



Study Of Fungal Contamination In Fruits And Vegetables

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ABSTRACT

Fungal contamination in fruits and vegetables represents a significant challenge to food safety, economic stability, and public health. The present study investigates the prevalence and diversity of fungi in rotten fruits and vegetables viz., Garlic (*Allium sativum*), Onion (*Allium cepa*), Cauliflower (*Brassica oleracea*), Papaya (*Carica papaya*), Tomato (*Solanum lycopersicum*), Ridge gourd (*Luffa acutangula*), Capsicum (*Capsicum annuum*), Beans (*Phaseolus*), Turnip (*Brassica oleracea gongylodes*), Carrot (*Daucus carota*), Ginger (*Zingiber officinale*), Potato (*Solanum tuberosum*), Chilli (*Capsicum frutescens*), Sapota (*Manilkara zapota*), Cucumber (*Cucumis sativus*). A comprehensive analysis was conducted using samples collected from local markets. Samples were prepared by ten serial dilutions and inoculated on potato dextrose agar media and incubated at room temperature for 4-5 days. Through microscopic examination using lactophenol cotton blue method and colony characteristics, the predominant fungal species were identified. *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus parasiticus*, *Corynespora* sp., *Alternaria* sp., *Fusarium* sp., *Mucor* sp., *Rhizopus microsporus* and *Rhizopus stolonifer* were among the most frequently isolated genera from the samples. The findings highlight the critical need for improved handling and storage protocols to mitigate fungal contamination and its associated risks. The present study provides valuable insights into the fungal ecology of decaying fruits and vegetables, contributing to the development of effective strategies for managing post-harvest fungal contamination.

KEYWORDS: Fungal Contamination, Fruits and Vegetables, Lactophenol cotton blue method, Colony characteristics, Fungal Ecology

INTRODUCTION

Fungi are unicellular or multi-cellular eukaryotic organisms that exist in all environments worldwide. From fungi visible to the naked eye, such as mushrooms, to microscopic yeasts and molds, they exist in a multitude of forms. Fungal diseases kill more than 1.5 million and affect over a billion people. However, they are still a neglected topic by public health authorities even though most deaths from fungal diseases are avoidable. Serious fungal infections occur as a consequence of other health problems including asthma, AIDS, cancer, organ transplantation and corticosteroid therapies. Although most fungi are harmless to humans, some of them are capable of causing diseases under specific conditions. Fungi reproduce by releasing spores that can be picked up by direct contact or even inhaled. Fungal infections are most likely to affect the skin, nails, or lungs. Fungi can also penetrate the skin, affect your organs, and cause a body-wide systemic infection. Fungal diseases (also known as mycosis) differ from most bacterial diseases: They tend to be chronic and kill the host slowly recalcitrant to therapy such that most invasive mycoses require treatment courses lasting months or longer. In contrast to bacterial and viral diseases, invasive human fungal infections are rarely communicable, and this has led to reduced interest by public health authorities in surveillance, so that we have relatively little information on the incidence and prevalence of mycoses. Morbidity rates linked to fungal infections represent an important health issue. For example, diseases such as chromoblastomycosis and eumycetoma lead to destructive deformations and debilitating conditions of the subcutaneous tissues, skin, and underlying bones, which result in social exclusion. Individual fungal diseases have profound impacts on human health: Recent global estimates found the following occurring annually: 3,000,000 cases of chronic pulmonary aspergillosis; 223,100 cases of cryptococcal meningitis complicating HIV/AIDs; 700,000 cases of invasive candidiasis; 250,000 cases of invasive aspergillosis; 100,000 cases of disseminated histoplasmosis; over 10,000,000 cases of fungal asthma; 1,000,000 cases of fungal keratitis. Around 220,000 new cases of cryptococcal

meningitis occur worldwide each year, resulting in 181,000 deaths concentrated in sub-Saharan Africa. More than 400,000 people develop Pneumocystis pneumonia annually and die without access to therapy. In Latin America, histoplasmosis is one of the most common opportunistic infections among people living with HIV/AIDS, and approximately 30% of patients diagnosed with histoplasmosis in that region die from this disease.

Fungi are increasingly implicated as the agents of spoilage of economically important fruits and vegetables. Fruits supply some necessary nutritional substances such as vitamins and essential minerals in human daily diet; this keeps the body in a good and healthy condition. Consumption of fruit and vegetable products has dramatically increased by more than 40% during the past few decades. It is also estimated that about 30% of all fruits and vegetables produced are lost each year due to spoilage. The prevalence of fungi as the spoilage organism of some edible fruits and vegetables abound in different locations. Fruits and vegetables are exposed to contamination by microorganisms through direct contact with soil, dust, water and by handling at harvest or during postharvest processing. This makes them to harbour a wide range of microorganisms including plant and human pathogens [2]. Microorganisms responsible for spoilage of fruits and vegetables exploit the host using extracellular lytic enzymes that degrade these polymers to release water and the plant's other intracellular constituents for use as nutrients for their growth. Fungi in particular produce an abundance of extracellular pectinases and hemicellulases that are important factors for fungal spoilage. Some spoilage microbes are capable of colonizing and creating lesions on healthy, undamaged plant tissue. Improper pre-harvest fungicide application, poor washing, and/or inadequate culling of fruits and vegetables usually lead to expanding infestation of spoilage microorganisms which can destroy a substantial portion of a stored lot of fruits

