

Incidence and Evaluation of Foodborne Fungal Pathogens

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ABSTRACT

Background: Fruits and vegetables provide an ideal condition for the growth of microorganisms. Cereals and bakery products serve as a valuable source of nutrients in the diet of many people and they provide an ideal condition for the growth of microorganisms too. Many fungal and bacterial species are responsible for the spoilage of fruits and vegetables. They contaminate fruits and vegetables and made them insufficient for consumption.

Objective: In our present study, an investigation has been carried out to isolate and identify the fungal species which are responsible for the spoilage of some food items particularly bread, cheese, lemon and onion.

Materials and method: A prospective study was conducted to know the incidence and to evaluate the common fungal pathogens are involved in the spoilage of different types of food products namely bakery product bread, milk product cheese, vegetables onion and lemon. The samples were collected from the spoiled bread, lemon, onion & cheese which were obtained from different shops in the local market. The samples were subjected to identification and isolation of fungal pathogens using the standard staining procedure and selective media. 5 fungal isolates were found to be most commonly occurring in all of the samples namely *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus*, *Mucor* and *Penicillium marneffeii*.

Keywords: *Aspergillus flavus*, *Aspergillus niger*, *Mucor* and *Penicillium marneffeii*.

INTRODUCTION

India is the second largest producer of fruits and vegetables in the world after China. Food and agricultural organization (FAO) data shows that India has produced about 76424.2 tonnes of fruits, 156325.5 thousand tonnes of vegetables and 388269.2 tons of food grains in 2011 [1]. Bread products, like bread has become an important staple food in many countries. Bread is made by mixing flour, salt, yeast and other ingredients which is followed by baking [1]. Most common source of microbial spoilage is due to mold growth. Bread molds like *mucor* and *rhizopus* are found to grow first during bread spoilage. This is followed by some other fungi like *Aspergillus*, *Penicillium* and *Fusarium* spp [2]. Among these *Penicillium* sp is the most common one through *Aspergillus* sp may be greater significance in tropical countries [3]. The colour of molds that grow on bread varies from white, golden yellow to green – gray, depending on the species and the degree of sporulation [4]. Onion is an important vegetable crop commonly grown in the world for consumption and economic value to farmers [5]. Onion is a valuable ingredient in the diet due to its content of sugars, vitamins and minerals [6]. Pathogens such as: *Pseudomonas* sp., *Staphylococcus* sp. and *Erwinia* sp., were identified with the often reduces germination, and yield of the crop [7]. Citrus is an important fruit crop worldwide, especially in tropical and

subtropical regions around the world, and citrus fruit contains many nutritional components beneficial to human health. During the postharvest stage, including handling, shipping, storing, and marketing, fruit is subjected to a series of biotic or abiotic stresses, and fruit decay and risks to food safety caused by postharvest fungal diseases are some of the most serious problems. Green mold and blue mold, caused by *Penicillium digitatum* and *Penicillium italicum*, respectively, are the two most important postharvest diseases in all citrus production areas. Citrus fruits are an important source of bioactive compounds, in particular flavonoids and vitamin C. The main flavonoids found in citrus species are hesperidin, narirutin, naringin, and eriocitrin [8][9]. In addition to antimicrobial activity, these naturally occurring flavonoids contain anti-inflammatory and antitumor compounds that can be used as effective therapeutic agents with low toxicity. Among the citrus fruit extracts, one of the most promising is lemon extract. Inflammatory and antitumor compounds that can be used as effective therapeutic agents with low toxicity [10][11][12][13]. Cheese is an important foodstuff introduced into the human diet in the early Neolithic Era. Cheese making began about 8000 years ago and has spread throughout the world; this spread has led to a remarkable diversity of cheeses that nowadays is estimated at more than 1000. Although cheeses may be categorized by the origin of their milk (e.g., goat, sheep or cow), the most usual classification of cheeses is based on their consistency; fresh and soft (e.g., Camembert, Maroilles or Munster), pressed and fresh, pressed and aged, pulled – curd and blue vein. Cheese – making increases the quality and duration of milk conservation and the digestibility of lactose. Contamination of these products may occur from the raw material or during manufacturing, storage and distribution. Cheese production subsequent development of filamentous fungi occurs during cheese ripening in most cheeses' fungi are present

only on the surface; the exception is blue cheeses, where the fungus also develops inside the blue “veins” of the cheese. In addition to fungal starters inoculated in the milk, indigenous molds of the ripening rooms may colonize the surface of cheeses. The level of fungal contamination as well as the identification of the main species is important, since they could give an indication of the food quality as well as of the potential due to the presence of mycotoxins. Conventional methods for detection and identification of the fungi in foods rely on microscopic or culture techniques, which are time consuming and laborious [14].

Objective: To investigate the incidence and evaluation of fungal pathogens in food items is the main objective of this study.

MATERIALS AND METHODS

Collection of samples:

The samples used in this study were collected from different shops in the local market. The spoiled samples namely bread, lemon, onion & cheese. The samples collected were brought in sterile polythene bags to the laboratory for analysis [Fig 1A-1D]. All the glassware used in this study were sterilized by autoclaving at 121°C for minutes.

