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SOME SAFE TREATMENT FOR CONTROLLING POST-HARVEST DISEASES OF VALENCIA ORANGE *(Citrus sinensus* L.) FRUITS

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ABSTRACT

Survey of naturally decay orange fruits at local markets from different governorates in Egypt revealed that green, blue moulds and brown spot caused by *Penicillium digitatum, Penicillium italicum* and *Alternaria citri* are the most important disease affecting orange fruits. They caused 56.3%, 25.7% and 12.9% infection of surveyed orange fruits respectively. All chitosan and citral concentrations used significantly reduced the linear growth and spore germination of three fungi. Complete inhibition was obtained with 6g/l chitosan for *Alternaria citri*, but at 8g/l for *P. digitatum* and *P. italiucum.* Citral at 8 ml/l also caused complete inhibition for three tested fungi. Bioagent *i.e Bacillis subtilis* and *Pseudomonas fluorescens* caused high reduction in the growth of pathogenic fungi (ranged between 85.5% to 100%). Orange fruits (Valencia cv.) coated with chitosan, citral or antagonist bacteria protected the fruits against post-harvest fungi. The most effective concentrations of chitosan and citral were 2%. Chitosan decreased diseases incidence by 14.7% 11.0% and 8.8% while citral by 16.2%, 13.0% and 12.6% for *P. digitatum, P. italicum* and *A. citri,* respectively as compared with the uncoated fruits after storage for 28 days. On the other hand, moderate protection was observed on orange fruits coated with *B. subtilis* which decreased incidence by 33.4%, 30.3 % and 20.9%, while *P. fluorescens* by 29.5%, 25.8% and 15.9% for *P. digitatum* and *P. italiucum* and *A. citri*, respectively. For fruits rotted part, 2.0% chitosan reduced the percentage of rotted fruit part by by 96.9%, 98.0 % and 98.7%, also citral at the same concentration by 95.4%, 96.4% and 96.6 %, while *B. subtilis* and *P. fluorescens* caused moderate effect on fruits infected by green, blue moulds and brown spot, respectively. The results suggest that these treatments can be safely commercially used specially chitosan as fruit coating for controlling post-harvest disease of Valencia orange fruits.

INTRODUCTION

Citrus occupies the greatest planted area among all grown fruit tree in Egypt. Valencia orange (*Citrus sinensus* L.) is one of important cultivar of citrus for exportation to the foreign markets (Mohamed *et al.*, 2003).

Green and blue moulds of citrus caused by *Penicillium digitatum* and *Penicillium italicum and* brown spot caused by *Alternaria citri* are the major post-harvest diseases affecting citrus fruits during handling, transportation, exportation and storage (Cacioni *et al.*, 1998; Abd-El-kareem *et al.*, 2002; El-Mohamedy *et al.*, 2002 and Ismail and Zhang, 2004). A number of fungicides

successfully controlled post-harvest decay pathogens of citrus fruits (Du and Sun, 1994 and Abd-El-Kareem *et al.*, 2002). However, chemical control programs facing treating problems. The use of chemical fungicides imposes a selective pressure upon the pathogen population and have residual harmful effect to the human causes dangerous diseases (Bower *et al.*, 2003). There is a growing need to develop alternative approaches for safe controlling post-harvest diseases of citrus fruits. Chitosan is biopolymer, which has numerous applications in agriculture and agroindustries. Coating fruits and vegetables with chitosan has some advantages for the long term storage of foods, because the film of chitosan provides a kind of an active package, which allows a gradual release of preservatives, thus inhibiting fungal growth and maintaining the external appearance of fruit for a longer time (Galed *et al.*, 2004). It has been shown to have fungicidal activeness against several fungi (Du and Sun, 1994). Abd- El-kareem *et al.* (2002) obtained complete inhibition with chitosan 6g/l for *Geotricum candidum* and at 8g/l for *P. digitatum* and *P. italicum*. He also found that Lime fruits coated with chitosan at concentration 1.5 and 2% they reduced soure rot by 89.3 and 91.9%, green mould by 85.4 & 88.7 % and blue mould by 88.7 % & 91.0 %, respectively.

