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Isolation and Characterization of Surface Fungi from Local Fruits of Meghalaya, India

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ABSTRACT: The diverse group of microbial communities especially fungi and bacteria colonize fruit surfaces are closely associated with the destruction of the quality, yield and food value. There are various environmental factors like moisture, temperature and humidity which are directly influence the occurrence of various surface fungi to infect the fruits. The present study was determined to study and characterize the diversity of surface fungi from some local fruits of Meghalaya. Six important types of fresh fruits and spoilt fruits were collected on the basis of highest consumption by the local people of Ri-Bhoi district, Meghalaya which include peach (*Prunus persica*), passion fruit (*Passiflora nepalensis*), pineapple (*Ananas comosus*), jackfruit (*Artocarpus heterophyllus*), pear (*Pyrus communis*), amla (*Emblica officinalis*). A total of eight fungal genera were identified which showed severe infection on the selected fruit surface. High temperature, high relative humidity and moisture can cause fungi to be present on the surface of fruits. Therefore, care must be taken to minimize the occurrence the occurrence of these disease for healthy consumption by the native people of this region. This preliminary data help us to identify various surface fungi associated with contaminated fruits thereby to provide suggestive approaches to the local people for safety consumption of these indigenous fruits.

Keywords: Surface fungi, mycotoxins, secondary metabolites, relative abundance, bio-control agents.

INTRODUCTION

Meghalaya is a popular state in North-Eastern region in India. The indigenous organic products, like plant seeds, tubers, shoots, plant are found to be regular routine of consumption for the local tribes of this region (Seal *et al*., 2020). It is being estimated that about 10% of the total geographical area of Meghalaya is used for cultivation of various fruits and vegetables to fulfil the demand of the local people. Thus, a large variety of horticultural crops including vegetables, fruits, spices flower and medicinal plants dependent on the favourable climatic conditions of this region (Dkhar and Rao 2019). Meghalaya is regarded as the popular state for its various indigenous fruits and vegetables which are not grown in any part of the country (Kumar and Kulshreshtha 2020). All the fruits contains a very rich sources of vitamins, minerals, carbohydrates, proteins and fats (Hazarika and Marak 2019; Nongbri *et al*., 2020). The most important fruits of the state includes peach, plum, pear, pineapple, banana, jackfruit, sohpieng, sohiong (black cherry), sohshang, sohphoh, sohlang, sohbrap (passion fruit), sohmon, etc. (Tafinta *et al*., 2013; Al-Hindi *et al*., 2011). This region is also famous worldwide for growing different varieties citrus fruits with a significant yield and export (Kayang, 2007; Dkhar and Rao 2019).

It has been recognized that fruits are commercially and nutritional important food product (Abdullah *et al*., 2016) required for proper growth and development of cells and tissues in man.The significance of fruits could also be traced to the high nutritional value and mineral content which are lifesaving species. (Odelade and Oladeji 2020). Fruits are a major source of macro-nutrients such as fibre and micronutrients, such as minerals and vitamin A, B, C, thiamin, riboflavin, B6 niacin, folate A and E. The oxidative damage of the cell caused by free radicals to cause retinal diseases, muscular degeneration can be reduced by the consumption of these fruits for having high content of their antioxidant compounds. These fruits are also used as nutritional remedies in many patients suffering from different ailments such as diabetes, constipation and stroke (Thliza *et al*., 2020). These local people used to display the indigenous fruits for selling in local markets which is very popular practice in this region. Now a days, due the high concentration of various sugars, minerals, vitamins, amino acids and low pH, various parasitic and saprophytic fungi grow and contaminate these fruits which is a major concern (Thiyam and Sharma 2013; Mukhtar *et al*., 2019). The kingdom Fungi is the most important group of micro-organisms contaminating food commodities (Ribes *et al*., 2018). The filamentous fungi grows very fast in highly perishable fruits and cause significant economic losses as the Fungi are ubiquitous organisms which grow well in high moisture content and favourable temperature (Sardella *et al*., 2016; Shen *et al*., 2018). Most of the fruits usually spoil during the storage conditions due to poor hygienic conditions.These fungi invade fruits by producing germ tubes and release various mycotoxins which are directly related with the consumption by human (Onyemata and Ibrahim 2018;

Muhammad *et al*., 2018; Mukhtar *et al*., 2019). *Aspergillus* spp. are known to produce several toxic metabolites, such as malformins, naphthopyrones and they can also produce ochratoxins, a mycotoxin, which is a very important toxin worldwide for causing various human diseases (Mukhtar *et al*., 2019; Saleh and Al-Thani 2019; Thliza *et al*., 2020). Some plant-associated microbes are found to be beneficial to the growth and development of the host while most of the species cause negative effect on disease development processes (Gomba *et al*., 2017; Mukhtar *et al*., 2019).

