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SURVEY, ISOLATION AND IDENTIFICATION OF POST HARVEST *Penicillium* MOULD OF SWEET ORANGE

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ABSTRACT

Sweet orange is grown in over 100 countries on six continents. Sweet orange fruits are affected by various post harvest diseases. It is essential to control post har vest diseases in order to maintain the quality and prolong the shelf life of fruit particularly in a market where transport from producer to consumer may take several weeks.This experiment was therefore, conducted to survey for post harvest diseases of fruits at different stages of marketing and to isolate, identify and prove pathogenecity of post harvest *Penicillium* mould of sweet orange during 2015-2016 at Agriculture College and Research Institute, Killikulum, Tamil Nadu. Green mould disease of sweet orange caused by *Penicillium* sp. (Pers.:) Fr., Sacc. is one of the most important post harvest disease frequently encountered during the survey made at Tirunelveli and Tuticorin markets. The extent of loss due to *Penicillium* sp.at whole sale market was 6%, at retail level was 24%, at farmer's market was around 20% and at consumer level was around 2% in locally cultivated fruits. In case of rainy season, the post harvest losses ranges from 50-60%. Sweet orange was found to be susceptible to *Penicillium* sp. Six isolates of the pathogen were isolated from different markets, three of them (isolate 1, 3 and 5) proved pathogenicity and one isolate (isolate 3) was highly virulent in the cultural characters.

(Key words: Sweet orange, *Penicillium* sps., survey, isolation, pathogenecity)

INTRODUCTION

Citrus species probably originated in north-eastern India in Burma and in the adjoining areas. Early in the spread of citrus, some species crossed into China where the sweet orange, the mandarins and Kumquat developed (Hill, 1952; Abayomi, 2004). Citrus (Sweet orange and Mandrin orange) is third most important fruit crop in our country after banana and mango. These fruits are rich in energy, minerals vitamins and dietary fiber (Kumar and Shrivastava, 2012). Leading growing areas in India are Maharashtra, Madhya Pradesh, Punjab.

The contribution of the sweet orange 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 sweet orange fruit is attributed to its diversified use, which is widely consumed either as fresh fruit or as juice (Talibi *et al.,* 2014).

Postharvest diseases played a major role in reducing the quantity and quality of sweet orange. Post harvest fungal decay might cause significant losses to the sweet orange industry worldwide (Holmes and Eckertm, 1999; Barkai Golan, 2001; Plaza *et al.*, 2003). The most common and serious diseases that affect sweet orange fruits were green and blue moulds caused, respectively, by *Penicillium digitatum* (Pers: Fr) Sacc. and *P. italicum* Wehmer (Caccioni *et al.,*1998; Palou *et al.,*2002). Injuries on sweet orange 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 (Palou *et al.,*2001; Skaria *et al.,* 2003; Plaza *et al.,*2004).

A single infected sweet orange can be the source of infection to other oranges during storage and on transit (Jay, 2003). Common air moulds such as *Penicillium* species may gain entry into the susceptible tissue and cause loss during packaging (Ronald, 1988). Green mould caused by the pathogen *Penicillium digitatum* (Pers: Fr.) Sacc., is the most economically important postharvest disease of sweet orange. Actual losses due to green mould depend upon the area of production, cultivar, weather and orchard conditions, and especially the extent of physical or mechanical injury to the fruit during harvest and subsequent handling. The conidia situated in injuries that ruptures oil glands or penetrate into the albedo of the peel usually bring irreversible infection within 48 h at 20-25^{0}C. Disease development was mediated by complex interactions between pathogen virulence mechanisms and host defense responses. For these reasons, any successful cost-effective post harvest disease management program for sweet orange fruit was primarily based on the control of green mould (Palou *et al.,* 2008).

