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ISOLATION, CHARACTERIZATION AND SPORULATION OF FUNGI FROM DECAYING VEGETABLES AND FRUITS OF LOCAL VEGETABLE MARKET IN HAZARIBAG INDIA

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ABSTRACT

A preliminary study was done to isolate fungal contaminants, particularly those responsible for spoilage of tomatoes, onions, cabbage and other vegetables sold at the local vegetable market of Hazaribag. The spoilt samples of tomato, onion, cabbage,, some other vegetables and some fruits were cut into pieces each with a sterile razor blade. The samples were then cultured on PDA and incubated at room temperature for 5 days after which the fungal growths were observed. The isolates were purified on Sabouroud's Dextrose Agar plates. A total of thirty five fungal isolates were obtained from the three samples. The fungi isolated and most commonly observed were *A. niger, R. stolonifer, A. flavus, Mucor Spp, Penicillium Spp., A. flavus* and some other common fungi were found to be associated with contamination of the above-mentioned vegetables and fruits. Based on these findings, it was observed that perishable food such as onion, tomato, and other vegetables and some fruits are susceptible to spoilage by fungi probably because the spores of these organisms are easily transmitted via the air which could lead to spoilage of these vegetables. This study, therefore, suggests that there is a need to wash as well as properly store these vegetables at the appropriate temperatures so as to minimize the level of contamination.
Keywords **:** Contaminants, isolates, vegetables, spoilage, perishable**.**

Introduction

Normally raw fruits and some vegetables are consumed as they are the comestible part of mature ovary of flowering plants (Ikhiwili, 2012). Fruit also includes many structures that are not commonly called fruits such as bean pods, corn kernels, tomatoes, and wheat grains (Ikhiwili, 2012). Fruits, as well as vegetables, are of great importance as they provide nutrition as well as essential growth factors such as vitamins and minerals which are necessary for proper body metabolism (Al-Hindi, 2011). Not only humans but also animals have become dependent on fruits as well as vegetables as a source of food (Lewis, 2002).

In comparison to vegetables, fruits are easily spoilt and usually have active metabolism during the storage stage (Singh, 2007). The high concentration of various sugars, minerals, vitamins, amino acids, and low pH also enhances the successful growth and survival of various parasitic and saprophytic forms of fungi (Droby, 2006). Reports state that almost 20% of fruits and vegetables produced are lost due to spoilage (Barth, 2009), especially during post-harvest stages (Singh, 2007). This has been associated with spoilage fungi which can be toxigenic or pathogenic (Zhu, 2006). Toxin-producing fungi have been identified and isolated from spoilt fruits especially (Al-Hindi, 2011). Allergic reactions as well as infections also occur due to pathogenic fungi (Monso, 2009). *Aspergillus spp*. especially *Aspergillus flavus, Aspergillus parasiticus* produces mycotoxins and other toxic metabolites which can be harmful to humans and animals globally (Afsah-Hejri, 2013; Petzinger, 2002).

Among the diverse group of micro-organisms, fungi are especially known to destroy fruits as well as vegetables and henceforth, reducing the quantity for consumption and the profits obtained from sales of fruits. In view of the above, we need to identify these micro-organisms especially those that are pathogenic to humans so as to reduce the risk of contamination and infection arising from handling and consumption of fruits as well as vegetables. Keeping in mind the above fact an initiative was und ertaken to isolate and identify fungi associated with spoilt fruits as well as vegetables commonly sold in Hazaribag main vegetable market.

Study area

The study was conducted in Hazaribag district of Jharkhand. The summers are much rainier than the winters in Hazaribag. The average ambient temperature remains 24.4℃,varies from 6.9 ℃to 39.2℃. The average relative humidity remains around 67.6%, varies from 10.8% to 98.3%. The annual rainfall is 1255 mm (Awowole, 2007).

Materials and Methods

Rotten fruits as well vegetables were collected in sterile polythene bags from the local vegetable market of Hazaribag. They were then brought to University Department of Biotechnology, Vinoba Bhave University, Hazaribag.

Further, studies were done to isolate and identify the fungus from the above samples.

Isolation of fungi

About 80 different rotten fruit samples as well as vegetable samples were undertaken for study. Some healthy fruits and vegetables were also examined .The fruits as well as vegetables were cut into small segments (3 mm in diameter) with a sterilized blade, surface sterilized in 1% hypochlorite for 2 min, plated on Potato Dextrose Agar(PDA) media aseptically and then incubated at 28°C for 5 days.

A pure culture was obtained and maintained by sub-culturing each of the different colonies that emerged onto the PDA plates and incubating at 28°C for 5 days.

As a control, each of the healthy fruits and vegetables was sterilized with 70% ethanol. The fruits were cut into small segments (3 mm in diameter) with a sterile blade, placed on PDA and then incubated at 28°C for 5 days.