Penicillium, Aspergillus, Alternaria species, Botrytis cinerea, Molinilinia lax and Rhizopus stolonifer are found to be popular fungi for causing post-harvest diseases in fruits. An abundance of extracellular pectinases and hemicelluloses produced by the fruits are found to be important factors for fungal spoilage (Aleme and Gupta 2017; Zhang *et al*., 2019). Pathogenic fungi mostly attacks the fruits and reduce the quality of fruits for consumption by human. Thus, it is very important to identify the micro-organisms which are associated with the surface contamination of the fruits by which the risk of contamination and infection of fruits can be prevented earlier in field conditions (Mailafia *et al*., 2017; Saleh and Al-Thani 2019). Therefore, the present work was carried out with the estimate the diversity of pathogenic fungi growing on some local fruits of Meghalaya. Keeping in view the importance of the local fruits, the present study has been undertaken to bring out a detailed account of the knowledge of the local fruits used by the local people of Meghalaya.

MATERIALS AND METHODS

Study site. Meghalaya, a hilly strip in eastern India, covers a total area of 22,429 km^2 (8,660 sq mi) lying between 25 47-26 10′N latitude and 89 45-92 47′E longitude. The percentage of literacy in this state is 75.84 and the languages mostly spoken are English, Khasi, and Garo. The present study was carried out in the Ri-Bhoi District of Meghalaya.

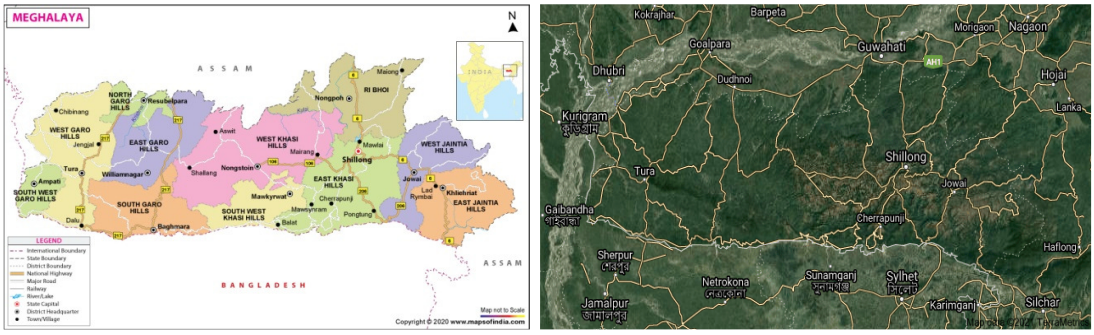


Fig. 1. Map and satellite image of the study site.

Table 1: Selected fruits with medicinal properties.

Sr. No.	Name of fruits	Vernacular name	Family	Medicinal properties
1.	*Prunus persica*	Peach	Rosaceae	Cough, asthma, etc.
2.	*Passiflora nepalensis*	Passion fruit	Passifloraceae	Rich in antioxidants, etc.
3.	*Ananas comosus*	Pineapple	Bromeliaceae	Weight loss, digestion, cancer fighting properties, etc.
4.	*Artocarpus heterophyllus*	Jackfruit	Moraceae	Fruits are edible, seeds are cooked as vegetable, unriped fruits used for pickling.
5.	*Pyrus communis*	Pear	Rosaceae	Mild digestion problems, diarrhoea, etc.
6.	*Emblica officinalis*	Amla	Euphorbiaceae	Fruit is rich in vitamin C very effective against blood pressure.

Table 2: Isolated fungi from the selected fruits.

Sr. No.	Macroscopic features	Microscopic features	Identified fungi
1.	Colony black with a woolly surface	Large conidia containing usually 4 cells	*Curvularia* sp.
2.	The colony is flat, surface is greyish white and greenish black at maturity.	Conidia are simple or branched with both transverse and longitudinal septa	*Alternaria* sp.
3.	Black powdered colony. Reverse white to yellow	Presence conidiophores that look like a vesicle	*Aspergillus niger*
4.	White cotton candy dense growth. Reverse white	rhizoids well developed at the point on the stolon and unbranched sporangiophore	*Rhizopus* sp.
5.	Pale greyish colony	Terminal chlamydospores with nonseptate hyphae , fusiform , slightly curved macroconidia	*Fusarium* sp.
6.	Green colony. Reverse usually white, but may be red or brown	Presence of conidiophores & appeared as in chains. Bears flask shaped phialides	*Penicillium* sp.
7.	Green powdered colony. Reverse goldish to red brown	Conidiophores hyaline and appear rough	*Aspergillus flavus*
8.	White cotton candy fluffy appearance	Branched sporangiophores and rhizoids absent	*Mucor* sp.

