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Effect of Precooling and Temperature on Citrus Green Mould Caused by *Penicillium digitatum*

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Abstract:
Post harvest losses during handling, transport, storage and distribution are the major problems in economy, especially in perishable fruits and vegetable. Post harvest management determines not only quality and safety of food but competitiveness on the market. Major constraints of post harvest management include inefficient handling and transportation, poor technologies for storage, processing and packaging. A study on the effect of precooling and temperature for storage was conducted for management of green mould caused by *Penicillium sps.* in citrus at AC&RI, Killikulum, TNAU during 2015-2016. The six different isolates of *Penicillium* sp. obtained from infected fruits of different locations were screened to identify the most virulent strain and found that isolate 3 was highly virulent. Fruits of sweet orange inoculated with *Penicillium* sp. isolate 3 were placed at different temperatures with precooling and without precooling to identify the temperature at which the fruits are more susceptible to the mould pathogen. The precooled fruits stored at 4^{0}C showed 0% infection even on the 28th day, whereas the non-precooled fruits stored at 4^{0}C showed 13.33% infection, which shows that low temperature storage delays the infection and low temperature storage combined with precooling can completely prevent infection for 28 days. Thus it is concluded from this study isolate 3 was highly virulent and the precooled fruits stored at 4^{0}C had 0% infection even on the 28th day revealing the fact that low temperature storage delays the infection and low temperature storage combined with precooling can completely prevent infection for 28 days.
(Key Words: Citrus, precooling, temperature, green mould)

INTRODUCTION

Fruits constitute an important item of our food and they play a significant role in the human diet through the supply of vitamins and minerals (Prabhakar *et al.*,2004*)*. There is a growing need of fruit and vegetables on world market. Post harvest losses during handling, transport, storage and distribution are the major problems in economy, especially in perishable fruits and vegetables. Besides resulting in low per capita availability and huge monetary losses, these increase transport and marketing costs also (Subramanyam,1986).

Citrus is one of the most widely produced fruit globally. It is grown commercially in more than 137 countries around the world (Ismail and Zhang,2004). The estimated annual production is above 105 million tons (FAO,2011).The contribution of the citrus industry to the world economy is enormous and it provides jobs to millions of people around the world in harvesting, handling, transportation, storage and marketing operations. The importance of citrus fruit is attributed to its diversified use, which is widely consumed either as fresh fruit or as juice (Talibi *et al.,* 2014).

Citrus is the second largest fruit crop worldwide (Spiegel-Roy and Goldschmidt, 1996) and it is one of the foremost export fruit crops in Libya and in the rest of the world. It is primarily valued for its fruit, which is either consumed (sour orange, sweet orange, tangerine, grapefruit etc.,) or used in processing industry. Citrus has many other uses including animal fodder and craft and fuel wood (Manner *et al.,* 2006). In addition, their essential oils are used in the cosmetic and pharmaceutical industries (Frazier and Westhofe, 1978) and a highly fermentable energy source with sweet taste and aroma (Lawal *et al.,* 2013). The citrus fruits are a rich source of vitamin “c” and a good source of vitamin “P” (Reddy *et al.,*2008).

Postharvest diseases played a major role in reducing the quantity and quality of citrus. Post harvest fungal decay might cause significant losses to the citrus industry worldwide (Plaza *et al.*, 2003). The most common and serious diseases that affect citrus fruits were green and blue moulds caused, respectively, by *Penicillium digitatum* (Pers: Fr) Sacc. and *P. italicum* Wehmer (Zhang *et al.*, 2005). Injuries on citrus fruit caused during harvest, provide entries to wound pathogens, including *Penicillium digitatum* Sacc. and *P. italicum* Wehmer. The mould invaded the fruit much more rapidly and predominated in mixed infections, causing approximately 60-80% of decay (Plaza *et al.,* 2004). Currently these moulds were controlled by post harvest application of synthetic fungicides (Kinay *et al.,* 2007). However, since the world trend was moving towards a reduction in their use, physical, chemical and biological approaches have been evaluated as safer alternatives (Palou *et al.,* 2008; Droby *et al.*, 2009).

Post harvest management determines not only quality and safety of food but competitiveness on the market. In developing countries, the horticultural supply chains lack sustainable post harvest management systems. Major constraints of post harvest management in these countries include inefficient handling and transportation, poor technologies for storage, processing and packaging. Hence, in this study the effect of precooling and temperature for storage were assessed.

