Journal of Applied Sciences Research, 9(8): 5086-5096, 2013
ISSN 1819-544X
This is a refereed journal and all articles are professionally screened and reviewed

ORIGINAL ARTICLES

Decay of Some Citrus Fruit Quality Caused by Fungi and their Control: III- Control Blue and Green mould decay by using some alternative fungicides.

[1]El-Sayed M. Embaby, [2]M. Hazaa, [3]Laila F. Hagag, [2]Talaat El-Sayed Ibrahim and [4]Faten S. Abd el-Azem

[1]Plant Pathology Dept., National Research Centre, Cairo, Egypt.
[2]Botany and Microbiology Dept., Fac. Of Science, Benha Univ.
[3]Pomology Dept., National Research Centre, Cairo, Egypt.
[4]Plant Dept., National Research Centre, Cairo, Egypt.

ABSTRACT

Green and blue moulds, due to the pathogenic action of *Penicillium digitatum* and *Penicillium italicum* respectively are the main cause of orange losses during storage. Under Mediterranean climate conditions, both together are responsible for 80% of total postharvest citrus fruit decay. They occur in all citrus growing countries, worldwide and may attack the fruits in packinghouses, in transit, in storage and in the market. Tested of some alternative fungicides *i. e*. Ascorbic acid, Benzoic acid, Citric acid and Potassium sorbate at 500, 1000, and 1500 ppm conc. on growth rate (cm) of *P. digitatum* and *P. italicum* indicated that, all tested alternative fungicides were found to be reduced significantly the growth rate of *P. digitatum* and *P. italicum* compared with untreated (control)**.** Data also presented that; Benzoic acid was the most effective than the others followed by potassium sorbate and Ascorbic acid while; citric acid was less. 1500 ppm concentration was more effective on the fungal growth rate than other concentrates. Also, both 1000 and/or 1500 ppm was better conc. for reducing the entire fungal growth rate compared with 500 ppm. *P. italicum* was the most affecting growth rate followed by *P. digitatum* comparing with non-treated control.

Key word: Citrus Fruit Quality, Fungi, Ascorbic acid, Benzoic acid, Citric acid and Potassium sorbate

Introduction

The commercial loss from decay could reach 30–50% in some susceptible citrus cultivars, and this represents a major limitation to the further expansion of the organic fruit market (Porat, *et. al*., 2000). Green and blue moulds, due to the pathogenic action of *Penicillium digitatum* and *Penicillium italicum* respectively are the main cause of orange losses during storage. Under Mediterranean climate conditions, both together are responsible for 80% of total postharvest citrus fruit decay. The type of orchard production system, field location with different types of climate and soil has a main influence on mineral composition of fruits. The mineral composition of fruits can have a significant impact on fruit quality and shelf life during postharvest period (Nunes, *et. al.,* 2010 and Munoz, *et. al.,* 2011).

The genus *Penicillium* comprises more than 150 species but only a minor proportion of them are economically important phytopathogens (Samson and Pitt, 2000; Barkai-Golan, 2001). The two fungi may appear together in the same lot or even on the same fruit. *Penicillium italicum* may sometimes develop in storage as a hyper- parasite over the green mold decay. Disease is characteristically initiated through wounds and mechanical injuries sustained during harvesting, packing and handling. The sporulating part becomes olive green in the case of *P. digitatum* and blue in the case of *P. italicum.* Under dry conditions the decayed fruit shrinks and becomes 'mummified'. The blue mold spreads directly from the decayed fruit into uninjured, healthy fruits, causing 'nesting'. The green mold typically infects adjacent fruits that have been injured. By producing and releasing ethylene, *P. digitatum* reduces the storage life of healthy fruits in the same container or sometimes even in the same room (Rivka, 2001).

Green mould (*P. digitatum Sacc*.) and blue mould (*P. italicum* Wehmer) are important post-harvest pathogens of Nagpur mandarin next to sour rot. However, green and blue mould rots occur in all citrus growing areas and often constitute the predominant type of decay. These pathogens are important post-harvest pathogens of Kin-now mandarin in Punjab State, California and other subtropical parts of the world (Snowdon, 1990 and Naqvi, 2004). *Penicillium digitatum* Sacc. (the green mold fungus) and *P. italicum.* Wehmer (the blue mold fungus) are the main wound pathogens of citrus fruits, causing the most common and the most devastating

Corresponding Author: El-Sayed M. Embaby, Plant Pathology Dept., National Research Centre, Cairo, Egypt.
E-mail: embaby.elsayed@yahoo.com

postharvest diseases. They occur in all citrus growing countries, worldwide and may attack the fruits in packinghouses, in transit, in storage and in the market.

Penicillium digitatum Sacc. and *Penicillium italicum* Wehmer are the two most significant and widely reported post-harvest pathogens in citrus. Blue mold is more harmful because it spreads in the box and healthy fruits are directly attacked, regardless of injury. Blue mold is a nesting-type pathogen, meaning that it produces enzymes that soften the adjacent fruit and thus allow fungus to enter. Green mold does not spread by nesting; thus, if a single fruit is affected it remains as such without contaminating adjacent fruit. However, spores lead to soiling of fruits and thus require repacking with a box change. Green mold infects fruit through wounds. Orchard and packinghouse sanitation is required to restrict sporulation of *Penicillium* on fruits in orchards and packinghouses to minimize decay losses (Milind, 2008). The postharvest fungal pathogens *Penicillium digitatum*, *P. italicum* and *P. expansum* are an increasing problem for the Mediterranean orchards and fruit industry (Munoz, *et. al.*, 2011).

