Prevalence of disease causing microorganisms in decaying fruits with analysis of fungal and bacterial species

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Abstract:

Fruits are colonized by fungi, bacteria and other agents causing different types of disease. Fruits, due to their low pH, high moisture content and nutrient composition are highly susceptible for the colonization of pathogenic fungi and bacteria. Microorganisms associated with decaying of stored fruits were studied using standard Microbiological methods. The analysis was done with Papaya, Banana, Guava, Orange, Apple, Custard apple, Mango, Sweet lime, Lemon, Gooseberry, Pomegranate, Pear and Grape. Aspergillus species (42%) had the highest rate of occurrence among the isolated fungi, followed by Candida species (36%) while Penicillium spp. (13%), Rhizopus spp. (6%) and Mucor spp. (3%) were the least predominant. It also showed that of the three bacterial species isolated from fruits, Klebsiella pneumoniae was highest (50%) followed by Pseudomonas aeruginosa 29% and Escherichia coli was the least (21%). It showed that fungal isolates were most predominant compared to the bacterial isolates. This investigation was undertaken to find out the association of fungi and bacteria with the decaying fruits from Thiruporur of Kancheepuram district and to identify them.

Key words: Bacterial cultures; Fruits; Fungal cultures; Microbiological methods; Thiruporur district

Introduction

Fruits play a vital role in human nutrition by supplying the necessary growth factors such as vitamins and essential minerals in human daily diet and that can help to keep a good health. Fruits are widely distributed in nature. One of the limiting criteria is the relatively short shelf-life period caused by pathogens attacked. It is estimated that about 20-25% of the harvested fruits are decayed by pathogens during post-harvest handling even in developed countries. It has been known that fruits constitute commercially and nutritionally important indispensable food commodity [1].

Fruits are highly perishable products; the quality is affected by post-harvest handling, transportation, storage and marketing. The improper

handling, packaging, storage and transportation may result in decay and production of microorganisms, which become activated because of the changing physiological state of the fruits [2].

Thus extra care should be taken during personnel handling of these fruits; such as harvesting, cleaning, sorting, packaging, transport and storage [3]. Pathogenic organisms can enter fruits and vegetables through damaged surfaces, such as punctures, wounds, cuts and splits that occur during growing or harvesting [4].

One of the factors influencing virulence of pathogens is their ability to produce enzymes capable of degrading their host's tissue. Microorganisms especially bacteria and fungi have been identified as major organisms causing deterioration of various fruits by the secretion of extracellular cell wall degrading enzymes [5].

Most of the reported outbreaks have been associated with bacterial contamination, particularly members of the Enterobacteriaceae [6]. A large number of lactic acid bacteria, coliforms, moulds and yeast have been reportedly implicated in food spoilage as they use the carbohydrate content of the foods for undesirable fermentation processes [7].

However, about two-third of the spoilage of these items are caused by mould of the genera Penicillium, Aspergillus, Botrytis and Rhizopus [8].Fruits contain high levels of sugars, nutrients and their low pH values make them particularly desirable to fungal decay [9].Generally, spoiling fungi are considered toxigenic or pathogenic. Toxigenic fungi have been isolated from spoilt fruits [10].

Use of untreated waste water and manure as fertilizers for the production of fruits and vegetables is a major contributing factor to contamination. In developing countries, food borne illnesses caused by contaminated fruits and vegetables are frequent and in some areas they cause a large proportion of illness [11].

The increase in food-related outbreaks prompted the U.S. Food and Drug Administration in 1995 to request the National Advisory Committee on Microbiological Criteria for Food (NACMCF) to investigate and characterize the association between cases of food borne illness and fresh produce to provide recommendations to reduce the risk of food borne outbreaks [12].

Contamination of fruits and vegetables takes place at all stages of fruit processing if proper sanitary and hygiene conditions are not maintained [13].

Thus this study is to create awareness among the consumers. Therefore, this investigation was carried out to study of various fungal and bacterial pathogens responsible for the decay and deterioration of economically important fruits from the Thiruporur, Kancheepuram district.

