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Isolation and characterization of Fungi associated with some post-harvest fruits of Darrang District, Assam

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Abstract

Fruits are the most important dietary and nutrient source for mankind of India and world-wide. The varied agro-climatic conditions prevailing in India are conducive for the cultivation of fruits around the year. Unfortunately, these fruits are naturally contaminated with microorganisms specifically by fungi in the field, during harvesting, drying, processing, transportation, storage and environmental pollution. The reduction in post-harvest losses is a complementary means for increasing the production of fruits. Thus, identification and characterization of post-harvest fungi associated with fruits, would help in developing proper measures required for improved availability of fruits both for the domestic consumption and export purposes. The present investigation was carried out to isolate and characterize the pathogenic fungi in some fruits of Darrang district of Assam. Five fruits namely Citrus sinensis, Musa sp, Carica Papaya, Vitis Vinfera, and Malus pumulis were selected. The infected fruit samples were collected from Dumunichowki market area, Mangaldai market area and Kharupetia market area of Darrang, Assam. A significant number of CFU was estimated and *Aspergillus niger, Aspergillus flavus, Peniciillium piscarium, Basidiolo* sp, *Mucor* sp, *Fusarium sp* were found to be most common pathogenic fungi in all the fruits. *Aspergillus niger* was found to the dominant fungus present in all the contaminated fruits. Thus, future study is recommended for the improvement of hygenic and post-harvest management of these fruits for safe consumption by the people of this region.

Key words : Pathogenocity, microfungi, isolation, characterization, *Aspergillus niger*.

India is the second largest producers of fruits in the world contributing 10% of the total production but the total production is quite below the requirements at the recommended dietary allowances of 90 gram of fruits per capita per day as laid by Indian Council of medical research. Accordingly, 90 million tones of fruits are required to feed the one billion population of India. Since, it is not possible to attain such a high target, as plant diseases are the major constraints in increasing the productivity of fruit crops. Fruits are a major source of nutrients for humans but it has been reported that globally around 45% of harvested fruits are wasted every year due to spoilage caused by contaminated growth environments, variety of bacteria, fungi and other microorganism that cause considerable economic losses by spoilage not only harvested fruits and vegetables but also crops in their fields. The identification of such spoilage microorganism is a crucial step toward controlling them. Some pathogenic strains specific to fruits are pathogenic to humans as well, especially those that produce toxins. During this period postharvest pathogens can develop rapidly and losses can reach staggering proportions, in particular in developing countries. The estimated annual losses can be significant reportedly ranging from 10 to 50 % [5,16]. Postharvest losses due to fungal decay are influenced by the perishability of the commodity, the environmental temperature and relative humidity which determine the natural course of decay, the length of time between harvesting and consumption, and practices of postharvest handling, storage and processing.

Postharvest losses refer to the losses that occur along the food supply chain due to pathogens infection, handling, storage, transportation and processing, thereby resulting in the reduction in quality, quantity and market value of agricultural commodities [12].Naturally occurring foods like fruits normally contain some microorganisms and may be contaminated with additional organisms during handling[21]. Food can serve as a medium for the growth of microorganisms and as a result, transmit diseases. Other microorganisms, if allowed to grow in certain food products, produce toxic substances that result in food poisoning when ingested[28,29,33,38]. Some diseases such as anthracnose caused by *Colletotrichum gloeosporioides* (mango, papaya and avocado) or *Colletotrichum musae* (banana) show symptoms after ripening and these types of infections are known as quiescent infections (Snowden, 1990). Grey mould rot (*Botrytis cinerea*) in strawberries is also a quiescent infection that causes significant postharvest damage during storage[22]. Apples (*Malus domestica*) that have been stored for a long time are largely wasted due to fungus diseases. Depending on the cultivar, season, and production region, among other things, fungi can result in significant post-harvest losses of apples. The post-harvest loss of banana is highest (22.00%) among all the fruits in the state of Assam[7,15]. From pathogenicity tests, *F. solani* and *F. semitectum* were pathogenic to both banana and papaya and *F. verticillioides* to banana[17,32,34,39].

Aspergillus niger, *A. avanaceum, P. digitatum, R. stolonifer, Saccharomyces* species, *A. flavus*, and *Fusarium solani* with *Aspergillus* niger. *Aspergillus niger, Aspergillus flavus, Mucor* species, *Mucor racemosus*, and *Fusarium* species were also isolated from spoilt fruits[24]. *Mucor* species had the highest frequency of 26% in Oranges together with *A. flavus* in Pineapple and Apple while *Mucor racemosus* had the least frequency in Oranges with frequency of 6% [10,40].

The most destructive postharvest disease of table grapes is grey mould caused by *Botrytis* spp. Infections often take place in the field, and the fungi remain latent until ripening[35]. Other diseases can occur during storage, including blue mould caused by *Penicillium* spp., some of which can grow even in refrigerated conditions[36]. Post-harvest losses due to fungal infections to grapes berries may be direct during ripening, packing, storage and transportation by a latent pathogen, thus becoming curious to grapes[8,14,18,19,20,30,31,37].