Control citrus fruit decay caused by fungi:

1- Disinfection and Cleaning:

Disinfection of water used in washers or for general cleaning purpose can be achieved by chlorination, ozone, and/or UV treatment. The most commonly used chemicals are sodium or calcium hypochloride for chlorination. Liquid soaps and detergents are also added to the wash water used for general sanitation of the packing house and for cleaning fruit. Fruit cleaners of various companies are available in the market. For example, FMC corporation, USA supplies fruit cleaner detergent (FMC fruit cleaner detergent 395) used as a foam application. The SOPP is commonly added to the water along with detergent before fruits are coated. Fruit cleaner, a biodegradable detergent, and FOMER (detergent _ 20 percent SOPP) are commercial products from the Fomesa Company of Spain (Zamani *et al*., 2008).

2. Surface Coatings:

Waxes and coatings for polishing and improving sheen and reducing water loss of citrus fruits are available in various forms such as solvent waxes, aqueous emulsions, and resin solutions. 'Wax' has become a generic term and any type of coating –whether it contains wax or not – is called wax. Common waxes are carnauba, paraffin, and oxidized polyethylene. The ability of these coatings to extend shelf life is due to differential permeability to CO2, O2, and water vapor, which reduce metabolic rate and water loss. Applying a water-wax emulsion by dipping was the most common method in the days before mechanization to extend the shelf life of citrus fruits. Paraffin wax offers good control of water loss while carnauba provides luster. Wax can be a carrier of fungicide to reduce rot or a carrier of anti-senescent PGR to slow aging. Water waxes give good results on clean fruit. Fruit should be at least damp (not completely dry) because a good bonding of water wax with fruit surface is necessary. Fruit should be clean to avoid 'sweating'. The organic acids, wetting agents, and oils are also added because they act as plasticizers. Washing and waxing tend to increase the carbon dioxide content and decrease the oxygen content; the degree of change depends upon the wax used. Washing increases weight loss in fruit and waxing reduces weight loss compared with that of unwashed fruit (Zamani *et al*., 2008).

3- Sodium bicarbonate (NaHCO3):

commonly known as baking soda was selected for integration with the biocontrol agent. It is a common food additive for pH adjustment, taste, texture modification, and spoilage control, and has been shown to have antimicrobial activity against *P. digitatum* on citrus fruit (Zamani *et al*., 2008). It is inexpensive, readily available, and could be used with minimal risk of injury to the fruit. However, it is a poor eradicante unable to kill spores and its inhibitory effect is not very persistent. Its inhibitory activity depends on the presence of salt residues within the wound infection sites occupied by the fungus and on interactions between this residue and constituents of the peel (Palou *et al*., 2001).

The aim of this study was:

Interestingly, this work centered on decay of some citrus fruit rots caused by *Penicillium* spp. affecting fruit quality (causing citrus fruit decay). Detection of citrus rotted fruits (fruit decay) compared with healthy once, Isolation and identification all fungal association, to assess the effect of some alternative fungicides (i.e. Ascorbic acid, Benzoic acid, Citric acid and Potassium sorbate) treatments to control the blue and green mold decay of orange cv. Valencia affecting quality characteristics during storage period under room temperature.

J. Appl. Sci. Res., 9(8): 5086-5096, 2013

Materials and Methods

Plant materials:

Citrus fruits were obtained from local orchards i.e. Nobarya region (Behera Governorate) and Kalubia governorate as, Egypt.

Effect of some chemical compound on the growth rate of pathogenic fungi:

I- In vitro conditions:

The efficacy of the tested chemical compounds as Ascorbic acid, Benzoic acid, Citric acid and Potassium sorbate, at different concentrations i.e. 500, 1000 and 1500 ppm were used to study the inhibitory effects on the growth rate of selected fungal pathogens i.e. *Penicillium digitatum and Penicillium italicum* were evaluated under *in vitro* conditions.

Chemical compounds were added to sterilized malt agar 2% medium, before solidifying to obtain the proposed concentrations i. e. 500, 1000 and 1500 ppm and then shacked for five minutes to ensure the equal distribution of the added chemical(s). After this, the concentrations were dispensed in sterilized Petri plates (9cm diameter). Malt agar medium free of chemicals was used as control treatment. All plates were inoculated at the center with discs (5mm diam.) of 10 days old culture of tested fungi. Three replicates were used for each treatment. Inoculated plates were incubated at 22±2^{o}C. The average of linear growth was measured when the fungi reached full growth with control treatment. Then, reduction (%) in mycelial growth was calculated in all treatments relative to the fungal growth (70 cm-diameters) in the control one.

II- In vivo tests:

The efficacy of the tested chemical compounds as Ascorbic acid, Benzoic acid, Citric acid and Potassium sorbate, at different concentrations i.e. 500, 1000 and 1500 ppm were used to study the inhibitory effects on selected test fungal pathogens i.e. *Alternaria citri, Penicillium digitatum and Penicillium italicum* with "Washington" Navel orange fruits under *in vivo*.

Preparation of fungal suspension:

All tested fungi were surface harvested after 10 days old culture from incubation. Spores were harvested by adding 9 ml of water sterile from the plate surface with a sterile glass rod, and passing through two layers of cheesecloth to remove the mycelial growth. The suspension was diluted with sterilized water to an optical density (OD) adjusted at 10^5 spores/ml (Plaza *et. al.,* 2004 and Zamani *et. al.,* 2009)

"Washington" Navel oranges was obtained from the field and disinfested with 70% ethanol. Calyces were removed from fruits before inoculation. Inoculation was performed by injecting 100 µl of conidial suspension (2×10^5 conidia/ml) into columella with syringe. For each isolates, six fruits were inoculated and incubated at 23 to 25°C for 21 days. Fruit rots were scored on a scale of 0 to 3 (Peever, *et. al.,* 2005 and Shideh and Naser 2012).

