 4% and 0.4% respectively.

Figures (4a, 4b and 4c) illustrated the effect of pre harvest dip and spray application of each of calcium nitrate and calcium chelate at the rates 4% or 0.4%, respectively, on cell wall structure of onions (from Ismailia) after harvest and storage for four months at room temperature. The figures show that cell wall of onions that received calcium chelate at 0.4 % (Fig. 4c) seems to have good cell wall structure with membrane integrity compared to that received calcium nitrate at 4% (Fig. 4b). While cells of control onions that were not treated with calcium show non supporting cell wall with no compactness (Fig. 4a).

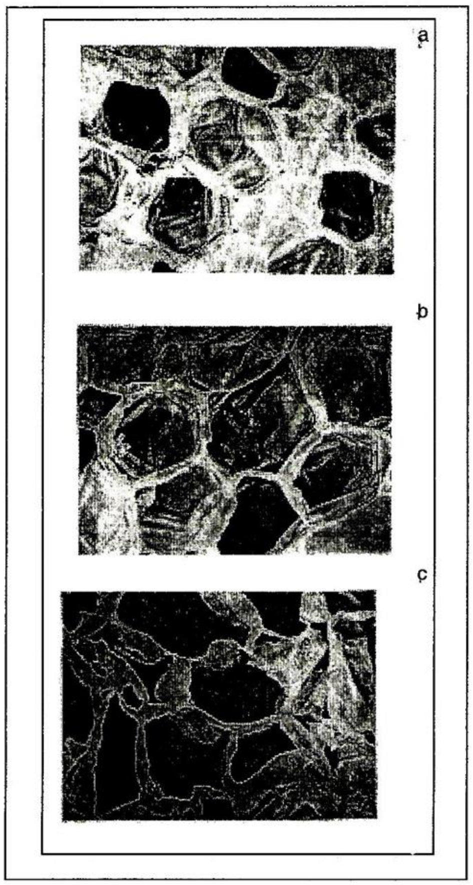


Fig. 4a, 4b and 4c show typical SEM photographs (magnification 350 x) of cell wall structure of outer flesh scales of onions produced at Giza governorate upon the effect of pre harvest calcium application. 4a (control onions), 4b [onions received Ca $(NO_3)_2$ at 4%] , 4c (onions received Ca chelate at 0.4%).

DISCUSSION

Dipping and spraying onion seedling with solution of Ca $(NO_3)_2$ and calcium chelate, caused significant reduction in black rot, neck rot and basal rot in naturally infected onion bulbs produced from plants treated at the tested rates in two locations.

Increasing the content of calcium in onion bulbs by supplementing with organic (Ca^{2+} chelate) and mineral [Ca $(NO_3)_2$] calcium fertilization resulted in a significant reduction of major post harvest rots after storage for four months. Non inoculated bulbs subjected to calcium treatments did not appear to be damaged after harvesting. At higher rates of calcium, bulbs remain free of decay up to four months. Tanaka and Nonaka (1981) found that the application of lime to the topped onion bulbs has reduced the losses caused by black rots in stores. The reduction in rots along with increasing calcium rate, however, was evident in bulbs collected from Ismailia governorate than in those collected from Giza governorate which perhaps due to the variability in soil nature, calcium content and soil pH.

In this study, calcium applied as a pre harvest dip and spray resulted in increasing of calcium content in outer flesh scales of onions collected from both governorates. The increase of Ca in bulbs collected from Ismailia was related to the lowest percentage of tested rots and can be realized with the high concentration applied (4% and 0.4%). However, the lowest percentage of black and basal rots on onions collected from Giza occurred only where calcium contents were at 2% and 0.2 % of Ca $(NO_3)_2$ and calcium chelate respectively.

The inverse relation between the highest calcium content in Ismailia bulbs and rot occurrence was explained by Krauss and Marschner (1971) who found that the reduction in bacterial soft rot was highly correlated with calcium increase in both peel and medullar tissue of the potato tuber. Direct movement of calcium into the tuber from the soil, however, may have helped the enrichment of the peel and accounted for the reduced gradient toward the centre.

