

**EFFECT OF SOME CALCIUM SALTS ON
CONTROLLING POST-HARVEST FRUIT
ROT OF MANGO CAUSED BY
Botryodiplodia theobromae Pat.**

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ABSTRACT: Effect of some calcium salts on growth, polygalacturonase (PG) activity, and infection of mango fruits by *Botryodiplodia theobromae* Pat. were determined. All salts tested except calcium carbonate, reduced growth of *Botryodiplodia theobromae* on amended potato-dextrose broth (PDB) medium after 10 days. Minimal growth occurred on PDB medium amended with calcium propionate. Calcium silicate and calcium chloride reduced growth by 15.9 and 13.3% respectively, compared with the control. Fungal PG activity was significantly decreased by all salts used in this study, except calcium carbonate. Greatest reduction in activity of PG was associated with calcium propionate followed by calcium sulphate. Both of the used concentrations of calcium salts and the dipping periods affected *Botryodiplodia theobromae* rot incidence on mango fruits. In this respect, reduction of fruit rot decreased gradually by the increase of calcium salt concentrations and the dipping period as well. Calcium chloride showed the greatest main effect on the reduction of rot on mango fruits. On the other hand, calcium carbonate increased rot than in control treatment.

Key words: *Botryodiplodia theobromae*, mango, fruit rots, calcium salts.

INTRODUCTION

Fruits are attacked by a wide range of microorganisms during post-harvest phase (Snowdon, 1990 and Ogawa and English

1991). Stem end rot caused by *Botryodiplodia theobromae* is a post-harvest fungal disease, which usually builds up in orchards as trees age. Sampaio *et al.*, (1981) reported that symptoms appear as fruit ripen. A brown discolouration and rot symptoms start at the fruit stem end and develop rapidly over the skin and through the flesh. In this stage it called botryodiplodia fruit rot. It can develop away from the stem end at any injury site on the fruit. Infected fruits have an unpleasant flavour. Also, fungus builds up on dead twigs, branches and leaf litter where it produces large numbers of spores. The spores prior to harvest can infect flowers and developing fruit rot. Reddy, (1975) detected that pre-harvest spray of copper oxychloride, used to control bacterial black spot, or mancozeb for anthracnose control, may reduce the incidence of stem end rot in fruits. Other methods of control are used to avoid rots. These methods including harvesting immature fruits cooling fruits immediately following harvest

and storage in well-ventilated containers. Post-harvest treatments dips can also give some control.

Post-harvest losses of mangos caused by stem end rot may be significant in some years, fungicide treatment of fruits after harvest is a routine procedure, especially if fruits are to be stored and/or shipped for long distances. With the recent concern regarding pesticide residues on fruit, there is a need for alternative post-harvest disease-management practices that will reduce risk to consumers (Anon, 1987). Bateman and Lumsden (1965) and Conway *et al.*, (1992) stated that increasing calcium content in fruits and vegetables treated with calcium salts has increased storage life, mainly as a role of calcium effect in changing physiological characters of plant to be unfavourable for colonization by the pathogens, reducing their pathological disorders. Research works on enhancing storage quality and reducing post-harvest decay with calcium supplementation has been done with apples, even

though mangoes have a much shorter storage life (Reddy, 1975). Thus, the aim of this study is to investigate the effect of several calcium salts on *in vitro* growth and polygalacturonase (PG) activity of *Botryodiplodia theobromae*. Rot reduction and colonization of harvested mango fruits were studied as well.

MATERIALS AND METHODS

Isolation and Identification

Samples of Mango shoots showing typical stem end rot symptoms were collected from four different governorates namely, Ismailia (Quntara Sharq), Giza (El-Aiaat), Alexandria (Nobareia) and Sharkia (Faqus). Samples were washed with tap water then surface sterilized by dipping in 1% sodium hypochlorite for 2 minutes then washed several times with sterilized distilled water and dried between two sterilized filter papers. By the aid of a sterilized razor the samples were cut into

longitudinal and horizontal sections approximately 0.5 cm long and placed on potato dextrose agar (PDA) medium in Petri-dishes then incubated at 27°C for 72 hours. A little portion from the resulted fungal growth was picked up from the resulted colony borders and transferred to other Petri-dish for single spore purification procedure (Kiett, 1915). The purified fungus then transferred to PDA slants and preserved in 4°C for further studies. The resulted fungi were identified according to Barnett and Hunter (1987).

