Detection of Mycotoxins from Bakery Food Samples by Thin Layer Chromatography (TLC)

Sowmya K. L[1]& Ramalingappa. B*

Research Scholar, Department of Microbiology, Davangere university, Davangere-577007, Karnataka, India

Professor, Department of Microbiology, Davangere university, Davangere-577007, Karnataka, India.

Corresponding author: **Ramalingappa B**

Abstract:

In mycotoxicology, TLC has developed into a very effective, quick, and in most cases, affordable separation procedure. This study involves screening of mycotoxins from some fungi like *Aspergillus* sp, *Penicillium* sp, *Fusarium* sp, *Mucor* sp, *Nocardia* sp, *Trichoderma* sp, *Curvularia* sp, *Bipolaris* sp, *Rhizopus* sp, *Alternaria* sp, throughThin Layer Chromatography (TLC) technique, an easy physicochemical laboratory process, to identify mycotoxins in bakery products. For the extraction of mycotoxins, various solvent systems were employed. The purpose of the further research on this work is to find out more about the specific mycotoxins present in the target fungal species.

Key words:*Aspergillus* sp, *Penicillium* sp, *Fusarium* sp, *Mucor* sp, *Nocardia* sp, *Trichoderma* sp, *Curvularia* sp, *Bipolaris* sp, *Rhizopus* sp, *Alternaria* sp, Thin Layer Chromatography, Mycotoxins.

1. Introduction

Mycotoxins are secondary metabolites produced by filamentous fungi, when consumed by higher animals and human beings, result in a harmful reaction (mycotoxicosis). The majority of the food we eat has been tainted by bacteria and their poisons. Mycotoxicosis (toxic sickness) is brought on by consuming food products contaminated with toxigenic fungus.A condition known as mycotoxicosis is brought on by eating certain foods that contain mycotoxins, which are generated by toxigenic fungus. 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(1).Aflatoxins (AF), fumonisins (FUM), deoxynivalenol (DON), ochratoxin A (OTA), zearalenone (ZEA), patulin (PAT), and citrinin (CIT) are the most prominent and commercially relevant mycotoxins (2).Mycotoxins, however, are often shown to be toxic. Numerous *Penicillium* species are also capable of producing a variety of poisonous substances, including citrin and citreoviridin (3-5). Aflatoxin B1 and B2 (AFB) and Aflatoxin G1 and G2 (AFG) were discovered by Awuchi *et al*. (6), as well as their metabolites Aflatoxin M1 (AFM1), Aflatoxin M2 (AFM2), Aflatoxicol (AFL), and Aflatoxin Q1 (AFQ1). The most dangerous aflatoxin, B1, is found in poorly preserved agricultural products such wheat, rice, cassava, cottonseed, millet, sesame seeds, sweet corn, chilli peppers, tree nuts and sunflower seeds.According to Nielsen *et al*.(7), the mycotoxins ochratoxin A (*Aspergillus carbonarius,* and *less commonly A. niger*), and fumonisin B2 (*A. niger*) are the most dangerous substances from the perspective of both human and animal safety. Because certain mycotoxins have therapeutic applications while others are detrimental in nature, it is crucial to discover the mycotoxins that may assist us classify these fungal species as toxic or non-toxic and/or either edible or non-edible. The following reports on mycotoxin extraction techniques may be found in the literature.While Turner *et al*. (8)also discussed the analytical techniques for determining mycotoxins, Zheng *et al*. (9) provided an overview for examination of mycotoxins using quick methods and traditional analytical methods. Through the use of two-dimensional TLC, highperformance TLC (HPTLC), quantization, and preparative TLC (PLC), Betina (10)explored broad technical elements of thinlayer chromatography of mycotoxins, including extraction and clean-up techniques, adsorbents and solvent systems, detection methodologies, and detection methods. Thin-layer chromatography was used by Scott *et al*. (11)to identify mycotoxins utilising acceptable general solvent systems and just one initial spraying

reagent.Before and after the reagent was sprayed, the toxins were seen under visible or ultraviolet light. The majority of Aspergillus, Penicillium, and Fusarium species are responsible for producing these mycotoxins.To extract mycotoxins, Hassan *et al*. (12)tested with *Aspergillus* species. Similar to this, Azliza *et al*. (13)investigated 30 fungal strains representing 12 *Fusarium* species, including *F. oxysporum, F. solani, F. semitectum, F. nelsonii, F. compactum, F. equiseti, F. chlamydosporum, F. proliferatum, F. subglutinans, F. sacchari, F. lateritium*, Using TLC and HPLC methods, this complex, which was isolated from wild grasses in Peninsular Malaysia, was shown to include four main mycotoxins: moniliformin (MON), fumonisin B1 (FB1), zearalenone (ZEN), and beauvericin (BEA).