Essential oils of citrus or their constituents are shown to have fungicides activities against post-harvest pathogens of citrus (Cacioni *et al.*, 1998) which may be more toxic against fungi than commercial fungicides(Singh *et al.*, 1993). The inhibitory effect of citral on post-harvest pathogens was reported by El-Mohamedy *et al.* (2002). Abd-El-Kareem and Abd-Alla (2002) stated that citral solutions at 8ml/l caused complete inhibition for linear growth of *P. digitatum* and *P. italicum* while dipping navel fruits in citral solution at 2% showed complete protection against green and blue moulds incidence.

Considerable attention has been given to the potential of biological control against post-harvest diseases of fruit and vegetables as a viable alternative to use synthetic fungicides (Pang *et al.*, 2002; El-Ghaouth *et al.*, 2002; Obagwu and Korsten, 2003). Microbial antagonists have been reported to protect a variety of harvested perishable commodities against a number of post-harvest pathogens (Wisinewski *et al.*, 2001). *Bacillus subtilis* was found to be effective against citrus fruits (El-Ghaouth *et al.*, 2002 and Obagwu and Korsten, 2003). Bull *et al.* (1997) found that controlled green and blue moulds on both lemon and orange caused by *P. digitatum and P. italicum* by using *Psedomonas syringae* .

The objective of this study is to evaluate the protective effect of chitosan and citral solution for coating fruits against post-harvest diseases incidence of Valencia orange. In addition, the effect of bioagents to avoid fungicides in the disease control .

MATERIALS AND METHODS

Estimation of naturally decayed Valencia orange fruits:

Valencia orange fruits collected from different local markets at Cairo, Giza, Qualyubia, 6th October and El-Oubor cities, during summer and winter seasons (2004),were classified into two groups apparently healthy to natural infection and that showing initial decayed symptoms. The two groups of fruits were surface disinfected

by dipping in 5% sodium hypocholorite for 2 min, washed with sterilized water and left to dry, then placed in sterilized moist desiccators and incubated for 5-7 days at 20 ± 2 °C and then examined. The appearing fungal colonies were picked up. The fungi purified by single spore technique described by Ezekiel (1930) then kept in refrigerator on potato dextrose agar PDA medium. Pure colonies of fungal isolates were examined microscopically and identified according to Kenneth *et al.* (1968), Ellis (1971) and Barnett and Hunter (1972). Verification of the identification was done by the Mycology Dept. Plant Pathology Research Center, Agricultural Research Center, Giza.

A-Effect chitosan and citral on pathogenic fungi *in vitro*:

1-Effect on linear growth:

Chitosan and citral obtained from Plant Pathology Dep., NRC. Chitosan solution was prepared by method described by El-Ghaouth *et al.*(1991). It dissolved in HCl and neutralized with NaOH. The precipitated chitosan was collected, washed with deionized water and subsequently lyophilized. Chitosan solution was added to conical flasks containing PDA medium to obtain the proposed concentrations, *i.e.* 0, 2, 4, 6 and 8 g/l, then mixed gently and dispensed in sterilized Petri plates (9-cm-diam.). Also citral solution was added as chitosan at the same concentrations ml/l. Plates were individually inoculated at the center with equal disks (5-mm-diam.) of 10 days old culture of *P. digitatum*, *P. italicum* and *A. citri*. Inoculated plates were incubated at 20 ± 2 °C. The linear growth was measured when the check plates reached full growth and the average linear growth of fungi was calculated. Each treatment was represented by three replicates.

