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DETECTION AND CHARACTERIZATION OF POSTHARVEST PATHOGENS OF ORANGE COLLECTED FROM MARKET IN NORTH EASTERN PART OF BANGLADESH

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ABSTRACT

Ahmed J, Muqit A, Asad-Ud-Doullah M (2022) Detection and characterization of postharvest pathogens of orange collected from market in north eastern part of Bangladesh. *Int. J. Sustain. Crop Prod.* 17(1), 17-21.

This experiment was carried out to identify and characterize the pathogens associated with postharvest loss of orange in fruit shops at Sylhet city, Bangladesh. A survey was conducted to determine the disease prevalence through collecting samples of sweet orange and mandarin orange from 10 fruit shops at various locations. About 7% of the total observed fruits in the market were found damaged due to postharvest fungal infection. Four different fungi namely, *Penicillium digitatum*, *Penicillium italicum*, *Geotrichum candidum*, and *Cladosporium cladosporioides* were isolated from the diseased and healthy fruit samples from different fruit shops after incubation of 6-7 days at room temperature. Tissue plating method was used to prepare the pure culture of each pathogen and Koch's postulate was performed to confirm pathogenicity of individual fungi. *P. digitatum*, *P. italicum*, *G. candidum*, and *C. cladosporioides* were cultured on potato dextrose agar (PDA) media and formed colonies of green, blue, creamy white, and dark black color. *Penicillium* sp. developed brush like phialides bearing circular conidia in chains, *G. candidum* produced subglobose or cylindrical conidia and *C. cladosporioides* developed lemon shaped, dark black conidia on the nutrient media. Each fungus showed similar symptoms in naturally infected fruits as well as artificially inoculated ones. Most of the pathogens took 3-4 fours days to develop respective symptoms and 6-8 days for total damage of the fruit.

Key words: *sweet orange, mandarin orange, postharvest diseases, Penicillium*

INTRODUCTION

Oranges are important citrus fruit that are grown commercially in tropical, sub-tropical, and warm temperate locations all over the world, making it the most widely grown fruit plant on the planet (Ehler 2011). In Bangladesh, it is one of the most promising citrus fruits where the soil and climate, with plenty of water and a humid temperature, is very conducive for growing citrus crops like oranges. It is mainly cultivated in hilly areas but also in some flatter and well-drained areas of Bangladesh. Sugars, citric, malic acids, pectin, essential oils, and glucosides etc. are the primary components of edible portion and rind. It is high in dietary value and a good source of nutrients, including vitamin C. Culled fruits produce oil which is used as flavoring extracts, and also in perfume and soap industries (Angew 2007).

The main constraint to orange productivity is postharvest damage. It causes direct or partial economic loss to farmers by making it unfit for consumption, posing a health risk to humans and animals. Oranges, like other citrus fruits is susceptible to a variety of pre and postharvest diseases that degrade fruit quality. Infections that occur between flowering and fruit maturity or during harvesting and subsequent handling and storage activities can cause postharvest losses and degradation in citrus fruits. Green fruits do not meet the nutritional requirements of the fungus. The enzymatic activity required for invading green fruits is more than for ripe fruits and is temporarily outside the pathogen's ability. Also some toxins present in green fruits disappear or become inactive in the ripe ones which permit easy infection in ripen fruits (Mehrota 2001).

Pathogens such as *Penicillium digitatum* Sacc, *Penicillium italicum* Wehmer, *Geotrichum citri-aurantii* (syn. *G. candidum* Link ex Pers), and *Geotrichum citri-aurantii* (syn. *G. candidum* Link ex Pers) cause most of the decay of citrus fruit worldwide through surface wounds induced during harvest and subsequent handling (Plaza *et al.* 2003). The most aggressively spreading postharvest disease, caused by *G. citri-aurantii*, is sour rot, which can be severe on fruits preserved at temperatures above 10^{o}C (El-Ghaouth *et al.* 2002). The principle of fungal infection in fruits suggests that a single affected orange might infect other oranges in a lot during postharvest handling, transport and storage (Jay 2003). For instance, *Penicillium* species are common air molds can enter into the susceptible tissue and cause loss during packaging (Ronald 1988).

In developing countries like Bangladesh, where fresh fruit is not properly stored or handled, losses during transit and storage are considerably higher, accounting for up to half of the produced fruit crops. It is a serious obstacle for long-term production and storage of oranges because these are particularly vulnerable to pathogenic fungus due to its low pH, higher water content, and nutritive value (Wisniewski and Wilson, 1992).

