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Epidemiology, Host Specificity and Integrated Control of Postharvest Green Mold Disease Caused by Penicillium digitatum on Citrus Fruits

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ABSTRACT

In study aerodynamic spore mechanism and epidemology of Penicillium digitatum on citrus fruits stadied : Orange varities (Blood , Sweet, Sour and Navel) , mandarine , lemon and grapefruits the results showed the spores
of Penicillium digitatum have low weight to became longtime before fall down , indicating that Penicillium digitatum have dry spores . The sedimentation of these spores on fruit surfaces was more vertically faster than horizontally, conferming the way of storage fruits above long distance from ground surface does not prevent the infection incidence .
From studying of lignification effect on peels of studied citrus varities after injuires and kept for 12 hours then injection showed the lignin formation do not prevent the infection but just lated about 6 days . From study specific different isolates showed all species have specific to infect the host fruit without the other . Through study the maturity of lemon fruits on enhance or lated infection with Penicillium digitatum show the mature lemon fruits infected earlier through 4 days compared with immature lemon fruits infected after 6 days.
Finally the results optained from programs to this disease on injected and non-injected fruits by selective two physical methods (Hot water – Sea water) , two natural oils (Eucalyptus oil – Olive oil) , one mineral oil (Johnson's baby oil) and wax , showed all of these method can not prevent the infection but just delated the infection period , wax test delay five days and the other two treatments were delay about three days only .

Keywords: *Penicillium digitatum, Citrus Fruits, Spore Dispersal, Fruit Infection, Disease Management.*.

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Introduction

Citrus fruits are an essential component of some of the human nutritional requirements like vitamins, minerals and organic acids. Preservation of these products, however, is one of the central problems encountered by producers worldwide. The postharvest losses of fruit and vegetable stands at 20-40% in the average (Irtwange, 2006) .
The genus Citrus, belonging to the Rutaceae or Rue family, comprises of about 140 genera and 1,300 species. Citrus sinensis (Orange), Citrus paradise (Grapefruit), Citrus limon (Lemon), Citrus reticulate (tangerine), Citrus grandis (shaddock), Citrus aurantium (sour orange), Citrus medica (Citron), and Citrus aurantifolia (lime) are some important fruits of genus Citrus (Kamal, et al., 2011) .

Reports on Citrus post-harvest fungi rots are extensive and world-wide. A number of fungal species including Phythium, Phytophthora, Botryodiplodia, Aspergillus, Penicillium, Rhizopus, Glasdosporium, Mucor, have been associated with Citrus fruit rot . Harvesting injuries, improper handling during transportation and poor storage facilities are general predisposing conditions for fruit rot development especially in economically stressed communities. In Nigerian markets, 20-90% fruits displayed for sale are reported to show symptoms of microbial infection . Due to environmental concerns, the control of plant diseases is now focusing on the use of natural products in place of traditional chemicals with hazardous effects. This study was therefore, conducted to identify the pathogenic fungi and also to determine the possibility of using water leaf extracts from A.

indica and C odorata as biocontrol agents of these pathogens (Edward Ntui Okey, 2015) .

Abd El-Moneim and Abd El-Mageed 2006 , studied that coating Washington Navel orange fruits with jojoba oil led to reduce fruit decay , weight loss and increasing fruits storage life . As storage days progressed total soluble sugar (TSS) increased , but the titratable acidity and ascorbic acid contents decreased . Abd-Allah et al., 2012 found that coating persimmon fruits with jojoba oil helped to delay ripening and reduced weight loss and decay percentage. Postharvest fungal decay may cause significant losses to the citrus industry. Injuries on citrus fruit caused during harvest, provide entries to wound pathogens, including Penicillium digitatum Sacc. and P. italicum Wehmer, causal agents of green and blue mould, respectively. These pathogens occur in almost all citrus growing regions of the world . The synthetic fungicide Imazalil, has been routinely used to control postharvest diseases including green and blue moulds . Attempts to find alternatives to chemical control have been ongoing for some time, and indeed many fungi have become resistant to commonly used fungicides. In the mean time, there is increasing consumer concern in respect to chemical residues on fruit products regarding health and environmental issues. However, alternative measures are generally less effective than fungicides. (Tarabih, et al., 2013).

