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Ferreting Out of Aflatoxigenic Production of *Aspergillus flavus* by UV Light and Ammonia Vapour Test from Bakery Samples

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A B S T R A C T

Bakery food products encompass a wide variety of items, ranging from traditional bread and pastries to innovative and specialty baked goods. Bakeries worldwide craft these products using a combination of basic ingredients such as flour, sugar, yeast, eggs, and butter, but the creativity of bakers often results in a diverse and delicious array of offerings. Contamination of bakery products with fungi is a serious concern, as it poses health risks to consumers. Fungi, including molds, can produce mycotoxins that are harmful if ingested. If you suspect that bakery products are contaminated with fungi, it's crucial to address the issue promptly. This research aims to investigate the aflatoxigenic potential of *Aspergillus flavus* in bakery samples using the UV light and ammonia vapor test. By employing this innovative methodology, we seek to streamline the detection process, providing a rapid and reliable means of identifying aflatoxin-producing strains. The findings of this study hold the potential to enhance food safety protocols in the baking industry, contributing to the production of uncontaminated and healthful bakery products. The results indicate that 35% of *A.flavus* isolates were aflatoxigenic, and proper handling and sanitation are emphasized to prevent spoilage and contamination of bakery products.

Introduction

For most people across the country and its cultures, baked products are staple meals. The most well-liked products include breads, buns, cupcakes, etc. The grains needed to manufacture bakery items provide us with about half of our daily protein requirements and most of our daily calories, making them a significant source of nutrients. Baked goods often contain the following ingredients: carbohydrates, proteins, lipids, vitamins, calcium, iron, minerals, starch, and energy. Bread products are the best candidates for fibre enrichment. As stated by Bartkiene *et al.,* (2008), Eggs, lipids, yeast, baking soda, baking powder, cornflour, milk, butter or margarine, honey, yoghurt, essence, cocoa powder, chocolate slabs, fruit jams, and sweetener are among the ingredients used in baking. Products from bakeries can have spoiling complications (Abellana *et al.,* 1996). Therefore, incorrect handling results in the loss of its commercial worth and economic significance. The potential for the creation of mycotoxins is one of the financial losses connected to bread goods. In fresh bread and other baked goods, baking often eliminates mould spores. Therefore, any bakery product that develops

mould must have come into contact with contaminated air, surfaces, tools, food handlers, raw materials, or during the chilling, slicing, or packaging processes that follow baking (Knight *et al.,* 2006). This implies that any mold-related spoiling issues must arise after baking.

Because of airborne contamination brought on by warmer temperatures and more humid storage conditions, fungal spore counts are greater in the summer than they are in the winter. Unwanted odours are created by fungus spoiling, which is frequently visible on the product's surface (Baily *et al.,* 1993).

The spoiling of bread can be attributed to many filamentous fungi, *including Aspergillus, Fusarium, Rhizopus* and *Mucor*, which can arise from inappropriate handling and sanitation. The percentage of bakery products lost owing to these fungi varies according on the season, product type, and processing techniques.

In recent years, there has been a rise in the prevalence of fungal-induced spoiling of wheat bread (Marin *et al.,* 2003). This is likely due to the increased production of bread without preservatives and the frequent addition of raw ingredients like bran and seeds. These bakery goods' potential for spoiling might pose a health concern and result in a minor foodborne disease. There is an association between eating these foods and food-borne illnesses.

Only strains of *Aspergillus* that are closely related *A. flavus, A. parasiticus, and A. nominius* are capable of producing aflatoxins. Aflatoxin-producing species include *A. bombycis* and *A. pseudotamari*, but their prevalence is lower. In addition to being an issue for several commodities, aflatoxin B1 (AFB1) has been shown to be carcinogenic in humans due to its acute toxicity, mutagenicity, and carcinogenicity (Gerez *et al.,* 2009).