Experiments:

Experiments were carried out on "Washington" Navel orange fruits obtained from Qalyoubia Governorate. Mature fruits free from injuries and infections were taken then placed in clean carton boxes. Fruits were surface sterilized by dipping into 1% Sodium hypochlorite for 3 min. The fruits were washed by rinsing 3 times in sterilized distilled water and then dried with sterilized filter paper. The fruits were dipped individually into water wax solution containing a mixture of each concentration of tested for 5 minutes. Then, the artificial infection was obtained by spraying the fruits with fungal spore suspension about 2 x 10^5 spores/ml of each tested fungus. In check treatment, fruits were dipped individually into water wax solution without any chemicals and then inoculated with pathogenic fungi.

Thereafter, all treated fruits were air dried at room temperature, placed into carton boxes with a capacity of 3 fruits/box with 90-95% relative humidity (RH), covered with plastic sheets to maintain, and stored in a fruit store at room temperature for 30 days. Three replicates were used for each treatment as well as cheeks. Three fruits were used per each replicate (Plaza, *et. al.,* 2004 and Zamani, *et. al.,* 2009).

J. Appl. Sci. Res., 9(8): 5086-5096, 2013

Diseases parameters:

Percentages of disease incidence and severity of decayed fruits were determined. The loss of yield weight was calculated at the end 7, 15. 21 and 30 days of storage period according to the following formula which calculated by Milind S. Ladaniya, (2008) and Rab, *et. al.,* (2012):

a- The percentage of weight loss was calculated using the following formula:

$$\text{Percent weight loss} = \frac{\text{Weight of fresh fruit} - \text{Weight after interval}}{\text{Weight of fresh fruit}} \times 100$$

Juice Percentage:

Percentage of juice is calculated as the weight of juice divided by the weight of fruit multiplied by 100. Juice is squeezed and measured in milliliters and percentage is calculated as the volume of juice divided by the volume of the fruit multiplied by 100 (Milind S. Ladaniya, 2008).

b - Chemical Attributes (Chemical Parameters):

These attributes of quality are measured by applying certain principles of chemistry and based on response of fruit internal parts/composition to chemical reactions. These quality attributes for citrus include total titratable acidity, ascorbic acid and pH (Milind S. Ladaniya, 2008).

c- Total (Titratable) Acidity:

Phenolphthalein is generally used as a visual end point indicator; it gives a pink-colored endpoint. Total acidity can be determined by using 0.1 NaOH (or 0.3125 N NaOH) for a large number of samples.

$$\text{Acidity (\%)} = \frac{\text{Titre} \times \text{Normality of alkali} \times \text{Volume made up} \times \text{Equivalent weight of citric acid (64)} \times 100}{\text{Volume of sample taken for estimation} \times \text{Weight or Volume of juice/pulp taken} \times 1000}$$

d- Total Soluble Solids:

(MI) was evaluated by the ratio: ºBrix / % Citric acid for each extracted juice (Porat,*et. al.,*(2000),Milind S. Ladaniya, (2008) and Nunes, *et. al.,* (2010).

e- Ascorbic Acid:

Ascorbic acid can be estimated using 2.6-dichlorophenol indophenol dye that is reduced by ascorbic acid. Ascorbic acid is calculated as:

$$\text{Ascorbic acid in mg/100 ml juice or 100 g pulp} = \frac{\text{Titre value} \times \text{Dye factor} \times \text{volume made} \times 100}{\text{Aliquote taken for estimation} \times \text{juice/pulp taken}}$$

Vitamin C was calculated according to following formula

$$\text{Ascorbic acid} = \frac{F \times T \times 10}{D \times S} \times 100$$

$$F = \text{Factor for standardization} = \frac{\text{Where, (ml of ascorbic acid)}}{\text{Ml of dye}}$$

T = ml of dye used for sample – ml of dye used for blank
D = ml of sample taken for dilution
S = ml of dilute sample taken for titration

Results and Discussion

I-Effect of some alternative fungicides on growth rate (cm) of Penicillium spp:

Tested of some alternative fungicides *i. e*. Ascorbic acid, Benzoic acid, Citric acid and Potassium sorbate at 500, 1000, and 1500 ppm conc. on growth rate (cm) of *P. digitatum* and *P. italicum* indicated that, all tested alternative fungicides were effective to reduce significant growth rate of the tested fungi (*P. digitatum* and *P. italicum)* compared with untreated fungi (control) **Table (1).** Data also presented that; Benzoic acid was the most effective than the others which record average 3.14% followed by potassium sorbate which gave 3.43% and Ascorbic acid with 4.17% while; citric acid substance was less effective 4.72%. The same table also show that 1500 ppm concentration was more effective on the fungal growth than other concentrates. Also, both 1000 and/or 1500 ppm was better conc. for reducing all the fungal growth rate compared with 500 ppm conc., which were stopped completely the linear growth and given zero percent equal hundred reduction. On the other hand data in this table show that, *P. italicum* had present the most effective growth rate which record 3.78 (cm) followed by *P. digitatum* with 3.70(cm) comparing with non-treated control which record 7.00(cm).

Table 1: Reducing linear growth rate (%R) of *P. digitatum and P. italicum* tested moulds using some alternative fungicides

Substrates	Conc. [ppm]	linear growth of tested fungi [cm]				
		P. italicum		*P. digitatum*		Mean
		G(cm	%R	G(cm)	%R	
Ascorbic acid	500	4.5 ef	35.7	4.1 fg	41.4	4.17 B
	1000	2.8 ijk	59.5	2.7 jk	61.9	
	1500	1.3 nop	80.9	1.7 mn	75.2	
Benzoic acid	500	5.7 c	19.00	5.7 c	19.1	3.14 D
	1000	0.0 q	100.00	0.0 q	100.0	
	1500	0.0 q	100.00	0.0 q	100.0	
Citric acid	500	5.5 cd	21.4	5.8 bc	16.7	4.72 A
	1000	2.7 jk	61.9	3.5 ghi	50.0	
	1500	1.7 mn	76.2	2.2 klm	69.0	
Potassium sorbate	500	3.8 fg	45.2	5.7 c	48.6	3.43 C
	1000	2.5 jkl	64.3	2.2 klm	69.0	
	1500	0.8 p	89.1	1.0 op	85.7	
Control		7.0 a	00.00	7.0 a	00.0	
Mean				3.78 B		3.70 B
Mean	500	4.95 A				
	1000	2.25 B				
	1500	1.25 C				