Green and blue moulds, caused by Penicillium digitatum (Pers. Fr.) Sacc. and Penicillium italicum (Wehmer) , respectively , are the most significant postharvest diseases of citrus in all production areas that like Spain, California (Eckert and Eaks 1989) . Both P. digitatum and P. italicum are severe wound pathogens that can infect the fruit in the orchard, the packinghouses and during allocation and marketing . They reproduce very quickly and their spores are ubiquitous in the atmosphere and on fruit exterior and are simple distributed by air currents . Therefore, the source of fungal inoculum in citrus orchards and packing houses is virtually continuous during the season (Kanetis, et al., 2007) . Citrus fruit can become " soiled" with conidia of the two fungi that are loosened in handling of diseased fruit . The conidia located in damage that laceration oil glands or penetrate into the albedo of the peel usually bring irreversible infection within 48 h at 20 – 25°C (Plaza, et al., 2003 , Lahlali, et al., 2006 and Stange, et al., 2002) . The germination of conidia of both Penicillium species inside rind wounds requires free water and nutrients (Tarabih and El- Metwally , 2013) .

Green mold caused by Penicillium digitatum is one of the major fungal decays of Florida fresh citrus. Injuries to the fruit peel are required for penetration of the fugus . Penetration by P. digitatum was inhibited in injuries to oranges and grapefruit where cells at the injured surface produced lignin before fungal entry. Accumulation of lignin occurred most rapidly at 30°C and at relative humidities above 90%. Under these conditions, lignin developed within 12 hours following injury. Lignification was delayed or inhibited by peel oil or desiccation which caused damage to cells near the injury. These injuries were easily penetrated by P. digitatum as were injuries into the albedo where cells were incapable of producing lignin (Eldon and Ismail , 1980) .

P. digitatum is a strict wound pathogen that can infect the fruit in the grove, the packinghouse, and during distribution and marketing (Palou, 2013) . It reproduces very rapidly and the spores are ubiquitous in the atmosphere and on fruit surfaces and are readily disseminated by air currents . In vitro investigation of the infection mechanisms and strategies of Penicillium digitatum done by (Alraaydi, et al., 2020) on citrus fruits illustrated all the used mechanism to stop the infection by this fungus just delayed the infection but didn’t completely prevent the infection . Furthermore, citrus fruit can become “soiled” with conidia that are loosened in handling of decayed fruit. For these reasons, any successful cost-effective postharvest disease management program for citrus fruit is primarily based on the control of green mold . The disease has been primarily controlled worldwide for many years by the application of conventional fungicides such as imazalil, sodium ortho-phenyl phenate, thiabendazole or, more recently, new active ingredients like pyrimethanil, azoxystrobin or fludioxonil . Different mixtures of these compounds are also commercially available. Postharvest treatments with these synthetic chemicals typically are relatively inexpensive, easy to apply, have curative action against pre-existing or established infections and persistent preventive action against potential new infections, and many also inhibit the sporulation from lesions on decaying fruit . However, concerns about environmental contamination and human health risks associated with fungicide residues periodically led to regulatory reviews and restrictions or cancellations, and export markets are increasingly more sensitive to the use of chemicals for disease control. Further, the widespread and continuous use of these synthetic fungicides has led to the proliferation of resistant biotypes of P. digitatum in commercial packinghouses that seriously compromises the effectiveness of these treatments (Palou, 2013) .

Biocontrol agents do not generally posses a broad spectrum of activity and they are not as effective as fungicides. Simultaneous application of several physical and chemical methods could provide more effective means of control and consistent results than that of one approach alone. Some physical treatments and exogenous substances, such as chitosan, amino acids, antibiotics, calcium salts, and carbohydrates have also been used to enhance biocontrol of antagonists against fungal pathogens . Pre–storage hot water dips of fruit at temperatures above 40ºC have been shown to be effective in controlling storage decay, not only by reducing the pathogen but also by enhancing the resistance of fruit tissue, influencing host metabolism and ripening . Postharvest dips are applied for a few minutes at high temperatures, because fungal spores and latent infections of the pathogen are either on the surface or in the first few cell layers under the peel of the fruit (Zamani, et al., 2009) .