2. Sample collection. Six types of Local fruits were collected in the selected area from different shops and fruit trees which include peach (*Prunus persica*), passion fruit (*Passiflora nepalensis*), pineapple (*Ananas comosus*), jackfruit (*Artocarpus heterophyllus*), pear (*Pyrus communis*), amla (*Emblica officinalis*). Fresh fruits as well as infected fruits were collected from these sites and kept in a sterilezed polythene bags (Tables 1 and 2).

Isolation of fungi: Petri dish, conical flask, cotton, test tubes and other glass wares were sterilized in autoclaved for 30 minutes at 121°C. After autoclaving all sterilized materials was dried in oven at 90°C. Potato Dextrose Agar (PDA) was used for fungal cultures growth followed by Spread plate method This technique was used for the enumeration of fungi from given sample. From each fruit sample the fungi was taken with the help of toothpick or needle. Then these were inoculated on sterile PDA plates in three replicates. The same was repeated for the rest and kept the plates for a week. (Mukhtar *et al*., 2019). The inoculated plates will be incubated at 30°C for 5 days and were observed for fungal growth and later sub-cultured for another 10 days at 30°C on nutrient agar. Resulting colonies will be then sub-cultured onto Potato dextrose agar (PDA) until pure isolates will be obtain. As a control, some healthy fruits will be also selected. A small portion of these healthy fruits will be cut (3mm) using a sterile scalpel, plated and inoculated onto a freshly prepared Nutrient agar. The inoculated plates will be then incubated for 5 days to observe for Fungi growth (Onyemata and Ibrahim 2018; Mailafia *et al*., 2017).

After 1 week of incubation, fungal isolates were identified using colonies and cell morphological features such as the thallus growth pattern, pigmentation, conidiophore and conidial morphology. Isolated fungi were identified using cotton blue in lactophenol stain. The morphology and characteristics of the conidia and conidiophores were used to classify the different types of fungus according to the standard taxonomic system (Muhammad *et al*., 2018, Saleh and Al-Thani 2019).

Identification of fungi: Colony growth, presence or absence of aerial mycelium, colony colour, presence of wrinkles and furrows, pigment production etc. were also recorded (Mc Lean and Ivimey 1965). Based on the morphology of the fungal culture colony or hyphae, the characteristics of the spores and reproductive structures, the pathogenic fungi are identified up to genus level (Barnett and Hunter 1998).

Macroscopic Identification: Colonial morphology, colour, texture and appearance of morphology Microscopic characteristics: Presence of reproductive structures and structure of conidia.

Extracellular Enzymatic Test: Fungi secrete an extracellular enzyme that facilitates the degradative properties in fungi. There are various enzymatic test to determine the degradative properties in fungi.

Starch Test: Starch test is done to determine the ability of an organism to hydrolyze starch and also differentiate organism based on their amylase activity. Using a sterile needle or toothpick inoculate the organism into starch agar plate, keep the plates for 2-3 days. The surface of the plate is flooded with iodine solution with a dropper for 30 seconds. The clear zone around the fungal growth indicated the positive test whereas no clear zone determines the negative result.

Carboxy Methyl Cellulose Test: Carboxy methyl test is carried out to determine the ability of organism that hydrolyzes cellulose and also differentiate organism based on their cellulase activity. By using sterile needle or toothpick, the organisms were inoculated on carboxy methyl agar plate for 2-5 days. The zone of clearance is examined by using 1% Congo red stain and 1M NaCl.

Prunus persica infected by *Curvularia* sp

Passiflora nepalensis infected by *Penicillium* sp

Ananas comosus infected by Fusarium sp

Artocarpus heterophyllus infected by *Aspergillus niger*

Pyrus communis infected by Alternaria sp

Emblica officinalis infected by *Penicillium* sp

Plate 1: Infected fruits showing symptoms caused by various surface fungi.