Pathogenicity Test

Pathogenicity test of twelve fungal isolates belonging to *Botryodiplodia theobromae* previously isolated from diseased mango plant parts was carried out. Healthy looking Kobaneia mango fruits obtained from the local market were wounded uniformly in four sites with a blunted nail that created a circle wound approximately 0.5 cm diameter. Fruits were inoculated with 0.5 cm diameter

disk "taken from 10 days old culture of each *B. theobromae* isolate" to cover each wound then incubated in adjusted growth chamber in the dark at 25°C and 90% RH. Rotted lesion diameter (mean of two measurements at right angles to each other) was measured after 48 h to determine the pathogenicity of the different isolates.

Effect of Various Calcium Salts on Fungal Growth and Polygalactronase PG Activity

One ml of *Botryodiplodia theobromae* conidio spores suspension (10^6 cfu) was prepared from 15 days old culture grown on potato dextrose broth (PDB) medium, and transferred to stationary 250-ml flasks containing sterilized 50 ml PDB medium that was either not amended or amended with one of the six chemically pure calcium salts (calcium nitrate, calcium chloride, calcium sulphate, calcium propionate, calcium silicate and calcium carbonate) to yield 1000 mg of calcium per

litre (1000 ppm). The pH value of the supplemented medium was determined before autoclaving and adjusted to be 5.8 with either 0.1 N HCl or NaOH. Inoculated flasks were incubated for 10 days at $25 \pm 2^\circ\text{C}$, then the growth was filtrated through Wattman No 1 filter paper to collect the fungal mass and assess the growth by determining the dry weight of the mycelium. The filtrates were collected and analyzed immediately for PG activity. The enzyme activity was tested on sodium polypectate at pH 4.5 in 0.1 M acetate buffer according to the method described by Sherwood (1966). The reducing groups were measured spectrophotometrically at 510 nm using arsenomolybdate method described by Nelson (1944) and modified by Naguib (1964). Standard curve of monogalacturonic acid was used as reference. One unit of PG enzyme activity was defined as mg monogalacturonic acid liberated / hour / ml enzyme source.

Effect of Calcium Salts on Mango Stem End Rot Infection

Ripe mango fruits from Kent cultivar were harvested and dipped for 30, 60 and 120 min in calcium solutions (calcium nitrate, calcium chloride, calcium sulphate, calcium propionate, calcium silicate and calcium carbonate) prepared at 2500, 5000, 10000 and 20000 ppm. Prior to dipping, each Kent mango fruit was wounded uniformly as previously mentioned in the pathogenicity test. Fruits were inoculated approximately one hour after being removed from the dip solutions with *B. theobromae* isolate G3. Fruits inoculations and incubation were carried out as previously mentioned in the pathogenicity test. Lesion diameter (mean of two measurements at right angles to each other) of each rotted part was measured after 48 hrs of incubation at $25 \pm 2^\circ\text{C}$. Control included fruits that were wounded, dipped in water, and then inoculated. The effect of the tested calcium compounds on rot reduction was measured

according to the formula [(mean control value – treatment value) / mean control value] x 100. The experiment was conducted with three replicates of 3 fruits per treatment.

Statistical Analysis

Data were statistically analysed by analysis of variance according to Snedecor and Cochran (1982) using system version 8 (SPSS, 1997). The differences between means were compared using the least square differences of the same system.

RESULTS AND DISCUSSION

Twelve fungal isolates recovered from the collected stem end rot samples are shown in Table 1. All the detected isolates were identified as *B. theobromae* Pat.. *In vitro*, pathogenicity test on mango fruits (Kobaneia cv.) showed different virulence levels. The highest virulence level was recorded by G3 isolate, this isolate was selected to be use in this work, and the lowest one recorded by IQs2 isolate. Symptoms of disease appeared

as a soft dark brown lesion surrounded by the inoculation sites. These results in harmony with those obtained by Spalding, (1982); Meah *et al.*, (1991) and El-Habbaa, (1995).