antimicrobial activity against P. digitatum on citrus fruit . It is inexpensive, readily available, and could be used with minimal risk of injury to the fruit. However, it is a poor eradicate 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 . Biocontrol agents which can persist for long periods may protect fruit from post-treatment infection . Combining heat treatment and chemical compounds with an antagonist, could possibly be synergistic . The antagonist is applied after hot water treatment as it cannot survive at 50ºC. Thus the problem with application of the biocontrol agent before hot water treatment is that it must be heat tolerant . The objective of this study was to determine if green mould on oranges could be reduced by combination of the biocontrol agent P. agglomerans along with sodium bicarbonate and hot water treatment. The experiments were designed to develop an integrated strategy to control postharvest decay on oranges caused by P. digitatum that would be as effective as chemical control (Zamani, et al., 2009) .

Material and Methods

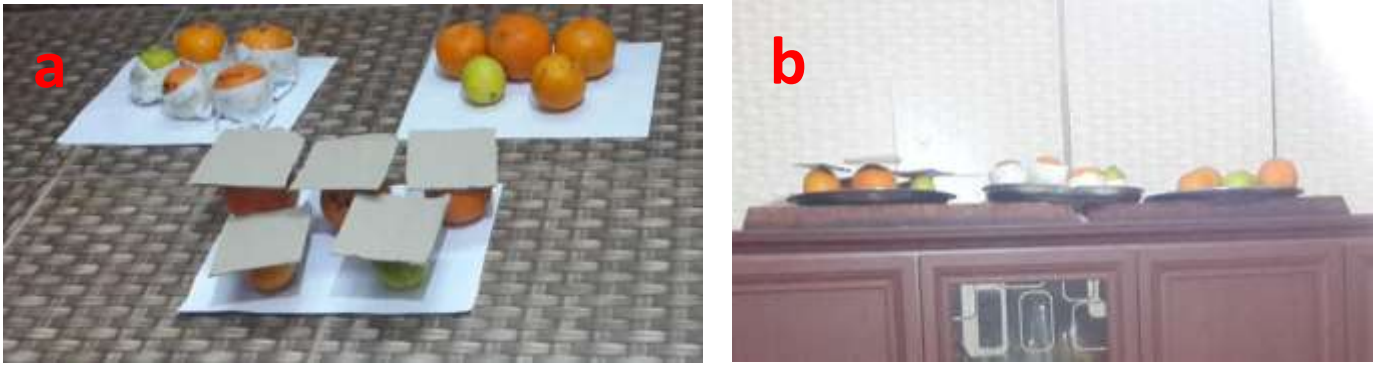
Study epidemology and aerodynamic mechanisms of post- harvest green mold disease in vitro .

These experiment were applied through collecting 1kg of healthy citrus fruit varities. Mechanical wounds were done by injuries the peel surface with sterile metal cork borer . Each type of these injuired fruits were covered from the upper surface with carton and the another covered from sides with clean paper cheet then kept in room temperatures

. Half of fruit samples were placed at distance 2.5 meter high from room surface and the another half samples putted direct in the room surface on clean paper cheet . Inoculum source of Penicillium spores were putted as full open petri dishes containing full growing Penicillium colonies in the ground center surface exposured directly to the air current for 2days after that the exposure fruits will kept for 7 days with daily observation (Fig. 1 and Fig.2) .

This current experiment was aimed to detect the aerodynamic of Penicillium spores and to know the effect of graphity on spore movement and distribution vertically or horizontally.

(Fig1) a: Cover fruits from sides in the initial experiment . b: Cover fruits from upper surface



(Fig.2) a: Samples in ground surface .
b: Samples putted in the distance

Study the effect of rapaid lignin formation of injuried citrus fruit peel on Penicillium infection . Injuried all 7 studied fruits in peels only with sterile metal borer then directly covering the injuried with sterile plastic and keeped these fruits for 12 hour then inject the injuried with first dilution (10^{-1}) conidia / ml from inoculum source and keeped to incubated in carton boxes to create a humid environment at 27°C to stimulated the injuried cell to produce lignin before fungal entry to delayed or prevent infect (Fig. 3) , (Eldon Brown and Ismail , 1980) .

