

Study of fungi from spoiled vegetables

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Abstract

A study to isolate fungal contaminants particularly those responsible for spoilage of tomatoes and onions sold at Tambaram Market in Chennai Tamilnadu. The spoilt samples of tomato and onion 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 (30) fungal isolates were obtained from the two samples. *Aspergillus. niger*, *Rhizopus. stolonifer*, *Aspergillus. flavus*, *MucorSpp* and *Penicillium Spp.* were found to be associated with contamination of tomato. *A.niger*, *R. stolonifer*, *A. flavus* and *Penicillium spp.* were found to be associated with contamination of onion. Based on these findings, it was observed that perishable food such as tomato and onion 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. The study recommends that thorough washing and storage of the vegetables at the appropriate temperatures should be carried out to minimize the level of contamination.

Keywords: Spoilage, Perishable, Fungi, Agar, Microorganisms

INTRODUCTION

Fruit and vegetables constitute commercially and nutritionally important indispensable food commodity. Fruit and vegetables play a vital role in human nutrition by supplying the necessary growth factors such as vitamins and essential minerals in human daily diet that can help to keep a good and normal Health (Al-Hindi *et al.*, 2011). Fruits and vegetables are widely distributed in nature. One of the limiting factors that influence the fruits and vegetables economic value is the relatively short shelf-life period caused by pathogens attack (Al-Hindi *et al.*, 2011).

It was estimated that about 20-25% of the harvested fruit and vegetables got decayed by pathogens during post-harvest handling even in developed countries (Droby, 2006; Zhu, 2006). In developing countries, post-harvest losses are often more severe due to inadequate storage and transportation facilities. Fungal fruit and vegetables infection may occur during the growing season, harvesting, handling, transport and post-harvest storage and marketing conditions, or after purchasing by the consumer (Al-Hindi *et al.*, 2011). Fruits and vegetables contain high

levels of sugars and nutrient elements and their low pH values make them particularly desirable to fungal decay (Singh and Sharma, 2007).

Micro-organisms are associated in a variety of ways with all the food we eat. They may influence the quality, availability, and quantity of our food. Naturally occurring foods such as fruits and vegetables normally contain some micro-organisms and may be contaminated with additional organisms during handling.

It is also estimated that about 20% of all fruit and vegetables is lost each year due to contamination (Barth *et al.*, 2009). Fruits and vegetables are vital sources of nutrients to human beings. They give the body the necessary vitamins, fats, minerals, and oil in the right proportion for human growth and development (Bajukaet *al.*, 2014.).

Fruits and vegetables however, have serious challenges to their existence; these include changes in climatic condition, pests and microbial attack (Bajukaet *al.*, 2014). Over the years, there has been an increase in the need to identify and isolate the fungi associated with the contamination, as a way of finding a means of control (Akinyele and Akinkunmi, 2012). Susceptibility of fruits and vegetables is largely due to differential chemical composition such as pH and moisture contents is associated with greater predisposition to microbial spoilage. The occurrence of fungal spoilage of fruits is also recognized as a source of potential health hazard to man and animal. This is due to their production of mycotoxins (naturally occurring toxic chemical often of aromatic structure) which are capable of producing aflatoxin in man, following ingestion or inhalation (Bajukaet *al.*, 2014).

These fruits and vegetables are usually displayed on benches and in baskets for prospective customers in the open markets until sold, thereby exposing them to further microbial infections beside those associated with these whole fruit and vegetables surface and those from adjacent infected fruits (Baiyewu *et al.*, 2007). Fruit and vegetables like Tomato and Onion are cultivated worldwide and have the greatest nutritional value (Thompson, 1998). Because they are seasonal food, their mycological study is necessary so as to identify the organisms associated with their contamination.

MATERIALS AND METHODS

The study area was Tambaram market in Chennai Tamilnadu, India. It is a major market in Chennai where different kinds of vegetables are sold.

Study Population

A total of thirty samples of tomato and onion were randomly collected from different sites of within the study area, where 10 samples of each vegetable were collected.

Sample collection

The samples were aseptically collected using sterile leather bags and disposable sterile hand gloves and taken immediately to the laboratory for analyses.

Sample Preparation and Inoculation

The contaminated portions of the samples were cut into pieces each with sterilized razor blade, sterilized in 1% sodium hypochlorite solution for 2 minutes and then placed gently on Potato Dextrose Agar plate (PDA) using spread technique as described by Akintobiet *al.*, (2011), Dimpka and Onugba (2010), Ebenezer and Seshi (2012) and Kutamaet *al.*, (2012).