MATERIALS AND METHOD

This experiment was conducted at AC&RI, Killikulum, TNAU during 2015-2016.

Screening virulent isolates

The various isolates of *Penicillium* sp. obtained from infected fruits of different locations were screened to identify the most virulent strain. The infected fruits were collected and washed with water, their surface were sterilized by exposing them in 1% sodium hypochlorite and then rinsed three times in sterile distilled water. Tissue segments of size 3-5mm were cut out from the margins of the rotted areas with a sterile scalpel and placed on potato dextrose agar (PDA) media and incubated at room temperature 28 ± 2°C (Zahara, 2014). Healthy oranges were surface disinfected and three 2mm holes were made with a flamed cooled wire loop. Two to three discs from the culture plate were inoculated on the fruits. Both inoculated and uninoculated oranges were incubated separately in plastic containers for 7 – 14 days at ambient temperatures of 25-30° C. The isolate that produced maximum mycelial growth on fruits within 96hours of inoculation was considered as the most virulent isolate.

Effect of precooling

Fruits of sweet orange inoculated with *Penicillium* sp. (isolate 3 since it was most virulent) were placed at different temperatures with precooling and without precooling to identify the temperature at which the fruits are more susceptible to the mould pathogen. The hydrocooling method described by Rab *et al.* (2013) was followed in this study to observe the effect of precooling on storage. Fruits of about the same size were selected immediately after harvest and were divided into two groups. One group of fruits were treated by dipping in water cooled to 10±2^{0}C for 15 min. to remove the field heat. After the treatment, the surface water was removed with a gentle air blower while the other group of fruits was left without precooling (control). Both the group of fruits were observed regularly upto 28 days at six different temperatures *viz.,*4°C, 10°C,15°C, 20°C, 25°C, 30°C respectively and percent disease incidence (PDI) was calculated.

RESULTS AND DISCUSSION

Screening of virulent isolates *Penicillium* sp. under *in vitro* condition and susceptibility of different citrus fruits

Different citrus fruits were used for testing their susceptibility against *Penicillium* sp. Isolate 1 to 6 were used and the result on their susceptibility are presented in Table 1. All the citrus fruits were highly susceptible to *Penicillium* sp. The disease development was estimated based on disease severity chart. The results indicated that after 24 hours of inoculation disease development was not observed by all the six isolates. After 48 hours of inoculation sweet orange was susceptible to isolate 3 (21-30%) followed by isolate 1and 5 (1-10%), while mandarin orange was susceptible to Isolate 3 (11-20%), followed by Isolate 1 (1-10%). Among the six isolates tested the isolates 1, 3 and 5 found to infect the fruits in 48 hours and produced symptoms. Other three isolates infected the fruit only after 72 hours. Hence the three isolates 1, 3 and 5 were taken for biochemical studies. Among the three isolates, isolate 3 was highly virulent as it covered more than 75% of the fruit surface in all the citrus fruits. Among the citrus fruits, sweet orange was found to be more susceptible as disease occurs at 41-50% after 48 hours of inoculation itself and reached above 50% after72 hours of inoculation itself (hence it was used for further studies). This was followed by lime in which disease occurred at 21-30% after 48 hours of inoculation and above 50% after 96 hours after inoculation. Hence, Isolate 3 was used further to study the effect of pre-cooling and temperature on the growth of *Penicillium sp,*

Effect of precooling and temperature on the growth of *Penicillium* sp. on fruits

Pre-cooling is the first step in good temperature management. Rapid cooling after harvest has been clearly shown to prolong the shelf life of freshly harvested produce. During busy harvest times, it is important to have practical systems in place to minimize the amount of field heat accumulating in harvested fruit, as well as having an efficient system for removing that heat at the cool store. Most storage rooms designed for holding produce under refrigeration do not have the refrigeration capacity, or the air movement needed for rapid cooling. Therefore, pre-cooling must be a separate operation using special equipment. Pre-cooling can be done using several methods including hydro cooling, vacuum cooling and forced air cooling. The choice of cooling method depends largely on the commodity and the cost benefit associated with it.