Materials and Methods

A total of twenty six economically important fruits like Papaya, Banana, Guava, Orange, Apple, Custard apple, Mango, Sweet lime, Lemon, Gooseberry, Pomegranate, Pear and Grape were collected from different markets in and around Thiruporur. Of these 13 fruits, a pair from each fruit type was selected and its decaying portion was subjected for fungal and bacterial identification.

Fungal identification:

Segments (3-5cm) of tissues from the margins of the decayed fruits were cut with a sterile scalpel and placed on the Sabouraud's dextrose agar in Petri dishes and incubated at 28+ 1°c for 5 days for fungus isolation. Fungal isolates from plates were prepared into mounts on microscopic slides. These were examined under the microscope for comparison of fungal morphology with descriptions given by Samson and Reenen-Hoekstra [14].

Bacterial identification:

The samples were plated out using Nutrient agar, Mac Conkey agar, Blood agar and Chocolate agar for bacterial identification. These were incubated for 24 hours at 37°c in the incubator. The discrete colonies from these sub cultured plates were identified by series of biochemical tests for proper characterization and identification by Sherman [15].

Results

A total of 45 organisms were isolated from this research which includes 31 fungal and 14 bacterial isolates. The fungal isolates were identified to the generic level while the bacterial isolates were identified to the species level (Table 1).

Fungi isolated were identified as Aspergillus spp. [from Papaya, Banana (Karpoora vazhai), Guava, Orange, Custard apple, Mango (Rumani, Banganapalli), Gooseberry, Pomegranate, Pear] , Candida spp. [from, Papaya, Banana (Poovum), Guava, Apple, Custard apple, Mango (Rumani, Banganapalli), Sweet lime, Gooseberry, Pomegranate] , Penicillium spp.[from Lemon, Pear and Grape], Rhizopus spp. [from Papaya, Grape] , Mucor spp. [from Apple] (Table 1 & Figures 1-5).

Table 1: The Fungal and Bacterial species isolated from Fruit samples

Fruits	Fungus	Bacteria
Papaya-1	*Aspergillus spp., Candida spp.*	*Pseudomonas aeruginosa*
Papaya-2	*Aspergillus spp., Rhizopus spp.*	-
Banana(poovum)-1	*Candida spp.*	*Escherichia coli*
Banana(karpoora vaazhai)-2	*Aspergillus spp.*	*Klebsiella pneumoniae*
Guava-1	*Aspergillus spp., Candida spp.*	-
Guava-2	*Aspergillus spp., Candida spp.*	*Klebsiella pneumoniae*
Orange-1	*Aspergillus spp.*	*Klebsiella pneumoniae*
Orange-2	-	-
Apple-1	*Mucor spp.*	*Pseudomonas aeruginosa*
Apple-2	*Candida spp.*	-
Custard apple-1	*Aspergillus spp.*	*Pseudomonas aeruginosa*
Custard apple-2	*Candida spp.*	*Klebsiella pneumoniae*
Mango(Rumani)-1	*Aspergillus spp., Candida spp.*	*Klebsiella pneumoniae*
Mango(Banganapalli)-2	*Aspergillus spp., Candida spp.*	*Escherichia coli*
Sweet lime-1	*Candida spp.*	-
Sweet lime-2	-	-
Lemon-1	*Penicillium spp.*	*Klebsiella pneumoniae*
Lemon-2	*Penicillium spp.*	*Escherichia coli*
Gooseberry-1	*Aspergillus spp., Candida spp.*	-
Gooseberry-2	*Aspergillus spp.*	-
Pomegranate-1	*Aspergillus spp.*	*Klebsiella pneumoniae*
Pomegranate-2	*Candida spp.*	-
Pear-1	*Aspergillus spp.*	*Pseudomonas aeruginosa*
Pear-2	*Penicillium spp.*	-
Grape-1	*Penicillium spp.*	-
Grape-2	*Rhizopus spp.*	-

Bacteria isolated were identified as Klebsiella pneumoniae [from Banana (Karpoora vazhai), Guava, Orange, Custard apple, Mango (Rumani), Lemon, Pomegranate], Pseudomonas aeruginosa [from Papaya, Apple, Custard apple, Pear], Escherichia coli [from Banana (Poovum), Mango (Banganapalli) and Lemon] (Table 1).

