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Research Article

Microscopic Characteristics as Preliminary Identification of Some Toxin Producing Fungi from Bakery Food Samples

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Abstract

Bakery food products, including bread, pastries, and cakes, are susceptible to fungal contamination. The presence of fungi in these products can affect their quality, shelf life, and safety. The isolation of fungi from bakery food products is a crucial step in ensuring the quality and safety of these bakery products. Various isolation methods, including direct plating, dilution series, filtration, and microscopic analysis, are used to identify and characterize fungal contaminants. Understanding the significance of fungal contamination in bakery products, including spoilage, mycotoxin production, allergenic reactions, and quality control, is essential for both the food industry and consumer safety. Fungi are prevalent in the environment and commonly isolated from bakery food samples. Microscopic characteristic of some toxin producing fungi are mainly defined by their conidia bearing structure. In most cases the structure is essential for rapid identification of species in the laboratory. In the present study, some toxin producing fungi isolated from bakery food samples were microscopically identified based on the characteristics. Microscopic observation was done by light microscope and scanning electron microscope. The fungi were identified as *Aspergillus sp, Bipolaris sp, Alternaria sp, Penicillium sp*. The results showed the preliminary identification of fungi.

Keywords: *Alternaria sp; Aspergillus flavus*; Bakery food samples; *Bipolaris sp*; Light microscope; *Penicillium sp*; Scanning electron microscope

Introduction

Bakery food products are beloved by people all over the world for their delicious taste and wide variety. However, like all food products, there is a potential for contamination, which can compromise the safety and quality of these items. Certainly, bakery food refers to a wide variety of food products that are typically prepared in bakeries. These products are often baked, and they include both sweet and savory items. To mitigate the risk of contamination, bakeries should implement strict hygiene protocols, educate staff on proper food handling, maintain good sanitation practices, and regularly inspect their facilities. Many bakeries follow Hazard Analysis and Critical Control Points (HACCP) systems to identify and control potential contamination points. Fungal contaminants in bakery food products can be a concern, as they can lead to spoilage, quality deterioration, and potential health risks. Common fungal contaminants found in bakery food products includes, mold, yeast, *Aspergillus, Penicillium, Rhizopus, Fusarium* [1].

Mold is a type of fungi that can grow on the surface of various bakery items, especially bread, cakes, and pastries. It appears as fuzzy or discoloured patches. Mold can affect the appearance, taste, and texture of the product. Some molds can also produce mycotoxins, which can be harmful if ingested. While yeast is intentionally used in baking to leaven bread and other products, unwanted yeast strains or overgrowth of yeast can lead to spoilage. Yeast can cause bread to become gummy, have an off taste, or develop an unusual texture [2]. *Aspergillus* species are commonly found in the environment and can contaminate bakery products. Some *Aspergillus* species can produce mycotoxins, which may be toxic if consumed in large amounts. *Penicillium* is a genus of fungi that can be found in various bakery items, especially those with a higher moisture content. *Penicillium* can produce mycotoxins and contribute to off-flavors and spoilage. *Rhizopus* is a mold commonly found on bread and other bakery products, particularly those with high moisture levels. It can lead to spoilage and affect the appearance and texture of the products. *Fusarium* is a genus of fungi that can contaminate grains and wheat, which are primary ingredients in many bakery items. Some *Fusarium* species can produce mycotoxins that are harmful to human health [3,4].

Some *Aspergillus* species, such as *Aspergillus flavus* and *Aspergillus parasiticus*, are known mycotoxin producers. These mycotoxins, including aflatoxins, can contaminate bakery products made from grains (e.g., wheat, corn) and pose health risks when consumed. Aflatoxins are potent carcinogens and can lead to acute and chronic health issues. Contamination by *Aspergillus* species may lead to visible mold growth on the surface of bakery products. This can appear as fuzzy, powdery, or discoloured patches, depending on the species involved and can affect the texture and taste of bakery products. Bread and other baked goods may become stale, moldy, or develop an unpleasant flavor. This can render the products unpalatable and unsuitable for consumption. Airborne spores (conidia) produced by some *Aspergillus* species can act as allergens, potentially causing allergic reactions in sensitive individuals who come into contact with contaminated bakery products [5]. *Aspergillus* contamination can lead to an increase in moisture content within bakery products. This can create a conducive environment for other microorganisms to thrive, leading to further spoilage and reduced shelf life.