2-Effect on spore germination:

Spores of 10 days old of cultures *P. digitatum, P. italicum* and *A. citri* were harvested in sterilized water containing (0.1 %tween 80) and adjusted to concentration of (10^6spore/ml). One ml of each prepared spore suspension was inserted into Petri plates. PDA media containing different concentration of 0, 2, 4, 6 and 8 g/l from chitosan and other containing the same concentration from citral ml/l were poured before solidification into the previous inoculated plates and rotated gently to ensure even distribution of fungal spores. Three plates as replicates were used for each treatment and inoculated plates were Incubated at 20°C for 24hr. The Germinated spores were counted microscopically and percentage of spore germination was calculated according to the following formula:

$$\text{Percentage of germination} = \frac{\text{No. of germinated spores}}{\text{Total number of fruits}} \times 100$$

B-Effect of bioagents on pathogenic fungi:

Bioagent *i.e Bacillus subtilis* and *Pseudomonas fluorescens* obtained from Plant Pathology Dept., NRC. were tested for their antagonistic capability against *P. italicum*, *P. digitatum* and *A citri* using dual culture technique (Ferreia *et al.*, 1991). Cultures of pathogenic fungi and antagonistic fungi growth on PDA medium for i0 days as well as bacterial cultures grown on nutrient broth for 48 hr were used in this test. Mycelial disks (5-mm-diam.) of pathogenic fungal tested growing on PDA medium were aseptically transferred singly to the center of the PDA plates. A loopfulls of each bioagent taken from 48 hr old nutrient broth cultures were placed

at each of the four corners of the plate in perpendicular positions. Three Petri plates were used as replicates for each bioagent tested. A set of plats inoculated only with pathogenic fungal disks was served as check. All plates were incubated at 20±2 °C for 7 days .The reduction in the fungal growth due to antagonistic effect of bioagent was calculate using the following formula:

$$\text{Growth reduction (\%)} = \frac{\text{Growth diameter in check-growth diameter in treatment}}{\text{Growth diameter in check}} \times 100$$

Effect of chitosan, citral and bioagents on post-harvest disease of Valencia orange fruits:

Fresh orange fruit (Valencia cv.) apparently free from physical damage and disease were surface disinfected with sodium hypochlorite (5%) for 2 min, then washed several times with sterilized water . Fruits were gently injured with sterilized needle and dipped in 0.5, 1.0, 1.5 and 2.0 % chitosan or citral at the same concentration and two bioagent *B. subtilis* and *P. fluorescens* suspension (10^4 spore/ml) for three minutes. Control fruits (non- treated) were dipped in sterilized water. The fruits treated and control (non- treated) were air dried for 2 hr in laminar flow. Inoculation of fruits was carried out by spraying them individually with spore suspension (10^6 spore /ml) of each *P. digitatum*, *P. italicum* or *A. citri*. Treated and non-treated fruits stored at 20 °C for 28 days. Orange fruits were examined daily for disease assessment. Each treatment was represented by 5 replicates with 10 fruits of each were used . Each experiment was repeated three time.

Disease assessment:

Percentage of severity of infection fruits was recorded after 7, 14, 21 and 28, days of storage as Fallik *et al.* (1996). Fresh weight of rotted tissue was recorded after 28 days of storage and percentage of rotted tissue in relative to the whole weight of fruit was calculated.

Statistical analysis:

Data were statistically analyzed using MSTAT-C computer program v.2.10 (1988).

RESULTS

Survey of orange decay:

Samples of Valencia orange fruits collected from different local markets were classified into two groups healthy to natural infection and decay fruits. Decayed fruits were stored at 20 °C for 5- 7 days then examined.

Results presented in Table (1) indicated that Valencia orange sensitive to natural decay which recorded as mean (23 .1%). Percentage of decay fruits in Qualyubia Governorate and 6th October city were relatively higher than percentage of decay fruits in Cairo, El-Oubour and Giza respectively. It clear that green mould is the most important disease affecting orange fruits(56.3%) from decayed fruits. Meanwhile, blue mould and brown spot were less effective represent 25.7and 12.9 % respectively. Physical damage represents only 5.1 % of decayed fruits. Isolation from decayed fruits proved that the causal agents of green mould, blue mould and brown spot are *P. digitatum*, *P. italicum* and *A. citri*, respectively.

Table (1): Survey of Valencia orange decay fruits caused by fungi and physical damage at different local markets.