The contamination of fruits and vegetables by fungi poses a significant threat to food security, economic stability, and public health. As these essential components of the human diet provide vital nutrients and contribute to overall health, ensuring their safety is paramount. However, fruits and vegetables are highly susceptible to fungal contamination due to their high moisture content and nutrient-rich environment, which are conducive to fungal growth. This contamination not only leads to substantial post-harvest losses but also raises serious health concerns, particularly with the potential production of mycotoxins by certain fungal species. Fungi such as *Aspergillus*, *Penicillium* and *Fusarium* are commonly associated with decaying produce and have been documented to produce mycotoxins that can have severe toxicological effects on humans and animals. These mycotoxins can cause a range of adverse health effects, including acute poisoning, immunosuppression, and carcinogenicity. Additionally, the economic impact of fungal contamination is profound, affecting growers, distributors, and consumers due to reduced shelf life and increased waste.

As processing and packaging technologies have improved during the last decade, microbiological spoilage or microbiological shelf life has become a major reason for sensory quality shelf life failure for most packaged fresh-cut fruits and vegetables, followed by surface discoloration (e.g., pinking of cut lettuce, browning of cut potato, greying and browning with processed pineapple, and grey discoloration with cabbage), water-soaked appearance or translucency (e.g., cut watermelon, papaya, honeydew, and tomatoes), moisture loss (e.g., "baby" carrots and celery sticks), off-aroma (e.g., broccoli florets and diced cabbage in low % O₂ and high CO₂ packages), flavor changes (e.g., cut kiwifruit), and texture changes (e.g., processed strawberry, grated celery, kiwifruit, and papaya). Microbial spoilage including off-flavor (e.g., fermented aroma with cut lettuce, sour taste with cantaloupe and bell pepper) formation, slimy surface (e.g., "baby" carrots), wetness and soft rot (e.g., cut bell pepper), discoloration (e.g., apple wedges), and visual microbial growth/colonies (such as apple wedges, cantaloupe chunks, and cored pineapple) has been used as a main or exclusive objective criterion to determine shelf life of fresh-cut products, microbial spoilage is a limiting factor for shelf life of fruit pieces stored under controlled atmosphere conditions. Shelf life, including microbial spoilage, results in 30- 50% shrinkage of fresh-cut fruits. Microbial spoilage has been used by quality assurance departments in the fresh-cut industry as the objective indicator for quality failure for more than 50% of fresh-cut vegetable commodities and almost 100% of fresh-cut fruit products that have been treated with preservatives (such as anti-browning reagents) and/or packaged properly using MAP technologies. Under equilibrium modified atmosphere (MA) conditions, mixed fresh-cut bell pepper (including green, yellow, and red bell pepper) was unacceptable by day 6 of storage at 7-C due to acidic flavor, water loss, and texture change. Processed Lollo Rosso lettuce had a shelf life of shorter than 7 days at 5-C due to high microbial counts and off-odor formation under MAP. Grated carrots became wet and slimy, lost firmness, and produced off-odors during storage at 10-C under MAP. The first indicator of changes in freshness for fresh-cut lettuce packed using active/passive MAP to prevent pinking or browning is fermented aroma formation. Studies of cut cantaloupe revealed evidence of visual spoilage, including presence of microbial colonies, slime, and turbidity in juice, within 15 days of storage at 4-C.

This study aims to investigate the prevalence and diversity of fungal contaminants in rotten fruits and vegetables and evaluate the potential health risks associated with these contaminants. By employing a combination of microscopic examination and colony characteristics, we seek to provide a comprehensive understanding of the fungal ecology in decaying produce. Furthermore, the study explores the effectiveness of various storage and handling practices in mitigating fungal contamination. Understanding the dynamics of fungal contamination in fruits and vegetables is crucial for developing effective management strategies and ensuring the safety and quality of fresh produce. This study not only

contributes to the scientific knowledge of fungal contaminants but also offers practical insights for improving post-harvest handling and storage practices to minimize the risks associated with fungal growth and mycotoxin production.

MATERIALS AND METHODS

❖ Sample collection

Samples of vegetables and fruits (healthy and infected) were collected from local market, Bengaluru, Karnataka. The samples were transported to laboratory in separate sterile plastic bags for fungal analysis.