Post harvest losses in fruits and vegetables in developing countries have been estimated to be 5 - 50 per

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cent. Even in the countries with most advanced technologies the post harvest losses are substantial. Loss due to post harvest disease is one of the major constraints faced during storage and transportation of the perishables. In developing countries, accurate information on the prevalence of post harvest diseases of perishables has not been made available to researchers and regulators involved in production and marketing of the produce. It is surprising that even survey methodologies have not been scientifically designed and too little is known about the economic losses caused by post - harvest diseases particularly in fruits. In some incidence, estimates of losses have been reported, but the data collected at the wholesale market indicates only a portion of total losses where as greater losses may occur in the retail and domestic markets, because of which attempts made on the estimation of losses due to post harvest diseases, have not been given much importance.

It is in view of this, research work was set up to work on survey, isolation and identification of post harvest *Penicillium* mould of sweet orange.

MATERIALS AND METHODS

Source and market survey for post harvest diseases of sweet orange

Fruits of sweet orange were collected for study from the wholesale and retail markets in Tirunelveli and Tuticorin and used as the source material for the study. A systematic survey was conducted to assess the extent of loss due to post harvest conditions in sweet orange at wholesale and retail market level.The losses due to fungal spoilage was assessed based on the quantity of fruits infected in each lot.

The disease development was estimated as per the standard grade chart given below and the per cent disease index (PDI) was worked out using the formula (Rose, 1974)

Percentage of fruit surface affected	Disease Grade
Nil	0
Up to 10%	1
11-20%	2
21-30%	3
31-50%	4
Above 50%	5

$$PDI = \frac{\text{Sum of individual ratings}}{\text{Total number of fruits observed}} \times \frac{100}{\text{Maximum grade}}$$

Isolation, identification and pathogenecity test of post harvest *Penicillium* mould

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 previously prepared potato dextrose agar (PDA) in Petri dishes and incubated at room temperature 28 ± 2°C (Zahara, 2014). The fungus was purified by single spore isolation technique (Ho and Ko, 1997) and the purified isolates were maintained on PDA slants for further studies. The pathogen that appeared first was primarily identified using cultural and morphological features under the light microscope.

The pathogenicity of isolated organisms was tested using matured fruits as per the methods described by Sharma *et al.* (1985). The fresh matured fruits free from any wounds were taken and they were thoroughly washed with running tap water, surface sterilized with 1 per cent sodium hypochlorite solution and finally rinsed in sterile distilled water. Injuries were made with sterile needle and 9 mm disc of mycelium of *Pencillium* sp. was placed over it. The inoculated area of the fruit were covered with cotton and the fruits were placed inside the perforated polythene bags sprinkled with water, the mouth was tied with rubber band and incubated under room temperature (28 ± 2°C).

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 3-5 mm 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 2 mm 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 96 hours of inoculation was considered as the most virulent isolate.

Susceptibility of sweet orange to *Penicillium* sp.

The reaction of sweet orange was assessed with *Penicillium* sp. Observations were recorded at 24, 48, 72 and 96 h after inoculation. The different types of healthy sweet orange fruits were surface sterilized with 1 per cent sodium hypochlorite solution and finally rinsed with sterile distilled water three times. Then the fruits were pin pricked with sterile needle under aseptic condition. The spore suspension was made by adding sterile water to the plates and then the suspension was poured on the pin pricked area. Then the fruits were transferred to trays covered with poly bag and incubated under room temperature. Proper control was also maintained.

The experiment was conducted in CRD replicated thrice for lab studies. The observations on screening and susceptibility were presented in terms of scores as given below:

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

RESULTS AND DISCUSSION

Survey for post harvest diseases of fruits at different stages of marketing

A systematic survey was conducted in different markets of Tirunelveli and Tuticorin (Table 1) to assess the extent of post harvest losses in sweet orange at different levels of marketing from August 2015 to April 2016. The survey showed that minimum loss was observed at wholesale market showing 6% loss followed by Farmers market which showed 20% loss and Retail market showing 24% loss. A loss of only 2.22 % was observed at consumer level.

