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Qualitative Analysis of Mycotoxins by Thin Layer Chromatography (TLC)

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Abstract: Mycotoxins are toxic secondary metabolites produced by various molds and fungi. While they are more commonly associated with crops such as grains, nuts, and fruits, they can also be found in bakery food products that use these ingredients as raw materials. The presence of mycotoxins in bakery products can pose health risks if consumed in large quantities. Mycotoxins are secondary metabolites generated by several species of fungus that have a negative impact on food quality and are dangerous for both people and animals. Aflatoxins (AF), Fumonisins (FUM), Deoxynivalenol (DON), Ochratoxin A (OTA), Zearalenone (ZEA), Patulin (PAT), and Citrinin (CIT) are the most prominent and commercially relevant mycotoxins TLC has become a remarkably efficient, fast, and, in most circumstances, inexpensive separation technique in mycotoxicology. In this study, mycotoxins from various fungi, including *Aspergillus* species, *Penicillium* species, *Fusarium* species, *Mucor* species, *Nocardia* species, *Trichoderma* species, *Curvularia* species, *Bipolaris* species, *Rhizopus* species, and *Alternaria* species, are screened using TLC (Thin layer chromatography) analysis metod, an easy physicochemical experiment, to determine whether they are present in bakery products. The extraction of mycotoxins used a variety of solvent systems. The study focused on the detection of mycotoxins in bakery food samples using Thin Layer Chromatography (TLC) technique. Fungal species, including *Aspergillus* sp, *Penicillium* sp, *Fusarium* sp, *Mucor* sp, *Nocardia* sp, *Trichoderma* sp, *Curvularia* sp, *Bipolaris* sp, *Rhizopus* sp, and *Alternaria* sp, were screened for mycotoxins. The TLC plates were visualized under visible light and UV light to identify the presence of mycotoxins. The study's ultimate objective is to find the precise mycotoxins that the targeted fungi species includes. Findings of this study can help create methods for preventing the formation of mould and extending the shelf life of bakery products.

Keywords: Mycotoxins, Thin Layer Chromatography, Fungi, *Aspergillus* sp, *Fusarium* sp, Fumonisins

1. Introduction

A chemical that is toxic to another creature is one that is produced by a species of plant, an animal, or a microorganism. Mycotoxins are poisonous byproducts of fungus, primarily saprophytic moulds that grow on a range of foods, including animal feeds, and several plant diseases. They might be dangerous to people and domestic animals. Early in the 1960s, it was determined that mycotoxins have been accountable for a number of disorders.

Mycotoxin is a combination of the Latin term "toxicum," which means poison, and the Greek word "mykes," which refers to a fungus. The name "mycotoxin" is often reserved for the few fungi that rapidly colonise crops in the field or after harvest and produce relatively tiny (MW 700), harmful chemical compounds known as secondary metabolites [1]. These substances might be effect to both animal and human health if consumed through food made from these ingredients. Contamination can occur either before or after harvest. For instance, *Fusarium* produced deoxynivalenol (DON) and T-2 toxin pre-harvest, and *Aspergillus*-produced ochratoxins (OTA) and aflatoxins (AFT), however aflatoxins contamination can also arise in the field [4].

Food products from bakeries that are left in storage for a long time are frequently prone to mould development and mycotoxin generation. Mycotoxins may exist in both

temperate and tropical settings, depending on the kind of fungus. The primary dietary components that are affected include cereals, nuts, dried fruit, coffee, chocolate, spices, oil seeds, dried peas, dried beans, and fruit, particularly apples. Due to the usage of tainted barley, other cereals, in the production of bakery food products may get contaminated with mycotoxins due to improper handling during processing of bakery food products. Although they can be generated by one or more fungi species, they are frequently genotypically specific. For instance, *A. ochraceus,* which is primarily found in tropical locations, and *P. verrucosum*, a common storage fungus in temperate areas, both generate OTA. In other instances, one species can also produce many mycotoxin forms [2]. Numerous analytical techniques have been developed to identify and measure mycotoxins in food samples as a result of all of these attempts to set mycotoxin limits and standards. Chromatographic methods, immunoassay-based techniques, and quick strip screening tests are some of the approaches that have been shown effective in the measurement of mycotoxins in products from bakeries [3]. Despite the enormous advancements achieved in this area, there are still many difficulties and drawbacks to these analytical techniques that need to be resolved. Special extraction, cleanup, and detection techniques are needed due to the mycotoxins' chemical diversity, co-occurrence, changing concentrations in food commodities, and complex food matrices that include mycotoxin contamination.