According to Jarvis *et al.,* (2001) blue fluorescence is a technique used to establish qualitative culture procedures for identifying aflatoxigenic *Aspergillus* species produced on appropriate conditions. These methods employ liquid media like aflatoxin producing-ability medium (APA) or solid media like coconut agar medium (CAM) and potato dextrose agar (PDA).

Aspergillus, a generator of aflatoxins, was identified using long-wave UV light (365 nm). Aflatoxigenic isolates may be quickly distinguished from non-aflatoxigenic isolates by this method: aflatoxigenic isolates look blue to blue-green fluorescent, but non-aflatoxigenic isolates do not exhibit fluorescence (Hara *et al.,* 1974). A fast and sensitive technique for identifying both aflatoxigenic and nontoxigenic strains of *Aspergillus* is the vapor-induced colour change approach. Using this technique, a single colony was grown in the Petri dishes.

When ammonia vapour from a drop of ammonia hydroxide was applied to the medium of aflatoxigenic *Aspergillus* strains, the reverse colony turned pink, while non-aflatoxigenic strains do not create colour. This study will concentrate on the quick identification of *Aspergillus flavus* strains that produce aflatoxin by using the UV light and ammonia vapour tests, as well as the appropriate control methods (Malkki *et al.,* 2000).

Materials and Methods

Collection, Isolation and identification of *A.flavus* and *A.niger*

Thirty-nine different kinds of baked products, including bread, toast, cupcakes, buns, cookies and so on, were gathered from several bakeries and used as inspiration for the decorated samples at the open market and samples were transported to the lab for examination in sterile polythene bags, where they were kept at temperatures between 25 and 27^0 degrees Celsius are needed for the 7-day fungal development.

Slides were made for fungal identification by scraping the materials. After samples of various baked products were collected, and the direct plate approach was used to inoculate the PDA plate. After seven days, the pure culture was incubated at 25 °C ± 2. Following incubation, lactophenol cotton blue was used to create separate macroscopic and microscopic colonies. According to Domsch, *et al* (1980); Klich (2002), the morphological traits of *A. flavus* and *A.niger* isolates were reported microscopically. Subcultures were conducted on both PDA and CAM, as reported by Davis *et al.,* (1987). Following that, the cultures were cultured for seven days at 25^0C ± 2. It was determined that the isolation frequency (Fr) was;

Frequency (%) =(ns/N) *100

[ns=number of samples where a genus or species of fungi occurred and N=samples]

Confirmation tests for aflatoxin production from *A.flavus* and *A.niger*

Coconut based medium test

For the confirmation test, fluorescence on coconut agar medium (CAM) has been used. A preliminary screening for the aflatoxin-producing *Aspergillus* was done based on the emission of blue to blue-green fluorescence upon UV light irradiation at 365 nm after the isolates were grown on the medium, as coconut agar is inductive of aflatoxin formation. Producer isolates can be identified using fluorescence on the other side of the culture CAM on glass Petri plates.

A five-millimeter-diameter sterile cork borer was used to create a hole in the centre of the CAM medium within a Petri plate. Using a cork borer, the isolate was placed with a large number of conidia into the CAM Petri dish's centre hole. The isolates were then cultured for seven days at 28°C. To compare with organisms that produce aflatoxin, non-aflatoxigenic *Aspergillus niger* has been employed as a control (Alkhersan *et al.,* 2016).

Ammonia vapor test

The fungal isolates were cultivated for seven days at 28°C in the dark after being inoculated as single colonies on plates. One or two drops of a concentrated ammonium hydroxide solution were applied to the interior of the Petri dish lid after the dish had been inverted. Next, the Petri dish with the control plate and ammonium hydroxide was inverted over the lid (Alkhersan *et al.,* 2016).