Owing to lack of comprehensive information about the post harvest losses due to the diseases, the survey was mainly carried out in different levels of marketing *viz.,* wholesale, retail farmer's and consumer level. The most preferable orange from survey was found to be Malta variety of Sweet orange in Tirunelveli and Tuticorin. Generally sweet orange tree gives fruit in five years. About 50% of surveyed producers reported that the qualities of sweet oranges are declining. According to survey this was due to over farming, improper packing during transporting etc. Losses due to *Penicillium* moulds ranged from 1 – 60% and the losses varied at different levels of marketing. According to the survey the spoilages at wholesale level was minimum with only 6% loss, the loss at farmer's market was around 20% and that at retail level was around 24%. The loss was minimum, only 2.22% at the consumer level. In support of the present study, Prabakar *et al.* (2004) reported that fungal spoilage of mandarin orange was higher at retail (27.9 to 40.3% in local mandarin and 23.6 to 35.6 per cent in Nagpur mandarin) and consumer level (15.1 to 22.1 per cent in local mandarin and 14.4 to 18.6 per cent in Nagpur mandarin) and minimum at wholesale (2.5 to 3.1 per cent in local mandarin and 5.1 to 7.1 per cent in Nagpur mandarin) and field level (1.3 per cent). Agrios (2005) reported that *Penicillium* rots caused up to 90 per cent losses in sweet orange fruits during storage, transit and after sales. Ashok *et al.* (2007) reported the yield loss of up to 21 per cent in Assam state due to green mould disease. In Ethiopia, the actual loss recorded in sweet orange storage was 46.7 per cent (Mekbib *et al.,* 2007).

Isolation and identification of post harvest *Penicillium* sps.

The causal agent of green mould disease *Penicillium* sp.was isolated from the diseased fruits using Czepek yeast extract agar (CYA), and sub cultured by the single hyphal tip method. The isolates from infected fruits were maintained in CYA slants for further studies. The diseased sweet oranges sampled from different markets of Tirunelveli and Tuticorin were found to be massively infected with six different isolates of fungi *Penicillium* sp. Different spoilage types were noticed on reinfection of healthy fruits with pure isolated species of the pathogens. All the isolates caused spoilage on re-infection. The disease development estimating based on disease severity chart indicated that, the symptom development started only after 24 hours after inoculation and maximum total area of fruit covered with mycelium was as high as > 75 per cent after 8th to 10th day. In the present study, it was found that sweet orange fruits were susceptible to the *Penicillium* sp. The difference in the extent of disease severity may be attributed to their ability to produce spores. Differences in storage conditions and varieties of these products in the different areas where they are produced may account for the variation in the isolates detected. Differences in the isolates observed may be due to varied geographical location where the fruits are grown and hence, variation of the microflora of those location. The presence of the fungi or their resistant spores is most likely to have originated from the farms where the fruits were harvested and some from the stores due to horizontal contamination by the already spoilt fruits. Most spoilage organisms may be present on fruits from the farm, during harvest operations, and this may result in post harvest contamination and spoilage of these fruits. The present and subsequent spoilage due to these fungi, if not checked could lead to serious economic lose and possible health hazard when these fruits are consumed.

In accordance to the present study Rodov *et al.* (1992) reported that *P. digitatum* are primarily pathogens of citrus sp. and related genera of the family *Rutaceae*. This narrow host range is typical for most pathogenic species of *Penicillium* and suggests unique adaptations to a particular host. The sweet orange peel is the natural habitat of *P. digitatum* and *P. italicum.* Bukar *et al.* (2009) in their study revealed that up to 90 % of the samples were infected with one or more fungal species. The most predominant pathogenic fungus isolated from the samples was *Aspergillus* sp. (32.5%), others include *Mucor* sp. (25%), *Penicillium* sp.(15%), *Rhizopus* sp. (15%), *Fusarium* sp. (7.5%) and *Alternaria* sp. (5 %). Similarly, Khokhar and Bajwa (2015) in their study reported that green mould was found to be the cause of post harvest rot of stored oranges. The pathogen was identified as *P. digitatum.*

Pathogenecity testing of post harvest *Penicillium* sps.