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Bipolaris contamination may lead to the visible growth of mold on the surface of bakery products. The appearance of *Bipolaris* mold can vary but often presents as dark, irregular patches, which may be gray, brown, or black. Bakery products affected by *Bipolaris* contamination may exhibit changes in texture and appearance. The product may become discolored, develop spots or irregularities, and lose its original texture and result in the spoilage of bakery items. Affected products may become unpalatable, with off-flavors, odors, and changes in texture, rendering them unsuitable for sale and consumption. While *Bipolaris* contamination in bakery products is more commonly associated with product quality and spoilage, certain species of *Bipolaris* may produce mycotoxins that could pose health risks if ingested in large quantities. However, this is not a common concern in the context of bakery products [6].

Alternaria species in bakery food products can have several distinct characteristics that affect the product's quality, safety, and appearance. *Alternaria* is a type of mold commonly found in the environment and is known to grow on various substrates, including baked goods. The most evident sign of *Alternaria* contamination is the growth of mold on the surface of bakery products. *Alternaria* mold typically appears as dark or black spots, sometimes with a fuzzy or powdery texture. Products contaminated with *Alternaria* may exhibit irregular discoloration in the form of dark, often brown or black, patches. This can lead to an unattractive appearance, making the products less appealing to consumers. *Alternaria* contamination can cause changes in the texture of bakery products and may develop off-flavors or off-odors. These changes in taste and smell can make the products unsuitable for consumption. Some *Alternaria* species are known to produce mycotoxins, which can pose health risks when ingested in large quantities. Mycotoxins such as alternariol and Alternariol Monomethyl Ether (AME) have been associated with certain *Alternaria* species [7]. However, mycotoxin production by *Alternaria* is generally not as common or widespread as with other molds like *Aspergillus* or *Penicillium*.

Penicillium species in bakery food products can exhibit specific characteristics that affect the quality and safety of the products. *Penicillium* is a genus of molds that can grow on a variety of substrates, including bakery items. The most apparent sign of *Penicillium* contamination is the growth of mold on the surface of bakery products. *Penicillium* molds typically appear as fuzzy or powdery patches and can be various colors, such as blue, green, or gray. Bakery products contaminated with *Penicillium* may experience changes in texture. The affected items can become softer or develop an altered texture, making them less appealing and potentially unpalatable and can lead to off-flavors or off-odors in bakery products. These changes in taste and smell can make the products unsuitable for consumption [8]. While *Penicillium* species are not typically known for producing mycotoxins, some species may produce certain mycotoxins under specific conditions. Mycotoxin production by *Penicillium* is generally less common than with other molds like *Aspergillus.*

Thus, the objective of present study was to identify several species isolated from bakery food samples using microscopic characteristics.

Materials and Methods

Isolation of Fungi from Bakery Food Samples

Bakery food samples were collected from different rural areas around Davangere district using sterile polythene covers. Isolation methods were based on the methods used by Patil and Kukade [8], Frazier *et al*. [9]; Aneja *et al*. [10], of which direct plating method, serial dilution method, spread plate methods were applied. Four different types of media used for the isolation method PDA (Potato dextrose agar medium), RBA (Rose Bengal agar medium), CZA (Czapec (dox) agar medium) and SDA (Sabouraud dextrose agar) amended with streptomycin was used for isolation purposes [11].

Identification of Fungi

For identification, species descriptions mainly by Patil and Kukade [8]; Zhang [12]; Mohamed Hashem [13]; Pundir and Jain [14], were used.

The microscopic characteristics were examined under a light microscope (Olympus) using 40X and 100X magnifications as well as Scanning Electron Microscope. Microscopic slides were prepared from cultures and observed under microscope.

Results and Discussion

Twenty-Six (26) bakery products were used for microbiological analysis according to Ogawa & Adachi [15]. During this the fungal isolates obtained from different bakery food products were identified as*, A. flavus, Penicillium* sp*, Curvularia* sp*, A. parasiticus, Fusarium* sp*, Trichoderma harzianum, A. niger, Rhizopus, Penicillium chrysogenum, Aspergillus* sp*, Bipolaris* sp*, Alternaria* sp (Figure 1).