Market locations	Decay fruit (%)	Causal agent (%)			
		Green mould	Blue mould	Brown spot	Physical damage
Cairo	22.9	52.9	28.5	12.9	5.7
Giza	18.6	50.0	27.1	15.7	7.2
Qualyubia	28.6	54.3	25.7	14.3	5.7
6th October city	25.7	62.9	24.3	10	2.8
El-Oubour	20.0	61.4	22.9	11.4	4.3
Mean	**23.1**	**56.3**	**25.7**	**12.9**	**5.1**

***In vitro* studies:**

Effect of chitosan and citral on linear growth and spore germination of post-harvest fungi:

Chitosan at four concentrations *i.e.* 2, 4, 6 and 8 g/l and citral solution at the same concentrations (ml/l) were tested against the linear growth and spore germination of the three fungi pathogenic fungi to Valencia orange fruits.

Results in Table (2) indicated that all tested concentrations chitosan and citral significantly inhibit the linear growth and spore germination of the three tested fungi compared with control. Inhibition was increased by increasing the concentration of citral and chitosan. At any concentration, citral was significantly more effective against *P. digitatum* and *P. digitatum* than chitosan,while the opposite trend was recorded against *A. citri*. Complete inhibition was obtained with chitosan at 6g/l for *A. citri*, and at 8g/l for *P. digitatum* and *P. italicum*, while citral at 8ml/l completely inhibited three tested fungi.

Table (2): Effect of different concentrations of chitosan and citral solution on linear growth (mm) and spore germination (%) of Valencia orange rot fungi.

Treatments and concentration	*P. digitatum*		*P. italicum*		*A. citri*	
	Linear growth	Spore germination	Linear growth	Spore germination	Linear growth	Spore germination
Chitosan (g/l)						
2.0	46.8b	40.2 b	53.1 b	41.3 b	35.9 d	31.5 c
4.0	38.5d	30.5 c	37.0 c	30.5 c	21.5 e	19.5 d
6.0	25.2f	20.0 e	18.0 e	16.5 e	0.0 f	0.0 e
8.0	0.0h	0.0 g	0.0 h	0.0 h	0.0 f	0.0 e
Citral (ml/l)						
2.0	42.0 c	30.0 c	25.5 d	18.8 d	55.0 b	53.5 b
4.0	27.3 e	25.8 d	12.5 f	10.5 f	40.5 c	31.8 c
6.0	14.5 g	12.9 f	7.3 g	4.2 g	21.0 e	20.1 d
8.0	0.0 h	0.0 g	0.0 h	0.0 h	0.0 f	0.0 e
Control	90.0 a	93.0 a	90.0 a	91.5a	90.0 a	90.5 a

The same letter in the same column are not significantly different.

Effect of different bioagents on the linear growth of tested fungi:

Data in Table (3) indicated that bioagents tested had inhibitory effect on the linear growth of the pathogenic fungi tested. Growth of *P. digitatum*, *P. italicum* and *A. citri* reduced by 95, 90 and 88.0 % respectively in presence *B.subtilis*, while the presence of *P. fluorescens* caused 100.0, 98.0 and 85.0% reduction in growth of the three pathogens, respectively.

Table (3): Inhibitory effect of two bioagents on the linear growth of *P.digitatum*, *P.italicum* and *A. citri*.

Tested bioagent	Growth reduction %		
	P.digitatum	*P. italicum*	*A. citri*
B. subtilius	95.0 b	90.0 a	88.0a
P. fluorescens	100.0 a	98.0 b	85.5a
Control	0.0 c	0.0 c	0.0 b

The same letter in the same column are not significantly different

Effect of chitosan, citral and bioagent on the post-harvest disease of Valencia orange fruits:

Four concentrations of chitosan and citral *i.e.* 0.5, 1.0, 1.5, and 2.0 % were tested against green, blue moulds and brown spot diseases incidence of Valencia orange.