(Fig. 4) a : Samples of Onion injected by Penicillium digitatum . b : Samples of citrus fruits injected by Penicillium allii

1.1.1 Study the specificity of different Penicillium isolates from infected vegetables fruits on citrus fruit infection .
Rotted onion (Allium cepa) bulbs were collected from vegetable Benghazi market infected with Penicillium allii , isolation of few spore by using sterile needle cultured on 9cm petri dishes containing Sabroid Agar then incubated at room temperature . Then three serious dilution have been done from Penicillium .spores (10^{-1} , 10^{-2} , 10^{-3} spores/ml) , three wounded samples for each type of studied fruits were injected with these prepared dilutions of Penicillium spores (10) with the same previous method (3.4)
The same procedures were repeated to the healthy onion bulbs through injected of onion bulbs with Penicillium digitatum spores-9isolated from infected citrus fruits . Inoculated onion and citrus fruits infected at 27°C for 7 days with daily observation . This experiment done to improve the infection specification of Penicillium isolates to differs fruits samples (Fig. 4).

(Fig.4) a: Samples of Onion injected by Penicillium digitatum . b : Samples of citrus fruits injected by Penicillium allii .

1.1.2 Study effect of maturity stages of citrus fruits on infection severit .
In this study 1kg of lemon fruit samples were collected from vegetable market showing differences in maturity colour yellow and green colour . The same artificial inoculation methods are repeat as mentioned previously . The inoculated injuired lemon fruit samples were inoculated at room temperature 27°C with daily observation . The aim of this study to explain the best time of harvesting (Lawal , et al., 2013) to avoide infection and prelonging shelf of life of citrus fruit to become healthy.

(Fig. 5) a : Immature lemon fruit (green color) , b : Mature lemon fruit (yellow color) .

1.1.3 Control of Penicillium green mold by using two physical methods (hot water and sea water) , two natural oils , mineral oil and wax .

At the beginning of this control experiment , media preparation from Sabrouraud Agar mixed with sea water instead of S.D.W , after sterilization media was pured in 9cm petri dishes . 7 plates were cultivated with 7 isolates from previous citrus fruits varities . All inoculated plates were placed at room temperature 27°C for 7 days with daily observation .

1.1.3.1 Test of olive oil , Eucalyptus oil and mineral oil (Johnson's baby)

The total tested fruit samples were 48 fruits represented from sour , navel orange , lemon and grapefruit . These samples fruits were divided into two parts . The first part fruit samples injured and contaminated then anointed with oils : olive oil , Eucalyptus oil and mineral Johnson's baby oil .

The other part of fruit samples were anointed with previous oils then injuried , contaminated and inoculated for 7 days at 27°C with daily observation .

1.1.3.2 Test of hot water :

The total 16 samples from studied fruits divided into two parts , first part of fruits injuried and contaminated, then soaked in hot water for 5 minute at 45 – 50°C , then removed these fruits from hot water , the another parts soaked in hot water firstly for 5 minute at 45 – 50°C , then removed from the hot water and kept to dry then injuried , contaminated and kept for inoculated for 7 days at 27°C with daily observation .

1.1.3.3 Test of using sea water :

By used 16 samples from fruits divided into two parts , first part of fruits injuried and contaminated, then soaked in sea water for 3 hours , then removed these fruits from sea water and kept to dry , the another parts soaked in sea water firstly for 3 hours firstly , then removed the fruits from the sea water and kept to dry then injuried , contaminated and kept for inoculated for 7 days at 27°C with daily observation .

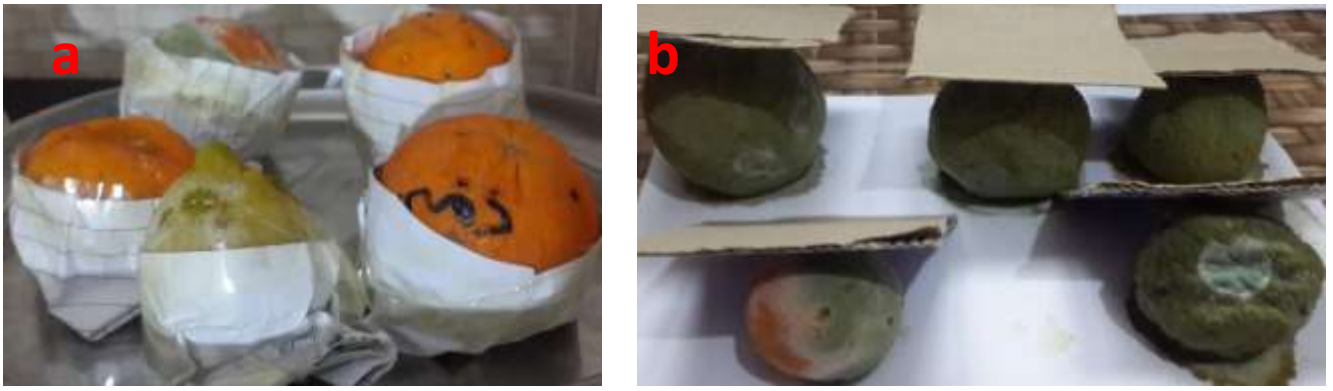
1.1.3.4 Test sterile wax :

