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Original Article

Isolation and Identification of Molds Causing Spoilage of Bread Sold within Damaturu Metropolis, Yobe State, Nigeria.

Mohammed Nuhu* and Rakiya Yusuf

Department of Biological Sciences, Faculty of Science, PMB 1144 Yobe State University, Damaturu

**Correspondence E-mail: nuhmoh5@gmail.com*

Abstract

Bread was purchased from different shops in Damaturu. The bread was kept in different laboratory environment for three days after which fungal growth was observed. The bread was sub-cultured in Potato Dextrose Agar in 15 petri dishes and incubated for four days after which a growth was observed. Five (5) different organisms were isolated which include *Aspergillus niger, Aspergillus. spp, and Rhizophus sp.* The fungi isolated from this study when consumed can cause toxigenic infections in man. Proper and more sanitized methods of packaging, transportation and storage has been reported to reduce the problems of spoilage of breads, thus controlling and reducing the possibility of infections by these spoilage organisms.

Keywords: - Fungi, Potato Dextrose agar, *Aspergillus niger, Aspergillus. spp, and Rhizophus sp.*

Introduction

Bread is one of the most staple foods consumed daily in different parts of the world (Hager *et al.,* 2012). Bread is a nutritive food supplement comprising of major macro and micronutrients essential for mans' health (Malomo, 2012; Chen., 2022). Bread has of recent becomes the second most globally consumed food product after rice in Nigeria and other countries in the world. (Odedeji and Adeleke, 2010; Komlaga *et al.,* 2012). According to Mason *et al.* (2012), Bread demand is expected to rise in the next few coming years.

Molds' spoilage of bread is usually due to contamination after baking (Kurahashi., 2020). Freshly baked loaves of bread are usually free of mold spores due to temperature inactivation during production (Ponte and Tsen, 2011). Bread can only become contaminated post baking due to the mold spores present in the atmosphere around the loaves during the cooling, cutting, wrapping, packing and storage process (Kurahashi., 2020). Major sources of microbial spoilage of bread are usually due to the growth of molds. According to Banwart, (2004), bread molds are just like *Mucor* and *Rhizopus nigricans which are* usually found growing around the bread during its first spoilage stage. *Rhizopus nigricans* has a white mycelium and dotted black spots of sporangia (Ruoyu *et al*., 2022; Li., 2023), which is then followed by other fungi such as *Aspergillus niger* having green or purple brownish to blackish conidial spores and yellowish colours spreading into the bread loaves (Banwart., 2004).

In Nigeria and many countries, bread is a staple food usually produced by baking the dough of wheat or cassava flour and water (Odedeji *et al.*, 2010). Bread has high dietary value and its consumption is gradually increasing because of its convenience as an easily ready- made food to eat (Iorizzo., 2021; Desrosier, 2006). Flour made from wheat is well suited for bread baking due to its glutamine and gladden composition. Glutamine and gladden combine with water to produce the gluten network which is essential for dough development during bread baking process (Dwyer and Hallow, 2009).

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Material and Methods

Materials used in the study are three different samples of bread, 15 petri dishes, Potato dextrose agar media, glass slide, cover slip, lactophenol cotton blue, inoculating needle.

Study area

Studies were carried out in Damaturu, the capital of Yobe State, Nigeria. Damaturu is located in the northeastern part of Nigeria with a latitude of 11'11'15^{o}N and longitude 30'05'^{o}S and has a total population of 88,014 (National Population Commission, 2016) occupation of Damaturu people are mostly civil servant, farmers and businessmen and women.

Collection of samples

A total of five (5) different samples of bread were collected from different shops at the local market within Damaturu metropolitan.

Preparation of media

39 (g) of PDA (Potato Dextrose Agar) was dissolved in 1 lltr of distilled water, this was then mixed, vortexed and heated until it is finally dissolved. The mixture was then sterilized in an autoclave at 121°C for 15 minutes and then cooled to 45-50°C, after which it was well shaken and poured into Petri dish.

Fungi pathogen Isolation

Fungi pathogen isolation from the three (3) different samples of bread was conducted in accordance with a method reported by Samson et al. (2001). The collected bread samples were taken and placed directly on the PDA and allow to incubate at 25°C for 5 - 7 days. Fungal colonies formed were then sub cultured on the PDA and incubated again at 25°C for 5 - 7 days for characterization and identification.