Results in Table (4) revealed that chitosan considered the superior treatment for decrease the diseases incidence of orange fruits comparing with control (non-treated) fruits followed by citral solution and then bacterial treatments after 28 days of storage. Orange fruits treated with 2% chitosan recorded 14.7, 11.0 and 8.8%, while those treated with 2% citral recorded 16.2, 13.0 and 12.6 % for green, blue moulds and brown spot incidence, respectively. On the other hand, moderate protective was observed on orange fruits coated with bacterial isolates *B.subtilis* at 10^4 spore/ml which caused decreasing incidence by 33.4, 30.3 and 20.9% but *P. Fluorescens* causing 29.5, 25.8 and 15.9% decreasing in green,blue moulds and brown spot incidence, respectively after 28 days of storage.

Effect of chitosan, citral and bioagent on fruit rotted tissue part of diseased orange fruits caused by green, blue moulds and brown spot after storage.

The data presented in Table (5) indicated that all treatments reduced the percentage of rotted tissues part of orange fruits infected by green, blue moulds and brown spot. High reduction in rotted tissues part was obtained in orange fruits which coated with 2% chitosan, causing 96.9 %, 98.0%, 98.7% reduction in rotted parts while coating with 2% citral reduced 95.4%, 96. 4% and 96.6 % of rotten parts, while *B. subtilis* reduced by 64.1%, 50.2% and 74.8%, in addition *P. fluorescens* reduced by 60.4%, 52.4 %and 7o.6% of rotten parts of orange fruit infected by green, blue moulds and brown spot, respectively.

Table (4): Effect of chitosan, citral concentration and bioagent on incidence of Valencia orange fruits decay caused by the three tested fungi after 28 days of storage.

Treatments	% severity of infection under artificially inoculation with											
	P. digitatum after Storage period (days)				*P. italicum* after Storage period (days)				*A citri* after Storage period (days)			
	7	14	21	28	7	14	21	28	7	14	21	28
Chitosan												
0.5%	9.0 c	27.3 c	35.0 c	40.2c	10.2d	27.0c	32.4c	42.0c	10.0b	16.3b	22.0 b	7.4 c
1.0%	6.7 d	18.4 e	23.7 e	30.7d	6.45 f	14.0d	21.3e	32.5f	6.1 f	8.3 d	14.6 c	16.9 f
1.5%	4.3 e	10.5 f	13.9 g	21.0 f	4.9h i	9.3 f	11.6h	26.2h	3.3 i	5.5 e	9.5 d	11.5 i
2.0%	3.8 e	8.4 g	10.0 i	14.7 j	4.25 i	7.0 g	8.2 i	11.0 j	2.0 j	4.3 f	7.3 e	8.8 k
Citral												
0.5%	10.7 b	30.5 b	37.0b	49.0b	18.3b	43.2b	48.5b	53.5b	9.45c	11.0c	23.1b	37.0b
1.0%	8.1 c	22.6 d	26.4d	30.2d	16.3c	27.8c	30.5d	37.5d	7.9 d	8.7 d	14.3 c	25.9d
1.5%	2.8 f	9.1 g	17.9 f	23.2e	8.4 e	13.5d	20.0 f	26.8h	5.2 g	5.7 e	7.9 e	15.7g
2.0%	1.0 g	3.3 i	10.2 i	16.2 i	4.6 i	7.0 g	12.5h	13.0 i	4.3 h	4.8 ef	6.9 e	12.6h
B.subtilis	2.8 f	6.5 h	11.7 h	33.4g	6.0 fg	11.3e	20.8ef	30.3g	7.3 e	10.9 c	14.4 c	20.9 e
P.flourescens	2.0 f	5.6 h	12.9 g	29.5d	5.0 hi	10.0f	18.4g	25.8h	2.0 j	4.8 ef	7.0 e	15.9g
Control	37.0a	65.0 a	92.0 a	100.0a	45.5a	91.0a	98.0a	100.0a	37.5a	72.0a	87.0 a	92.3 a

The same letter in the same column are not significantly different.

Table (5): Effect of coating with chitosan, citral or bioagent on wight of rotted tissues in diseased Valencia orange fruits caused by green, blue moulds and brown spot after 28 days of storage at 20 °C.