Results and Discussion

Of thirty-nine samples, seventeen isolates of A. flavus were taken into consideration. Using UV light and the ammonia vapour test, *Aspergillus flavus* was found to be both aflatoxigenic and non-aflatoxigenic in bakery products made with grains and sweeteners. Of the isolates, 6 (35.29%) were aflatoxigenic (positive) for both methods, while 11 (64.70%) were non-aflatoxigenic (negative) (Table 1). Based on the calculation of isolation frequency percentage (%) and incidence of relative density, *Aspergillus flavus, Rhizopus, Penicillium,* and *Fusarium* are the most common species. *A. flavus* and *A. niger* microscopic views were taken. In contrast to non-aflatoxigenic colonies, which were non-generating fluorescent colonies akin to control isolates of non-aflatoxigenic *A. niger*, the detection by UV light at 365 nm identified aflatoxigenic colonies by forming blue-green fluorescent colonies in the middle of the glass Petri dish of CAM (Figure 1). Ammonia vapour was used to identify aflatoxigenic *A. flavus*, and the results were reported.

Six isolates (35.29%) out of the total of 17 isolates of *A. flavus* were shown to be aflatoxigenic by employing the UV light method. These isolates were coloured blue-green fluorescence (positive) on the back of a glass Petri dish of CAM, whereas non-aflatoxigenic isolates produced no colour (negative) findings and were regarded as negative. The same ammonia vapour detection results as previously released showed that the colony of aflatoxigenic *A. flavus* became pink, while the non-aflatoxigenic isolates did not exhibit any colour change.

This indicates that the amount of ammonia vapour on CAM and UV light at 365 nm produced an equivalent number of aflatoxigenic isolates of *A. flavus*. This outcome is comparable to the findings of Zarari *et al.,* 2013; Nair *et al.,* 2014 and Yazdani *et al.,* 2010. While this study differs from that of Fani *et al.,* 2014 which reported only 25.6% of positive isolates of aflatoxigenic *A. flavus* by fluorescence detection on CAM, and only 12% of isolates were identified as aflatoxigenic using ammonium vapour detection, there was a difference with Nair *et al.,* 2014 who confirmed that the cultures of aflatoxigenic *Aspergillus* were tested for bright orange-yellow colony reverse colouring and 365 nm UV light fluorescence.

In developing countries, where contaminated food products may easily find their way into households and grocery shops, aflatoxin contamination is a serious concern. It's critical to understand that aflatoxin affects animals in ways beyond just causing symptoms.

Compared to other fungi, the percentage of aflatoxigenic *A. flavus* in bakery food items is high. The study's main objective was to identify *Aspergillus flavus* aflatoxigenic synthesis in bakery samples by employing ammonia vapour and UV light tests. According to the findings, six of the seventeen isolates of *A. flavus* were aflatoxigenic. This research highlights how crucial it is to handle baked goods carefully and maintain hygienic conditions to avoid contamination and spoiling. The study also emphasised how important it is to manage *A. flavus* contamination in order to assure the production of wholesome and uncontaminated bakery products.

Figure.1 Frequency percentage of fungi isolated in bakery food products for aflatoxin production