Samples included; Garlic (*Allium sativum*), Onion (*Allium cepa*), Cauliflower (*Brassica oleracea*), Papaya (*Carica papaya*), Tomato (*Solanum lycopersicum*), Ridge gourd (*Luffa acutangula*), Capsicum (*Capsicum annuum*), Beans (*Phaseolus*), Turnip (*Brassica oleracea gongylodes*), Carrot (*Daucus carota*), Ginger (*Zingiber officinale*), Potato (*Solanum tuberosum*), Chilli (*Capsicum frutescens*), Sapota (*Manilkara zapota*), Cucumber (*Cucumis sativus*)

❖ Isolation and identification of fungi

From each of serially diluted tubes 1 ml was inoculated onto plates of Potato Dextrose Agar (PDA). The plates were allowed to solidify and incubated at room temperature for 2-5 days. The plates were incubated at 28 +1°C for five days. The fungal colonies that appeared were primarily identified using morphological features. The fungal isolates were purified by sub- cultured transplanting to new set using potato dextrose agar. The pure strains of isolated fungi were identified using fungal identification keys.

❖ Microscopic Examination of fungal isolates

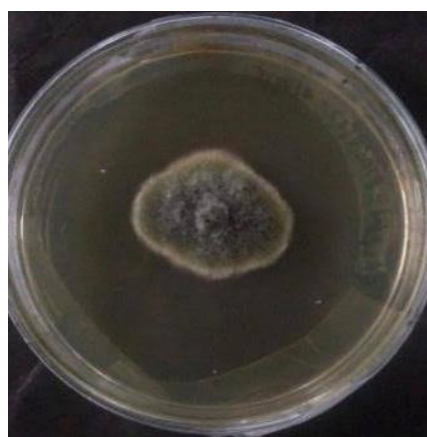
Lacto phenol cotton blue was dropped on a glass slide and small portion of fungal colony from the sub-structure plates was taken, using a sterile inoculating needle and transferred to a glass slide, it was then emulsified with a sterile needle and then covered with a cover slip gently, to avoid air bubbles. Observation under low and high power objective lens was carried out, the observation include, searching for different features of fungi including, the hyphae, conidia, sporangiophore (reproductive structure), and identification was carried out microscopically by examining the colony.

RESULTS AND OBSERVATIONS

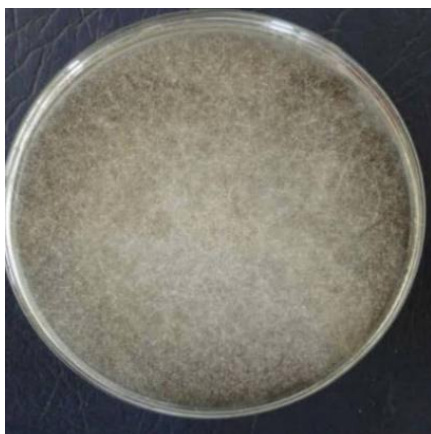
In the present study it was observed that *Allium sativum* was contaminated with *Aspergillus niger*. *Allium cepa* with *Aspergillus fumigatus*. *Solanum tuberosum* was contaminated with *Alternaria sp.*, *Mucor sp.*, and *Rhizopus stolonifer*. *Zingiber officinale* was contaminated with *Fusarium sp.* Contamination of *Corynespora sp.*, was observed in solanaceae members (tomato, chilli, brinjal and capsicum), Tomato was also contaminated with *Rhizopus sp.* *Fusarium sp.*, was also isolated from chilli and *Aspergillus niger* from capsicum. *Phaseolus vulgaris* was contaminated with *Rhizopus microsporus*. Carrot was contaminated with *Rhizopus sp.* *Fusarium sp.*, was isolated from raddish, *Luffa acutangula* and sapota. Cauliflower was contaminated with *Fusarium sp.*, *Rhizopus sp.*, and *Mucor sp.* Papaya was contaminated with *Rhizopus sp.*, and *Mucor sp.* *Aspergillus flavus* and *Aspergillus parasiticus* was isolated from capsicum. No fungal contamination was seen in turnip, lemon and cucumber.



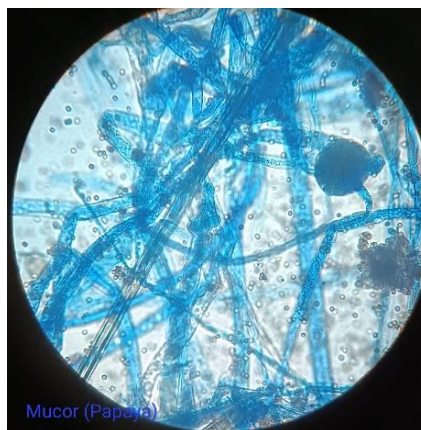
Infected Papaya



Mucor sp.,



Rhizopus sp.



Mucor sp.

