



(RESEARCH ARTICLE)



ISOLATION, IDENTIFICATION, AND CHARACTERIZATION OF FUNGAL ORGANISMS ASSOCIATED WITH CORN SPOILAGE

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ABSTRACT

Maize (*Zea mays*) is a major staple crop in Nigeria, widely used for human consumption, livestock feed, and industrial purposes. This study was conducted to isolate, identify, and characterize fungal organisms associated with maize spoilage. A total of twenty (20) maize samples, comprising ten yellow maize and ten white maize samples, were purchased from Elele Market, Rivers State, Nigeria. The samples were stored under ambient conditions to promote fungal growth before microbiological analysis was carried out. Fungal isolation was performed using Potato Dextrose Agar (PDA), while identification was based on cultural, morphological, and microscopic characteristics using lactophenol cotton blue staining. The fungal load varied among the maize samples, with yellow maize generally showing higher fungal counts than white maize. A total of four fungal genera were isolated and identified: *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp., and *Mucor* spp. Percentage occurrence was used to determine the distribution of the fungal isolates. *Aspergillus niger* (28.89%) recorded the highest percentage occurrence, followed by *Aspergillus flavus* (24.07%), *Penicillium* (19.26%), while *Mucor* spp. (12.59%) had the lowest occurrence. The predominance of *Aspergillus* species indicates that maize sold in the study area was highly susceptible to fungal contamination, which may reduce grain quality and pose potential health risks due to possible mycotoxin production. The study concludes that maize sold in Elele Market can easily be contaminated with spoilage fungi and recommends proper drying, hygienic handling, suitable storage conditions, and regular microbiological monitoring to minimize fungal contamination and ensure the safety and quality of maize for human consumption.

Keywords: Maize, Fungal contamination, Spoilage, Fungal load, *Aspergillus*, *Penicillium*, *Rhizopus*, *Mucor*

1 INTRODUCTION

Freshly harvested corn on the cob is one of the foods most closely associated with the summer season (Odebunmi *et al.*, 2017). Corn grows in structures known as *ears*, each consisting of rows of kernels protected by silk-like threads called corn silk and enclosed within a husk (Mbanaso *et al.*, 2018). Scientifically, corn is known as *Zea mays* as named by Carl Linnaeus (1753). This name reflects its traditional designation as maize, a crop widely cultivated and consumed by Native Americans and many other cultures across the world. Although corn is commonly associated with the yellow

variety, it exists in several other forms with diverse colours, including red, pink, black, purple, and blue (Shutt *et al.*, 2022). While corn is presently available in markets throughout the year, locally grown varieties obtained during the summer months are often fresher, more flavorful, and relatively inexpensive (Odebunmi *et al.*, 2017).

Maize (*Zea mays*), commonly known as corn, is one of the most widely cultivated cereal crops in the world (Oladeji *et al.*, 2022). It is a member of the Poaceae family and serves as a staple food, livestock feed, and industrial raw material (Oluwajobi *et al.*, 2022). Originating from Central America, maize was domesticated by indigenous people, thousands of years ago and has since spread globally due to its adaptability to diverse climatic and soil conditions (Ajayi *et al.*, 2013; Oladele *et al.*, 2015). Today, it is cultivated in almost every continent and is a major source of calories and nutrients for millions of people, particularly in Africa, Latin America, and parts of Asia (Obadina *et al.*, 2015).

Maize is valued for its high carbohydrate content, moderate protein levels, and essential vitamins and minerals, making it a key component of human diets (Oladipo *et al.*, 2022). In addition, it is extensively used in the production of animal feed, biofuels, starch-based products, and processed foods (Odebunmi *et al.*, 2017). Its versatility in both food and industrial applications has contributed to its economic importance worldwide.

The physical structure of maize consists of kernels arranged on an *ear*, protected by a husk and silk threads (Coronell-Tovar, 2019). Kernels can vary in color, including yellow, white, red, blue, black, and purple, depending on the variety (Coronell-Tovar, 2019). This diversity in kernel color and composition is important not only for nutritional value but also for resistance to pests and diseases (Oladele *et al.*, 2015).