Identification of isolated fungi

The isolated fungi were later on identified. The isolates were then identified using cultural and morphological features such as colony growth pattern, conidial morphology, and pigmentation (Tafinta, 2013). Lactophenol using cotton blue stain was used to identify the fungi using the technique of Oyeleke and Manga (Oyeleke, 2008). The fungus identification was done by placing a drop of the stain on clean slide with the aid of a mounting needle, where a small portion of the aerial mycelia from the representative fungi cultures was removed and placed in a drop of lactophenol. The mycelium was then spread on the slide with the needle. A cover slip was gently placed on it. Care was taken to avoid pressure to eliminate air bubbles. The slide was then mounted and observed under the light microscope with ×10 and ×40 objective lenses. The morphological characteristics and appearance of the fungal organisms seen were identified in accordance with Adebayo-Tayo *et al*., 2012; Onuorah *et al*., 2015; Klich, 2002; Samson and Varga, 2007.

Results

The work was conducted to isolate and identify different fungus from isolated decaying vegetables and fruits of local vegetable market of Hazaribag.

The table below shows the frequency of occurrence of fungi in the various fruits and vegtables. *Aspergillus niger* had the highest occurrence in vegetables as well as fruits with a frequency of 40%. *Fusarium avenaceum* followed with the frequency of occurrence of 20% in fruits as well as vegetables while *Penicillium digitatum* and *R. stolonifer* had the least frequency of 5% each in vegetables as well vegetables. Other fungal species were identified as yeast (*Saccharomyces species*) (10%), *F. solani* (10%) and *Aspergillus flavus* (10%).

Table 1 : Frequency of occurrence of fungal species:

Fungi isolate	Source	Frequency occurrence (%)
Aspergillus niger	Fruits & vegetables	40
Fusarium avenaceum	Fruits & vegetables	20
Yeast (*Saccharomyces* spp.)	Fruits & vegetables	10
Fusarium solani	Fruits & vegetables	10
Aspergillus flavus	Fruits & vegetables	10
Penicillium digitatum	Fruits & vegetables	5
Rhizopus stolonifer	Fruits & vegetables	5

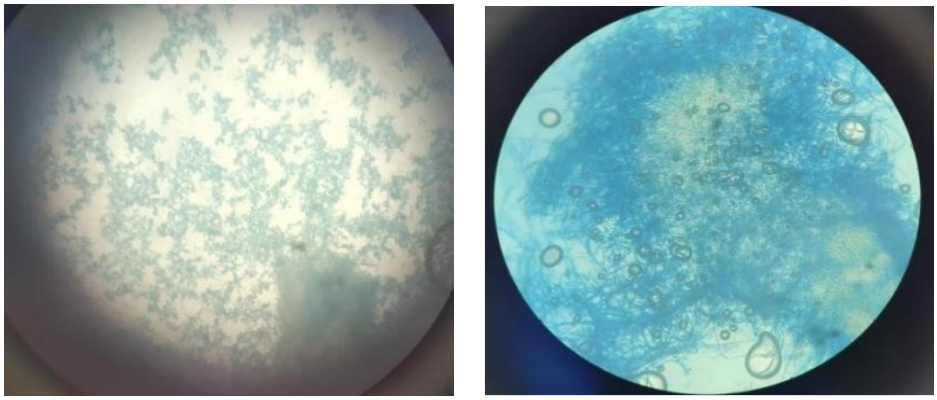

Fig. 1 : Photograph of fungal colonies isolated on PDA media from decaying vegetables and fruits of local vegetable market of Hazaribag

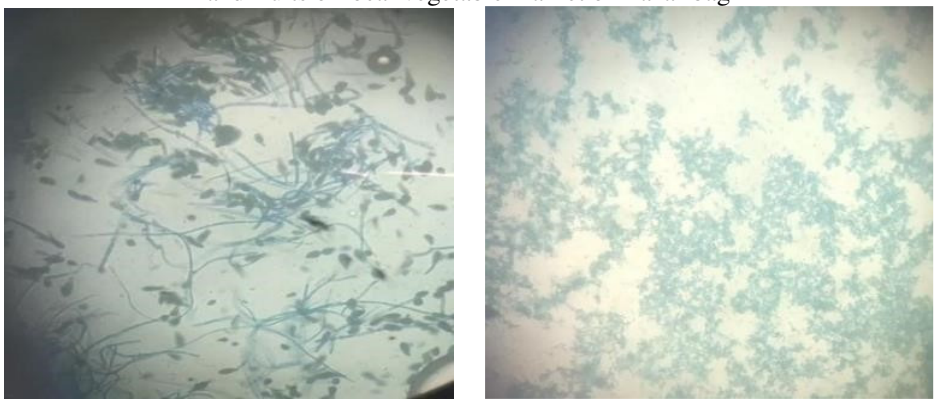

Fig. 2 : Microphotographs of fungi stained with Lactophenol cotton blue grown on PDA media.