RESULTS AND DISCUSSION

A total of 8 fungal species occur i.e. the *Curvularia* sp., *Alternaria* sp, *Aspergillus niger*, *Rhizopus* sp, *Fusarium* sp, *Penicillium* sp., *Aspergillus fumigatus*, *Rhizopus* sp were isolated and identified from the selected diseased fruits. Amylase activity and cellulose test were also carried out to study the degradative property of the isolated fungal strains (Tables 2 and 3).

Table 3: The Degradative properties of isolated fungi.

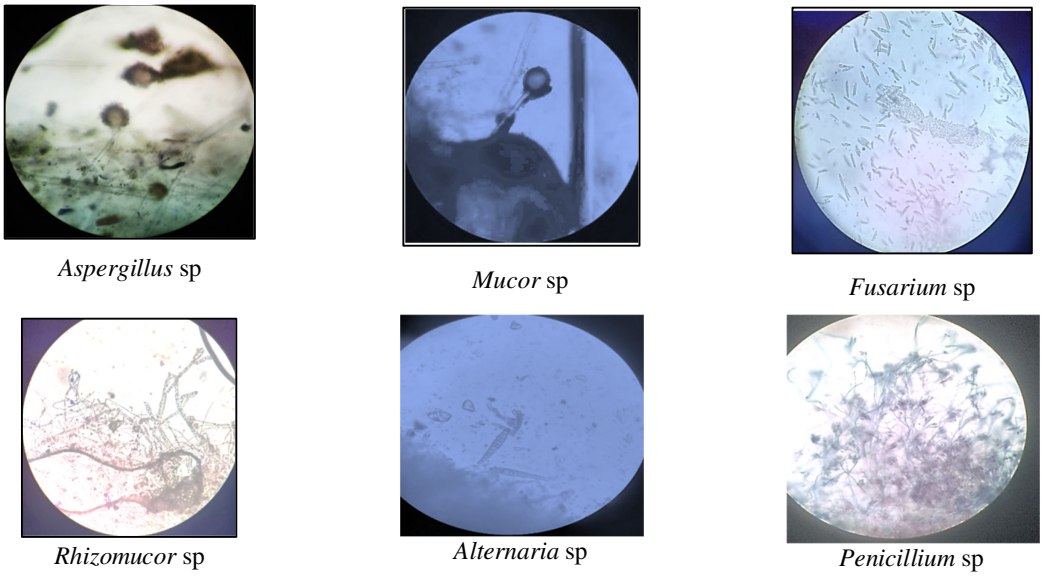
Identified species	Amylase activity	Cellulose test
Curvularia sp		+
Alternaria sp	+	+
Aspergillus niger	+	+
Rhizopus sp	+	+
Fusarium sp.	-	+
Penicillium sp.	+	+
Aspergillus flavus	+	+
Mucor sp.	+	+

Black rot, brown spot, fruit rot, yeast fermentation, powdery mildew and blue mold fungal mycelium were recorded on collected fruits. Based on microscopic characteristics and observations, the causal organisms were identified. The decayed tissue of the selected fruits were found to be soft, watery and the lesion showed sharp margin between diseased and healthy tissues (Lino *et al*., 2016; Parveen *et al*., 2017; Naeem *et al*., 2018; Parashar *et al*., 2018; Muscat *et al.,* 2020).

An important mycotoxins like aflatoxins, produced by *A. niger* and other related species found to cause severe damage in fruits and vegetables. Ochratoxin A *produced by Alternaria* spp. also showed notably toxicity, such as mutagenicity, carcinogenicity, induction of DNA damage in infected person (Escriva *et al*., 2017; Waithaka *et al*., 2019; Duan *et al*., 2019).

According to the present work, the species of *Aspergillus* (*A. niger*, *A. flavus*), *Fusarium*, *Mucor*, *Rhizomucor* sp., *Penicillium* sp and *Alternaria* sp, showed highest occurrence with high relative abundance in all the selected fruits (Thiyam and Sharma, 2013; Fulgence *et al*., 2019; Gandhi *et al*., 2020; Wang *et al*., 2021; Zakaria, 2022) (Photoplate 2). The relative abundance of all the eight isolated fungal species were also estimated *Aspergillus niger, Aspergillus fumigatus, Mucor, Penicillium* sp. and *Alternaria* sp. showed a remarkable relative abundance among all the isolated fungi (Fig. 2).