The calcium content of treated bulb scales collected from Giza was often higher but lower in untreated bulbs than that of Ismailia.. The greater uptake of calcium into bulbs grown at Giza, in particular at lower rates, might be due to certain soil conditions related to soil content, pH as well as soil nature. This was correlated with the absence of neck rot caused by *Botrytis allii*. An opposite effect occurred in

Ismailia samples. These findings agree with those obtained by McGuire and Kelman (1984) who found that as the potato tuber calcium was increased, the percent surface area of the tubers decayed by *Erwinia carotovora pv. atroseptica* under mist chamber conditions was reduced significantly. Previous findings also indicated that calcium was able to regulate aspects of the post harvest metabolism of many fruits. The findings of Wills *et al.* (1977) and Wills and Rigney (1979) suggested that higher uptakes of calcium obtained by dipping in high concentrations at reduced pressure retarded ripening of tomato and greater calcium additions would retard tomato fruit respiration. McGuire and Kelman (1984) have found the same response in potato tubers by increasing calcium fertilization. In other work with peaches, spray with Ca $(NO_3)_2$ resulted in sufficient uptake of Ca by the fruit that could reduce physiological weight loss and respiration, maintain firmness and reduce decay of naturally infected fruits (Bhullar et. al. 1981; Gautanm *et. al.*, 1981 and Singh *et. al.*, 1982).

The examination of tissue sections of calcium-treated onion flesh scales revealed that calcium prevented cell wall disintegration as indicated by preservation of cell structure. These observations indicate that calcium could strengthen the cell walls, thus interfering with penetration and fungal development within the tissues and might probably stimulate defence mechanisms by eliciting the biosynthesis of phytoalaxins in treated tissue. Confirmed findings was reported by Ng and Tigchelaar (1977) who reported that the presence of high concentrations of calcium might reduce the activity of PE and PG enzymes and hence reduce the rate of breakdown of tomato cell walls, and thereby inhibit further ripening. Dimitrey *et. al.* (1996) also found that treatment of onion cell suspension cultures with abiotic elicitor derived from the fungus *Botrytis cinerea* resulted in the synthesis and accumulation of two phytoalexins, and the removal of extra cellular Ca^{2+} by calcium chelator abolished the elicitor mediated phytoalexin synthesis. They added that calcium channel blockers caused similar effects, whereas the addition of the ca^{2+} ionophore enhanced the accumulation of phytoalaxins in the absence of the elicitor. Increase in the cytoplasmic Ca^{2+} concentration in elicitor- treated cells was observed as monitored by the fluorescent calcium indicator. These observations suggest that Ca^{2+} acts as a second messenger in the regulation of phytoalexin synthesis in cultured onion cells

These explanations indicate that a large increase in calcium in onion bulbs could reduce the occurrence of post harvest rots by accumulating calcium in flesh tissue. The associated inhibition of the breakdown of the cell wall as well as possible effect on enzyme activities and or formation of certain phytoalaxins are possibly involved.

Acknowledgement:

The authors appreciate the excellent technical assistance provided by the assistant researcher Hamam EL- Din Heniesh Youness, Department of post harvest, Plant Pathol. Res. Int. ARC, Giza, Egypt.

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تأثير معاملات ما قبل الحصاد بأملاح الكالسيوم علي إصابة البصل بأعفان المخزن

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نفـذت في كل من محافظتي الإسماعيلية والجيزة تجربتان بالحقل علي البصل صنف جيزة ٢٠ لدراسـة تأثـير معاملات غمر البادرأت والرش قبل الحصاد لنباتات البصل بصورتين لأملاح الكالسـيوم (نترات كالسيوم وكالسيوم مخلبي) عند معدلات محددة وذلك علي جذوث الاعفان (عفن الرقبة والعفن الاسود وعفن القاعدة) علي الأبصال بالمخزن بعد ٢ و ٤ شهور من التخزين.

وجد أنة بزيادة معدلات الرش بأملاح الكالسيوم يتناقص معدل حدوث أعفان المخزن علي الأبصال في كل من التجربتين.

وفـي محافظة الإسماعيلية وجد أن أعلي محتوي للكالسيوم يكون بالأبصال نتيجة الرش بالمعدلات المرتفعة من الملحين والتي عندها يحدث أقل نسبة للأعفان عند كل من فترتي التخزين.

وفي محافظة الجيزة يحدث فقط أقصي إنخفاض في حدوث أعفان المخزن بعد أربعة شهور وعـند المعـدلات المتوسـطة للأملاح بالإضافة لغياب حدوث عفن الرقبة. كما وجد أن عند هذين المعدليـن يكون أقصي إختزال لكل من عفن القاعدة والعفن الأسود مع وجود علاقة بأعلي محتوي للكالسيوم .

أوضـحت ملاحظات الميكروسكوب الألكتروني ظهور إندماج تراكيب جدر الخلايا للأبصال كنتيجة للرش بالمعدلات المرتفعة لإملاح الكالسيوم.