G= growth rate (cm) %R= reduction percent
L.S.D. Fungi = 0.1626 L.S.D. Concentration = 0.1877
L.S.D. Substrates = 0.1877 L.S.D. F*S*C = 0.6503

II- Controlling Green mould disease caused by P. digitatum using some alternative fungicides on "Washington" Navel orange fruits:

1- Reducing disease incidence (%) of Green mould disease:

Data in **(Table 2)** presented that, all alternative fungicides were found to decrease significantly green mould decay comparing with untreated control. Data also showed that, benzoic acid had higher effective than others which gave average mean 26.56 **%** of disease incidence (infection) after 30 days from inoculation, comparing with non-treated control which was record 88.89 **%** of disease incidence (infection) after 30 days of storage at room temperature followed by ascorbic acid which record 20.83**%** of disease incidence (infection) after the same period time. Potassium sorbate had moderate effect and gave 25.00**%** of disease incidence, while citric acid was less affected which record 31.94**%** of disease incidence (infection). Also, 1500 ppm was the best concentration for all substances, followed by 1000 and 500 ppm which recorded 6.94, 18.06 and 43.06**%** of green mould disease incidence (infection) respectively. Benzoic acid substance was found to significantly decrease green mould decay incidence from 33.33, 44.44, 66.67 and 77.78**%** with un-treated control after 7, 15, 21 and 30 days storage at room temperature to 11.11, 22.22 and 33.33**%** after the same period at 500 ppm respectively, it give hundred percent reduction with both 1000 and 1500ppm. Marketing ability was increased from 7 until 30 days when treated fruits with 1000 and 1500 ppm by using benzoic acid under room temperature. All alternative fungicides substance was found to increase significantly Marketing ability of "Washington" Navel orange fruits for 30 days under room temperature.

- Reducing disease Severity (%) of green mould:

Reducing green mould disease severity (**%**) on "Washington" Navel orange fruits in response to different concentrations of some alternative fungicides substance was studied. Data in **(Table3)** indicated that, all alternative fungicides were found to significantly decrease green mould severity, compared with untreated control. Benzoic acid had higher effect controlling green mould severity which record 10.56 **%** of disease

severity compared with other substances followed by potassium sorbate and ascorbic acid which gave 11.67 and 13.47 **%,** respectively. Citric acid was less affected which gave 16.25 **%** of disease severity comparing with non-treated control which gave 53.34 **%**. Also, 1500 ppm was the best concentration with all substances followed by 1000 and 500ppm which recorded 1.81, 5.42 and 14.17 **%** of green mould disease severity, respectively. On the other hand, both 1000 and 1500 benzoic acid conc. were found to reduced hundred percent of disease severity and protected Navel orange citrus fruits for 30 days under room temperature. All alternative fungicides substance was found to significantly increase marketing ability of "Washington" Navel orange fruits for 30 days under room temperature.

Table 2: Reducing disease incidence (%) of Green mould disease on "Washington" Navel orange fruits caused by using some alternative fungicides *in vitro*

Treatments	Conc. [ppm]	Disease incidence (%)				Mean
		7	15	21	30	
Ascorbic acid	500	00.00 f	11.11 ef	55.56 bc	77.78 ab	27.79 B
	1000	00.00 f	33.33 cde	55.56 bc	55.56 bc	
	1500	00.00 f	00.00 f	11.11 ef	33.33 cde	
Benzoic acid	500	00.00 f	33.33 cde	44.44 cd	55.56 bc	11.11 C
	1000	00.00 f	00.00 f	00.00 f	00.00 f	
	1500	00.00 f	00.00 f	00.00 f	00.00 f	
Citric acid	500	44.44 cd	55.56 bc	77.78 ab	88.89 a	37.04 A
	1000	00.00 f	22.22 def	44.44 cd	55.56 bc	
	1500	00.00 f	00.00 f	22.22def	33.33 cde	
Potassium sorbate	500	00.00 f	11.11 ef	44.44 cd	77.78 ab	12.96 C
	1000	00.00 f	00.00 f	00.00 f	22.22 def	
	1500	00.00 f	00.00 f	00.00 f	00.00 f	
Control		44.44 CD	55.56 BC	77.78 AB	88.89 A	
Mean		11.11 D	22.22 C	42.36 B	53.47 A	
Mean	500	43.06 A				
	1000	18.06 B				
	1500	6.94 C				

L.S.D Treatments= 6.145 L.S.D concentration = 6.145
L.S.D Days = 6.145 L.S.D T*C*D = 24.58

Table 3: Reducing disease severity (%) of green mould on "Washington" Navel orange fruits

Treatments	Conc. [ppm]	Disease severity (%)				Mean
		7	15	21	30	
Ascorbic acid	500	00.00 g	4.45 fg	13.33 bcdef	20.00 bcde	13.47 B
	1000	00.00 g	11.11 cdefg	15.55 bcdef	17.78 bcdef	
	1500	00.00 g	00.00 g	4.45f g	6.67 efg	
Benzoic acid	500	00.00 g	11.11 cdefg	15.55 bcdef	20.00 bcde	10.56 C
	1000	00.00 g	00.00 g	00.00 g	00.00 g	
	1500	00.00 g	00.00 g	00.00 g	00.00 g	
Citric acid	500	15.55 bcdef	17.78 bcdef	22.22 bcd	26.67 b	16.25 A
	1000	00.00 g	8.89 defg	13.33 bcdef	15.55 bcdef	
	1500	00.00 g	00.00 g	6.67 efg	11.11 cdefg	
Potassium sorbate	500	00.00 g	13.33 bcdef	20.00 bcde	26.67 b	11.67 BC
	1000	00.00 g	00.00 g	00.00 g	4.45f g	
	1500	00.00 g	00.00 g	00.00 g	00.00 g	
Control		17.78 bcdef	24.45 bc	26.67 b	53.34 a	
Mean		5.416 D	10.28 C	13.61 B	22.64 A	
Mean	500	14.17 A				
	1000	5.42 B				
	1500	1.81 C				