Despite its global importance, maize is highly susceptible to microbial contamination, particularly by fungi (Rao *et al.*, 2016). Fungal infection can occur at various stages, including during cultivation, harvesting, processing, and storage. Such contamination may lead to spoilage, nutrient loss, reduced market value, and production of toxic metabolites known as mycotoxins (Melese & Negussie, 2015). Common fungal genera associated with maize include *Fusarium*, *Aspergillus*, and *Penicillium*, all of which pose health risks to humans and animals if consumed (Obadina *et al.*, 2015).

Environmental conditions such as humidity, temperature, drought, and insect infestation, along with handling practices, strongly influence the growth of spoilage fungi on maize (Mairami *et al.*, 2018; Ugwuwananyi *et al.*, 2014). Effective management of these factors through proper harvesting, drying, storage, and monitoring is essential to maintain the quality, safety, and economic value of maize. (Obadina *et al.*, 2015).

Overall, maize remains an essential crop globally due to its nutritional, economic, and industrial significance. Understanding the factors that affect its quality, particularly microbial spoilage, is crucial for improving food security, reducing post-harvest losses, and protecting human and animal health.

Since the early stages of human civilization and the domestication of crops, food spoilage fungi have posed serious challenges to food preservation. The presence of moulds appearing as fuzzy, powdery, or slimy growths in colours such as white, black, green, orange, red, and brown are visible signs of microbial invasion. These organisms are capable of fermenting, acidifying, discoloring, and decomposing food materials, thereby rendering them unpalatable or unsafe for consumption (Mbanaso *et al.*, 2018). More so, Pitt and Hocking (2020), emphasized the potential dangers associated with common spoilage fungi. Since the 1960s, an increasing number of pathogenic and toxigenic fungi have been identified, assuming that “it is only a mould” is unacceptable to microbiologists, health inspectors, and consumers alike. Consequently, the need for accurate identification and proper characterization of food spoilage fungi has become increasingly important. This research work therefore focuses on corn (*Zea mays*) and the fungi associated with its spoilage.

The increasing global concern about food safety has heightened interest in fungal contamination and its effects on agricultural products. Fungal infection in food crops causes deterioration of quality, reduction in nutritional value, and the production of toxic secondary metabolites known as mycotoxins, which can seriously affect human and animal health. Corn (*Zea mays*), being one of the most important dietary staple foods in the world, has attracted particular attention due to its widespread consumption and economic importance (Okpala *et al.*, 2020).

Corn is a major raw material used in both food and feed production. However, it is highly susceptible to contamination by fungi and the subsequent accumulation of mycotoxins, which can result in significant nutrient loss, reduction in palatability, and potential health hazards for animals and humans (Mbanaso *et al.*, 2018). This contamination can occur at various stages, from cultivation to storage, and may severely impact crop yield and economic returns. Factors such as drought, high humidity, temperature fluctuations, insect infestation, and rough handling during harvesting and transportation have all been identified as contributing to the presence of fungi and their toxins in agricultural commodities like cereals (Adebayo *et al.*, 2017).

Several genera of fungi are commonly associated with corn during both pre and post-harvest periods (Mubarak *et al.*, 2021). Among these, *Fusarium*, *Penicillium*, and *Aspergillus* are notable for their ability to produce important mycotoxins (Mubarak *et al.*, 2021). Fungal spoilage not only reduces the nutritional content and palatability of corn but also increases its allergenic potential and the risk of mycotoxin contamination (Scudamore *et al.*, 2018). Other fungal species that are frequently isolated from corn include *Mucor*, *Epicoccum*, *Cladosporium*, and a variety of yeast species, all of which may contribute to spoilage under suitable environmental conditions (Morrison *et al.*, 2019). Some species, such as *Aspergillus fumigatus*, have been specifically linked to mycotic infections in cattle, highlighting the broader implications of fungal contamination in both human and animal health (Adebayo *et al.*, 2017).