The objective of the current investigation was to identify various mycotoxins. For example, *Penicillium* sp, *Fusarium* sp, *Aspergillus* sp, *Mucor* sp, to determine if there is a difference between mycotoxins that occur and those that are likely to exist, mycotoxins were identified and assessed based on the colours that were produced on plates by thin layer chromatography [5].

2. Materials and Methods

2.1. Isolation and Identification of Fungi

Fresh samples of various bakery foods were collected from rural areas and stored in polythene covers for later processing. Samples were inoculated on sterile medium (PDA, SDA, RBA) plates for the isolation of fungi from bakery foods, and after the fungi had grown, identify them by macroscopic and microscopic observation [13, 14].

2.2. Mass Production and Extraction of Fungi

For the majority of the production of fungal cultures, subculturing was used to get pure cultures from different cultures. The suitable growth medium, Potato Dextrose broth (PDB), was then used to assist mass culturing. Pure fungal colonies were introduced in broth under sterile conditions. Flasks were incubated to promote the development of the desired fungus. Using Whatman filter paper, broth was filtered after 1-2 weeks of incubation, and two sections of the media were used for separation. Both culture and liquid are included. Later that, samples of fungi were extracted using the separating funnel, fungal mycelium was extracted in the ratio 1:1 mixture of ethyl extract and culture filtrate. Finally, thin layer chromatography (TLC) was employed to identify mycotoxins from processed extracts [15].

3. Results and Discussion

3.1. Isolation and Identification of Fungi

Fifteen fungi (15) were isolated from 73 bakery food samples using different media like PDA, SDA and RBA medium by serial dilution method, direct plate method and spread plate method and fungi were identified using lactophenol cotton blue stain under microscope as shown in the Figure 1 and Figure 2.

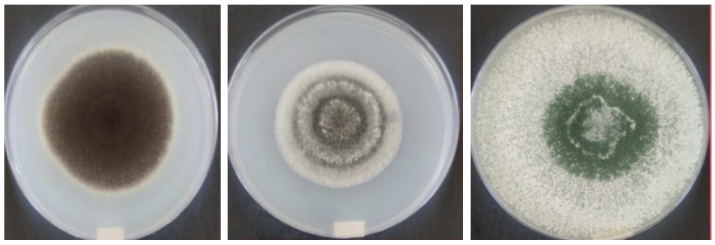
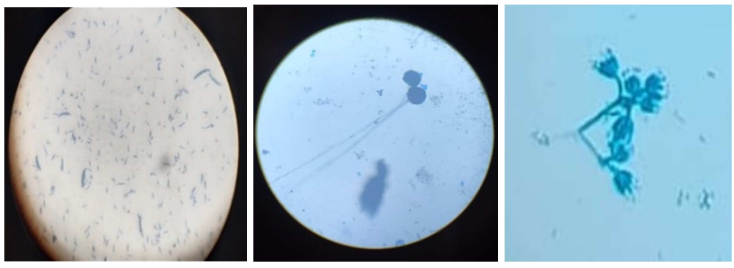


Figure 1. *Fungal species isolated from Bakery food products.*



Fusarium sp *A. niger* *A. flavus*

Figure 2. *Identification of Fungi from bakery food samples under 40X.*

3.2. Submerged Fermentation and Extraction Method

Nine (9) fungal species were selected for detection of mycotoxins by Thin Layer Chromatography technique. Observing the essential macro and micromorphological traits of cultures of various fungus on various media is a standard method for classifying fungi [7]. After inoculation to the potato dextrose broth, flasks were incubated for 7-14 days for mat formation. A thick mat was obtained as shown in the Figure 3.