Data in Table 2 show the effect of the calcium salts tested on *B. theobromae* growth *in vitro* on potato dextrose broth supplemented with five different calcium salts. All calcium salts, except calcium carbonate, significantly reduced growth of *B. theobromae* on amended PDB medium after 10 days as shown in Table 2. Calcium propionate was the most effective salt tested, *i.e.* reducing growth on PDB medium by 20.51%. Other calcium salts, calcium nitrate, calcium chloride and calcium silicate, reduced growth by approximately 11.79, 13.33 and 15.9%, respectively in comparison with the control. Meanwhile, calcium sulphate recorded the lowest growth reduction, being 3.59%. On the other hand, calcium carbonate stimulated the fungal growth by +14.36% compared to the control treatment. This study

has demonstrated the general toxicity of calcium salts against growth of *Botryodiplodia theobromae in vitro*. Five of the 6 salts tested on PDB medium, were differed from slightly to strongly affected substances, reducing growth by 3.59 to 20.51%. The obtained results in Tables 2 and 3 indicate that polygalacturonase produced by *B. theobromae* play an important role in the progress of the infection on mango fruits (Aspinali, 1980). The activity of PG significantly decreased by all salts tested in this study, except calcium carbonate. The greatest reduction percentage of PG activity was indicated by amending growth medium with calcium propionate (33.03%), followed by calcium sulphate, calcium chloride, calcium silicate and calcium nitrate. These salts reduced PG activity by approximately 6.91–27.03% (Table, 3) while, calcium carbonate stimulated the PG activity by +9.01% than the control treatment. Four of these five salts significantly decreased PG activity by 6.91 to 33.03%. In addition, the effect of

Table 1: Pathogenicity test on Kobaneia mango fruits inoculated with *Botryodiplodia theobromae* isolates obtained from diseased mango trees grown in four different governorates

Governorate	location	isolate code	Rotted area on mango fruit mm ²
Giza	El Aiaat	G1	48.6
		G2	52.6
		G3	60.3
Alexandria	Nobaria	AN1	55.4
		AN2	55.8
		AN3	54.3
Ismailia	Quntara Sharq	IQs1	57.6
		IQs2	42.3
Sharkia	Faqus	SF1	55.4
		SF2	54.6
		SF3	57.4
		SF4	55.6

Table 2: Mycelial dry weight of *Botryodiplodia theobromae* isolate G3 after 10 days of incubation at 25 ± 2 °C on PDB medium amended with various calcium salts 1000 mg of calcium per litter

Calcium salts	Mycelium dry weight mg	
	Dry weight	% of reduction
Calcium nitrate	172	11.79
Calcium chlorid	169	13.33
Calcium sulphate	188	3.59
Calcium porpionate	155	20.51
Calcium silicate	164	15.90
Calcium carbonate	223	-14.36
Control	195	0.00

L.S.D 0.05 for dry weight: 5.57

calcium salts on pathogen dry weight was correlated with PG activity. Conway *et al.* (1987 and 1994) and Blodgett *et al.* (2002) found that mango fruits that had been sprayed with calcium chloride had 70% more calcium content than untreated controls, but did not resist decay, whereas fruits that were dipped and pressure infiltrated had two to four times more calcium and resist infection but were physically injured by the pressure treatments. In the present study, calcium salts including calcium chloride, reduced fungal growth and PG activity, suggesting the possibility that the observed effects of calcium *in vivo* may result partly from suppressed pathogen activity (Singh *et al.*, 2000)

Incidence of fruit rots on wounded mango fruits (Kent variety) was 100% for all salts. The effect of salt on disease severity was more variable than that on disease incidence. Infection and colonization of wounded fruits by *B. theobromae* progressed rapidly; thus, 2 days incubation period

was chosen as limit time to accurate determination of disease severity (El-Habbaa, 1995). All calcium salts, except calcium carbonate, reduced lesion diameter relatively to the control (Fig 1). Calcium chloride provided the most reduction in percentage of disease severity being 59.3% as shown in Table 4 and Fig.2. The lowest reduction of rotten area was recorded with calcium nitrate treatment while, the other calcium salts recorded a rotted area ranged from 38.8 to 35.6 mm² which indicated a percentage of rot reduction ranged between 30.2% - 35.9%. Calcium carbonate enhanced the rotted area to 7.5% more than the control treatment. Calcium chloride also found to have inhibitory effect against colonization of excised mango twigs by *Leucostoma personii* (Biggs and Peterson, 1990). However, the association of calcium chloride with fruit injury (Conway *et al.*, 1987) and foliar injury may limit its use in commercial mango production. Preharvest sprays of calcium nitrate have reduced decay

Table 3: Polygalacturonase activity unit/ml/hour of *Botryodiplodia theobromae* after 10 days incubation at 25 ± 2 °C on PDB medium amended with various calcium salts 1000 mg of calcium per liter