Processing of samples [15]:

Cultivation:

The samples were subjected to cultivation and staining was done in fungal culture medium Sabrauds dextrose agar. Sabrauds dextrose agar (SDA) which is a common medium to grow fungi was used in this study. 64 g of SDA was dissolved in 1000ml of distilled water. Each sample was inoculated on the center of respective Sabouraud dextrose agar plate which was labelled with the sample name. The plates were kept at 37°C incubator for 2 days. The fungal growth was seen in the plates. The colonies are picked from the plates and stained using Lactophenol cotton blue.

Staining procedure:

A small portion of sub cultured colony was cut using a sterile scalpel. It was placed on a sterile glass slide using a sterile forceps. The slide was covered with a coverslip and a coverslip and placed in a petri dish. These petri dishes were left at 30⁰ C for 5 days. The cover slips were taken with forceps and placed on slides containing cotton blue. The excess stain was removed and observe it under microscope, by 10 X and then in 40 X magnification power. The morphology i.e. shape, structure of conidia, conidiophores, pigmentation, shape of sporangia, sporangiophores were recorded. The identification was based on the standard keys available [15].

RESULTS

The morphological and cultural characteristics and the frequency of the isolated fungi were shown in Table 1.

Based on morphology, *Aspergillus flavus* was grown in bread and lemon which showed yellow to yellowish green colonies with distinct margin and morphological characteristics like conidiophores arise from a foot cell. Club shaped vesicles were found at top of the conidiophores. Conidia were found in chains. *Aspergillus niger* was grown in onion and cheese which had

produced black colonies with distinct margin & morphological characteristics like Conidiophores raised from a foot cell. Club shaped vesicles and conidia were found in chains.

Mucor was isolated from lemon, bread and onion which had shown like large white colonies which turned into black later, structural morphology shown erect sporangiophores and sporangiophore had swollen at the tip to form sporangia which were globular shaped. Columella was present.

On cheese, *Penicillium marneffeii* was grown in cheese which shows cultural characteristic like Fast growing colonies in green colour with dense conidia & morphological characteristic like Branched conidiophores with chains of conidia looks like a brush.

From the above study, fungi *Aspergillus flavus* was found in bread and lemon, *Aspergillus niger* was grown on onion and cheese. *Mucor* was grown on lemon, bread and onion and *Penicillium marneffeii* was found in onion, lemon and cheese. The frequently isolated fungi were *Aspergillus* spp (31% & 27%) contrast to that *Penicillium* had shown least frequency about 4%. [Table 1] [Fig 2A-D].

FUNGAL ISOLATE	CULTURAL CHARACTERISTICS	MORPHOLOGICAL CHARACTER	ISOLATED FROM	FREQUENCY
<i>Aspergillus flavus</i>	Yellow or yellowish green colonies with distinct margin.	Conidiophores arise from a foot cell. Club shaped vesicles at top of the conidiophores. Conidia are found in chains.	Bread and Lemon	15 (31%)
<i>Aspergillus niger</i>	Black colored colonies with distinct margin.	Conidiophores with club shaped vesicles are seen. Conidia are found in chains.	Onion and Cheese	13 (27%)
<i>Rhizopus</i>	Deep grey to black colored colonies with rhizoids.	Colonies were had haploid, multinuclear, coenocytic hyphae	Bread and Cheese	11 (22%)
<i>Mucor</i>	Large white colonies which turns into black later.	Erect sporangiophores are formed. Sporangiophore swells at the tip to form sporangia which are globular shaped. Columella is present.	Lemon, Bread and Onion	8(16%)
<i>Penicillium marneffeii</i>	Fast growing colonies in green colour with dense conidia.	Branched conidiophores with chains of conidia looks like a brush.	Onion, Lemon and Cheese	2 (4%)
Total = 5 spp.,				49 (100%)

COLLECTION OF SAMPLES:

FIG 1



A) BREAD



B) ONION



C) LEMON



D) CHEESE

ISOLATED FUNGI:

FIG 2



A) BREAD



B) ONION



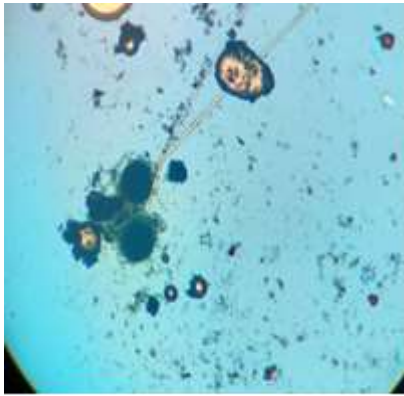
C) LEMON



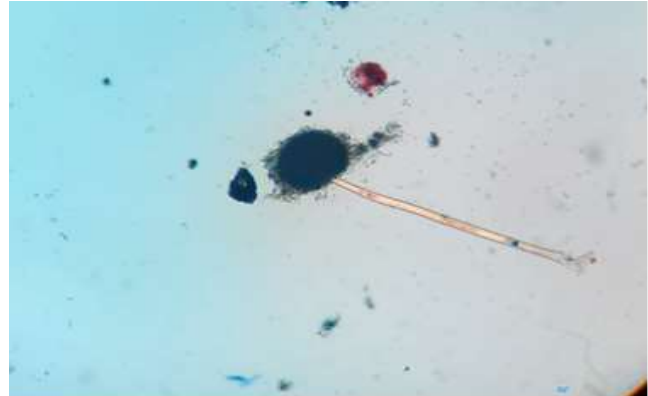
D) CHEESE

IDENTIFIED FUNGI:

FIG 3



A) BREAD



B) ONION



C) LEMON



D) CHEESE

DISCUSSION

Raja *et al.*, (2012) reported that *Pseudomonas* sp. and *Bacillus* sp. as dominant bacteria isolated from spoiled fruits and vegetable collected from local and supermarket [16]. Chaudhary and Dhaka (2016) reported that *Bacillus* sp. was found to be dominant in spoiled fruits and vegetable [17]. Tafuinta *et al.*, (2013) reported that *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger* and *Rhizopus stolonifer* were dominant fungi isolated from Sweet orange (*Citrus sinensis*) in Sokoto state [18].

CONCLUSION

Different genus of fungi was isolated and identified from different types of spoiled food items. The fungal problem in foods is a

cause for concern and its control is a major challenge for food industries too. A story of strategies, including an effective sanitization of environment and the use of antifungal compounds may reduce the incidence of fungal pathogens in foods.

Declaration by Authors

Ethical Approval: Approved

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REFERENCES

1. Nirmala Ravimannan, Pathmanathan Sevel, Selvaratnam Saarutharshan .Study of fungi associated with spoilage of bread. *International Journal of Advanced Research in Biological Sciences*.3(4):165-167(2016).
2. Banwart , G.J. Basic Food Microbiology. A Westport publication.505-544.
3. Legan, J.D . Mold spoilage of bread : the problem and some solutions. *International Biodeterioration and Biodegration* , 32:33-53.
4. Bordei, D. Tehnologia moderna a panificatiei. Editura Agir, Bucuresti.2004.
5. Jay MJ, modern food microbiology. 4 th ed.Chapman and Hall. Newyork .p.187 2005.
6. Ole H , Torben L, Lars PC, Ulla K , Nazmul H, Shakuntala Ha, Contents of onions , zinc and Beta carotene in commonly consumed vegetables in Bangladesh . *J Food Composit Anal* , 17:587-595, 2004.
7. Dimka SON, Onuegbu BA , Mycoflora of copra and effect of bringing on some properties of copra in Nilgeris. *Agric Biol J North Am*,1(3):391-394, 2010.
8. Mouly,P.P.,C.R. Arzouyan ,E.M. Gaydou, and J.M.Estienne.Differentiation of citrus juices by factorial discriminant analysis using liquid chromatography of flavonone glycosides. *J.Agriculture Food Chemistry*.42:70-79, 1994.
9. Schieber,A.,F.C.Stintzing , and R.Carle . By products of plant food processing as a source of functional compounds-recent developments. *Trends Food Science Technology* ,12:401-403,2001.
10. Deschner,E.E.,J. Ruperto, G.Wong , and H.L. Newmark.Quercetin and rutin as inhibitors of azoxymethanol-induced colonic neoplasia.*Carcinogenesis* 7:1193-1196, 1991.
11. Havsteen, B.Flavonoids, a class of natural products of high pharmacological potency. *Biochemistry Pharmacol*.32:1141-1148,1983.
12. Landolfi, R.,R.L.Mower, and M.Steiner. Modification of platelet function and arachidonic acid metabolism by bioflavonoid: structure-activity relations. *Biochemistry Pharmacol*. 33:1525-1530,1984.
13. Limasset,B.C.L.Doucen,J.C.Dore .T.Ojasoo,M.Damon, and A.C.D.Paulet .Effects of flavonoids on the release of reactive oxygen species by stimulated human neutrophils : multivariate analysis of structure-activity relationships (SAR).*Biochemistry Pharmacology* 46 :1251-1271,1993.
14. Banwart,G.J. Basic of Food Microbiology . A Westport publication .Pp505-544.2004.
15. Legan ,J.D. Mold spoilage of bread : The problem and some solutions.*International Bioderiation and Biodegeradation* 32:33-53 1993.
16. Raja, M. M., A. Raja, M. S. Hajee and S. A. Mohamed (2012). Screening of bacterial compost from spoiled vegetables and fruits and their physiochemical characterization. *International Food Research Journal*, 19(3) : 1193- 1198.
17. Chaudhary,L. and T.S. Dhaka, (2016) Isolation and identification from some spoiled fruits. *Plant Archives*, 16(2): 834-838
18. Tafinta I.Y, K. Shehu ,H. Abdulganiyyu , A.M.Rabe , A.Usman (2013) Isolation and identification of fungi associated with the spoilage of sweet orange (*Citrus sinensis*) fruits in Sokoto State. *Nigerian Journal of Basic Appied. Science* ,21(3):193–196.

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