The first stage in developing an active control strategy to prevent losses due to deterioration or infections is to isolate and characterize the microorganisms associated with it (Singleton *et al.* 1992). As the orange industry in Bangladesh is still in an initial stage, there is very few research information available regarding postharvest diseases of oranges, particularly in Sylhet which is one of the major cultivating region.

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There is also no information available on postharvest losses. The present study is carried out to assess the prevalence of postharvest diseases in oranges through surveying selected markets and also to identify and characterize the pathogens involved.

MATERIALS AND METHODS

This experiment was carried out at the laboratory of Department of Plant Pathology and Seed Science in Sylhet Agricultural University. At first a survey was conducted in different fruit shops in the Sylhet city. Ten fruit shops were randomly selected for assessment of postharvest rots in orange. Sixty fruits (30 mandarin and 30 sweet orange) were observed for postharvest disease. Prevalence of disease in the market was calculated using the following formula:

$$Disease\ incidence\ (\%) = \frac{\text{No. diseased fruits}}{\text{Total no. fruits observed}} \text{ x } 100$$

Fruit samples, both healthy and infected mandarin and sweet orange, were collected and kept in the laboratory at room temperature for developing disease symptoms. After an inoculation period of 7 days, symptoms on the diseased fruit were recorded. Tissue plating method was used to isolate the fungi from diseased fruits and pure culture of each fungus was prepared. Morphological and cultural characteristics of the four isolated fungi were studied using a compound microscope. Koch's postulate was performed for pathogenicity test of the isolated fungi.

For artificial inoculation few wounds were made on each of the healthy fruits using sterile toothpick and inoculation was carried out by detached fruit technique. Mycelial Droplet Inoculation Technique (MDIT) was used to test the pathogenicity of the pathogens. Inoculum suspension containing spores and mycelia were sprayed over the artificially injured fruits. During the incubation period average temperature and humidity were 25-30^{o}C and 80-85%, respectively. Time required for first disease appearance, growth rate, and disease symptoms were recorded until the entire fruit surface was covered with infection. All the four pathogens were re-isolated again after artificial inoculation and identification was confirmed according to Barnett and Hunter (1998).

RESULTS AND DISCUSSION

Survey on postharvest diseases

During survey, both type of orange were collected from different fruit shops in the Sylhet city area (Table 1). Both types of oranges were kept for incubation under natural condition. After 5-7 days some of the fruits seemed to develop characteristics disease symptoms. Table 2 represents different diseases observed in both sweet oranges and mandarin oranges of different origins.

Table 1. Survey on postharvest diseases on orange

Name to shop	Location	Total no. of fruits observed	No. of infected fruits	DI (%)
Faruk Miah Fruit Shop	Amberkhana	60	7	11.67
Nasim Foler Dukan	Amberkhana	60	3	5.00
Sweety Foler Dukan	Tilaghor	60	5	8.33
Fresh Fruit Shop	Rikabibazar	60	3	5.00
Tajafoler Dukan	Rikabibazar	60	4	6.67
Rakib Miar Foler Bhandar	MejorTila	60	6	10.00
Shila Fruit Ghor	Bondor bazar	60	5	8.33
Swapno super shop	Zindabazar	60	2	3.33
Shahin Mia Fruit Store	Bondor bazar	60	4	6.67
Ali Ahmed Foler Vandar	Bondor bazar	60	3	5.00

*DI = Disease Incidence (%)

Table 2. Diseases observed on the fruit samples

Orange	Observed Diseases			
Mandarin orange (China)	Sour rot	Green mold		
Mandarin orange (Indian)	Sour rot	Green mold	Blue mold	Sooty spot
Mandarin orange (Local)	Sour rot	Green mold	Blue mold	Sooty spot
Sweet orange	Sour rot	Green mold		

Results indicated that postharvest rot is present in all the shops. It varied from 3 to 11% with an average of 7%. The lowest disease incidence (3.33%) was found Swapno chain store. It might be due to low temperature and overall cleanliness. The survey conducted through observation of total 60 fruits including both mandarin (30) and sweet orange (30), revealed that about 7% of the fruits at different shops are discarded due to postharvest

infection. However, Wisniewski and Wilson (1992) and Hassan *et al*. (2014) described postharvest loss in citrus fruits ranges within 30-80%. Feliziani and Romanazzi (2013) reported that postharvest losses by *Penicillium italicum* and *Penicillium digitatum* in citrus fruits can cause more than 50% loss in the stored fruits in a single year. As this study was carried with fruit samples collected from fruit shops, most of the fruits might be treated with postharvest fungicides which led to such low level of incidence. Postharvest handling and fruit shop environment also could be an important factor for determining variation in disease condition. In the present study four diseases sour rot, green mold, blue mold and sooty spot were found. In mandarin orange (China) and sweet orange only two diseases were found while mandarin orange (Indian) and local were found to be infected by four diseases. Results are in agreement with the findings of several authors (Feliziani and Romanazzi, 2013; Saito and Xiao, 2017; Bashir *et al*. 2020). Bashir *et al*. (2020) reported that distribution of fungal infection varies with citrus species. They found that lime had the highest fungal counts followed by lemon and sweet orange. They found *Aspergillus* sp. as the major pathogen but in the present study it was not observed.