Table 2: Percentage of fungal and bacterial isolates from fruit samples

Fruits Sample	Total Number Of Samples	Fungal species in each fruit	Percentage Of fungi in fruits	Bacterial species in each fruit	Percentage Of bacteria in Fruits
Papaya	2	4	**13**	1	**7**
Banana	2	2	**6**	2	**14**
Guava	2	4	**13**	1	**7**
Orange	2	1	**3**	1	**7**
Apple	2	2	**6**	1	**7**
Custard apple	2	2	**6**	2	**14**
Mango	2	4	**13**	2	**14**
Sweet lime	2	1	**3**	-	**-**
Lemon	2	2	**6**	2	**14**
Gooseberry	2	3	**10**	-	**-**
Pomegranate	2	2	**6**	1	**7**
Pear	2	2	**6**	1	**7**
Grape	2	2	**6**	-	**-**

Table 3: Percentage of occurrences of fungal spp. and bacterial spp. associated with fruits decaying

Fungal species	Total No. of Fungal species	Percentage of Fungi in fruits	Bacterial species	Total No. of Bacterial species	Percentage of Bacteria in fruits
Aspergillus	13	**42**	*Klebsiella*	7	**50**
Candida	11	**36**	*Pseudomonas*	4	**29**
Penicillium	4	**13**	*Escherichia coli*	3	**21**
Rhizopus	2	**6**	**-**	-	-
Mucor	1	**3**	-	-	-

Figures showing fungal isolates from fruits:
Figure 1: *Aspergillus spp.*   **Figure 2:** *Candida spp.*   **Figure 3:** *Penicillium spp.*
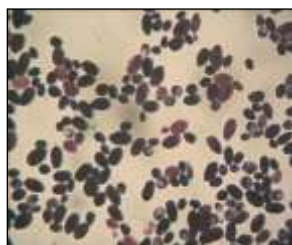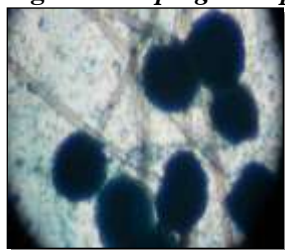
Figure 4: *Rhizopus spp.*   **Figure 5:** *Mucor spp.*
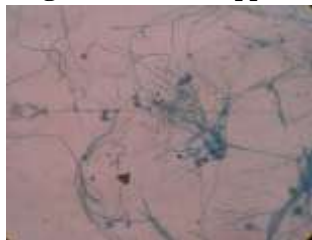

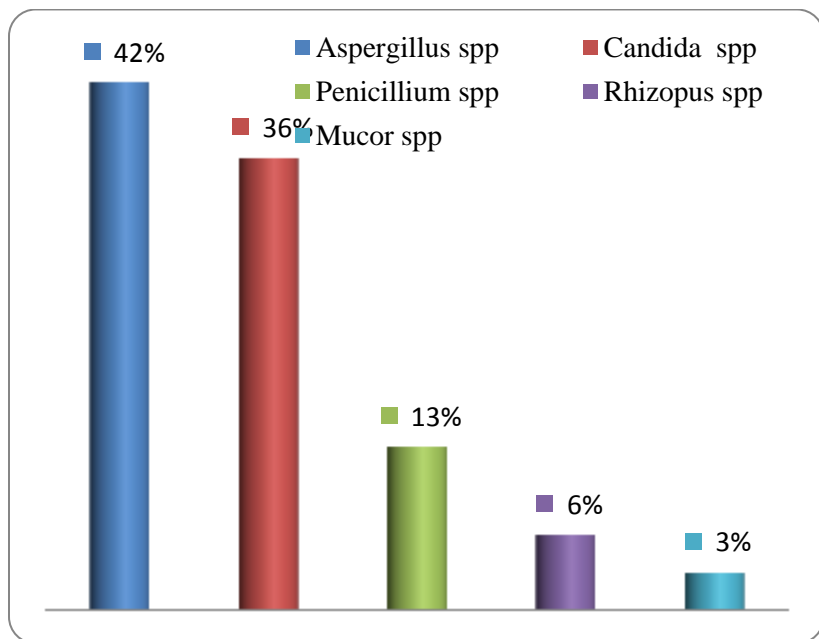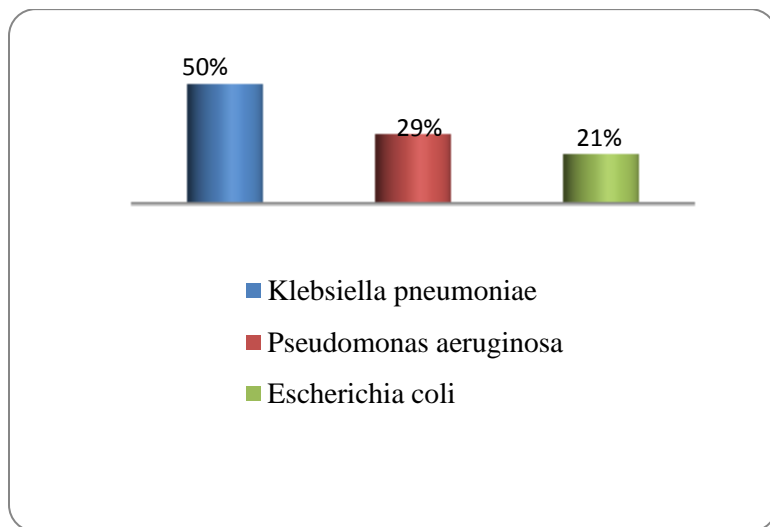**Figure 6: Prevalence of Fungal species in fruits**