By used 16 samples from studied fruits divided into two parts , first part of fruits injuried then contaminated, then covering with liquid sterile wax , second parts of fruits covering with liquid sterile wax firstly , then injuried , contaminated and kept for inoculated for 7 days at 27°C with daily observation .

Results

4.1 In the study of the epidermology of disease and aerodynamic characteristic of Penicillium digitatum spores which maintained previous appears the following results :

After 48 hours first early symptoms appears in samples putted in the upper surface with distance 2.5 meters high from the surface of the ground (Fig. 6) . While the other samples in the ground surface appears the symptoms after 5 days this affirmation the spores becomes suspended in the air before fall-down on the ground surface . In addition to that the samples which have been covered from the sides only was infected early after passing 48 hours . While the other samples covered from upper side the fruits was infected after 6 days . This affirmation the movement of spores Penicillium mold are vertically faster than horizontally in the same room condition.



The results obtained in injuired peels of all studied fruits which kept for 12 hours injected inoculum density 10-¹ spores / ml caused delaying the symptoms of green mold about 6 days from initial fruit injection , but does not prevent the growth of Penicillium digitatum green mold (Fig. 7)

(Fig. 7) a: Appear the initial symptoms of green mold after 6 days from injection with Penicillium digitatum spores .
b : Spread olive green spores with retain white margin after 9 days in the grapefruit .

4.3 The result of host specification of different Penicillium isolates .

Isolated Penicillium allii from different infected plant material (vegetable) chosen onion . After inoculation onion bulb with Penicillium digitatum isolated from citrus fruit showed there is no infection exposure for long time and the vice versa (Fig. 8,9 and 10).

(Fig. 8) Growth Penicillium allii on Sabroid agar .

(Fig. 9) Onion after 5 days from injection by Penicillium digitatum .

4.4 The effect of maturity stage of lemon fruits .

The experiments of testing the fruit maturity stages effect on enhance or delay infection by Penicillium digitatum . After selection mature and immature lemon fruits samples , inoculation both samples with similar (density) potential (10 -9 spores / ml) and kept under the same condition . The result obtained from this experiment show that the effect of maturity of inoculated immature lemon fruits was delaying the infection about 6 days , while the inoculated mature lemon fruits was infect earlier after 4 days from inoculation time .

4.5 Control of post-harvest citrus green mould disease .

No.	Treatments	Varities		Time of applied treatment	Appearance of symptoms
1	Hot water	1	Blood	5 minutes At 45 – 50 °C	After 3 day from inoculated with two methods of treatment
		2	Sweet		
		3	Sour		
		4	Navel		

		5	Mandarine		
		6	Lemon		
		7	Grapefruits		
2	Sea water	1	Blood	3 hours At ± 27 °C	After 3 day from inoculated with two methods of treatment
		2	Sweet		
		3	Sour		
		4	Navel		
		5	Mandarine		
		6	Lemon		
		7	Grapefruits		

Discussion

The aerobiology study of Penicillium digitatium spor after isolation and identification this fungus by (El-Majberi, et al., 2020) in our research was a new approach in post-harvest disease epidemiology . There are three steps needed to transport the air spore through are source take off dispersal deposition and effect many study of spore dispersal have been made in out-door environments . But our study was conducted in indoor environments to identify sources of Penicillium contamination in post-harvest citrus fruits . The aerodynamic characteristics of fungal spores .As fungal spores are much denser than air they will naturally fall through the air under the force of gravity . The rate at which they fall plays an important role in the dispersal and deposition of air borne spores . Spores that are fall quickly will tend to be less efficiently dispersal and more readily deposited than those that fall slowly . Any object falling through the air will eventually reach a " steady speed " called the settling speed fall speed . The settling speed of a spore depends on its physical properties : mass , size , and shape . However , environmental factors such as temperature or humidity can have small effects by altering the density air or spore . It self the gravitational forces acting on a spore are determined by its mass , while the drag forces depend on the size and shape of spore .