Mycotoxigenic fungi can be classified as either field or storage organisms. *Fusarium* species, for example, are primarily field pathogens or endophytes, while *Alternaria* species are generally epiphytic saprophytes or weak pathogens (Bilgrami *et al.*, 2018). In contrast, *Aspergillus* and *Penicillium* are often considered post-harvest spoilage fungi; however, under favorable conditions such as hot, dry weather and insect damage, members of these genera may also be present in the field and contribute to toxin contamination (Pitt *et al.*, 2015; Marsh *et al.*, 2020).

The development of strategies to reduce toxin contamination in corn depends on accurate identification and characterization of the fungi present. Isolation of these organisms typically relies on selective culture media, which promote the growth of target fungi while inhibiting unwanted organisms. Nonetheless, this approach has limitations, as fastidious species may fail to grow, slow-growing fungi may be masked by faster-growing species, and antagonistic interactions among fungi in culture can make it difficult to recover sensitive organisms (Adebayo *et al.*, 2017). A wide variety of culture media are used to optimize fungal growth and observe characteristic colony morphology, pigmentation, and sporulation patterns, all of which are important for accurate identification (Oladele *et al.*, 2015).

Understanding the diversity, behavior, and toxigenic potential of fungi associated with corn spoilage is essential for developing effective control measures. The study of these fungi not only informs proper storage and handling practices but also contributes to safeguarding the nutritional quality, safety, and economic value of corn for both human and animal consumption.

1.1 Study area

The study was conducted in Elele, Ikwerre Local Government area of Rivers State, Nigeria. Elele is located in the south-south geopolitical zone of Nigeria and lies approximately at latitude 5.10°N and longitude 6.81°E. The town is strategically situated along the East-West Road, serving as an important commercial and agricultural centre within the region. Elele usually experience a humid tropical rainforest climate, characterized by high rainfall, high relative humidity, and warm temperatures throughout most of the year. These environmental conditions favour the growth, survival, and proliferation of fungal organisms associated with food spoilage. The inhabitants of Elele are predominantly engaged in farming, trading, and other commercial activities with agricultural produce such as maize, cassava, yam, vegetables, and fruits forming a major part of the local economy. The maize samples used in this study were obtained from Elele market, one of the major markets where cereals and other food commodities are sold and distributed.

The laboratory analyses, including the isolation, identification, and characterization of fungal isolates, were carried out at the Microbiology Laboratory, Department of Microbiology, Madonna University, Elele, Rivers State, Nigeria.

1.2 Source of Raw Material

The raw corn (*zea mays*) samples used for this research were purchased from Elele Market in Rivers State, Nigeria.

1.3 Storage Condition

The sample were stored according to the method by Oladipo *et al.* (2022). in a cool dry place inside an empty room at ambient temperature ($28 \pm 2^\circ\text{C}$) by leaving it in a black polythene bag to undergo spoilage

1.4 Sample Preparation

The samples were purchased from Elele and transported to the laboratory in a sterile black nylon bag. The samples were stored at room temperature ($28 \pm 2^\circ\text{C}$) and observed daily for fungal growth. Visible fungal growth began to appear between the 3rd and the 5th day of storage. The spoiled maize samples showing fungal growth were then used for subsequent isolation, culturing, and identification of the associated fungi.

1.5 Microbiological Analysis of Maize Samples

The total viable count of the flour samples was determined using the pour plate technique as described by Giwa *et al.*, (2012). A sterile scalpel was used to aseptically cut the unspoiled portion of each corn kernel. The cut portion was wrapped in sterile aluminium foil to avoid contamination. 25g of the sample was weighed using an analytical balance.

The weighed sample was transferred into a sterile mortar and macerated into a fine paste. The macerated corn was then placed in a sterile beaker and covered with sterile aluminium foil. 500ml of sterile distilled water was measured with a measuring cylinder and added to the beaker. The mixture was stirred thoroughly with a sterile glass rod until a uniform suspension was formed (Oluwajobi *et al.*, 2022).