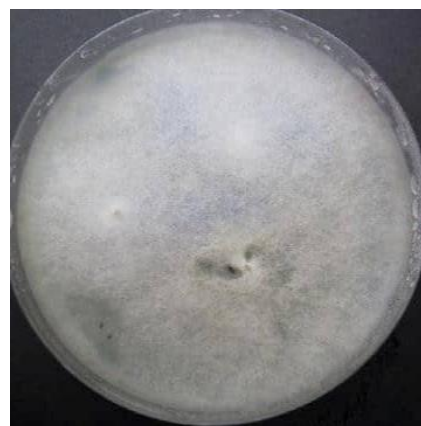


Rhizopus stolonifer

Fig 1: Fungal contamination in Papaya



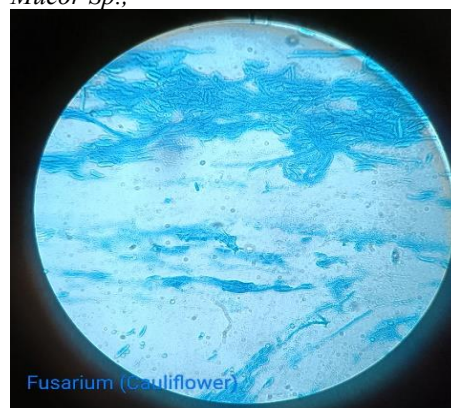
Infected Cauliflower



Mucor Sp.



Fusarium oxysporum

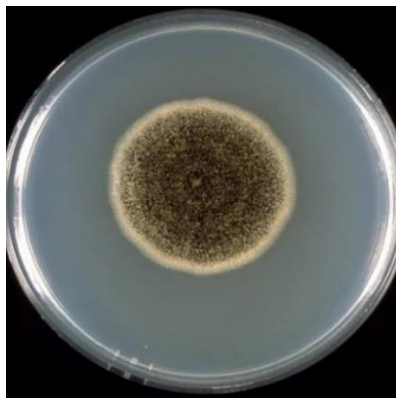


Fusarium oxysporum

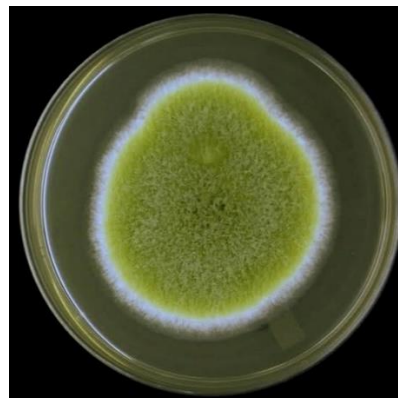
Fig 2: Fungal contamination in Cauliflower



Capsicum (*Capsicum annuum*)