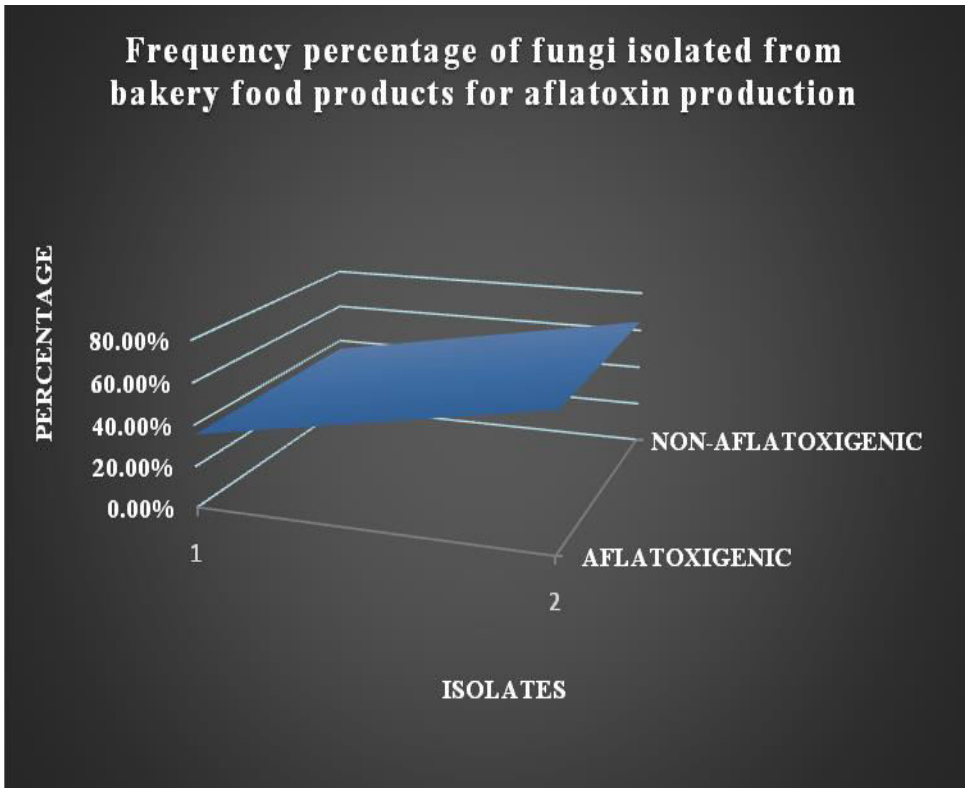
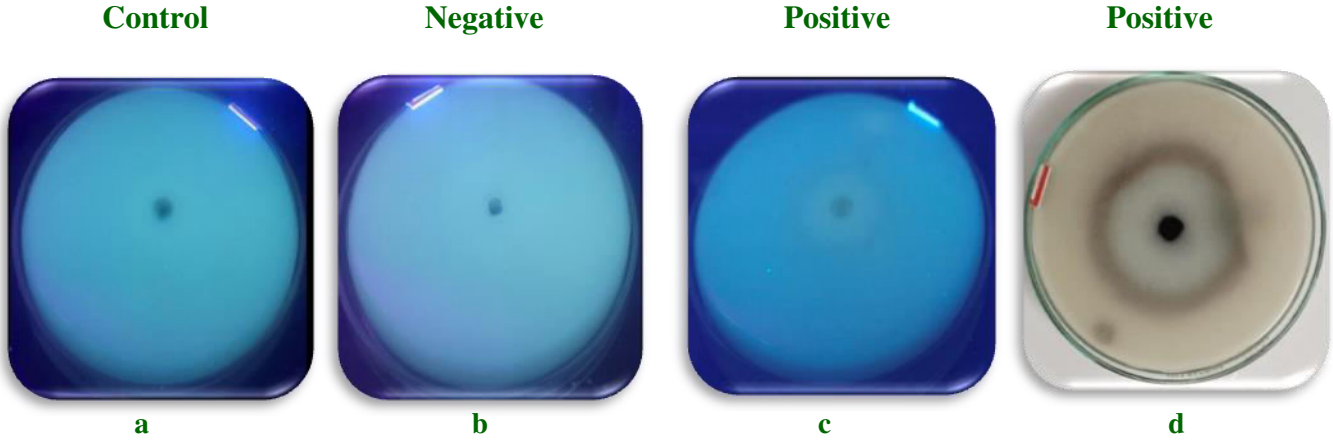


Figure.2 Showed the detection of aflatoxigenic *A.flavus* by coconut agar medium (CAM) under UV light. (a) Control (b) non-aflatoxigenic isolate of *A.niger*, (c) aflatoxigenic *A.flavus* isolate showing a blue green fluorescent around the colony (d) aflatoxigenic *A.flavus* isolate showing a red colour around the colony.