Figure 7: Prevalence of Bacterial species in fruits

A total of 31 species of fungi were isolated from 26 samples of decaying fruits. The 13% of fungal species were isolated from Papaya, Guava and Mango, 10% from Gooseberry and 6% from Banana, Apple, Custard apple, Lemon, Pomegranate, Pear, Grape and 3% from Orange and Sweet lime(Table 2). In these fruits, Aspergillus spp. was highest (42%) and Mucor spp. was lowest (3%). This reveals that the species of Aspergillus spp. were the predominating fungi in many fruits (Table 3). Mucor spp. was isolated from apple fruit alone (Table 1). This was followed by Candida spp. (36%), Penicillium spp. (13%) and Rhizopus spp. (6%) (Table 3 & Figure 6).

Like fungus, a total of 14 species of bacteria were isolated from 26 samples of decaying fruits. 14% of bacterial species were isolated from Banana, Custard Apple, Mango and Lemon, 7% from Papaya, Guava, Orange, Apple, Pomegranate and Pear (Table 2). Among this fruits Klebsiella pneumoniae was highest (50%), Pseudomonas aeruginosa (29%) and Escherichia coli was the least (21%) (Table 3 & Figure 7). The presence of these bacteria may be due to the unhygienic environmental conditions and poor handling.

Both fungal and bacterial species were commonly isolated from Papaya, Banana (Poovum, Karpoora vazhai) Guava, Orange, Apple, Custard Apple, Mango (Rumani, Banganapalli), Lemon, Pomegranate and Pear (Table 1). It showed that fungal isolates were most predominant compared to the bacterial isolates.

The results of this experiment suggest that decaying fruits nourishes various types of fungi and bacteria which may cause harm to the consumers. Decaying of fruits pose a serious problem to the health conscious consumers.

Discussion

Aspergillus niger and Candida tropicalis were found to be associated with deterioration of orange; this is in line with the work of [Nijis, Dee Van H.P. Egmond, F.M. Rombouts, and S.H.W. Notermans (1997)] [16], who reported that Aspergillus spp. is the predominant organism associated with the spoilage of orange. The isolation of Aspergillus flavus and Candida tropicalis from pineapple is in line with the work of [Effiuvwevwere, B.J.O.(2000)] [17], who reported that Aspergillus spp. and Candida spp. is responsible for rotting of pineapple. Whereas both fruits and vegetables are highly susceptible to microbial spoilage, there is a variation in the susceptibility which is largely due to the differential chemical composition such as pH and moisture contents. Thus, the lower pH and moisture contents of the fruits makes them more prone to fungal spoilage [Effiuvwevwere, B.J.O. (2000)] [17], also reported that high moisture and relative humidity led to greater fungal growth in agricultural produce and thus low storability of fruits and vegetables.