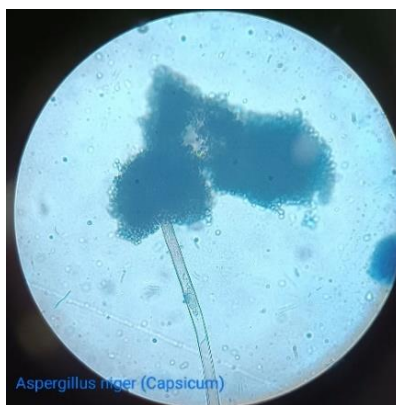
Aspergillus niger from Capsicum



Aspergillus flavus from Capsicum

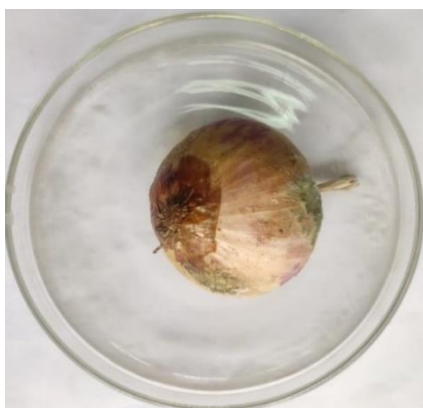


Aspergillus parasiticus from Capsicum

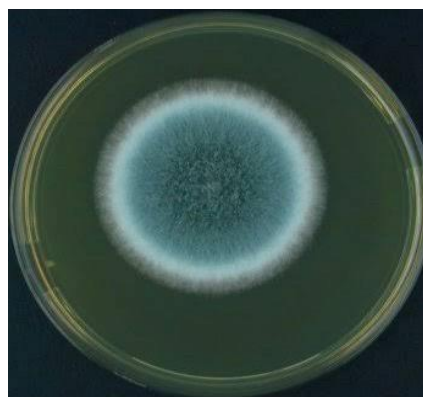


Aspergillus niger from Capsicum

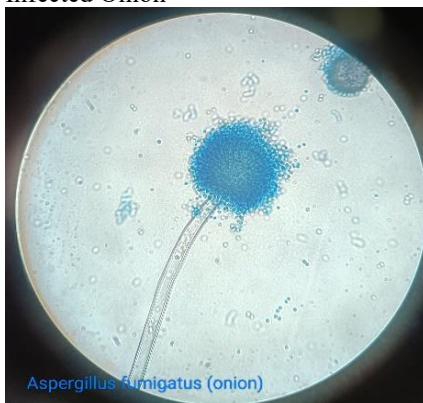
Fig 3: Fungal Contamination in Capsicum



Infected Onion



Aspergillus fumigatus from onion

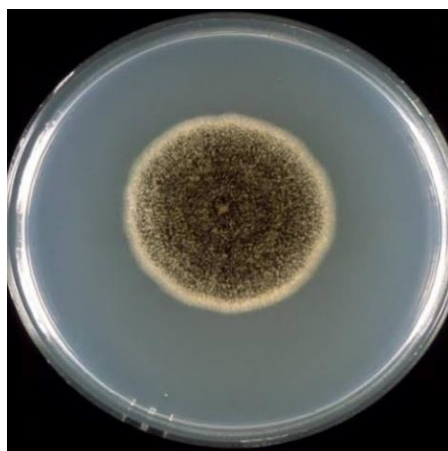


Aspergillus fumigatus from onion

Fig 4: Fungal Contamination in Onion



Infected Garlic



Aspergillus niger

Fig 5: Fungal contamination in Garlic



Infected Beans

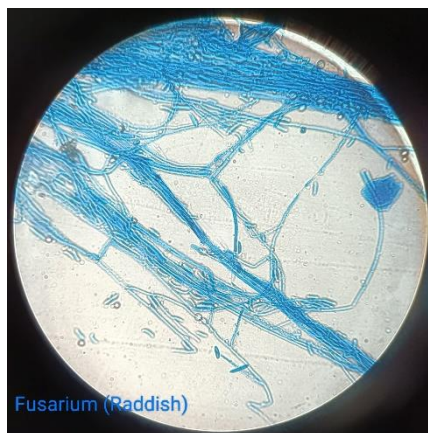


Rhizopus microsporus

Fig 6: Fungal Contamination in Beans



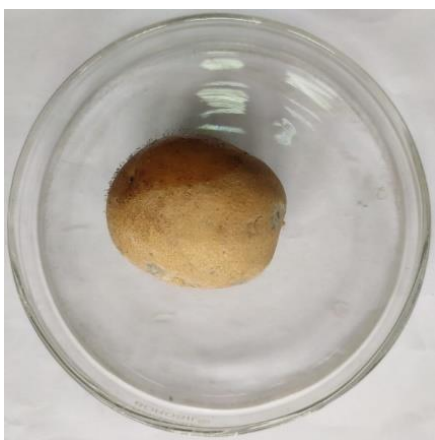
Infected Raddish



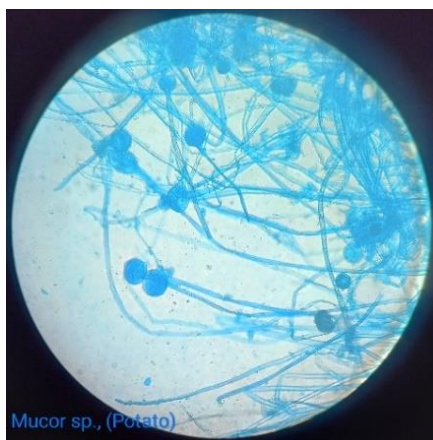
Fusarium (Raddish)

Fusarium

Fig 7: Fungal Contamination in Radish

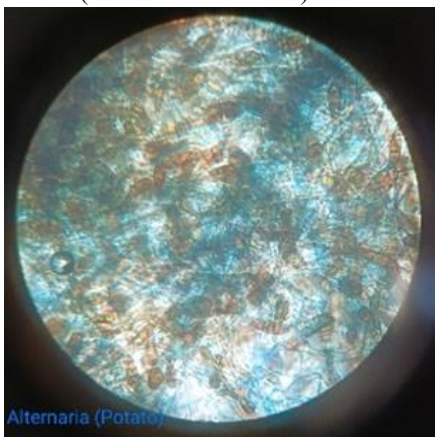


Potato (*Solanum tuberosum*)



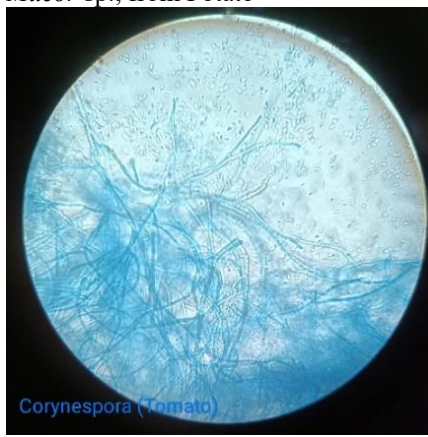
Mucor sp., (Potato)

Mucor sp., from Potato



Alternaria (Potato)

Alternaria from Potato



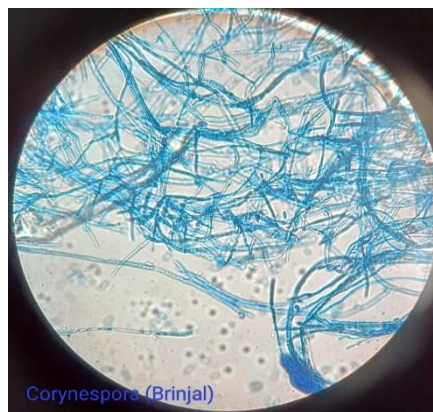
Corynespora (Tomato)