Fruits of sweet orange inoculated with *Penicillium* sp. (isolate 3 since it was most virulent) were placed at different temperatures with precooling and without precooling to identify the temperature at which the fruits are more susceptible to the mould pathogen. The non-precooled fruits placed at 25°C were most susceptible to the *Penicillium* attack with 93.33% of the fruit being covered. The precooled fruits placed at 25°C showed 60% infection on fruit on the 28th day. (Table 2). This was followed by 30°C (66.67%)in non-precooled fruits and 53.33 % infection in precooled fruits at 30°C.The least incidence of disease (0.00%) was observed in precooled fruits on 28th day and 13.33 % in non-precooled fruits on 28th day at 4°C.

Therefore, in this study the hydro cooling method was followed to observe the effect of pre-cooling on storage. The fruits treated by dipping in water cooled to 10 ± 2⁰C for 15 min to remove field heat and stored at 25⁰C had minimum infection (60%) on the 28th day whereas the non-precooled fruits stored at 25⁰C had maximum infection (93.33%) on the 28th day. Similarly, the precooled fruits stored at 4⁰C had 0% infection even on the 28th day, whereas the non-precooled fruits stored at 4⁰C showed 13.33% infection, which shows that low temperature storage delays the infection and low temperature storage combined with precooling can completely prevent infection for 28 days.

In accordance to this result Rab *et al.* (2013) reported the favorable effect of pre-cooling in tomato fruits. Wijewardane and Guleria (2013) also found that pre-cooling on apple storage was the most effective by retaining better physicochemical characteristics, in addition significantly lowering the disease incidence.

Thus it is concluded from this study that out of the six isolates of the pathogen isolated from different markets, one isolate (isolate 3) was highly virulent, hence used for further management studies. The precooled fruits stored at 4⁰C had 0% infection even on the 28th day revealing the fact that low temperature storage delays the infection and low temperature storage combined with precooling can completely prevent infection for 28 days.

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Table 1. Screening of different isolates of *Penicillium sp.* and susceptibility of different citrus fruits.

Fruits	Storage period (h)	Mycelial growth					
		Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6
Sweet orange (Malta variety)	24	-	-	-	-	-	-
	48	+	-	++++	-	+	-
	72	++	+	+++++	+	++	+
	96	+++	++	+++++	++	+++	++
Mandarin orange	24	-	-	-	-	-	-
	48	+	-	++	-	+	-
	72	++	++	++++	++	++	+
	96	+++	++	+++++	+++	++++	++
Lime	24	-	-	-	-	-	-
	48	+	-	+++	-	++	-
	72	++	++	++++	+++++	++	+
	96	+++	++	+++++	+++++	++++	++

Note:		**Per cent fruit covered with mycelial growth**
-	:	no growth
+	:	1-10 %
++	:	11-20 %
+++	:	21-30 %
++++	:	31-50 %
+++++	:	> 50 %

Table 2. Effect of temperature and precooling on the growth of *Penicillium sp.*(isolate 3) on fruits

Different temperature	% disease incidence on fruits							
	Pre cooled fruits				Non pre cooled fruits			
	7th day	14th day	21st day	28th day	7th day	14th day	21st day	28ths day
4 °C	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	6.67 (14.97)	13.33 (21.41)
10 °C	0.00 (0.0)	0.00 (1.28)	0.00 (1.28)	6.67 (14.97)	0.00 (1.28)	6.67 (14.97)	13.33 (21.41)	20.00 (26.57)
15 °C	0.00 (0.0)	0.00 (1.28)	6.67 (14.97)	13.33 (21.41)	0.00 (1.28)	6.67 (14.97)	20.00 (26.57)	40.00 (39.23)
20 °C	0.00 (0.0)	0.00 (1.28)	6.67 (14.97)	20.00 (26.57)	0.00 (1.28)	6.67 (14.97)	26.67 (31.09)	46.67 (43.09)
25 °C	13.33 (21.41)	26.67 (31.09)	40.00 (39.23)	60.00 (50.77)	20.00 (26.57)	33.33 (35.26)	73.33 (58.91)	93.33 (7503)
30 °C	20.00 (26.57)	26.67 (31.09)	33.33 (35.26)	53.33 (46.91)	26.67 (31.09)	33.33 (35.26)	46.67 (43.09)	66.67 (54.74
SEm ±	**0.14**	**0.19**	**0.24**	**0.41**	**0.14**	**0.30**	**0.45**	**0.49**
CD 5%	**0.41**	**0.57**	**0.70**	**1.22**	**0.41**	**0.91**	**1.35**	**1.46**

*Mean of three replications Values in parenthesis are arscine values

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