Treatment	Rotted tissue part					
	Green mould		Blue mould		Brown mould	
	Fresh weight of rot part %	Reduction %	Fresh weight of rot part %	Reduction %	Fresh weight of rot part %	Reduction %
Chitosan						
0.5%	23.6 d	73.8	14.4 f	82.7	13.8 g	82.1
1.0%	12.5 e	86.1	11.5 h	86.1	9.8 h	87.3
1.5%	4.8 g	94.7	2.6 i	96.9	2.3 j	97.0
2.0%	2.8 h	96.9	1.6 j	98.0	1.0 k	98.7
Citral						
0.5%	32.0 c	64.4	46.2 b	44.3	33.9 b	56.1
1.0%	23.2 d	74.2	30.1 e	63.7	28.7 c	62.9
1.5%	10.1 f	88.8	12.5 g	84.9	15.4 f	80.1
2.0%	4.1 g	95.4	3.0 j	96.4	2.6 j	96.6
B.subtilis	32.3 c	64.1	41.3 c	50.2	19.5 e	74.8
P. flourescens	35.6 b	60.4	39.5 d	52.4	22.6 d	70.6
Control	90.0 a		83.0 a		77.3 a	

The same letter in the same column are not significantly different

DISCUSSION

Green, blue moulds and brown spot caused by *Penicillium digitatum*, *P. italicum* and *A. citri* are important post-harvest diseases which affecting Valencia orange during storage and exportation (Cacioni *et al.*, 1998; Abd-El-Kareem *et al.*, 2002 and Ismail and Zhang, 2004). Survey of the naturally occurrence of Valencia orange diseases indicate that the most dominant fungus is *P. digitatum* (56.3%) followed *P. italicum* (25.7%) and (12.9 %) for *A. citri*, while physical damage was less effective (5.1). Chitosan, citral and microbial biocides were used in the present study against green, blue moulds and brown spot under *in vitro* and *in vivo* conditions. Chitosan is the soluble form of chitin, and its derivatives have plant protective and antifungal properties. They can trigger defensive mechanism in plants against pathogenic attacks at very low concentrations(Pramila and Douby, 2004). Chitosan caused complete inhibition of linear growth and spore germination at 8gm/l for *P. digitatum*, *P. italicum* and *A. citri*, while citral caused complete inhibition at 6ml/l for *A. citri* and 8ml/l for *P. digitatum*, *P. italicum*. Simlarly reported by (Abd-El-kareem and Abd-Alla, 2002 and Abd-El-Kareem *et al.*, 2002).

The mechanism of chitosan or citral coating in reducing post harvest dieases of orange fruits appears to be related to its fungistatic property (El-Ghaouth *et al.*, 1992 and Rodove *et al.*, 1985). The mode of action proposed to explain the antifungal activity of chitosan: frist, the activity of chitosan is related to its ability to interfere with the plasma membrane function (Leuba and Stossel, 1986) and second

the interaction of chitosan with fungal DNA and RNA is the basis of its antifungal effect (Hadwiger and Loschke, 1981). Coating orange fruits with chitosan, citral or bacteria provide preventive effect against infection by green, blue moulds and brown spot and reduced fungal infection and delay disease development under artificial inoculation during storage period up to 28 days. Chitosan at 2% caused the highest decreased in percentage of severity of infection of three tested fungi and showed the lowest percent of rotted tissues part comparing with the control treatment followed by citral and bacteria .

Antagonistic bactria were used for controling post-harvest diseases (El Ghaouth *et al.*, 2002 and Obagwu and Korsten, 2003). The results indicated bacteria *Bacillius subtilis* and *Pesdomonas flourscences* inhibited the linear growth of three tested fungi, also it significantly reduced the green, blue and brown spot incidence on Valencia orange. These finding are in harmony with those reported by(Bull *et al.*, 1997; Pang, 2002; El-Ghaouth, 2002 and Obagwu and Korsten, 2003). Obagwu and Korsten, (2003) evaluated that the *B. subtilis* F1, L2, and L-5 isolates each alone or in combination with sodium bicarbonate(SB) or hot water(45°C) for treatment on"Valencia" and "Shamouti" orange artificially inoculated with *P. digitatum* and *P. italicum* and stored for four weeks at 10±1°C. When applied alone, all isolates performed significantly better than the water control in checking the incidence of both green and blue moulds. The controling ability of bacteria against pathogen may be related to competation or nutrients and space antibiotics production and / or direct parasitism and induced resistance(Wilson and El-Ghaouth, 1993) .