Calcium salts	Polygalactrunase	
	activity Unit/ml/hour	% of activity reduction
Calcium nitrate	3.10	6.01
Calcium chlorid	2.86	14.11
Calcium sulphate	2.43	27.03
Calcium porpionate	2.23	33.03
Calcium silicate	2.90	12.91
Calcium carbonate	3.63	-9.01
Control	3.33	0.00

L.S.D 0.05 for enzyme activity: 0.12

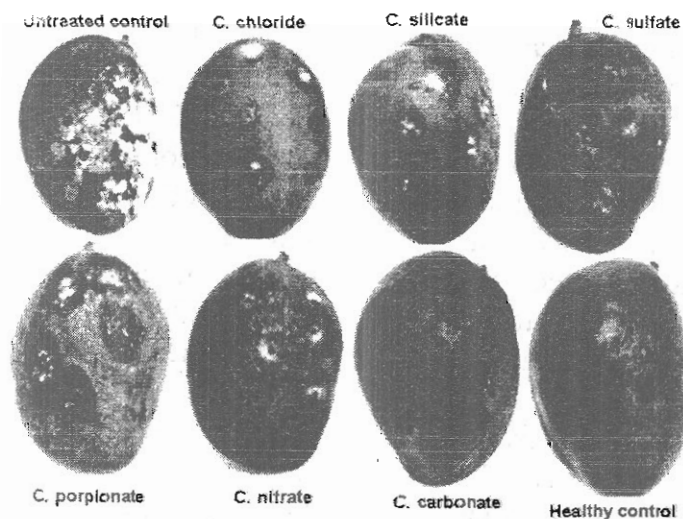


Fig. 1: General effect of dipping treatments with six different calcium slats on rotted mango fruits.

during storage in mangoes naturally infected by *Rhizopus stolonifer* (Singh *et al.*, 1982). The results obtained in their study revealed that calcium nitrate moderately reduced the growth of *B. theobromae* besides decreasing PG activity. While, calcium silicate was slightly differed from those reported by Adaskaveg *et al.*, (1992), which showed that this salt had no effect against the fungus *Monilinia fructicola*. They added that two of the three mango genotypes examined, calcium formate and calcium silicate controlled *M. fructicola* on fruits, and control of disease by calcium formate was equal to that of the fungicide iprodione.

Increasing calcium salt concentrations used for dipping fruits markedly affected the rotted area diameters. The overall mean of rotted areas developed on the fruits dipped in 2500 ppm was 47.24 mm² decreased to be 44.3 mm² with the concentration 5000 ppm, 40.84 at 10000 ppm and 38.26 mm² at 20000 ppm. These results indicating a percentage of rot reduction for each

concentration 17.4%, 23.5%, 30.79% and 36.22%, respectively as shown in Table 4. On the other hand, the dipping periods showed a remarkable effect on the formed rot area and the rot reduction compared to the control treatment. The overall mean effect of the tested dipping periods on rot reduction indicate that the percentage of rot reduction increased with the increase of the dipping period of the different calcium salts tested (Fig. 3). Data presented in Table 4 show that, the highest mean of rot reduction was obtained when mango fruits dipped in 20000 ppm of calcium chloride for 120 minutes (5.4 mm²) followed by calcium silicate at the same concentration and dipping period (20 mm²). In the present study, the effect of calcium silicate on *B. theobromae* growth in liquid culture demonstrated the possibility that the Ca²⁺ ion stimulates the synthesis of phytoalexins and/or phenols (Kohle *et al.*, 1985) or, alternatively, that Ca²⁺ reduce the effectiveness of fungal PG activity by forming cation cross

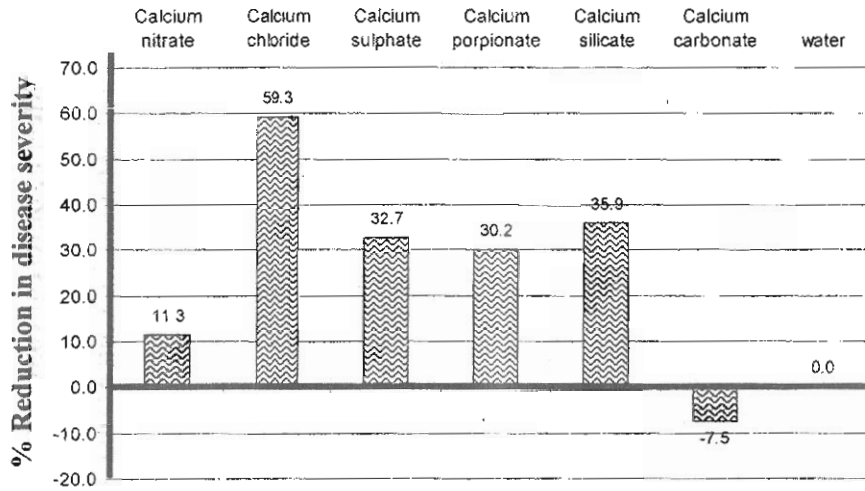


Fig. 2: Percentage of mango fruit rot overall reduction incited by six different calcium salts (dipping treatment)