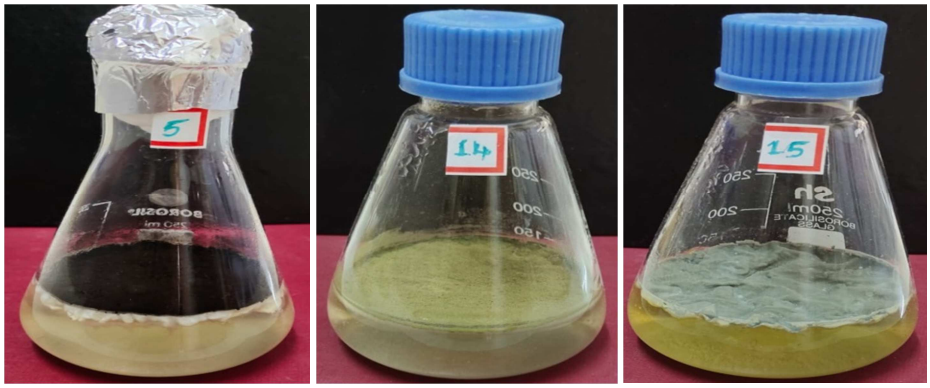


Figure 3. *Submerged fermentation method.*

Extraction of fungal samples was carried out by separation method. Using a separating funnel, mycelium from fungi was extracted in the 1:1 ratio with ethyl acetate (Figure 4). Finally, processed extracts were used for detecting mycotoxins by Thin Layer Chromatography (TLC) method (Figure 5).

Figure 4. *Ethyl acetate extraction method.*

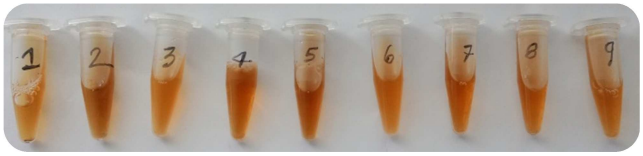
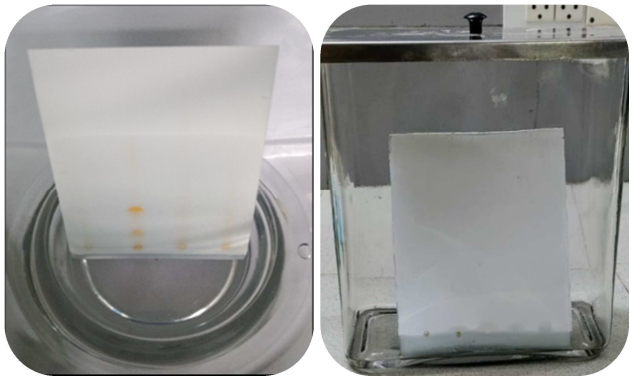


Figure 5. *Ethyl acetate extract.*

3.3. Mycotoxins Determination by TLC Method (Thin Layer Chromatography)

In order to determine the biosynthesis of mycotoxins from the fungal strains that were isolated, a TLC analysis was conducted. The resulting ethyl acetate fungal extract was then dried and the residue was resuspended in 100 µl of ethyl acetate. Next, 20 µl of the sample was loaded on thin layer chromatography plates. The standard solvent systems, consisting of toluene-ethyl acetate-90% formic acid (6:3:1; TEF), (Figure 6) was used for the separation process. Finally, the spots were visualized under UV light (transilluminator) (Figure 7).