L.S.D Treatments = 2.737 L.S.D concentration = 2.737
L.S.D Days = 2.737 L.S.D T*C*D = 10.95

3- Efficacy of alternative fungicides in reducing some physical characteristics of stored Navel orange fruits caused by P. digitatum fungus:

All changes in physical characteristics i.e. weight loss (%), juice (%) and Fiber (g for the whole fruit) of stored Navel orange fruits affected by *P. digitatum* fungus compared with non-treated control **(Table 4)**. Data in this table presented that, significant differences were recorded in between the three tested concentration of alternative fungicide substances. 1500 ppm was the most affecting on all physical determined than others.

On the other hand, data in this table show that, benzoic acid substances had higher effect and significantly decreased weight loss (%) of Navel orange fruits compared with other tested substances which recorded 1.6 %, comparing with non-treated control, followed by ascorbic acid which gave 1.8%.Potassium sorbate and citric acid was less effective which gave 2.2% and 2.3%, respectively, with no significant differences.

All alternative fungicide substances were found to significantly increase juice (%) of Navel orange fruits compared with non-treated control. Benzoic acid was the most effective and significantly increased juice (%) of Navel orange fruits which gave 66.56 % followed by potassium sorbate with 60.90% and citric acid with 63.25%, respectively. Ascorbic acid was the less effective one which record 53.79%

Also, the same table indicated that, all alternative fungicide substances were found to significantly increase fiber content (g for the whole fruit) of Navel orange fruits compared with non-treated control. Benzoic acid and potassium sorbate were the most effective and increased fiber content (g for the whole fruit) with no significant differences which gave 85.08 g and 81.72 g respectively, followed by ascorbic acid with 75.08 g and citric acid with 70.99, g respectively, with no significant differences.

Table 4: Efficacy of alternative fungicides in reducing some physical characteristics of stored Navel orange fruits caused by *P. digitatum* fungus

Compounds	Conc. (ppm)	Weight loss (%)	Mean	Juice (%)	Mean	Fiber (g for the whole fruit)	Mean
Ascorbic acid	500	1.85 c	1.880 B	48.5 ef	53.79 C	68.85 e	75.08 B
	1000	0.58 e		52.00 ef		74.87 de	
	1500	0.03 f		69.00 cd		91.6 bc	
Benzoic acid	500	1.35 d	1.618 C	66.64 cd	66.56 A	83.87 bcd	85.08 A
	1000	0.05 f		68.24 cd		87.59 bc	
	1500	0.01 f		85.68 a		103.84 a	
Citric acid	500	2.47 b	2.315 A	66.24 cd	63.25 AB	65.74 e	70.94 B
	1000	1.28 d		67.86 cd		69.51 e	
	1500	0.44 e		73.23 bc		83.51 cd	
Potassium sorbate	500	2.34 b	2.217 A	54.67 e	60.90 B	82.62 cd	81.72 A
	1000	1.4 d		63.59 d		84.42 bcd	
	1500	0.06 f		79.69 ab		94.82 ab	
Control		5.07a		45.67 f		65.02 c	
Mean	500	2.003 A		64.13 B		75.27 B	
	1000	0.8258 B		62.05 B		79.10 B	
	1500	0.1342 C		72.65 A		93.44 A	

4- Efficacy of alternative fungicides in reducing some chemical characteristics of stored Navel orange fruits caused by P. digitatum fungus:

All tested alternative fungicides were found to protect all chemica**l** characteristics determined i.e. TSS (%), TA (%), TSS/TA and Vitamin C (mg/100 ml juice) of stored Navel orange fruits affected by *P. digitatum* fungus compared with non-treated control as we see in **(Table 5).** Data in this table presented that, significantly differences in between the three tested concentration of alternative fungicide substances. 1500 ppm was the most affecting on all chemical characteristics determined than others. No significant in between 1000 and 1500 ppm.

Alternative fungicide substances were found to increase the average of TSS (%) were benzoic acid and potassium sorbate were the most effective substances which recorded 11.67 and 11.58%, with no significant differences, followed by ascorbic acid which gave 11.17 %, respectively. Citric acid was the less effective one which gave 10.75%.

Alternative fungicide substances were found to decrease the average of TA (%) which benzoic acid and potassium sorbate were the most effective substances which recorded 0.39 and 0.39% with no significant differences, followed by ascorbic acid which gave 0.46 %, respectively. Citric acid was the less effective one which gave 0.57%.

Alternative fungicide substances were found to significantly increase the average of TSS/ acid ratio where benzoic acid was the most effective substances which recorded 77.88%, followed by potassium sorbate which gave 56.81 %. Ascorbic and citric acid were the less effective one which gave 35.65% and 24.19% respectively, with no significant differences.

Alternative fungicide substances were found to significantly increase the Vitamin C which benzoic acid was the most effective substances which recorded 62.50% followed by ascorbic acid and potassium sorbate which gave 56.6% and 55.17%, respectively with no significant differences. Citric acid was the less effective one which gave 52.92%.