Fungi Identification

The fungi isolated were then identified by the comparison of their micro and macro morphological characteristics by using the standard taxonomic as reported by Samson *et al*., (2001).

Macroscopic identification

Lacto phenol cotton blue was stained on the inoculated glass slide that has the sample on it and viewed using microscope at x10 and x40 objective lens magnification.

Table 1 Macroscopic, microscopic characteristics and name of organism

S/N	Macroscopic characteristics	Macroscopic characteristics	Name of organism
1	Black Sponge-like colonies after 5 days of incubation at room temperature.	Thick walled, septale hyphae with conidia on sporangiospore	*Aspergillus niger*
2	Green Spores on PDA	Green conidiospores with septate hyphae	*Aspergillus flavus*
3	Black Sponge-like colonies on PDA	Thick walled, septate hyphae with conidia on sterigina	*Aspergillus niger*
4	Green Spores on PDA after 3-5 day at room temperature	Green conidia with septate hyphae under x40 objectives lens	*Aspergillus spp.*
5	Large soft whitish milky colonies that later changes to black as culture progresses.	Aseptae hypha with sporangiosphores inter wooven by stolons and rhizophus	*Rhizophus stolonifer*

Results

Fungal growth was seen on day two of incubation. On the third day, all three samples had scanted and dispersed fungal count. However, from the fourth day all the samples showed positive growth. Similarly, the fungal colonies continue to increase progressively with days. Therefore, after four days

of storage period, the highest fungal count was noticed in all samples. The total fungal count of the bread samples after incubation over a storage time of four days are shown in figure below;