The present results suggested that citral and chitosan as fruit coating can be considered as an applicable and effective safely technique for controlling post-harvest disease of Valencia orange fruits. Moreover it can replace all fungicidal treatments. Further studies are needed for biological control due to its culmination of complex interactions among the host, pathogen, antagonist and environment .

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بعض المعاملات الآمنة لأمراض ما بعد الحصاد لثمار البرتقال الصيفي

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تم عمل حصر لأهم الأمراض التى تصيب ثمار البرتقال الصيفي من الأسواق التجارية المختلفة فى جمهورية مصر العربية وجد أن العفن الأخضر والأزرق والتبقع البنى المتسبب عن بنسيليوم ديجيتاتم و بنيسيليوم إيتالكم والترناريا سيترای هى أهم الأمراض التى تصيب ثمار البرتقال الصيفى حيث أن نسبة الإصابة كانت ٥٦,٣٪، ٢٥,٧٪، ١٢,٩٪،على الترتيب. أدى أستخدام الكيتوزان بتركيز ٦جم /لتر إلى تثبيط كامل لكل من نمو وإنبات الجراثيم لفطر الترناريا سترای و٨جم/لتر للبنسيليوم ديجيتاتم وبنسيليوم إيتالكم. وكذلك زيت السترال بتركيز ٨ مل / لتر أدى إلى التثبيط الكامل لنمو وإنبات جراثيم الفطريات المختبرة. ومن ناحية أخرى أظهرت العوامل الحيوية باسيلس ساتلس و سيدموناس فلورسنت قدرة تضادية عالية تتسبب فى تثبيط الفطريات الممرضة بنسبة تتراوح ما بين ٨٥,٥ ٪ إلى ١٠٠٪ .

أدت تغطية ثمار البرتقال الصيفى بالكيتوزان بتركيز ٢ ٪ إلى خفض نسبة الإصابة بنسبة ١٤,٧٪ ، ١١,٠٪ ، ٨,٨٪ وكذلك السيترال عند ٢ ٪ بنسبة ١٦,٢٪، ١٣,٠٪، ١٢,٦ ٪ للفطريات بنسيليوم ديجيتاتم وايتالكم والترناريا سيترای على الترتيب، وكذلك أدت تغطية الثمار بالبكتيريا المضادة إلى تقليل حدوث المرض

بنسبة٣٣,٤ ٪ ،٣٠,٣٪ ، ٢٠,٩٪، للباسيلس ساتلس ولكن سيدوموناس فلورست بنسبة ٢٩,٥٪ ، ٢٥,٨٪ ، ١٥,٩٪ للفطريات المختبرة بالترتيب وذلك بعد التخزين لمدة ٢٨ يوم .أدت المعاملة بالكيتوزان عند تركيز ٢ ٪ إلى إختزال نسبة الأنسجة المتعفنة بنسبة ٩٦,٩٪ ، ٩٨,٠٪ ، ٩٨,٧٪ من الثمار المصابة بالفطريات الثلاثة الممرضة فى حين أن الستراك أختزل بنسبة ٩٥,٤٪ ، ٩٦,٤٪ ، ٩٦,٦٪ بينما كان معدل أختزال نسبة الأنسجة المتعفنة متوسط عند استخدام المقاومة الحيوية ببكتيريا باسيلس ساتلس وسيدوموناس فلورست ضد الفطريات المسببة للعفن الأخضر والأزرق والتبقع البنى على التوالى .

تشير هذه الدراسة إلى إمكانية إستخدام هذه المعاملات الأمنة وخاصة الكيتوزان لمكافحة أمراض ما بعد الحصاد.