Briefly, 10g of each corn sample was homogenized in 9ml of sterile peptone water in a sterile 500ml gas jar cylinder. The solution was agitated vigorously for a few minutes to 10^{-2} dilution and then allowed to settle. A ten-fold dilution series was performed to obtain a colony count of 10^{-5} (CFU/mL). A fresh sterile pipette tip was used for each transfer to prevent cross-contamination. Furthermore, one ml of the diluted sample was poured into an empty sterile 9ml Petri dish. Potato dextrose agar (PDA) was poured into the prepared plates for fungi counts, incubated at for 72 hours (Spencer and Ragout de Spencer, 2001). All plates were prepared in duplicate. Colonies were counted and recorded after incubation period. The different colonies on each plate were isolated, purified and stored on Potato Dextrose Agar slants (for fungi) for further characterization and identifications.

1.6 Characterization and Identification of isolated bacteria and fungi

Purified selected fungal isolates were identified using their cultural, morphological and microscopic characteristics as described by Oluwajobi *et al.*, (2022).

1.7 Statistical Analyses

The data obtained was analysed using descriptive statistics. The frequency of occurrence of each fungal isolate was determined, and the percentage of occurrence was calculated using the formula: Percentage occurrence (%) = (Number of occurrence of fungal isolate / Total number of fungal isolates) x 100 as described by (Samson *et al.*, 2020).

2 RESULTS AND DISCUSSION

Table 1 shows the cultural and morphological characteristics of the fungal species isolated from both yellow and white maize samples. Based on the colonial characteristics observed on the culture medium, five probable fungal isolates were identified, namely, *Aspergillus niger*, *Aspergillus Flavus*, *Penicillium spp.*, *Rhizopus spp.*, and *Mucor spp.* The isolates exhibited distinct colony characteristics, including variations in colour, texture, elevation, margin, and growth pattern, which aided their identification.

Table 1: Morphology of fungal isolates from cassava flour and enriched product

Macroscopy	Microscopy	Parable organism
White powdery surface, bluish-green with white border. Reverse is white and non-crack	Separate hyphane with branched conidiospores that have secondary branches known as metulae	<i>Penicillium spp.</i>
Brown/black powdery surface with a velvet whitish surrounding, the reverse is cream and crack colony	Non-septate Non—branched sporangiophores no stalons and rhizoids	<i>Mucor spp.</i>
Irregular, at first white, then turn to yellow and finally to green	Branched mycelium, condiophores are enlarged at the tip forming a rounded vesicle.	<i>Aspergillus spp.</i>
White colony at first, becoming grey to black unto age. Reverse: White pale to grey	Colony is circular, fluffy, with abundant and abundant serial growth and a smooth edge.	<i>Rhizopus spp.</i>

Table 2 shows the total fungal colony count of isolates recovered from the yellow maize samples. *Aspergillus niger* recorded the highest fungal load (7.5×10^{-6}) followed by *Aspergillus Flavus* (6.5×10^{-6}), *Penicillium spp.* (5.2×10^{-6}), *Rhizopus spp.* (4.1×10^{-6}), and *Mucor spp.* (3.4×10^{-6}), which had lowest fungal load. This result indicates that *Aspergillus spp.* was the predominant fungal contaminants of the yellow maize samples analyzed.

Table 2: Total Fungal Colony Count of Yellow Maize

Organism	Dilution Factor	No. Of Colonies	Volume (ml)	CFU/g
<i>Aspergillus niger</i>	10 ⁻⁵	78	1	7.8 x 10 ⁻⁶
<i>Aspergillus flavus</i>	10 ⁻⁵	65	1	6.5 x 10 ⁻⁶
<i>Penicillium spp.</i>	10 ⁻⁵	52	1	5.2 x 10 ⁻⁶
<i>Rhizopus spp.</i>	10 ⁻⁵	41	1	4.1 x 10 ⁻⁶
<i>Mucor spp.</i>	10 ⁻⁵	34	1	3.4 x 10 ⁻⁶

Table 3 presents the percentage occurrence of the fungal isolates recovered from the yellow maize samples. *Aspergillus niger* recorded the highest percentage occurrence (78%), followed by *Aspergillus Flavus* (65%), *Penicillium spp.* (52%), *Rhizopus spp.* (41%), and *Mucor spp.* (34%), which had lowest percentage occurrence. This indicates that *Aspergillus* spp. was the most dominant fungal contaminants of the yellow maize samples analyzed.