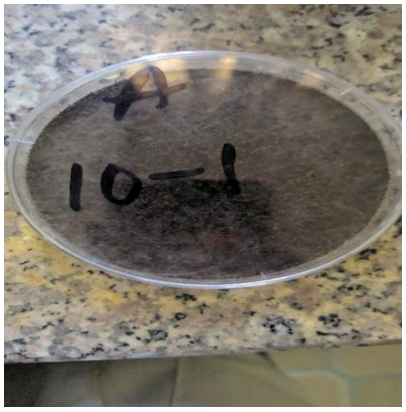

Plate 1: Aspergillus niger

Plate 2: Aspergillus flavus

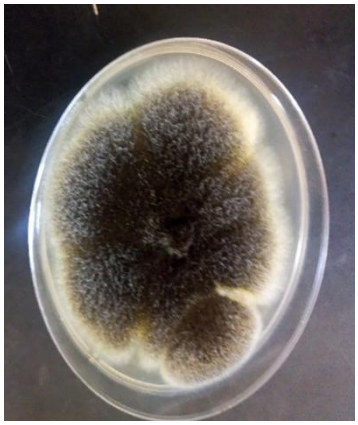

Plate 3: A micrograph of Aspergillus Niger

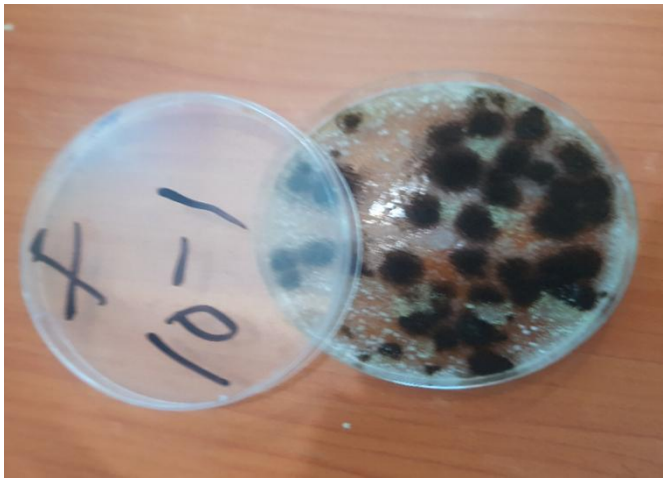

Plate 4: Culture of Aspergillus Spp

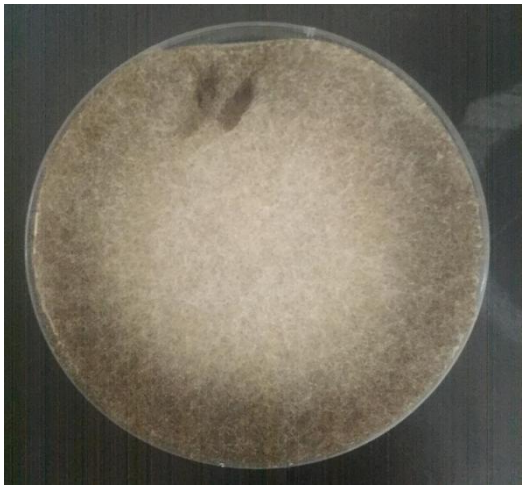

Plate 5: Culture of Rhizophus stolonifer and micrograph

Discussion

Fungal infestation is the main cause of spoilage which causes many damages and economic losses in bakery products and also responsible for many public health issues. (Legan, 1993). The occurrence of the molds *Aspergillus spp, Aspergillus niger., Rhizophus sp.* In the bread could be due to the fact that they are spore formers. Contamination of the bread could have resulted from inappropriate processing, incomplete heating, or secondary contamination via contact with contaminated utensils, (Oranusi, 2013). The incidence of molds in bakery products in Damaturu metropolis might be very much on the increase probably due to the hot and wet climate conditions couples with the baking process. According to (Gerez *et al.,* 2009), the rates of growth of fungi and bacteria are markedly influenced by the storage environment and the lower temperature (and humidity), the slower growth of these microorganisms, and the smaller the likelihood of new infections. Not only is the climate , cooling and packaging environment important in the spoilage of bread in Damaturu, but also the conditions under which the bread is held temporarily or offered for sale. The conditions under which the cereal grains (for making flour i.e the most important raw material in the production of bread) are stored also plays a vital role in the fast spoilage of these finished products (breads) as Damaturu has extremely high or extremely low temperature, as not all of the fungal spores that were harvested along with the grains are usually killed during baking procedure. The high occurrence of *Aspergillus* species identified to be responsible in the spoilage of bread can be attributed to the ability of its spores to contaminate the cereal grain while still on the plants in the farm (Oranusi, 2013).

From the results of this study, there are five organisms isolated that corroborate with the findings of Taulo et al. (2008) in their work on fungi causing the spoilage of bread in Enugu state, Nigeria, where these fungal organisms were fingered in the contamination process of ready-made foods and the organisms isolated were *Aspergillus spp., Aspergillus Niger,* and *Rhizophus sp.* Fungi common to bread contaminations were reported by Ogundare and Adetuyi, (2003) as known to produce mycotoxins, which is not advisable to consume spoiled bread or any bread that have been stored for a long time as many of the organisms responsible for the spoilage are pathogenic to health. Some of the pathogenic effect of the fungal species isolated from this study like *Aspergillus Niger* cause fungal ear infection, lung infection, intestinal problems as well as liver and lung cancer, *Aspergillus Niger*. Cause respiratory disease, and black bread mold which is *Rhizophus sp.* Cause fatal infections.

Conclusion

Considering the data obtained from this study, molds spoilage of bread will continue to increase if not fully controlled. There is occurrence of various isolates which are *Aspergillus* and *Rhizopus* species, the worst agents of spoilage bread. On the other hand, *Aspergillus niger* specie isolated has been found to be pathogenic to man. In other words, it can cause health problems to man. It is therefore advisory that controllable methods such as freezing or the use of preservatives during the production process should be used to control or reduce the spoilage of bakery products. When this microbial spoilage is reduced, more of these breads will be in circulation for human consumption.
It is advisable not to consume spoilt breads so as to avoid fatal infections, for example *Aspergillus* species when consumed can cause toxigenic infections in man. Spores of *Aspergillus* can survive the baking procedure during the production of bread. Proper and more sanitized methods of packaging, transportation and storage have been reported to reduce the problems of spoilage of breads, thus controlling and reducing the possibility of infections by these spoilage organisms.

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Conflict of Interest:

The authors declare no conflict of interest.

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