III- Controlling blue mould disease caused by P. italicum using some alternative fungicides on "Washington" Navel orange fruits:

1- Reducing disease incidence (%):

Data were recorded in **(Table 6).** Data presented that all alternative fungicides were found to significantly decrease blue mould decay comparing with untreated control. Data also show that, benzoic acid substance was higher effective than others which gave average about 26.56 **%** of disease incidence after 30 days from inoculation, comparing with non-treated control which record 88.89 **%** of disease incidence after 30 days of storage at room temperature followed by ascorbic acid which record 20.83**%** of disease incidence after the same period time. Potassium sorbate substance was moderate affecting and gave 25.00**%** of disease incidence, while citric acid was less affected which recorded 31.94**%** of disease incidence. The best concentration was obtained with 1500 ppm with all substances, followed by 1000 and 500ppm which recorded 1.39, 8.33 and 37.50 **%** of blue mould disease incidence, respectively. Benzoic acid substance was found to significantly decrease blue mould decay incidence from 33.33, 44.44, 66.67 and 77.78**%** with un-treated control after 7, 15, 21 and 30 days storage at room temperature to 11.11, 22.22 and 33.33**%** after the same period at 500 ppm respectively, while was given hundred percent reduction with both 1000 and 1500ppm. Shelf life storage was increased from 7 until 30 days when treated fruits with 1000 and 1500 ppm by using Benzoic acid substance under room temperature. All alternative fungicides substance was found significantly increase s storage shelf life of "Washington" Navel orange fruits for 30 days under room temperature.

Table 5: Efficacy of alternative fungicides in reducing some chemical characteristics of stored Navel orange fruits caused by *P. digitatum* fungus

Compounds	Conc. (ppm)	TSS (%)	Mean	TA (%)	Mean	TSS/TA	Mean	L-ascorbic acid (mg/100 ml juice)	Mean
Ascorbic acid	500	11.00 c	11.17 B	0.49 c	0.46 B	23.83 efg	35.65 C	48.00 f	56.67 B
	1000	11.67 b		0.3 defg		40.38 cdefg		62.00 cd	
	1500	12.00 b		0.19 gh		66.67 c		69.00 ab	
Benzoic acid	500	11.67 b	11.67 A	0.38 cde	0.39 C	30.7 defg	77.88 A	65.33 bc	62.50 A
	1000	12.00 b		0.23 fgh		52.44 cde		66.00 bc	
	1500	13.00 a		0.06 i		216.67 a		71.00 a	
Citric acid	500	10.33 d	10.75 C	0.73 b	0.57 A	14.26 fg	24.19 C	47.33 f	52.92 C
	1000	11.00 c		0.43 cd		25.94 defg		56.00 e	
	1500	11.67 b		0.26 efg		44.87 cdef		60.67 d	
Potassium sorbate	500	11.67 b	11.58 A	0.35 def	0.39 C	31.58 defg	56.81 B	45.33 f	55.17 B
	1000	12.00 b		0.21 gh		55.73 cd		60.00 de	
	1500	12.67 a		0.11 hi		128.21 b		67.67 ab	
Control		10.00 d		0.87 a		11.71 g		47.67 f	
Mean	500	11.17 C		0.49 A		25.09 C		51.50 C	
	1000	11.67 B		0.29 B		43.62 B		61.00 B	
	1500	12.33 A		0.15 C		114.1 A		67.08 A	

Table 6: Disease incidence (%) on navel orange fruits in response to different concentrations of some alternative fungicides

Treatments	Conc. [ppm]	Disease incidence (%)				Mean
		7	15	21	30	
Ascorbic acid	500	11.11 de	22.22 cde	55.56 ab	66.67 a	27.78 A
	1000	00.00 e	22.22 cde	22.22 cde	33.33 bcd	
	1500	00.00 e	00.00 e	00.00 e	11.11 de	
Benzoic acid	500	00.00 e	33.33 bcd	44.44 abc	55.56 ab	20.83 B
	1000	00.00 e	00.00 e	00.00 e	00.00 e	
	1500	00.00 e	00.00 e	00.00 e	00.00 e	
Citric acid	500	22.22 cde	33.33 bcd	44.44 abc	55.56 ab	25.69 AB
	1000	00.00 e	00.00 e	22.22 cde	22.22 cde	
	1500	00.00 e	00.00 e	00.00 e	11.11 de	
Potassium sorbate	500	00.00 e	33.33 bcd	55.56 ab	66.67 a	22.92 AB
	1000	00.00 e	00.00 e	00.00 e	11.11 cde	
	1500	00.00 e	00.00 e	00.00 e	00.00 e	
Control		33.33 bcd	44.44 abc	55.56 ab	66.67 a	
Mean		10.42 D	20.14 C	29.17 B	37.50 A	
Mean	500	37.50 A				
	1000	8.33 B				
	1500	1.39 C				

L.S.D Treatments = 4.990 L.S.D Concentration = 4.990
L.S.D Days = 4.990 L.S.D T*C*D = 19.96

2- Reducing blue mould disease severity (%):

Reducing blue mould disease severity (**%**) on "Washington" Navel orange fruits in response to different concentrations of some alternative fungicides substance was studied *in vivo*. Data in **(Table7)** indicated that, all alternative fungicides were found to significantly decrease blue mould severity, compared with untreated control. Benzoic acid substance was higher significantly affected in controlling blue mould severity which record 7.64 **%** of disease severity compared with other substance used followed by potassium sorbate and citric acid which gave 8.75 and 9.17 **%**, respectively. Ascorbic acid was less affected which gave 10.28 **%** of disease severity comparing with non-treated control 26.67 **%**. Also, 1500 ppm was the best concentration with all substances, followed by 1000 and 500ppm which recorded 0.42, 2.08 and 12.78 **%** of blue mould disease severity, respectively. On the other hand, both 1000 and 1500 Benzoic acid conc. were found to reduced hundred percent of disease severity and protected Navel orange citrus fruits for 30 days under room temperature. All alternative fungicides substance was found to significantly increase storage shelf life of "Washington" Navel orange fruits for 30 days under room temperature.