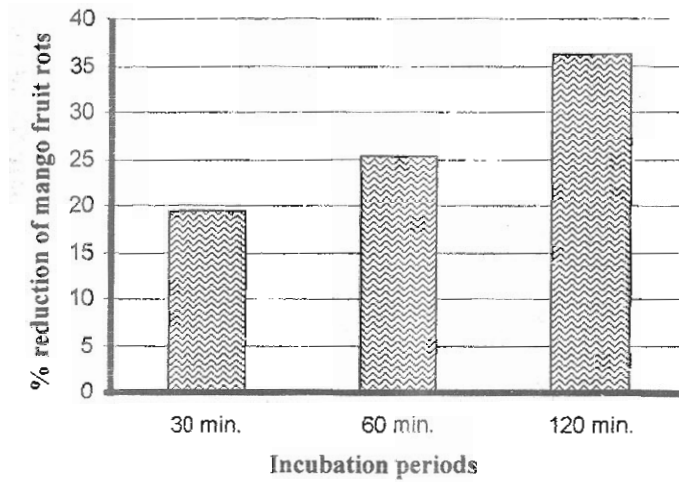


Fig. 3: The overall fruit rot reduction caused by different dipping periods in all tested calcium salts.

Table 4: Effect of different concentrations of various calcium compounds and the dipping duration on the mean of rotted area in mm² as well as the rot reduction percent after 48 hours incubation at 25°C on Mango fruits variety Kent inoculated by *Botryodiplodia theobromae*

Calcium salts	Average of rotted area in mm ²												Mean	Mean % of reduction
	Concentrations in ppm.													
	2500			5000			10000			20000				
	dipping time min.													
	30	60	120	30	60	120	30	60	120	30	60	120		
Calcium nitrate	49	55	53	48	60.2	46.4	48.6	58	38.6	48.4	56.6	28.8	49.2	11.3
Calcium chloride	35.8	31.2	29	32	25.8	21.8	27.4	18.2	10.2	20.6	13.8	5.4	22.6	59.3
Calcium sulphate	51.2	42.4	33.8	53.2	39.6	33.8	46.2	31.6	28.2	40	25	23.4	37.4	32.7
Calcium porpionate	43.4	42.8	40.4	43.8	40	37.4	41.8	32	33.2	43.8	33.2	33.2	38.8	30.2
Calcium silicate	42.6	43.6	44.2	38.2	37.4	34.6	39	36	28	28.6	34.8	20	35.6	35.9
Calcium carbonate	66.6	61.2	60	61.4	56.4	54.2	61.4	57.8	55.2	63.2	61.8	56.6	59.7	-7.5
Water control	55.4	56	55.4	55.4	55.4	55.4	55.4	55.4	55.4	55.4	55.4	55.4	55.5	0.0
Mean of dipping time	49.14	47.46	45.11	47.43	44.97	40.51	45.69	41.29	35.54	42.4586	40.09	31.83		
Mean of Concentrations	47.24			44.30			40.84			38.26				
Mean reduction % of Concentrations	17.40			23.50			30.79			36.22				

L.S.D 0.05 for: Salts S= 1.96, Concentration C=0.96, Period P= 0.59, SxC= 2.55, SxP= 1.58, CxP = 1.19 and S x C x P= 3.16.