Morphological and Cultural Characteristics

Colony and morphological characteristics of the isolated fungus cultured on PDA media were studied through microscopic observation. Colony characteristics on the PDA media are showed in the Table 3 and Figure 1. *Penicillium digitatum* and *Penicillium italicum* produced green and bluish colored colony, brush like conidiophore bearing subglobose shaped, single celled conidia in chains. The conidia sized varied within (4 -7 x 6–8µm) and (4.0–5.0 × 2.5–3.5µm) for both fungi. The growth of the pathogen was moderate. These findings are in agreement with Barnett and Hunter (1998); Sharma (2005); and Raper and Thorn (1984).

The fungus *Geotrichum candidum* causes sour rot in mandarin orange and sweet orange. In pure culture preparation, this fungus produced creamy white colored colony on the PDA media. Conidia were single celled, hyaline, cylindrical or subglobose shaped, and 4.5-12.5 x 2-5µm in size. Nazerian and Alian (2013) recorded similar morphological characteristics of *Geotrichum candidum* var. *citri-aurantii.*

On PDA media, *Cladosporium cladosporioides* produced brown to black colored colony. The growth rate was faster than other postharvest pathogens isolated from orange. In microscopic observation, conidia appeared as single celled, brown to dark black colored, lemon shaped which was mostly grown in chains.

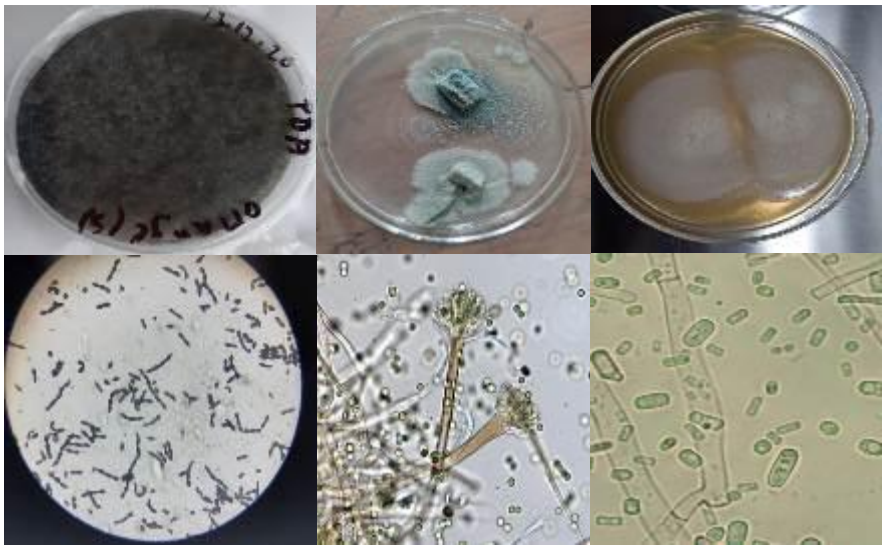

Fig. 1. Colony, conidiophore, and conidia of *Cladosporium* sp., *Penicillium* sp. and *Geotrichum* sp.

Table 3. Colony characteristics on the PDA media

SL No	Name of pathogen	Colony diameter (cm) in hours				Mean growth (mmh^{-1})	Colony characteristics		
		48	72	96	120		Growth	Color	Shape
1	*Penicillium digitatum*	2.3	3.8	4.7	6.1	0.51	+++	Greenish	Roughly Circular
2	*Penicillium italicum*	1.9	3.4	4.3	5.9	0.49	+++	Bluish	Roughly Circular
3	*Geotrichum candidum*	0.8	1.7	4.3	5.2	0.43	++	Creamy white	Circular
4	*Cladosporium cladosporioides*	2.7	4.3	5.9	7.2	0.60	++++	Dark black	Circular

**Growth + = Poor, ++ = moderate, +++ = Good, ++++ = Very good

Pathogenicity test and time required for total damage of the fruit in artificial inoculation