Table 7: Disease severity (%) on navel orange fruits in response to different concentrations of some alternative fungicides

Treatments	Conc. [ppm]	Disease severity (%)				Mean
		7	15	21	30	
Ascorbic acid	500	2.22 ij	6.67 ghij	22.22 abc	26.67 a	10.28 A
	1000	00.00 j	4.45 hij	4.45 hij	11.11 efgh	
	1500	00.00 j	00.00 j	00.00 j	4.45 hij	
Benzoic acid	500	00.00 j	6.67 ghij	15.55 cdef	17.78 bcde	7.64 C
	1000	00.00 j	00.00 j	00.00 j	00.00 j	
	1500	00.00 j	00.00 j	00.00 j	00.00 j	
Citric acid	500	6.67 ghij	11.11 efgh	15.55 cdef	17.78 bcde	9.17 AB
	1000	00.00 j	00.00 j	4.45 hij	6.67 a	
	1500	00.00 j	00.00 j	00.00 j	2.22 ij	
Potassium sorbate	500	00.00 j	8.89 fghi	20.00 abcd	26.67 a	8.75 BC
	1000	00.00 j	00.00 j	00.00 j	2.22 ij	
	1500	00.00 j	00.00 j	00.00 j	00.00 j	
Control		13.33 defg	17.78 bcde	24.45 ab	26.67 a	
Mean		3.89 D	6.81 C	11.25 B	13.89 A	
Mean	500	12.78 A				
	1000	2.08 B				
	1500	0.42 C				

L.S.D Treatments= 1.344 L.S.D concentration = 1.344
L.S.D Days = 1.344 L.S.D T*C*D = 6.206

3- Efficacy of alternative fungicides in reducing some physical chemical characteristics of stored Navel orange fruits caused by P. italicum fungus:

All changes in physical characteristics i.e. weight loss (%), juice (%) and Fiber (g for the whole fruit) of stored Navel orange fruits affected by *P. italicum* fungus compared with non-treated control **(Table 8)**. Data in this table presented that significant difference in between the three tested concentration of alternative fungicide substances. 1500 ppm was the most affective concentration in all tested parameters. On the other hand, data in this table show that benzoic acid, potassium sorbate, ascorbic acid and citric acid substances were higher effective and decreased the weight loss (%) of Navel orange fruits, compared to other tested substances where no significant differences. All alternative fungicide substances were found to significantly increase juice (%) of Navel orange fruits, compared to non-treated control. Both benzoic acid and potassium sorbate were the most effective which increase juice (%) of Navel orange fruits, with no significant differences to 41.89 and 41.10%, followed by ascorbic acid and citric acid being, 39.06 and 39.12% with no significant differences.

The same table also indicated that all alternative fungicide substances were found to increase fiber content (g for the whole fruit) of Navel orange fruits compared to non-treated control. Both benzoic acid and potassium sorbate were the most effective which increase fiber content (g for the whole fruit) of Navel orange fruits to, 85.08 and 81.72 g where no significant differences were recorded, while ascorbic acid and citric acid were 75.08 and 70.94 (g for the whole fruit).

4- Efficacy of alternative fungicides in reducing some chemical characteristics of stored Navel orange fruits caused by P. italicum fungus:

All tested alternative fungicides were found to protected all chemica**l** characteristics determined i.e. TSS (%), TA (%), TSS/acid ratio and L-ascorbic acid (mg/100 ml juice) of stored Navel orange fruits affected by *P.*

italicum fungus compared with non-treated control as we see in (**Table 9).** Data in this table presented that significantly differences in between the three tested concentration of alternative fungicide substances. 1500 ppm was the most affecting on all Chemical determined than others. No significant in between 1000 and 1500 ppm. Alternative fungicide substances were found to increase the average of TSS (%). Both Benzoic acid, ascorbic acid and Potassium sorbate were the most effective which increase TSS (%) of Navel orange fruits with no significant differences being, 11.85, 11.33 and 11.33%, followed by citric acid being, 10.9%.

Alternative fungicide substances were found to decrease the average of TA (%). Both benzoic acid and potassium sorbate were the most effective which increase TA (%) of Navel orange fruits with no significant differences being, 0.41 and 0.41% followed by ascorbic acid being, 0.47%, while citric acid was the less effective one being, 0.54%. Alternative fungicide substances were found to increase the TSS/acid ratio. Benzoic acid was the most effective which significantly increase TSS/acid ratio of Navel orange fruits (103.7%) followed by potassium sorbate (74.1%).While ascorbic and citric acids were the less effective with no significant differences (46.08 and 44.42%), respectively. Alternative fungicide substances were found to increase the L-ascorbic acid mg/100 ml juice. Benzoic acid was the most effective which significantly increase L-ascorbic acid of Navel orange fruits being 66.50%, followed by potassium sorbate being 64.75%.While ascorbic and citric acids were the less effective with no significant differences being 60.08 and 57.75%, respectively.

Table 8: Efficacy of alternative fungicides in reducing some physical chemical characteristics of stored Navel orange fruits caused by *P. italicum digitatum* fungus

Compounds	Conc. (ppm)	Weight loss (%)	Mean	Juice (%)	Mean	Fiber (gm)	Mean
Ascorbic acid	500	2.23 b	2.51 A	42.01 cd	39.06 B	84.92 de	75.08 B
	1000	2.19 b		42.61 bcd		96.88 c	
	1500	0.52 bc		43.28 abc		109.92 ab	
Benzoic acid	500	5.06 a	2.55 A	45.83 ab	41.89 A	91.05 cde	85.08 A
	1000	0.28 c		46.65 a		96.37 c	
	1500	0.05 c		46.74 a		112.18 a	
Citric acid	500	2.14 b	2.17 A	39.53 d	39.12 B	83.51 e	70.94 B
	1000	0.99 bc		44.11 abc		92.82 cde	
	1500	0.72 bc		44.49 abc		93.99 cd	
Potassium sorbate	500	1.95 b	2.03 A	45.01 abc	41.10 A	95.08 cd	81.72 A
	1000	0.99 bc		45.22 abc		101.14 bc	
	1500	0.33 c		45.84 ab		101.49 bc	
Control		4.83 a		28.34 e		68.73 f	
Mean	500	2.84 A		43.09 B		88.60 C	
	1000	1.11 B		44.65 AB		96.80 B	
	1500	0.47 B		45.06 A		104.4 A	