The present study was aimed to detect the mycotoxins from some fungi. For example, *Fusarium*sp, *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.

2. Materials and Methods

2.1. Isolation and Identification of Fungi

Fresh samples of different bakery food products were collected from rural areas and kept in polythene covers for further processing. Samples were inoculated on sterile medium (PDA, SDA, RBA) plates for isolation of fungi from bakery food products. After the growth of fungi, the identification was done by macroscopic and microscopic observation followed by Ellis and Ellis (14).

2.2. Mass production and Extraction of fungi

Sub culturing was done to get pure culture from different cultures. Then, mass culturing was facilitated using Potato Dextrose broth (PDB) as the appropriate growth medium. Under sterile conditions pure fungal colonies were inoculated in broth. Flasks were incubated for the desired growth of fungi. 1-2 weeks of incubation, broth was filtered using Whatman filter paper and separation was carried in two parts of the media. They include culture and liquid part. Then, extraction of fungal samples was carried out by Soxhlet method.Soxhlet fungal mycelium was extracted with chloroform and methanol in 1:1 ratio using Soxhlet extractor for 5-7 hours. Finally, processed extracts were used for detecting mycotoxins by Thin Layer Chromatography (TLC) (15)(10).

3. Results and Discussion

Nineteen (19) 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. The morphological Characterization was conducted in this study to emphasise the necessity of such fundamental identifying procedures for the fast screening of isolates in the majority of underdeveloped nations, where access to uncommon instruments is a significant challenge.Paying attention to crucial morphological characteristics enabled reliable fungal identification. Physical characteristics of isolates were observed, including colony colour, texture, and edges. The colonies frequently have floccose centres and velvety or murky surfaces. The isolates in this inquiry displayed colony morphology that was comparable to various fungus, according to Klich's review of taxonomic descriptions. Different types of these fungi were common in bread products. Culturing of different fungal species namely*Aspergillus* sp, *Penicillium* sp, *Fusarium* sp, *Mucor* sp, *Nocardia* sp, *Trichoderma* sp, *Curvularia* sp, *Bipolaris* sp, *Rhizopus* sp, *Alternaria* sp, were carried out under aseptic conditions. Sub culturing was done to get pure colonies from different fungal cultures**(Figure 1)**. After mass culture, the fungal extracts from different fungal species were used for the detection of mycotoxins by Thin Layer Chromatography.