Aspergillus is a genus of filamentous fungi that includes a wide variety of species, some of which are commonly found in the environment and can also be pathogenic to humans and animals. The microscopic characteristics of *Aspergillus* species can vary somewhat between different species, but there are some common features that can help in their identification. *Aspergillus* was characterized by the conidiophores, conidia, conidiogenous cells, septation, colour, sporulation pattern. *Bipolaris* is a genus of filamentous fungi within the family Pleosporaceae. These fungi are known for causing various plant diseases, and they have distinct microscopic characteristics that can aid in their identification. The microscopic features, especially the two-celled, bipolar conidia, are key identifiers for *Bipolaris* species. However, accurate identification of a specific *Bipolaris* species may require additional characteristics and molecular techniques such as DNA sequencing to distinguish closely related species within the genus. *Alternaria* is a genus of filamentous fungi commonly found in soil, plants, and indoor environments. These fungi are known for causing plant diseases and are associated with various allergies and respiratory issues in humans. It's important to note that *Alternaria* is a large and diverse genus, and accurate species identification often requires a combination of morphological characteristics, growth conditions, and molecular techniques, such as DNA sequencing. *Penicillium* is a diverse genus of filamentous fungi known for its wide distribution in the environment and its significance in various applications, including the production of antibiotics (penicillin) and various food products. Additionally, different *Penicillium* species may have different growth requirements and characteristics, and some are associated with food spoilage and mycotoxin production [16-18].

Aspergillus Flavus: The distinctive characteristics of the isolates that belonged to *Aspergillus* species were the production of thick-walled rough conidiophores, the vesicle shape was globose to sub globose. *Aspergillus flavus* produced uniseriate phialides and the conidial head was hyaline, coarsely roughened. Vesicles are elongated when young, later becoming sub globose or globose. Based on these

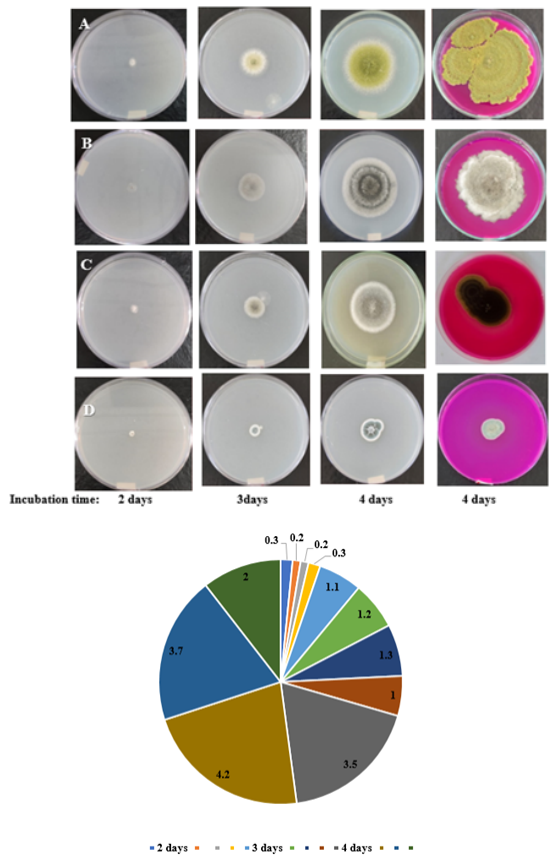

Figure 1: Macroscopic morphology of A) *A.flavus, B) Bipolaris sp, C) Alternaria sp and D) Penicillium sp* on PDA (Potato Dextrose Agar) medium plates and RBA (Rose Bengal Agar) medium plates with different length of incubation and its graphical representation.

characteristics, one member of *Aspergillus* species was identified as *Aspergillus flavus*. The microscopic characteristics of *A.flavus* observed in this study fit the descriptions by Rodrigues *et al*. [19]. The arrangement of conidia was smooth walled when observed under light microscope (Figure 2A) but the observations under SEM showed a finely roughened texture (Figure 2B, 2C, 2D).

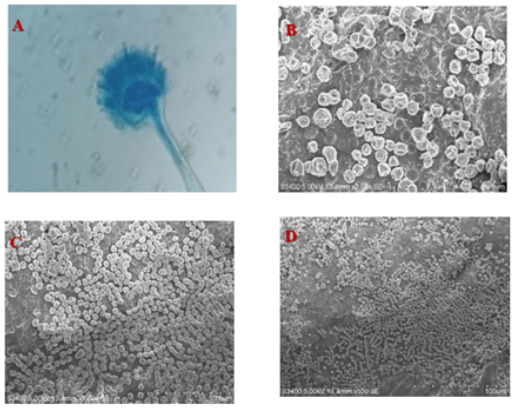

Figure 2: Microscopic characteristics of conidial head conidia of *A.flavus*. **(A)** Globose vesicle (40X). **(B)** Rough walled, sub globose conidia observed using SEM scale bar 20µm. **(C)** Rough walled, sub globose conidia observed using SEM scale bar 50µm. **(D)** Rough walled, sub globose conidia observed using SEM scale bar 100µm.