Sterilization

All apparatuses or materials used for the research were sterilized using an autoclave at 121°C for 15 minutes. This was done to avoid contamination during the media preparation as well as the sample processing.

Media Preparation

All the media used were weighed, prepared and sterilized according to the manufacturer's instruction. The inoculated plates were incubated at room temperature for 5-7 days under light to enhance fungal growth and sporulation (Okigbo and Osuinde, 2003).

Purification of the Fungal Isolates

After the appearance of a mixed growth, each colony was sub-cultured in a fresh Sabouraud Dextrose Agar in order to obtain a pure culture by using streak method as described by Cheesbrough (2006). Identification and characterization of the various isolates were based on macroscopic and microscopic examination outlined in Kutama and Aliyu (2007).

Macroscopic Examination

This was done on the basis of the nature appearance of the colony and morphological characteristics of the isolated fungi using Color Atlas of Diagnostic Microbiology by Luis *etal.*, (1997).

Microscopic Examination

Slides of the mycelium observed from different isolates were prepared as follows: A few drops of lacto phenol cotton blue solution was placed at the centre of clean grease free slides. A small portion of the unidentified fungal isolates were picked with sterile wire loop. The portions were placed in the lacto phenol cotton blue droplet on the slides and emulsified out with a sterile wire loop. Cover slips were placed at the centre of the slides for viewing and examining the structure

of the mycelia, spore structure and fruiting bodies were identified with the help of Standard Color Atlas of Diagnostic Microbiology by Louis *et al.*, (1997).

Results

The fungal contaminants associated with the spoilage of tomato and onion were identified based on colony appearance, morphology and cellular characteristics as shown in Table, I. However, all the control experiments of fresh tomato and onion showed no any fungal growth.

Table 1: Colonial and Morphological characteristics of fungal contaminants associated with spoilage of Tomato and Onion.

Colony appearance	Morphology and cellular characteristics	Fungi isolated
Yellow fluffy mycelia and some black sporangiospores	Septate hyphae with filamentous structure	<i>Aspergillus flavus</i>
Colonies with loose white mycelium rapidly becoming dark brown to black on the development of conidia	The conidiospore are large with septate hyphae	<i>Aspergillus niger</i>
White to grey and fast growing with some black sporangiospore	The sporangiosphores have terminal sporogaea containing round sporangiospores and columella was well developed with non septate hyphae	<i>Mucor spp.</i>
Green fluffymycelia with some white sporangiospore	Septate hyphae with filamentous structure	<i>Penicillium spp.</i>
Colonies light grey, growing rapidly and filling the petridish with dense cottony mycelium, producing mass of sporangia	Large sporangiophores with non septate hyphae were formed.	<i>Rhizopus stolonifer</i>

Discussion

Vegetables are important farm produce that can get infected at the point of harvest, during handling, processing and storage or at point of sale. This has caused serious economic impact on the farmers, consumers and the society at large. Environmental conditions such as temperature, humidity, acidic pH and other factors contribute to spoilage of these perishable items.

The most commonly encountered fungi associated with tomato contamination were; *Aspergillus flavus*, *Aspergillusniger*, *Mucor spp.* *Penicillium spp.* and *Rhizopusstolonifer*. This is in agreement with the finding of Akintobiet *al.*, (2014) who isolated *A. niger*, *R. stolonifer*, *Mucor spp.* and *A. flavus* from microorganisms associated with deterioration of tomato. This could probably be due to mechanical injuries such as cuts that occur during harvesting, postharvesting

or storage periods which could provide infection sites for spoilage fungi, improper handling and lack of good storage facilities, ability of the fungi to produce spores and their ubiquitous nature, environmental conditions such as temperature and relative humidity etc. *A.niger*, *R. stolonifer*, *A.flavus* and *Penicillium* spp. were found to be associated contamination of onion. This is in contrast with the finding of Samuel and Ifaenyi, (2015) who isolated *A. niger* *R. stolonifer* and *Penicillium* spp. from onion bulb sold in some markets of Nigeria. Some of the fungi isolated from this research have been reported to produce secondary metabolites which are potentially harmful to humans and other animals (Baiyewuet *al.*, 2007).

Conclusion

Vegetables sold in Tambaram market were found to be contaminated with different species of fungi which are responsible for the spoilage of these vegetables. Findings from this research revealed that tomato and onions had the highest contamination from *A. niger*, *R. stolonifer* and *A. Flavus* respectively.

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