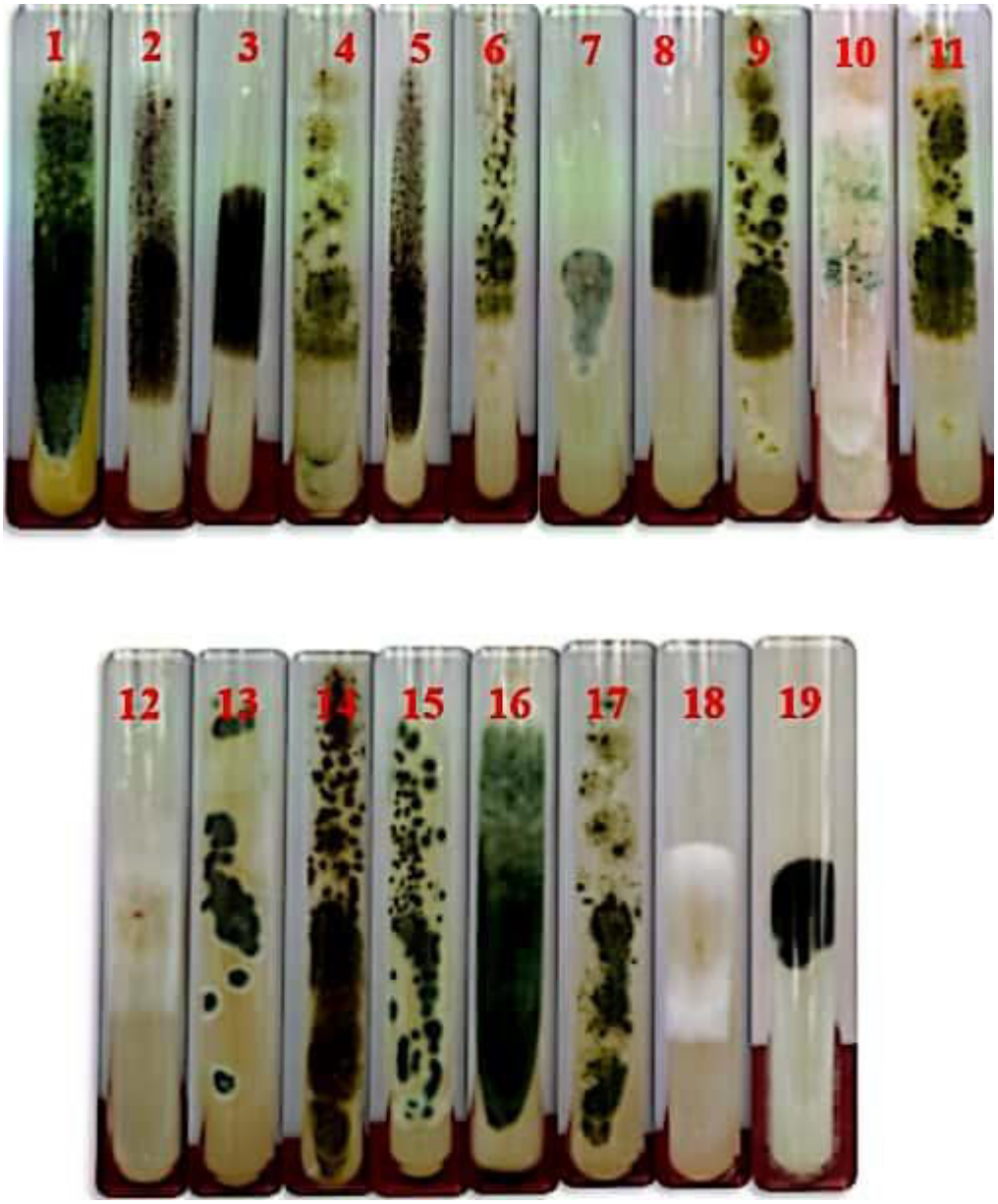

Figure 1: Pure cultures of mycotoxin producing fungi grown on Potato dextrose agar slants.

(1-*Trichoderma harzianum,* 2-*A.niger,* 3-*Biporalis,* 4- *A.oryzae,* 5-*Rhizopus,* 6-*A.flavus,* 7-*Penicillium sp,* 8-*Alternaria sp,* 9-*Mucor sp,* 10-*Curvularia sp,* 11-*A.parasiticus,* 12-*Fusarium sp,* 13-*P.chrysogenum,* 14-*Aspergillus sp,* 15-*Penicillium sp,* 16-*Trichoderma sp*, 17-*Aspergillus sp,* 18-*Fusarium sp,* 19-*unidentified).*

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 spotted on TLC plates. The standard solvent systems, consisting of toluene-ethyl acetate-90% formic acid (6:3:1; TEF), was used for the separation process. Finally, the spots were visualized under visible light and UV light(transilluminator)**(Figure 2) and (Figure 3).**