Pathogens cultures prepared from the infected fruits in the first stage were inoculated in healthy fruits. After certain time pathogens developed typical diseases symptoms and proved pathogenicity of the inoculated pathogens. Most of the pathogens developed symptoms on the fruit after inoculation within 2–4 days. The infection rate was different in three fruit. The infection rate for individual pathogens and time required first symptoms to total damage for both types of orange is represented in the Figure 2 and 3. Infection from *Penicillium* sp., *Cladosporium cladosporioides*, and *Geotrichum candidum* took 3-4, 4, and 8 days respectively to cover the entire fruit surface of mandarin orange, whereas in case of sweet orange it was 4-6 days on average. In both cases, *Geotrichum* took more time due to its overall slower growth rate than other pathogens. Results are in agreement with the rate of mycelial growth observed which is presented in Table 3.

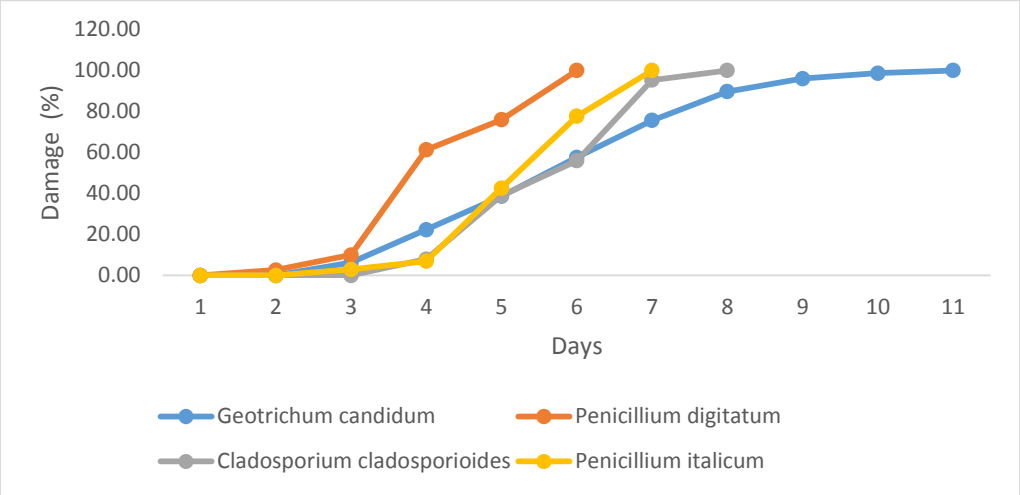

Fig. 2. Infection rate on inoculated fruit surface (Mandarin orange)

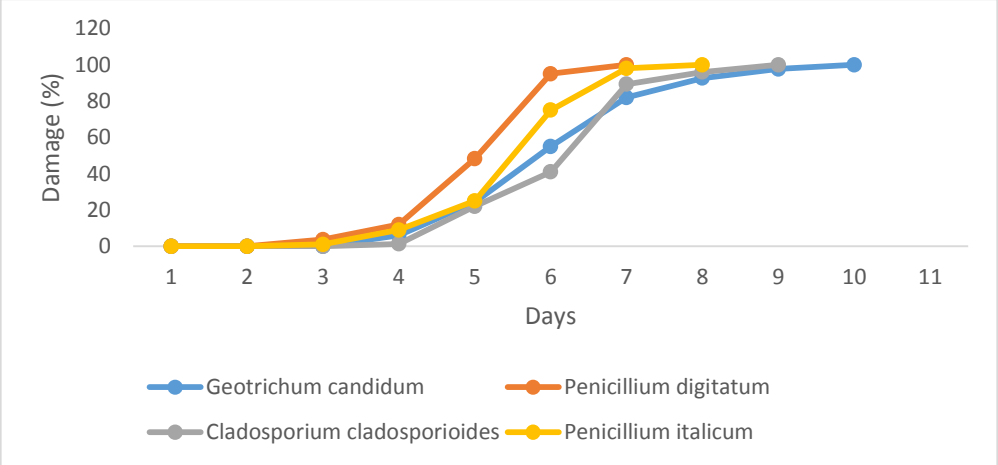

Fig. 3. Infection rate on inoculated fruit surface (Sweet orange)

CONCLUSION

Survey on postharvest diseases revealed that four fungal species namely *Penicillium digitatum*, *Penicillium italicum*, *Cladosporium cladosporioides*, and *Geotrichum candidum* are causing postharvest rots in sweet and mandarin orange. Postharvest rot in different shops varied from 3 to 11% with an average of around 7%. It took 3 to 8 days from initial infection to total damage of individual fruit depending on the fungal species.

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