Release of Penicillium spores in air has three distict phases , take off , flight and landing so spores can be passively or actively released into the air . In our experiments the result show the aerodynamic mechanisms of Penicillium spore under indoor environment indicate the high capacity and movement of released spore to reach wounded orange , lemon and grapefruits quickly through change or alternation of spore direction vertically and Penicillium spores become long time suspended in the air before fall-down because these spores it's dry and have low weight , therefore fall slowly , the samples putted on high distance infected earlier after 48 hours while samples putted on the ground surface infected later after 5 days . Penicillium species produce conidia in chain so they are mainly most of the fungi that employ an active spore release mechanism are basidiomycetes and ascomycetes (Goldbach, et al., 1997) .Which all the Penicillium spores are released passively by wind force and air current , unfortunately , natural winds (and many indoor air flows) are turbulent which caused the concentration of spores in a spore plume diluted as the plume expands downwind . Because of this it difficult to define the " dispersal distance " for wind born spores .

From the experiment of lignification of injuries to citrus fruits studied and susceptibility to green mold caused by P. digitatum , the result shows of the injuired fruit surface which kept for 12 hours the peel cells were produce lignin before fungal entry then inoculated with Penicillium spores and kept at room temperature resulted in delaying the infection about 6 days but does not prevent the infection with P. digitatum . Accumulation of lignin occurred most rapidly at 30°C and relative humidity above 90% under these conditions lignin developed within 12h following injuiry , we found disagree results with Brown and Ismail (1980) that lignification was delayed or enhanced by peel oil or desiccation which caused damage to cells near the injury . These injuries were easily penetrated by P. digitatum . The emerging spread and severe damage mold diseases on citrus fruits caused by P. digitatum species are increasingly recognized as presenting a worldwide threat to citrus production , quality and citrus industry .

This is not new problem and Penicillium fungus have long been known to constitute a wide spread threat to citrus fruits , despite different control measures were applied in vivo and vitro Penicillium will develop a new resistant strains for survival. (Abd-Allah, et al., 2012).

The control measure in this experiments were carried out in the work by using hot water , sea water , natural oils , mineral oils and wax show no significant effect and are non-significant important to reduce (minimize) or aberuncate of citrus infection by inoculated and non inoculated citrus fruits samples in vitro . The results in this study just delaying the infection for 5 days by wax and 3 days by used natural oils, mineral oils , sea water and hot water . Does not significant effect on growth of treated of the 5 treatments this does not agree with the other results reported by (Tarabih and El-Metwally, 2013 and Carla Nunes, et al., 2007) . The absent of plant breeding for disease resistance , improving cultural method measurement and proliferation of resistant strains of the pathogen are the major problems associated with the used of these natural oil (olive oil , Eucalyptus oil) , mineral oil , wax and physical treat (hot water – sea water)

There is no one way to control plant diseases . The ever increasing of fungal pathogens to fungicides and growing concern about environmental pollution required an integrated complex of control measures . It became evident that the problem of Penicillium attack on citrus fruits can't be solved by any one system . Now days , develop a unique technique strategy to control postharvest diseases on fruits and vegetables by using safety nontoxic and environment friendly materials are urgent necessary . present study was aimed to test different physical and chemical methods natural plant extract oils which may reduce and minimize the Penicillium mould on citrus fruits .

All control measures (methods) are aimed at protecting plants from becoming diseased rather than of curing them after they have become diseased . As long as plants and pathogens can be kept away from one another , no disease will be develop . The effective control of citrus post-harvest decays could maintain fruit quality , enhance fruit shelf life reduces the losses and increase grower's economic returns . The control of one or more diseases by using a single treatment method is not always effective . More effective control could be achieved using an integrated approach to prevent , reduce , and/or eradicate pathogen infections and disease development during pre-and post- harvest stages .

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