The isolated pathogen was tested for pathogenicity by inoculating the pathogen in the healthy sweet oranges. The inoculated fruits were observed for growth of fungal colonies. After four days of inoculation fungal colonies started growing on inoculated sweet oranges. A soft water soaked area developed on the peel on the 4th day which was followed by the development of a circular colony of white mould, up to 4 cm diameter, on the 6th day at 25°C. Green asexual spores (conidia) formed at the

Table 1. Survey for post harvest losses at different levels of marketing in sweet orange

Levels of market	*Losses observed									
	August	September	October	November	December	January	February	March	April	Mean
Wholesale market	4.00 (11.54)	6.00 (14.18)	4.00 (11.54)	6.00 (14.18)	6.00 (14.18)	8.00 (16.43)	6.00 (14.18)	6.00 (14.18)	8.00 (16.43)	**6.00 (14.18)**
Retail market	28.00 (31.95)	26.00 (30.66)	24.00 (29.33)	22.00 (27.97)	22.00 (27.97)	24.00 (29.33)	24.00 (29.33)	22.00 (27.97)	24.00 (29.33)	**24.00 (29.33)**
Farmers market	20.00 (26.57)	16.00 (14.18)	20.00 (26.57)	24.00 (29.33)	22.00 (27.97)	18.00 (25.10)	20.00 (26.57)	18.00 (25.10)	22.00 (27.97)	**20.00 (26.57)**
Consumer	2.00 (8.13)	2.00 (8.13)	2.00 (8.13)	4.00 (11.54)	2.00 (8.13)	2.00 (8.13)	2.00 (8.13)	2.00 (8.13)	2.00 (8.13)	**2.22 (8.57)**

*Mean of three replicates Value in parenthesis are arcsine value

Table 2. Screening of different isolates of *Penicillium sp.* and susceptibility of sweet orange fruits

Storage period (h)	Mycelial growth					
	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6
24	-	-	-	-	-	-
48	+	-	++++	-	+	-
72	++	+	+++++	+	++	+
96	+++	++	+++++	++	+++	++

centre of the colony surrounded by a broad band of white mycelium on the 7th and 8th day. The fruit rapidly spoiled and collapsed, shrink and mummified on the 10th day. (Plate 1). The isolated pathogen was identified as *Penicillium* sp. on the basis of morphological characteristics. Pathogenicity tests conducted on healthy fruits under laboratory conditions showed typical rot symptoms after 6 to 8 days. The pathogen from the inoculated oranges was re-isolated on medium. The morphological characteristics of the re-isolated organisms were compared with the original pathogen. The pathogen was identified from all infected orange samples.

Present findings are coinciding with those reported by other investigators previously with constant presence of *Penicillium* on rotted fruits from different areas in world (Roman *et al.,* 2005; Nevarez *et al.,* 2008 and Katleen *et al.,* 2008). Similarly, Khokhar and Bajwa (2015) reported in their study that *Penicillium digitatum* was the cause of post harvest rot of stored oranges. Pathogenicity tests conducted on healthy fruits under laboratory conditions showed typical rot symptoms after seven to fourteen days.

Screening of virulent isolates *Penicillium* sp.under *in vitro* condition and susceptibility of sweet orange fruits

Different sweet orange 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 2 and figure 1. The sweet orange 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%).

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. Among the three isolates, isolate 3 was highly virulent as it covered more than 75% of the fruit surface.

It is summarized from this study that, in survey the loss in the wholesale market was recorded to be around 6% and at retail market was 24%. Loss recorded at farmer's market was around 20% and at consumer level the loss was around 2%. Six isolates of the pathogen were isolated from different markets, three of them (isolate 1, 3 and 5) proved pathogenicity and one isolate (isolate 3) was highly virulent in the cultural characters.

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