Colonization of fruits and vegetables by the invading microorganism is a critical phase in the microbial spoilage of produce. The colonization process involves the ability of the microorganism to establish itself within the produce. This is initiated when the microorganism (following adhesion and release of enzyme) degrade certain specific cell wall polymers such as protopectin, the cementing substance of the produce. The magnitude of the symptoms of the induced disease is a reflection of the extent of colonization [Chuku, E.C., D.N. Ogbonna, B.A. Onuegbu and M.T.V. Adeleke (2008)] [18].

Certain fungi such as Aspergillus, Fusarium, and Penicillium spp. are commonly occurring filamentous fungi and their growth may result in production of toxins known as mycotoxins, which can cause a variety of ill effect in human from allergic responses to immunosuppression and cancer [Pitt JI, AD Hocking (1996)] [19].

A. niger is a fungus commonly found on grapes [Chulze SN, Magnoli CE, Dalcero AM(2006)] [20] and apples [Oelofse D, Dubery IAM, Arendse S, Mm S, Gazendam I (2006)] [21]. [Bali RV, Bindu MG, Chenga RV, Reddy K (2008)] [22]. reported that black mould A.niger caused post harvest spoilage in sweet orange and acid lime at field. The group of diseases caused by Aspergillus is called aspergillosis; the symptoms include fever, cough, and chest pain. Usually, only patients with already weakened immune system or who suffer from other lung conditions are susceptible. Penicillium spp. has been implicated in fermentation of fruits and other food items. Penicillium species could be useful for the appropriate food and drug industries. [Battock.M and Azam-Ali, S (1998)] [23].

The bacterial isolates identified in this study include Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa. This is consistent with the findings of previous studies. The bacteria found to spoil the fruits were, Klebsiella, Bacillus, Escherichia coli, Staphylococcus, Pseudomonas, Salmonella and Lactobacillus [Ashok Kumar, Varun Bhushan (2011)] [24], and [Nwachukwu E. and Chukwu C. M (2013)] [25].

Presence of E. coli indicates recent contamination by fecal matter and possible presence of other enteric pathogens known to be causative agents of food borne gastroenteritis and bacterial diarrhoeal disease [Jiwa SFH, Kiovacek K, Wadstorm T (1981)] [26].

A number of studies from different countries have shown presence of E. coli, coliforms and a variety of microorganisms like Streptococcus pyogenes, Streptococcus equi, Pseudomonas aeruginosa, Staphylococcus spp.,Micrococcus spp.etc (Moyer et al.,1993)] [27]. [Vieira RHSF, de Souza OV and Patel TR (1997)] [28]. [Nichols G, Gillespie I, and de Louvois J (2000)] [29]. [Lateef A, Oloke JK, Kana EB and Pacheco E (2006)] [30]. [Amusa NA, Ashaye OA (2009)] [31]. Klebsiella pneumoniae is a potent enteroinvasive food borne pathogen that causes serious illness [Sabota JM, Hoppes WL, Ziegler JR, DuPont H, Mathewson J, Rutecki GW (1998)] [32].

Conclusion

Fruits are very important and have high dietary and nutritional qualities. They can also be used in the preparation of jellies, juice, jams and fruit salad.

Microorganisms are naturally present on all food stuff and can also be brought in by outside elements (wind, soil, water, insects, animals, human handling). They can become contaminated during growing, harvesting and transport of the raw materials, and/or processing into edible products [Lelieveld HLM, MA Mostert, J Holah and B White (2003)] [33].

The prevalence of some fungi and bacteria demand that appropriate control measures should be employed if farmers expect a beneficial outcome of their product. Adequate Microbiological knowledge and handling practices of these products would therefore help to minimize wastes due to deterioration of fruits.

Proper measures should be adopted while handling fruits to limit the level of microbial contamination. It is therefore important that the farmers and stake holders properly transport the fruits into bags, the marketers and consumers take necessary precaution to prevent contamination and also try to create an environment that will not encourage the growth and multiplication of microorganisms.

This study has shown that microorganisms causing diseases are present in the decaying fruits and this is of public health importance.

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