bridges between pectic acids in the plant cell walls, thus making the cell walls more resistant to digestion (Conway and Sams, 1984; El-Habaa, 1995 and Joyce *et al.* 2001). However, the high concentrations of calcium must be present in host cell walls in order to provide effective cross bridging in apple tissues at approximately 800 $\mu\text{g/g}$ of dry weight (Byrde 1969 and Singh *et al.*, 2000). Data concerning the effects of selected calcium compounds on PG activity, suggested that, Ca^{2+} may act directly on the pathogen and reduced virulence or, in the extreme, fungistatic effect (Smilanick *et al.*, 1996). Calcium propionate was the most effective treatment for reducing activity of PG by *Botryodiplodia theobromae*; however, the results of the other four salts resulted in reduction of PG activity by approximately 75%. None of the calcium salts in the present study were fungicidal at the concentrations examined. Calcium propionate, the hemicalcium salt of propionic acid (a three-carbon organic acid), has been

extensively used as a food additive and is well known as an inhibitor of certain molds and bacteria in stored grains (Milward 1976; Tsai *et al.*, 1984 and Raeker *et al.*, 1992), hay (Nash and Easson 1977 and Draughon *et al.*, 1982), and bread (Byrde, 1969 and Doores 1983) and has the potential for widespread use in the preservation of other foods (Byrde, 1969). Punja and Gaye (1993) and Biggs *et al.* (1997) demonstrated the utility of calcium propionate dips in reducing black root rot, caused by *Chalara elegans*, on fresh market carrots. The mechanism of action of calcium propionate is the result of fungistatic effect and is thought to be caused mostly by the molecule in its undissociated state (Byrde, 1969 and Byrde and Willets 1977). The lipophilic, undissociated molecule is readily soluble in cell membranes and may interfere with the permeability of the microbial cell membrane, causing uncoupling of both substrate transport and oxidative phosphorylation from the electron transport system. In

less acidic environments where a portion of the molecule is dissociated, the Ca^{2+} moiety may act to inhibit fungal growth and PG enzyme production in addition to possibly enhancing host resistance. Short chain, organic acids could have a role as disease control agents for wound pathogens, however, individual compounds must be examined for activity against specific pathogens. If a compound is found to be fungistatic against an appropriate pathogen, this property could provide the time required for host defence mechanisms to provide more effective resistance. Alternatively, a substance such as calcium propionate that has no activity against yeasts could be used to supplement biological control by yeasts. Additional research should address optimal concentrations of calcium salts, the use of additives or synergists (*i.e.*, organic acids), pH effects, and carriers that would maintain effective concentrations of materials for an effective time period (Conway and Sams,

1984; Conway *et al.*, 1987; Biggs *et al.*, 1997 and Biggs, 1999 and 2004).

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تأثير بعض أملاح الكالسيوم على مقاومة أعقان ما بعد الحصاد لثمار الماتجو
المتسبية عن الفطر بوتريوديبلوديا ثيبرومي

محمد سامح شلبي

قسم الإنتاج النباتي - معهد الكفاية الإنتاجية

جامعة الزقازيق - مصر

تم تقدير تأثير بعض أملاح الكالسيوم على نمو الفطر ونشاط انزيم البولي جلاكتورونيز وإصابة ثمار الماتجو بالفطر بوتريوديبلوديا ثيبرومي. و قد اختزلت كل الأملاح المختبرة نمو الفطر بوتريوديبلوديا ثيبرومي بعد فترة تحضين ١٠ أيام على بيئة مرق دكستروز البطاطس المدعمة بالأملاح المختبرة (فيما عدا عند استخدام كربونات الكالسيوم المشجع لنمو الفطر) حيث سجل ملح بوربيونات الكالسيوم أقل معدل لنمو الفطر عند تنميته على بيئة مرق دكستروز البطاطس المدعمة به. و قد اختزلت الأملاح سليكات الكالسيوم و كلوريد الكالسيوم نمو الفطر بمعدل ١٥,٩ و ١٣,٣% على الترتيب مقارنة بتجربة المقارنة غير المعاملة. وقد أحدثت جميع الأملاح المختبرة ما عدا كربونات الكالسيوم خفضا مغفويا في نشاط انزيم البولي جلاكتورونيز المنتج عن طريق الفطر. وكان اعلي معدلات الخفض في الإنزيم عند استخدام بوربيونات الكالسيوم و سلفات الكالسيوم على الترتيب. كذلك أثرت تركيزات الأملاح المختبرة و كذلك فترات الغمر للثمار على شدة إصابة ثمار الماتجو بعفن الفطر بوتريوديبلوديا ثيبرومي. وقد زاد معدل تثبيط إصابة الثمار بالفطر بزيادة تركيزات الاملاح و كذلك فترات غمر الثمار. و اظهرت المعاملة بكلوريد الكالسيوم اعلي معدل تثبيط الإصابة مقارنة بالأملاح الأخرى. و من ناحية أخرى فقد أدت المعاملة بكربونات الكالسيوم إلى زيادة معدل العفن عن المعدل المسجل من تجربة المقارنة.