The fruits were collected from different places of market. The selected fruits for investigation for study were:

1. Orange (*Citrus sinensis*)
2. Banana (*Musa paradisiaca*)
3. Papaya (*Carica papaya*)
4. Grapes (*Vitis vinfera*)
5. Apple (*Malus pumilis*)

Samples were collected during the month of January to May 2024 from different market areas of Darrang district.At each market, the sample was collected per available type of Banana, Orange, Apple, Papaya and Grapes. Samples were handled aseptically and were assigned serial numbers. Samples were kept in the sterile bags and at a storage temperature approximating that of the supermarket until transferred the laboratory the next day. A separate polyethylene bag was used for each type of infected fruits in all cases.

Samples were collected from 3 sites of the Darrang district of Assam for isolation of contaminating fungi from the selected fruits. The three sites were Mangaldai, Khandajan and Kharupetia. From these sites the sample were collected per available type of Banana, Orange, Apple, Papaya and Grapes. Altogether 245 CFU's were isolated from the contaminated fruit samples. Highest number of CFU's were recorded from apple followed by orange and banana. Lowest number of CFU's were recorded from papaya (table-4).In case of orange and grapes highest number of CFU's were recorded from site I followed by site II. Whereas, in case of apple papaya and banana the highest number of CFU's were recorded from site III followed by site II (table-5).

A total of 8 fungi were isolated from the contaminated fruits. The name of the isolated fungi were *Aspergillus niger*, *Aspergillus flavus*, *Penicillium piscarium*, *Basidobola* sp., *Mucor* sp., *Rhizopus* sp., *Fusarium* sp. and *Penicillium verrucosum*. The relative abundance (%) was also calculated among the isolated fungi and highest relative abundance (%) was recorded in case of *Aspergillus niger* followed by *Aspergillus flavus* (Figure 1). They were isolated from all

Table-1. Site wise count of CFU's:

Name of the fruit	CFU's in Site I	CFU's in Site II	CFU's in Site III
Orange	23	17	15
Apple	22	26	34
Grapes	13	10	09
Papaya	06	08	10
Banana	13	16	23

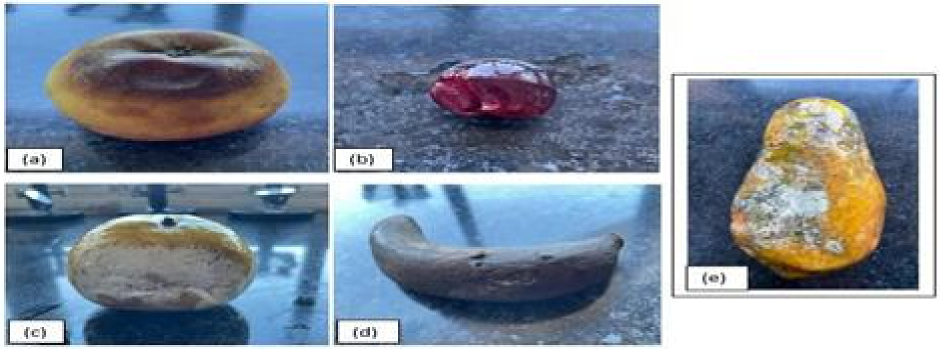
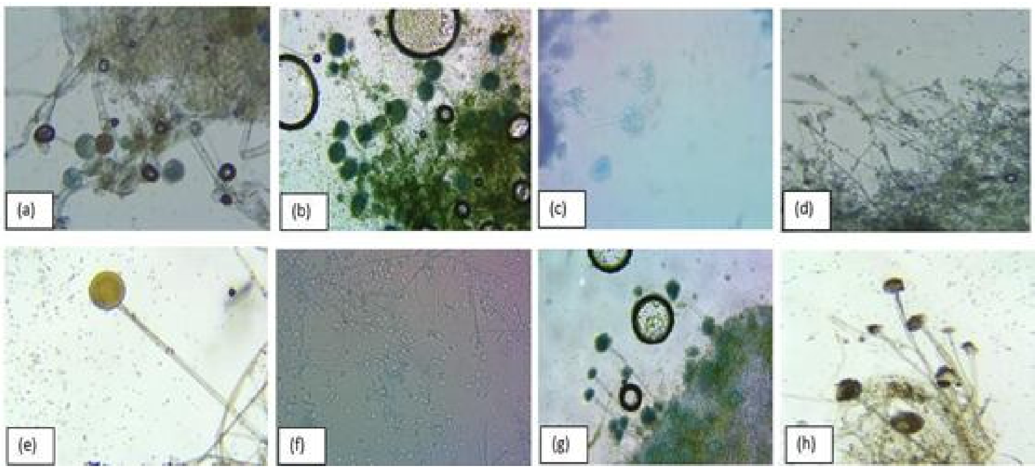


Fig. 2. Photographs showing the collected contaminated fruits
(a) Apple, (b) Grapes, (C) Orange, (d) Banana, (e) Papaya



(a) *Basidobola* sp., (b) *Aspergillus niger*, (c) *Penicillium verrucosum*, (d) *Penicillium Piscarium*, (e) *Mucor* sp., (f) *Fusarium* sp., (g) *Aspergillus flavus*, (h) *Rhizopus* sp.

the investigated fruit samples. Lowest relative abundance (%) was recorded in *Fusarium* sp. which was isolated only from banana.