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Author Contribution

K. L. Sowmya: Investigation, formal analysis, writing—original draft. B. Ramalingappa: Validation, methodology, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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References

Abellana M, sanchis U. and Ramos A J. Effect of water activity and temperature on growth of three *Penicillium* Sp. and *Aspergillus flavus* on a spongy cake analogue. International Journal of Food Microbiology, 1996.71: 3151-3157. https://doi.org/10.1016/s0168-1605(01)00596-7

Raed Najeeb Alkhersan, Mohammed H.Khudor, Basil A. Abbas. 2016. Rapid detection of aflatoxigenic producing atrains of *Aspergillus flavus* from poultry fees by uv light and Ammonia Bas.J.Vet.Res.15(4): 169-178.

Bailey C P. and Holy A V. *Bacillus* spore contamination associated with commercial bread manufacture, Food Microbiology, 1993. 10: 287- 294.

Bartkiene E G, Juobeikiene and Vidmantiene D. Evolution of deoxynivalenol in wheat by acoustic method and impact of starter on its concentration during wheat bread baking process. Food Chemistry and Technology, 2008. 42: 5-12.

Carla Lucian Gerez, Mariaines Torino, Prevention of bread mold spoilage by using Lactic acid bacteria with antifungal properties. Journal of Food Science, 2009. 20: 144-148. https://doi.org/10.1016/j.foodcont.2008.03.005

Davis N D, Iyer S K. and Diener U L. Improved method of screening for aflatoxin with a coconut agar medium. Appl. Environ. Microbiol. 1987.53(7):1593–1595. https://doi.org/10.1128/aem.53.7.1593-1595.1987

Domsch K H, Gams W. and Anderson T H. Compendium of soil fungi. Academic press. London, New York, Toronto, Sydney, San Francisco, 1980.1:859.

Fani ,S.R.; Moradi ,M.; Probst ,C.; Zamanizadeh, H. R.; Mirabolfathy, M. and Haidukowski,M.(2014). A critical evaluation of cultural met hods for the identification of atoxigenic *Aspergillus flavus* isolates for aflatoxin mitigation in pistachio orchards of Iran. Eur. J. Plant Pathol.,140(4):631-642.

Hara S, Fennell D I. and Hesseltine C W. Aflatoxin-producing strains of *Aspergillus flavus* detected by fluorescence of agar medium under ultraviolet light. Appl. Microbiol, 1974. 27(6):1118-1123. https://doi.org/10.1128/am.27.6.1118-1123.1974

Jarvis B. Mould spoilage of food. Process biochemistry, 2001. 7: 11-14.

Knight R A. and Menlove E M. Effect of the bread baking process of destruction of certain mould spores. Journal of the Science of Food and Agriculture, 2006. 10: 653-660.

Klich,M.A.(2002).Identification of common Aspergillus species. Centraalbureau voor Schimmel cultures, Utrecht, The Netherlands.pp:46

Malkki Y. and Rauha O. Mould inhibition by aerosols. Bakers Digest, 2000; 52: 47-50.

Marin S, Guynot M E. Sanchis. *Aspergillus flavus, Aspergillus niger* and *Penicillium coryophilum* spoilage prevention of bakery product by means of weak acid preservatives. Journal of Food, 2003.

Nair ,S. C.; Bhagobaty, R.K. ;Nmpoothiri,K.; Kalaighandhi ,V. and Menon, K.R.K. (2014). Detection of aflatoxin production by fungi in spice samples using HPLC and direct visual cultural methods. Innovative Romanian Food Biotechnology, 14, 1-12.

Yazdani, D.; Zainal Abidin, M. A.; Tan, Y. H. and Kamaruzaman, S.1(2010). Evaluation of the detection techniques of toxigenic *Aspergillus* isolates. Afr. J. Biotechnol., 9 (45): 7654-7659.

Zarari,T.J.O. (2013). Detection of aflatoxin from some Aspergillus ssp. isolated from wheat seeds. Journal of Life Sciences, 7 (10): 1041-1047.

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