Corynespora from Potato

Fig 8: Fungal Contamination in Potato



Brinjal (*Solanum melongena*)



Corynespora sp., from Brinjal

Fig 9: Fungal Contamination in Brinjal



Tomato (*Solanum lycopersicum*)



Corynespora sp.,

Fig 10: Fungal contamination in Tomato



Chilli (*Capsicum frutescens*)



Corynespora from Chilli

Fig 11: Fungal Contamination in Chilli

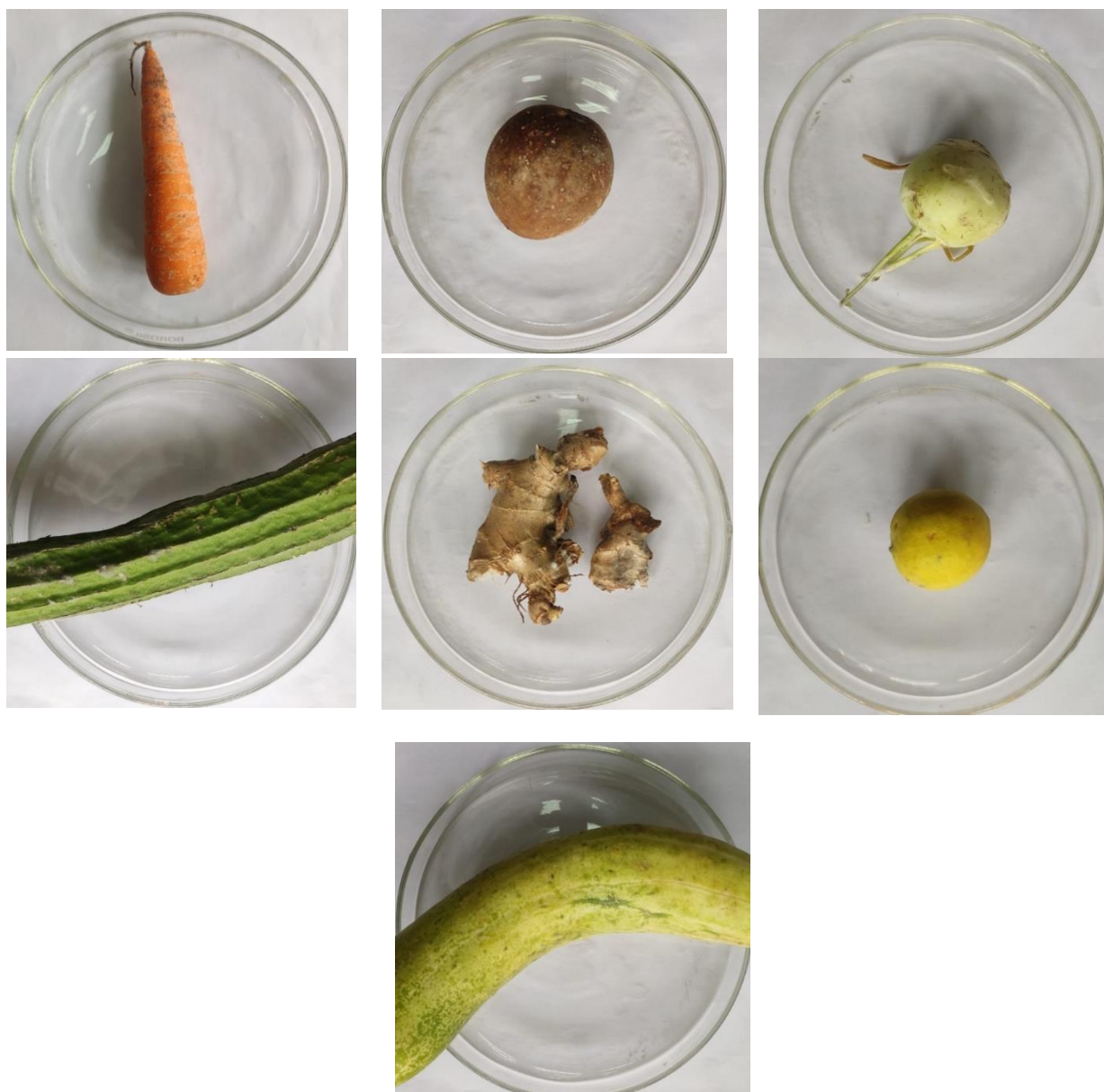


Fig 12: Infected Carrot (*Daucus carota*), Sapota (*Manilkara zapota*), Turnip (*Brassica oleracea gongyloides*), Ridge gourd (*Luffa acutangula*), Ginger (*Zingiber officinale*), Lemon (*Citrus limon*), Cucumber (*Cucumis sativus*)