Table 9: Efficacy of alternative fungicides in reducing some chemical characteristics of stored Navel orange fruits caused by *P. italicum* fungus

Compounds	Conc. (ppm)	TSS (%)	Mean	TA (%)	Mean	TSS/TA	Mean	Vitamin C (mg/100 ml juice)	Mean
Ascorbic acid	500	11.67 d	11.33 A	0.47 c	0.47 B	14.91 e	46.08 C	56.00 cde	60.08 B
	1000	12.00 cd		0.24 de		41.99 de		65.33 bc	
	1500	12.33 bc		0.15 ef		118.49 bc		70.00 b	
Benzoic acid	500	11.67 d	11.58 A	0.47 c	0.41 C	24.97 e	103.7 A	56.00 cde	64.75 A
	1000	12.33 bc		0.08 f		169.66 ab		63.00 bc	
	1500	13.00 a		0.06 f		211.11 a		91.00 a	
Citric acid	500	10.67 e	10.92 B	0.72 b	0.54 A	25.44 e	44.24 C	52.33 de	57.75 B
	1000	11.67 d		0.28 d		53.1 de		63.00 bc	
	1500	12.00 cd		0.13 f		89.47 cd		67.67 b	
Potassium sorbate	500	11.00 e	11.33 A	0.38 c	0.41 C	31.17 e	74.13 B	60.67 bcd	66.50 A
	1000	12.33 bc		0.13 f		92.31 cd		70.00 b	
	1500	12.67 ab		0.08 f		164.1 ab		86.33 a	
Control		9.33 f		1.04 a		8.94 e		49.00 e	
Mean	500	11.25 C		0.51 A		24.12 C		56.00 C	
	1000	12.08 B		0.18 B		89.26 B		65.33 B	
	1500	12.50 A		0.11 C		145.8 A		78.75 A	

J. Appl. Sci. Res., 9(8): 5086-5096, 2013

References

Barkai-Golan, R., 2011. Postharvest Diseases of Fruits and Vegetables. Development and Control. 1st edition, Elsevier Science B.V., Amsterdam, Netherlands.

Milind, S.L., 2008. Citrus fruit biology, technology and evaluation. First ed book, Copyright © Elsevier Inc. All Rights Reserved Academic Press is an Imprint of Elsevier.

Munoz, A.M., B.L. Garcia, A.V. Candelas and J.F. Marcos, 2011. Comparative analysis of the sensitivity to distinct antimicrobials among *Penicillium* spp. causing fruit postharvest decay. Phytopathol Mediterr, 50: 392-407.

Naqvi, S.A.M.H., 2004. Diseases of Fruits and Vegetables, Volume I, 339-359. Kluwer Academic Publishers. Printed in the Netherlands.

Nunes, C., A. Duarte, T. Manso, J. García, J. Cayuela, K. Yousfi, M. Martínez, C. Weiland and M. Salazar, 2010. Relationship between post-harvest diseases resistance and mineral composition of citrus fruit, Acta Hort. pp: 868.

Palou, L., J. Smilanik, J. Usuall and I. Vinas, 2001. Control of postharvest blue and green moulds of oranges by hot water, sodium carbonate, and sodium bicarbonate. Plant Dis., 85(4): 371-376.

Peever, T.L., B.L. Carpenter, L.W. Timmer, L.M. Carris and A.M. Bhatia, 2005. Citrus black rot is caused by phylogenetically distinct lineages of *Alternaria alternata*. Phytopathology, 95: 512-518.

Plaza, P., J. Usall, R. Torres, M. Abadias, J. Smilanik and Viñas, 2004. The use of sodium carbonate to improve curing treatments against green and blue moulds on citrus fruits. Pest Manag Sci., 60: 815-821.

Porat, R., A. Daus, B. Weiss, L. Cohen, E. Fallik and S. Droby, 2000. Reduction of post-harvest decay in organic citrus fruit by a short hot water brushing treatment. Post-harvest Biology and Technology, 18: 151-157.

RIVKA, B.G., 2001. Postharvest Diseases of Fruits and Vegetables Development and Control. First ed. Elsevier Science B.V. Sara Burgerhartstraat 25 P.O. Box 211, 1000 AE Amsterdam, the Netherlands.

Samson, R.A. and J.I. Pitt, 2000. Integration of modern taxonomic methods for *Penicillium* and *Aspergillus* classification. 1st ed, Taylor and Francis Inc., Amsterdam, Netherlands.

Shideh, M. and S. Naser, 2012. Phylogenetic analysis of *Alternaria* species associated with citrus black rot in Iran. Plant Pathology & Microbiology, 3: 1-7.

Snowdon, A.L., 1990. A Colour Atlas of Post-Harvest Diseases and Disorders of Fruits and Vegetables. Volume 1: General Introduction and Fruits. London, UK: Wolfe Scientific Ltd, pp: 302.

Zamani, M., A. Sharifi-Tehrani, M. Ahmadzadeh, K. Behboudi and V. Hosseininaveh, 2008. Biological control of *Penicillium digitatum* on oranges using *Pseudomonas* spp. either alone or in combination with hot sodium bicarbonate dipping. Australasian Plant Pathology, 37: 605-608.

Zamani, M., A. Sharifi-Tehrani, M. Ahmadzadeh1, V. Hosseininaveh and Y. Mostofy, 2009. Control of *Penicillium digitatum* on orange fruit combining *Pantoea Agglomerans* with hot sodium bicarbonate dipping. Plant Pathology, 91(2): 437-442.