The isolation and characterization of contaminated fungi in spoilt fruits in Darrang district, Assam, India is a novel discovery which exposed array of fungi which causes serious loss in the supply chain. The identified fungal organisms associated with spoilt fruits in the study area include *Aspergillus niger*, *Aspergillus flavus*, *Penicillium piscarium*, *Basidobola* sp, *Mucor* sp, *Rhizopus* sp., *Fusarium* sp. and *Penicillium verrucosum* suggesting that these fungal organisms could be responsible for the fruit spoilage. This finding is in conformity with previous works which reported isolation of *A. niger*, *F. avenaceum*, *R. stolonifer* and yeast from pawpaw in Nigeria[2,6].

Previous literature indicates that processes such as harvesting, storing, packing and transporting, fruits may encounter physical injury that increases post-harvest loss and the possibility of fungal contamination. In addition, the problem can be enhanced from poor management of fruits in different market areas of Darrang, Asaam, India. Market conditions that favour contamination can be worsened by poor hygiene of the vendors, using microbial unsafe container poor handling practice and poor environmental conditions such as sanitarily unsafe marketing environment. The consequence of the problems could be increased loss of fruit due to microbial spoilage and the existence of some human pathogens[13,25].

Most of the fungal organisms isolated in this study play a pivotal role in the deterioration of food and feed systems and some of them are also able to produce toxic compounds for humans and animals. The mycotoxins produced by these fungi can cause serious health hazards including cancerogenic, immunotoxic, teratogenic, neurotoxic, nephrotoxic and hepatotoxic effects, and Kashin-Beck disease[27]. *P. digitatum* causes a destructive fruit rot of citrus. It is generally considered the most important agent of post-harvest spoilage in the most citrus species. Early symptoms include a soft water-soaked area on the peel, followed by the development of a circular colony of white mold, up to 4 cm diameter after 24- 36 h at 24°C. *R. stolonifer* is a significant agent of fruit disease. It is a threadlike mold and a heterotrophic species; it depends on sugar or starch for its source of carbon substances for food. It uses food matter, mostly soft fruits, like grapes or strawberries, as a food source for growth, nutrition and reproduction[9].

In this study, fungal organisms were isolated from Orange, apple, grapes, papaya and banana *A. niger* and *A. flavus* were more widespread among all the spoilt fruits examined followed by *P. piscarium*, *Basidobola* sp., *Mucor* sp., *Fusarium* sp., *P. verrucosum*. Similar findings on the isolation fungal pathogens from some fruits stored and sold in the market have been reported by earlier researchers[11]. *A. niger* was found to be the cause of post-harvest spoilage in sweet orange and acid lime at field[3]. *A. niger*, *Alternaria species*, *Botryodiplodia theobromae*, and *Colletotrichum gloeosporioides* were isolated from the spoilt mangoes[26]. *Rhizopus nigricans*, *A. flavus*, *A. niger*, *Fusarium* spp., and *Mucor* sp. are the common pathogens for causing the losses of market quality and hygiene of fruits, resulting in major economic problem in the world at large[6].

Fresh fruits recently have been identified as a significant source of plant and human pathogens and chemical contaminants that pose a potential threat to human health worldwide. Because it is likely to be eaten raw by scavenging animals especially ruminants, humans also stands risk of getting infected with pathogenic fungi from fruits as a results of poor processing methods. More so, fresh fruits pose potential food safety hazard and poor type of microbiologically lethal processing regime could lead to potential food safety problems. Poor handling can damage fresh fruits, rendering their products susceptible to the growth or survival of spoilage and pathogenic microorganisms[1,4,23].

This study has shown that *A. niger*, *A. flavus*, *P. piscarium*, *Basidobola* sp., *Mucor* sp., *Rhizopus* sp., *Fusarium* sp. and *P. verrucosum* were isolated from spoilt Orange, apple, grapes, papaya and banana. However, except banana other fruits such as Orange, apple, grapes, and papaya, are free from contamination with *Fusarium* sp. These fungal species associated with fruits spoilage are of economical and public health significance. *Aspergillus niger* was found to the dominant fungi present in all the contaminated fruits. Thus, care must be taken during handling of these fruits, technology based modern preservative methods such as pasteurization, vacuum packing, radiation, and pulsed electric field electroporation, high-pressure food preservation, and bio preservation are suggested to enhance the quality of the fruits.

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