DISCUSSION

Fungal contamination in fruits and vegetables is a critical issue affecting food safety, shelf life, and economic value. Our study identified various fungal contaminants in different produce, reflecting both common and unique fungal associations. In our study, *Allium sativum* (garlic) was contaminated with *Aspergillus niger*, and Capsicum (bell pepper) showed contamination with *Aspergillus niger* and *Aspergillus flavus*. The occurrence of *Aspergillus niger* in garlic is consistent with findings by Kumar et al. (2018), who noted that *A. niger* is a common post-harvest pathogen in garlic, often leading to black mold disease. This pathogen thrives in conditions of high humidity and temperature, which are typical in storage environments. *Aspergillus flavus* contamination in capsicum is particularly concerning due to its ability to produce aflatoxins, potent carcinogens. Reddy et al. (2019) reported that aflatoxin contamination in peppers is a significant health risk, emphasizing the need for stringent monitoring and control measures. The study by Reddy et al. also highlighted that aflatoxin contamination can occur at any stage from pre-harvest to storage, necessitating a comprehensive approach to prevention. Our findings showed that *Solanum tuberosum* (potato) was contaminated with *Alternaria* sp., *Mucor* sp., and *Rhizopus stolonifer*. *Alternaria* species are well-known for causing early blight in potatoes, leading to significant yield losses. Research by Koley et al. (2020) documented the widespread presence of *Alternaria* in potato fields and its impact on crop health. *Mucor* and *Rhizopus stolonifer* are common soil-borne fungi that cause soft rot in potatoes. These pathogens are particularly problematic during storage, as they can spread rapidly under moist conditions. The study by Koley et al. emphasized that effective management of storage conditions is crucial to preventing outbreaks of soft rot caused by these fungi. *Zingiber officinale* (ginger) in our study was found to be contaminated with *Fusarium* sp. *Fusarium* species are notorious for producing a range of mycotoxins, including fumonisins and trichothecenes, which pose serious health risks. Tan et al. (2017) reported that *Fusarium* infections in ginger can occur both in the field and during storage,

highlighting the need for integrated pest management strategies to mitigate these risks. Our study also identified *Fusarium* sp. in *Raphanus sativus* (radish), *Luffa acutangula* (ridge gourd), and sapota. The widespread presence of *Fusarium* across different produce underscores its adaptability to various environmental conditions. Studies by Tan et al. (2017) and others have shown that *Fusarium* can survive in soil for extended periods, making crop rotation and soil treatment essential components of effective management strategies. Within the Solanaceae family, our results indicated that *Corynespora* sp. contaminated tomato, chili, brinjal (eggplant), and capsicum. Additionally, tomato was contaminated with *Rhizopus* sp., while chilli showed contamination with *Fusarium* sp., and capsicum was contaminated with *Aspergillus niger* and *Aspergillus flavus*. Gupta et al. (2021) noted similar patterns of fungal contamination in Solanaceae crops, emphasizing that these crops are highly susceptible to fungal infections due to their tender skin and high moisture content. *Rhizopus* species are known to cause soft rot in tomatoes, leading to rapid decay. Gupta et al. (2021) reported that *Rhizopus* infections can significantly reduce the marketability of tomatoes, highlighting the importance of proper handling and storage practices to minimize contamination.

Our study found that *Allium sativum* (garlic) was contaminated with *Aspergillus niger*, while Capsicum (bell pepper) was contaminated with both *Aspergillus niger* and *Aspergillus flavus*. Similar findings were reported by Chavan et al. (2020), who observed that *A. niger* is a common post-harvest contaminant in garlic, leading to significant spoilage due to its ability to thrive in humid storage conditions. Additionally, *A. flavus* in capsicum is particularly concerning because of its aflatoxin production, a potent carcinogen, as noted by Kebede et al. (2021). These aflatoxins can contaminate crops during both pre- and post-harvest stages, posing serious health risks and economic losses. Our research identified *Alternaria* sp., *Mucor* sp., and *Rhizopus stolonifer* in *Solanum tuberosum* (potato). *Alternaria* species are known to cause early blight, a common and damaging disease in potatoes, corroborated by the findings of Leiminger and Hausladen (2018). The presence of *Mucor* and *Rhizopus stolonifer* further complicates post-harvest management due to their role in causing soft rot, as discussed by Tsrer et al. (2021). These pathogens are particularly challenging to control because they can rapidly proliferate in humid and poorly ventilated storage conditions. In our study, *Zingiber officinale* (ginger) was contaminated with *Fusarium* sp., a genus notorious for producing mycotoxins such as fumonisins and trichothecenes. Our findings align with the research by Sumanth et al. (2018), who documented the prevalence of *Fusarium* species in ginger and their significant impact on both crop yield and quality. The widespread occurrence of *Fusarium* in *Raphanus sativus* (radish), *Luffa acutangula* (ridge gourd), and sapota observed in our study is consistent with findings by Singh et al. (2020), highlighting the broad host range and environmental adaptability of *Fusarium* species. Our study detected *Corynespora* sp. in several members of the Solanaceae family, including tomato, chili, brinjal (eggplant), and capsicum. Additionally, tomato was contaminated with *Rhizopus* sp., while chili showed contamination with *Fusarium* sp., and capsicum with *Aspergillus niger* and *Aspergillus flavus*. The susceptibility of Solanaceae crops to multiple fungal pathogens has been well-documented. For instance, Liu et al. (2019) reported similar fungal profiles in Solanaceae crops, emphasizing the need for effective management strategies to mitigate fungal infections, which can significantly impact crop yield and quality. Our study found *Rhizopus microsporus* in *Phaseolus vulgaris* (common bean) and *Rhizopus* sp. in carrot and papaya. *Rhizopus* species are well-known for causing soft rot in a wide range of produce, leading to rapid spoilage under favorable conditions. Similar observations were made by Zhang et al. (2018), who highlighted the rapid spread and significant post-harvest losses caused by *Rhizopus* species in various fruits and vegetables. Effective control measures, such as maintaining low humidity and temperature during storage and employing antifungal treatments, are crucial to minimize these losses.