Table 3: Percentage Occurrence of Fungal Isolates from Yellow Maize Samples.

Fungal isolates	No. Of Colonies	Percentage Occurrence (%)
<i>Aspergillus niger</i>	78	28.89
<i>Aspergillus flavus</i>	65	24.07
<i>Penicillium spp.</i>	52	19.26
<i>Rhizopus spp.</i>	41	15.19
<i>Mucor spp.</i>	34	12.59

Table 4. shows the fungal load (CFU/g) of fungal isolates recovered from the yellow and white maize samples. Five fungal species were isolated from the yellow maize samples, whereas only three fungal species were recovered from the white maize samples. Generally, the yellow maize samples exhibited greater fungal diversity than the white maize samples.

Table 4: Total Fungal Colony Count of White Maize

Organism	Dilution Factor	No. of Colonies	Volume (ml)	CFU/g
<i>Aspergillus niger</i>	10 ⁻⁵	60	1	6.0x 10 ⁻⁶
<i>Rhizopus spp.</i>	10 ⁻⁵	55	1	5.5 x 10 ⁻⁶
<i>Mucor spp</i>	10 ⁻⁵	40	1	4.0x 10 ⁻⁶

Table 5: Percentage Occurrence of Fungal Isolates from White Maize Samples.

Fungal isolates	No. Of Colonies	Percentage occurrence (%)
<i>Aspergillus niger</i>	60	38.71
<i>Rhizopus spp.</i>	55	35.48
<i>Mucor Spp.</i>	40	25.80

Table 6: Comparison of Fungal Loads (CFU/g) of Fungal Isolates Recovered from Yellow and White Maize Samples

Fungal Isolate	Yellow Maize (colony count)	White Maize (colony count)
<i>Aspergillus niger</i>	7.8×10^{-6}	6.0×10^{-6}
<i>Aspergillus Flavus</i>	6.5×10^{-6}	-
<i>Penicillium spp.</i>	5.2×10^{-6}	-
<i>Rhizopus spp.</i>	4.1×10^{-6}	5.5×10^{-6}
<i>Mucor Spp.</i>	3.4×10^{-6}	4.0×10^{-6}

3 DISCUSSION

The present study investigated the fungal species associated with the spoilage of yellow and white maize, using cultural, morphological and microscopic characteristics. The findings revealed that the maize samples were contaminated with several fungal species, such as; *Aspergillus niger*, *Aspergillus flavus*, *Penicillium spp.*, *Rhizopus spp.* and *Mucor spp.* This confirms that maize is highly susceptible to fungal contamination during storage, especially under conditions of increased moisture and favorable temperature. These findings are consistent with those of Okpala et al. (2020), who reported that maize serves as a suitable substrate for the growth of spoilage fungi because of its rich nutrient composition.

Among the yellow maize samples, *Aspergillus niger* recorded the highest percentage occurrence (28.89%), followed by *Aspergillus flavus* (24.07%), *Penicillium spp.* (19.26%), *Rhizopus spp.* (15.19%) and *Mucor spp.* (12.59%). Similarly, *Aspergillus niger* showed the highest percentage occurrence (38.71%) among the white maize samples, followed by *Rhizopus spp.* (35.48%) and *Mucor spp.* (25.80%). The predominance of *Aspergillus* species observed in this study agrees with the findings of Mubarak et al. (2021), who reported that species of *Aspergillus* are among the most frequently isolated fungi from maize and other cereal grains. Their ability to grow under relatively low moisture conditions and warm storage environments contributes to their widespread occurrence in stored agricultural products.

The isolation of *Aspergillus flavus* from the yellow maize samples is of particular concern because this species is well known for producing aflatoxins, which pose serious health risks to humans and animals. This observation supports the report of Mbanaso et al. (2018), that noted that fungal contamination of maize is often associated with mycotoxin production, resulting in deterioration of grain quality, nutrient loss and potential public health hazards. The presence of *A. flavus* therefore highlights the importance of proper drying and storage of maize after harvest.