***Bipolaris* sp:** *Bipolaris* is a genus of filamentous fungi known for its plant pathogenic species. While many species of *Bipolaris* share common characteristics, there can be distinctions that help identify them. One of the most distinctive features of *Bipolaris* species is the conidia, which are usually two-celled ("bipolar"). The conidia have a central transverse septum, creating two distinct cells, often referred to as "beak cells" and "body cells." This bipolar conidium is a hallmark feature of the genus. *Bipolaris* conidia are typically showed brown to dark brown in colour. The specific shade can vary, but the dark pigmentation is a distinguishing characteristic for identification. The conidia are usually elongated or cylindrical, with the beak cell being more slender than the body cell. The beak cell typically tapers toward the tip [20,21] (Figure 3A).

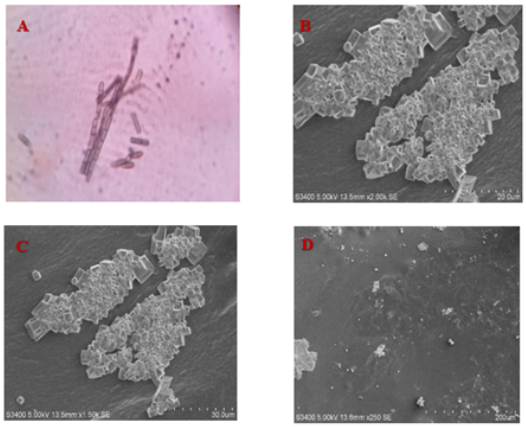

Figure 3: Microscopic characteristics of conidial head conidia of *Bipolaris sp*. **(A)** *Bipolaris sp* under light microscope (40X). **(B)** Rough walled and crystal-like structure observed using SEM scale bar 20µm. **(C)** Rough walled and crystal-like structure observed using SEM scale bar 30µm. **(D)** Rough walled and crystal-like structure observed using SEM scale bar 200µm.

Bipolaris conidiophores are simple or branched, and they usually arise directly from the substrate they may have dark pigmentation and provide support for the conidia. Some *Bipolaris* species may produce sclerotia, compact and darkened structures. It is important to note that *Bipolaris* is a diverse genus, and accurate species identification often requires a combination of morphological characteristics, knowledge of the host, and molecular techniques, such as DNA sequencing, to differentiate closely related species, but the observations under SEM showed a finely roughened and crystal-like texture (Figure 3B, 3C, 3D).

***Alternaria* sp:** Microscopic observations of *Alternaria* sp isolates was shown (Figure 4A). Conidiophores appeared pale brown, simple and branched, bearing conidia at the apex and apical fertile parts. Conidia looks like dark brown, cylindrical shaped and having longitudinal walls. Under SEM observations, Conidiophores are short, straight or flexures, and germinated in single or bushy heads (Figure 4B, 4C, 4D) exhibits the olive-brown ellipsoidal conidia with typically vertical and transverse septa. Conidia were catenated. *Alternaria* was identified in the present study in accordance with existing descriptions of the fungi [22,23].

Alternaria is a genus of fungi that are known for their saprophytic nature in decomposing soil and plants. *Alternaria* **sp** on PDA plates grows as dark brown or black colonies with a velvety texture. *Alternaria* sp on PDA plates grows well at a temperature range of 20-30°C. The colonies are usually flat, but can also be raised or

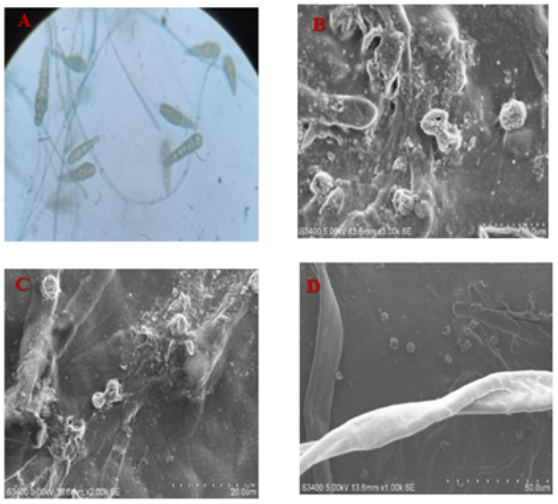

Figure 4: Microscopic characteristics of conidial head conidia of *Alternaria sp.* **(A)** *Alternaria sp* under light microscope (40X). **(B)** Hyphae and conidia observed using SEM scale bar 10µm. **(C)** Hyphae and conidia observed using SEM scale bar 20µm **(D)** Hyphae and conidia observed using SEM scale bar 50µm.