TLC chamber Separation of components in TLC

Figure 6. *Separation of samples (Ethyl acetate extract) in TLC plates using toluene-ethyl acetate 90% formic acid (6:3:1; TEF).*

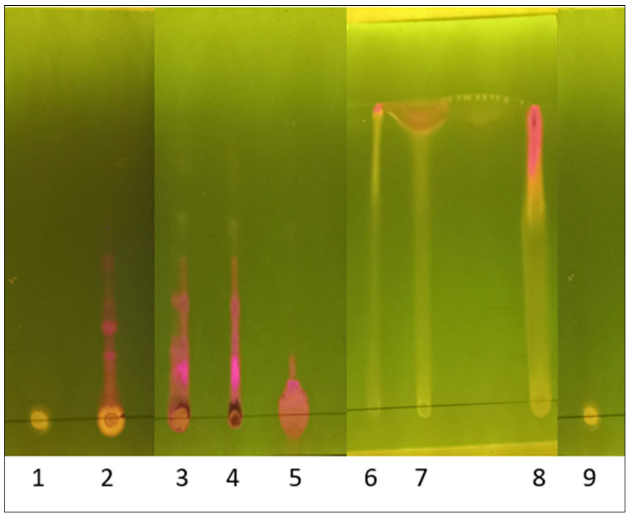


Figure 7. *Illumination of mycotoxins of fungi in TLC plates under UV light.*

All of the plates were passed through the same solvent solution, and since some cultures reflect colour while others do not, no colour was seen. This demonstrates the ability of fungi in the production of toxic substances. *Biporalis* sp. (3), *A. oryzae* (4), *Rhizopus* sp (5), *A. flavus* (6), *Penicillium* sp (7), and *Fusarium* sp (8) were reflected and appeared dark pink/reddish in colour. Hassan *et al*., (1995) [12] provided a standard note and key for the detection and preliminary identification of all lighted mycotoxins based on the colour that emerged on the TLC plates. Isolates that reflect light may generate toxins.

Turner *et al*., [9] also covered analytical methods for identifying mycotoxins, while Zheng *et al*., [8] presented an overview of rapid and conventional analytical methods for looking at mycotoxins. Betina [10] investigated the broad technical aspects of thin layer chromatography of mycotoxins, including extraction and clean-up methods, adsorbents and solvent systems, detection methodologies, and detection methods. They used two-dimensional TLC, high performance TLC (HPTLC), quantization, and preparative TLC (PLC). Scott *et al*., [11] employed one initial spraying reagent and a suitable general solvent solution to detect mycotoxins using thin-layer chromatography. The mycotoxins generated by the reflected isolates have been determined and tentatively identified based on the colour appearance on the TLC plates.

The intense pink/reddish colour seen on the TLC plates suggested that the fungus may create toxic substances. The study effectively identified mycotoxins generated by different fungus that can be harmful to human health if ingested in high amounts using the Thin Layer Chromatography (TLC) technique, which was used as an easy and effective approach for the qualitative investigation of mycotoxins in bakery food samples.

Overall, the study highlights the importance of monitoring mycotoxins in bakery food products and the efficacy of Thin Layer Chromatography (TLC) as a technique for their detection.

4. Conclusion

Mycotoxins are harmful compounds made by certain moulds (fungi) that may infect a range of food items, including baked products. These toxins pose health risks to humans and animals when ingested in sufficient quantities. While mycotoxin contamination is more commonly associated with grains, nuts, and other staple foods, bakery products can also be affected if the raw ingredients used in their production are contaminated. The present study focuses on the detection of mycotoxins in bakery food products using the Thin Layer Chromatography (TLC) technique. The study involved the isolation and identification of various fungal species from bakery food samples, followed by the extraction of mycotoxins using different solvent systems. The mycotoxins were then analysed using TLC plates and visualized under UV light. The results showed the presence of mycotoxins in certain fungal species, indicating their ability to produce toxins. The findings of this research can contribute to the development of strategies to prevent mold growth and ensure the safety of bakery products.

It's important to note that mycotoxin contamination in bakery products is relatively rare compared to other food categories, but it is still a concern for food safety. Both food producers and regulatory agencies work to ensure that bakery items and other food products meet safety standards and pose minimal health risks to consumers.

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Conflicts of Interest

The authors declare no Conflict of Interest.

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