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measures, such as maintaining low humidity and temperature during storage and employing antifungal treatments, are crucial to minimize these losses.

Our study provides a comprehensive overview of fungal contamination in various fruits and vegetables, underscoring the consistency of our findings with broader research trends. The comparative analysis with existing literature highlights the need for integrated management strategies that address fungal contamination at multiple stages, from pre-harvest to storage. Understanding the specific associations and broader implications of fungal contaminants is crucial for developing effective control measures and ensuring food safety.

CONCLUSION

This study underscores the pervasive issue of fungal contamination in fruits and vegetables collected from local markets in Bengaluru, Karnataka. The extensive range of fungal pathogens identified in our research highlights the significant threat these organisms pose to food safety, shelf life, and economic value. Our findings reveal a diverse spectrum of fungi, including *Aspergillus niger*, *Aspergillus flavus*, *Alternaria sp.*, *Mucor sp.*, *Rhizopus stolonifer*, *Fusarium sp.*, and *Corynespora sp.*, each with unique implications for produce contamination and public health. The detection of *Aspergillus niger* in garlic and *Aspergillus flavus* in capsicum is particularly concerning due to the production of aflatoxins by these fungi. Aflatoxins are potent *carcinogens*, and their presence in food products poses serious health risks. The widespread occurrence of these fungi in commonly consumed vegetables calls for stringent monitoring and control measures. This aligns with previous studies highlighting the significance of these contaminants in food safety (Agrios, 2005; Frisvad et al., 2007). *Fusarium* species were found to contaminate ginger, radish, ridge gourd, and sapota, indicating a broad host range and environmental adaptability. These fungi are known for producing mycotoxins such as fumonisins and trichothecenes, which can lead to severe health issues upon consumption. The frequent detection of *Fusarium* in these vegetables underlines the need for effective agricultural and post-harvest practices to mitigate contamination and ensure food safety (Nelson et al., 1993). The study also identified *Rhizopus stolonifer* and *Mucor sp.* in potatoes, carrots, and papayas. These fungi are notorious for causing soft rot, leading to rapid spoilage under humid conditions. Their presence necessitates the implementation of improved post-harvest handling and storage techniques to reduce spoilage and economic losses. These findings corroborate the challenges posed by these pathogens in maintaining the quality and shelf life of produce (Snowdon, 1990; Pitt & Hocking, 2009). *Corynespora sp.* was detected in several Solanaceae crops, including tomato, chili, brinjal, and capsicum. These fungi cause leaf spots and other diseases, significantly impacting crop yield and quality. The prevalence of *Corynespora* species in these crops highlights the importance of targeted disease management strategies to protect Solanaceae crops from fungal infections (Sinclair & Lyon, 2005).

The findings from this study emphasize the need for comprehensive management strategies to address fungal contamination in fruits and vegetables. Effective measures should include: Employing crop rotation and resistant varieties to reduce the incidence of fungal infections, Implementing integrated pest management (IPM) practices to minimize fungal contamination, Ensuring proper sanitation and handling during harvesting and transportation to reduce fungal spore spread, Utilizing appropriate storage conditions, such as controlled humidity and temperature, to inhibit fungal growth, Conducting regular surveillance and testing for fungal contaminants in produce to detect and address issues promptly, Implementing stringent quality control measures in markets and storage facilities, Educating farmers, handlers, and consumers about the risks associated with fungal contamination and the best practices to mitigate these risks, Promoting awareness about the importance of consuming properly stored and handled produce to avoid health risks associated with mycotoxins.

Continued research and monitoring are essential to develop more effective control measures against fungal contamination. Future studies should focus on: Breeding and genetic modification of crops to enhance resistance against common fungal pathogens, Exploring the use of biocontrol agents and natural fungicides as sustainable alternatives to chemical treatments, Improving detection techniques for early identification of fungal contaminants to prevent the spread and establishment of infections, Investigating the impact of climate change on the prevalence and distribution of fungal pathogens to adapt management strategies accordingly.

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