Discussion

The isolation and distribution of fungi in spoilt fruits in Hazaribag is a novel discovery which exposed array of fungi which are pathogenic to man and animals. The identified fungal organisms associated with spoilt fruits in the study area include *A. niger, A. flavus, F. solani, F. avenaceum, P. digitatum, R. stolonifer,* and yeast (*Saccharomyces* species) suggesting that these fungal organisms could be responsible for the fruit spoilage. This finding is in conformity with previous works of Baiyewu *et al*., 2007 and Chukwuka *et al*., 2010 which reported isolation of *A. niger, F. avenaceum, R. stolonifer* and yeast from pawpaw in Nigeria.

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 as well as vegetables in Hazaribag market (Gultie, 2013). Market conditions that favor 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 (Okojie, 2014; Gultie, 2013).

Out of fungi isolated in this study, *A. niger* which causes a disease called black mold on certain fruits and vegetables and produces potent mycotoxins called ochratoxins that can be harmful to human beings and animals had the highest occurrence (40%) followed by *F. avenaceum* (20%) while *P. digitatum* (5%) and *R. stolonifer* (5%) had the lowest frequency of occurrence. However, Tafinta *et al*., 2014 reported a frequency of occurrence of 36%, 25%, 22%, and 17% for *R. stolonifer, A. flavus, A. fumigatus,* and *A. niger*, respectively, from sweet oranges. These differences could be attributed to number and type fruits examined in both studies.

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 (Tafinta *et al.*, 2014). *F. avenaceum* is well-known for causing ear blight and root rot of cereals, blights of plant species within genera as diverse as *Pinus* and *Eustoma*, as well as post-harvest storage rot of numerous crops, including potato, broccoli, apple and rutabaga. *F. avenaceum* has also been described as an endophyte, and an opportunistic pathogen of animals (Sorensen, 2009; Lysoe, 2014; Varvas, 2013 and Yacoub, 2012). *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. *Penicillium* species are common fungi in the environment and are often considered non-pathogenic to humans (Yacoub, 2013). *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 (Foody, 2008).

In this study, fungal organisms were isolated from pineapple, watermelon, pawpaw, orange and tomato. *A. niger* and *F. avenaceum* were more widespread among all the spoilt fruits examined followed by *F. solani* and yeast (*Saccharomyces species*). *P. digitatum* and *A. flavus* were isolated from only tomato. Similar findings on the isolation fungal pathogens from fruits stored and sold in the market have been reported by earlier researchers (Bali, 2008). Bali *et al*. stated that *A. niger* was the cause of post-harvest spoilage in sweet orange and acid lime at field. Okereke *et al*.; 2010 reported that *A. niger, Alternaria species, Botryodiplodia theobromae* and *Colletotrichum gloeosporioides* were isolated from the spoilt mangoes. Chukwuka *et al.*, 2010 implicated *Rhizopus nigricans*, *A. flavus*, *A. niger*, *Fusarium spp*., and *Mucor spp*. in pawpaw fruit spoilage from a farm in Oyo state, Nigeria.

Fungal pathogens are causing losses of marketable quality and hygiene of fruits, resulting in major economic problem in Nigeria and the world at large. Fruit spoilage can be prevented using physical (Boyer, 2009) and chemical methods (Msagati, 2012), but no efficient strategy has been proposed so far to reduce the microbial growth ensuring public health safety. Lactic acid Bacteria (LAB) can play a vital role as natural preservatives. The protection of fruits or fruit products using LAB is mainly because of the production of antifungal compounds such as carboxylic acids, fatty acids, ethanol, carbon dioxide, hydrogen peroxide, and bacteriocins (Pawlowska, 2012).

The control experiment showed no fungal growth on PDA after healthy fruits were sterilized with 70% ethanol indicating that the isolated fungi were introduced postharvest from farms through fruit vendors and finally to consumers (Singh, 2007). 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 and vegetables 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 (Gultie, 2013).

Conclusions

This study has shown that *A. niger, A. flavus, F. solani, F. avenaceum, P. digitatum, R. stolonifer* and yeast (*Saccharomyces species*) were isolated from spoilt pineapple, watermelon, pawpaw, orange, and tomato. However, some fruits such as pineapple, watermelon, oranges, and pawpaw are free from contamination with fungi such as *A. flavus, P. digitatum,* and *R. stolonifer*. These pathogenic fungi species associated with fruits spoilage are of economical and public health significance. Care should be taken during handling of these fruits, technology based modern preservative methods such as pasteurization, vacuum packing, radiation, pulsed

electric field electroporation, high-pressure food preservation, and bio preservation are suggested to enhance the keeping quality of fruits.

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