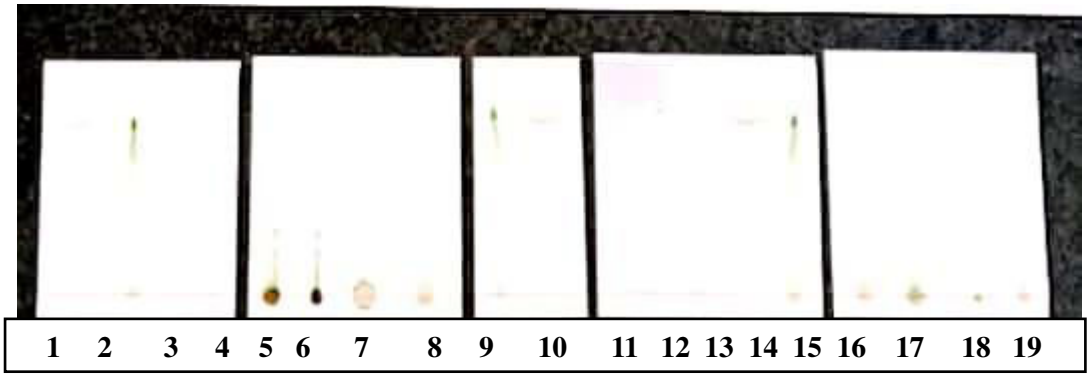

Figure 2: Illumination of mycotoxins of fungi in TLC plates under visible light

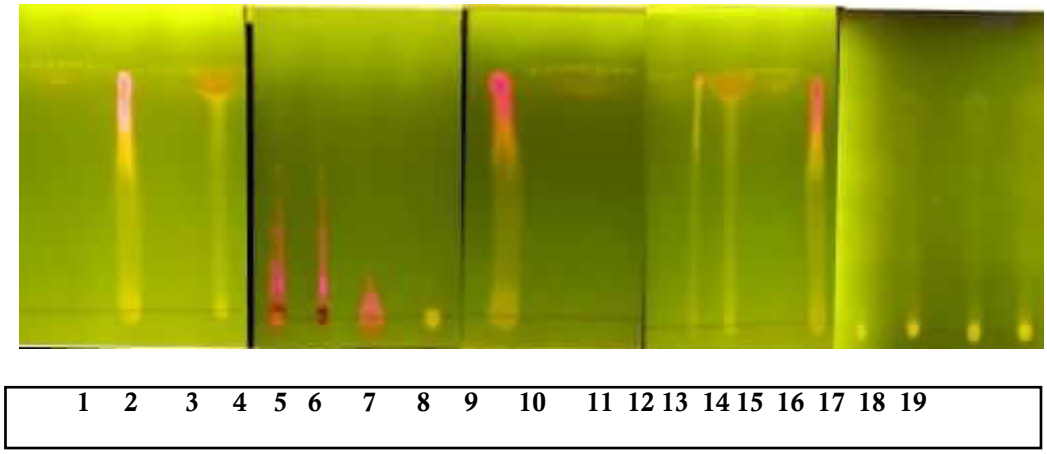

Figure 3: Illumination of mycotoxins of fungi in TLC plates under UV light

All plates were run in same solvent system, some cultures reflected colour where as some are not reflected, thus no colour was observed. *Biporalis* sp (3), *A.oryzae* (4), *Rhizopus* sp (5), *A.flavus* (6), *Penicillium* sp (7), *Mucor* sp (9), *Fusarium* sp (12), *Penicillium chrysogenum* (13) and *Penicillium* sp (15)were reflected and appeared dark pink/ reddish colour, this shows the ability of fungi in the producing toxins. Based on the colour appeared on the TLC plates, detection and tentative identification of all illuminated mycotoxins was done as per standard note and key available (11). Beauvericin (BEA) was found to be highest frequency in *Fusarium* species followed by other mycotoxins like ZEN, FB1 mycotoxins (13). Screening technique for the detection of mycotoxins used by Scott *et al*.

Conclusion

Mycotoxins are very important metabolites produced by the fungi. However, fungi have the power to destroy the bulk of baked goods. It is regrettable that bread-related fungal spoilage has gained more attention. The primary factors affecting the fungi that damage bread products, as well as their origins in bakeries, have been identified. Mould degradation continues to have a significant negative influence on the shelf life of many high and intermediate moisture bread products. The losses from mould degradation have caused the baking enterprises to lose money. Since there is a growing need for global consumption, methods to stop mould growth and extend the shelf life of baked goods are essential for the baking industry. The best options include extra efforts like keeping bakeries extremely clean and, if required, additional post-packing heat treatments or changed environment packaging.

The present study that *Trichoderma harzianum*, *A. niger*, *Rhizopus*, *A. flavus*, *Penicillium sp*, *Curvularia sp*, *A. parasiticus*, *Penicillium chrysogenum*, *Aspergillus sp*, *Fusarium sp*, *Trichoderma sp*, *Alternaria sp* and *Mucor sp* are the common genera of molds generally isolated from the bakery food products during the present investigation. A standard technique Thin Layer Chromatography was used for the screening

of mycotoxins based on the colour appearance on TLC plates by using toluene-ethyl acetate-90% formic acid (6:3:1; TEF) solvent system.The colours produced by thin layer chromatography, which were used to identify probable mycotoxins, it might help us in classifying these fungal species as toxic or not toxic, edible or not edible. The future prospect of this study is to identify the particular mycotoxins available in the target fungal species.

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