Aspergillus flavus was not isolated from the white samples, although it was recovered from the yellow maize samples. This difference may be due to variations in the physicochemical characteristics of the yellow maize and white maize varieties, which can influence the ability of fungal species to colonize the grains (Mubarak et al., 2021). The absence of *Aspergillus flavus* in the white maize samples is noteworthy because these species are well-known producer of aflatoxins that pose significant health risks to humans and animals (Mbanaso et al., 2018).

The occurrence of *Penicillium spp.* in the yellow maize samples also agrees with the reports of Pitt et al. (2015) and Marsh et al. (2020), who classified *Penicillium* as one of the major post-harvest fungi commonly associated with stored cereals. The relatively lower occurrence of *Penicillium spp.* compared with *Aspergillus spp.* in this study may be related to differences in environmental conditions and the competitive ability of the fungi during storage.

The absence of *penicillium spp.* in the white maize samples may be attributed to differences in the composition of the maize kernels, moisture availability, or other environmental conditions that influenced fungal spoilage. Studies have shown that the occurrence of fungal species on maize is influenced by factors such as moisture content, temperature, nutrient availability, and competition among fungi (Pitt & Hocking, 2020; Magan & Aldred, 2021). These factors may have limited the growth of *penicillium spp.* in the white maize samples.

The isolation of *Rhizopus spp.* and *Mucor spp.* from both maize types further demonstrates the diversity of spoilage fungi associated with stored maize. Morrison et al. (2019) similarly reported that members of the genera *Rhizopus* and *Mucor* are frequently recovered from cereals exposed to favourable environmental conditions. Although these fungi are generally regarded as secondary spoilage organisms, their rapid growth contributes to visible deterioration, discoloration and loss of market quality of stored maize.

The fungal colony counts obtained in this study indicate a considerable level of fungal contamination in both yellow and white maize samples. This finding supports the reports of Adebayo et al. (2017), who stated that high humidity, temperature fluctuations, insect infestation, poor handling and inadequate storage practices significantly increase fungal colonization of cereals. Since the maize samples used in this study were allowed to remain under conditions favourable for fungal growth, the observed contamination was expected. The cultural and microscopic characteristics observed during the study were useful in the identification of the fungal isolates. Colony colour, texture, growth pattern and microscopic structures such as septate and non-septate hyphae, conidiophores, vesicles, phialides, sporangia and rhizoids corresponded with standard identification characteristics described by Pitt and Hocking (2020) and Samson et al. (2019). This confirms the reliability of conventional morphological methods for the identification of common fungi associated with maize spoilage.

Finally, the results obtained from this research, along with some studies affirm that spoiled maize can harbor diverse fungal species capable of reducing grain quality and posing significant health risks through the possible production of mycotoxins. The predominance of *Aspergillus* species underscores the need for proper post-harvest handling, adequate drying and appropriate storage conditions to minimize fungal contamination and ensure the safety and quality of maize intended for human consumption and animal feed.

4 CONCLUSION

This study successfully isolated, identified and characterized the fungal organisms associated with the spoilage of yellow and white maize samples. The fungal isolates identified includes; *Aspergillus niger*, *Aspergillus flavus*, *Penicillium* spp., *Rhizopus* spp. and *Mucor* spp. The predominance of *Aspergillus* species in both maize types indicates that these fungi are the major contaminants of stored maize under favourable environmental conditions, the percentage occurrence of the fungal isolates showed that *Aspergillus niger* was the most frequently isolated fungus in both yellow and white maize samples, while *Mucor* spp. was the least isolated among the yellow maize samples. The presence of *Aspergillus flavus* in particular, is a red flag, because of its ability to produce aflatoxins, which may pose serious health risks to consumers if contaminated maize is consumed. The findings of this study confirm that maize is highly susceptible to fungal contamination during storage, especially when exposed to high moisture content, poor handling practices and unsuitable storage conditions. Therefore, proper drying, handling and storage of maize are essential in reducing fungal contamination, maintaining grain quality and protecting public health.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

Authors have declared that no competing interest exist.

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