folded. Conidiophores are septate and brown in color, occasionally producing a zigzag appearance. Conidia produced by *Alternaria* species are typically one-celled and vary in shape and size. They are often elongated and can be oblong, elliptical, or spindle-shaped. Conidia can also be quite variable in size, typically ranging from 10 to 150µm in length. The color of the conidia may vary, but it is often dark brown to black. The distinctive shape and dark coloration make Alternaria conidia easily recognizable. *Alternaria* conidia are typically septate, with one or more transverse septa. These septa divide the conidia into individual cells. The number and arrangement of septa can be used as an identifying characteristic. The hyphae (filamentous structures) of *Alternaria* species can vary in thickness and branching patterns. They are often septate, and the arrangement of septa can be used for identification [24].

***Penicillium* sp:** *Penicillium* sp was identified based on the colony morphology, produced conidiophores, having septate hyphae. The conidiophores are simple and branched. Brush like clusters were observed at the end of the conidiophores. Conidia looks like round to ovoid (Figure 5A).

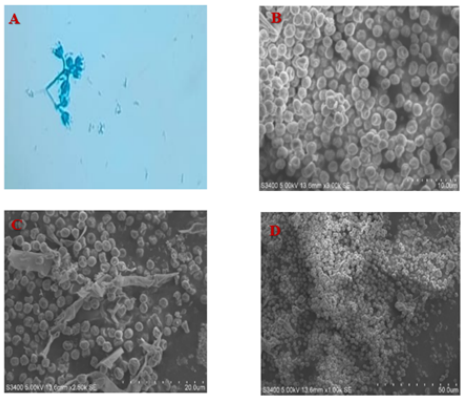

Figure 5: Microscopic characteristics of conidial head conidia of *Penicillium sp.* **(A)** *Penicillium sp* under light microscope (40X). **(B)** Hyphae and conidia observed using SEM scale bar 10µm. **(C)** Hyphae and conidia observed using SEM scale bar 20µm **(D)** Hyphae and conidia observed using SEM scale bar 50µm.

Penicillium is a diverse genus of filamentous fungi with numerous species, and identifying specific species within this genus can be challenging due to their similarities. When grown on appropriate culture media, *Penicillium* colonies often have distinct characteristics. These can include colors ranging from blue-green, green, or yellow, with various textures such as powdery, velvety, or granular, the rate of colony growth, which can vary among different species. Some *Penicillium* species produce distinctive conidial heads or structures that can be used for identification. *Penicillium* species produce conidiophores, which are structures that bear conidia (asexual spores) [25]. These conidiophores can be simple or branched and vary in size and shape. Conidia are the asexual spores of *Penicillium*. They are typically one-celled, and their shape and size can vary among species. They are often produced in chains or heads at the tips of conidiophores. Both conidia and hyphae in *Penicillium* species are typically septate (Figure 5B, 5C, 5D). Some *Penicillium* species are known for their ability to grow on specific substrates or in certain environments, such as indoor environments or on decaying organic matter. Few *Penicillium* species are known to produce mycotoxins, which can be relevant in food safety and health considerations.

Conclusion

Microscopic characteristics are used as a preliminary identification method for some toxin-producing fungi in bakery food samples. The presence of fungi in bakery products can affect their quality, shelf life, and safety. Various isolation methods, including direct plating, dilution series, filtration, and microscopic analysis, are used to identify and characterize fungal contaminants. The fungi identified in this study include *Aspergillus* sp, *Bipolaris* sp, *Alternaria* sp, and *Penicillium* sp using light microscope and scanning electron microscope and compared their features with existing descriptions in the literature and discussing the significance of fungal contamination in bakery food products such as spoilage, mycotoxin production, allergic reactions. Understanding the significance of fungal contamination in bakery products is crucial for ensuring food safety and quality control.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Data Availability

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

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