Due to poor infrastructure in storage mechanism, a good number of indigenous fruits of Northeast India usually become prone to physical damage as well as shrinkage due to the poor management practices (Sawian *et al*., 2007; Mahapatra and Panda 2012). Various physical conditions like temperature, pH, moisture content also extracellular environment greatly affects the growth and enzymatic activities of the microorganisms in the different fruits as reported by Hyde *et al*. (2019). The identified genera of *Aspergillus, Fusarium, Penicillium, Mucor* are also associated with human and animal infections due to consumption, inhalation etc. These surface fungal pathogens produce various mycotoxins and other fungal metabolites which might be very dangerous for consumption by the people (Onuorah *et al*., 2015; Kavya *et al*., 2020; Thambugala *et al*., 2020; Gangaraj *et al*., 2021; Shi *et al*., 2022).



Aspergillus sp | *Mucor* sp | *Fusarium* sp

Rhizomucor sp | *Alternaria* sp | *Penicillium* sp

Plate 2: Microphotographs of some isolated fungi from infected fruits.

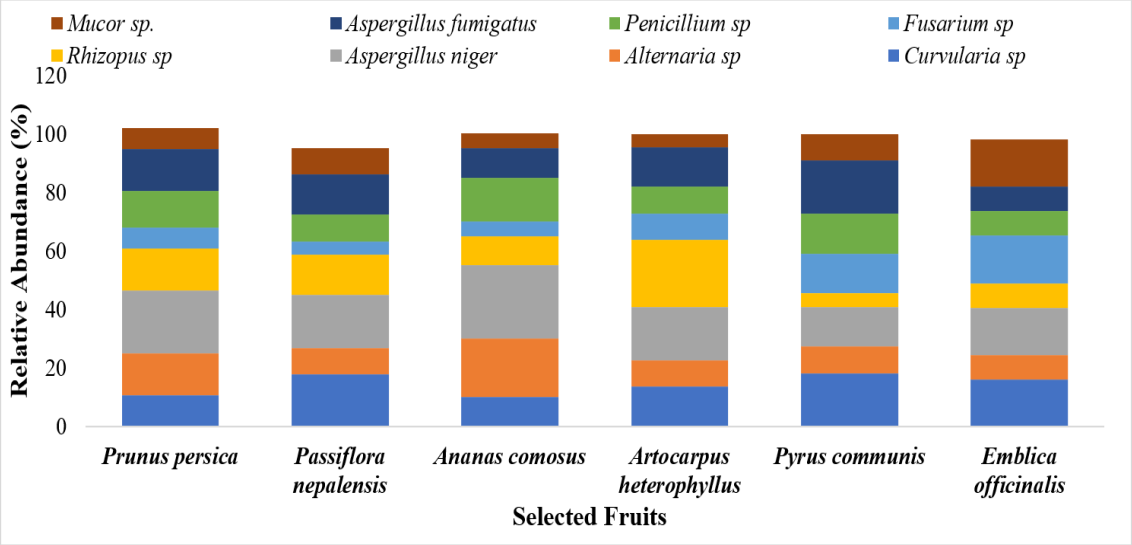


Fig. 2. Relative abundance of isolated fungi in selected fruits.

CONCLUSIONS

The present study was carried out to isolate and characterize the pathogenic fungi growing in local fruits which showed a significant occurrence of various pathogenic fungi. Due to high relative humidity and favorable conditions, the isolated fungi strains showed significant value of relative abundance and positive amylase activity and cellulose test. Therefore, there is a need to keep the fruits in a good place of storage condition for proper care to get rid of the attack of various pathogenic fungi which can lead to various human illness. Care should also be taken to consume the fruits with proper scientific knowledge by knowing the pathogenic fungi which may contaminate and thereby spoiled the fruits.

Thus, present work help us to identify and estimate the abundance of surface fungi especially in the fruits growing in Meghalaya on basis of which future preservation. A suggestive approach must be carried out among the local people for the healthy consumption of these fruitsbased on proper scientific preservation methods and thereby to maintain the quality of fruits. The future work can be extended for molecular characterisation of the fungi and to estimate the percentage of pathogenesis with control measures for safety consumption and socio-economic aspects of this region.

FUTURE SCOPE

The indigenous fruits of Meghalaya is a great source of vitamins, minerals with variety of medicinal values. These fruits are used widely by the tribes of this region but due to short shelf life and poor storage and transportation facilities, the availability of many fruits are reduced now a days. So with proper knowledge of disease control, efficient marketing strategies with proper educational campaigns among the all people will definitely enhance the socioeconomic value of the people of this region. Thus, the knowledge of disease types and causal pathogens is fundamental to develop suitable disease management practices in the field as well as appropriate post-harvest preservation.

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Conflict of Interest. None.

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