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Isolation and Identification of Fungi from Spoiled Vegetables and Fruits of Rythubazar, Kurnool, Andhra Pradesh, India

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Abstract

The present investigation was carried out to isolate and identify fungal contaminants associated with the spoilage of fruits and vegetables obtained from Rythubazar, C-Camp, Kurnool, Andhra Pradesh, India. A variety of visibly spoiled produce samples were collected and subjected to standard microbiological techniques for fungal isolation using Potato Dextrose Agar (PDA) medium. Morphological and microscopic examination facilitated the identification of six distinct fungal genera: *Aspergillus*, *Fusarium*, *Mucor*, *Rhizopus*, *Penicillium*, and *Candida*. Among these, *Aspergillus* spp. were found to be the most predominant, whereas Candida spp. were the least frequently encountered. Pathogenicity tests were performed by reinoculating healthy fruits and vegetables with isolated fungal strains, which reproduced characteristic spoilage symptoms, thereby confirming their role in post-harvest decay. The study highlights the diversity and prevalence of fungal contaminants in local market produce and emphasizes the importance of proper post-harvest handling, storage, and fungal management practices to reduce economic losses and health risks.

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Introduction

The isolation and identification of fungi from fruits and vegetables constitute a critical aspect of food safety and quality control. Traditional methods for identifying fungi involve morphological and physiological assessments, which remain prevalent despite advancements in molecular techniques.[1] Fungi, including yeasts and molds, are ubiquitous in various environments and can contaminate food products, potentially producing mycotoxins even in the absence of visible growth.[2,3]

In medical mycology, the processes of isolating and identifying fungi play a crucial role in managing fungal infections effectively.[4] With more than 200 fungal species known to infect humans, precise identification is vital for determining the appropriate treatment. These procedures are essential for diagnosing and treating fungal infections, despite facing obstacles such as expenses and contamination risks. They are particularly important in addressing the increasing concern of antifungal-resistant pathogens Furthermore, the rise of resistant fungi has become a

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major threat to public health, necessitating additional research to comprehend and combat these pathogens Martins-Santana et al., 2023).[5,6] This study aids in early detection and control of fungal contamination and there by supporting safer food handling practices in local markets.

Materials & Methods

Sample Collection

A total of ten different fruits and vegetables showing signs of fungal infection were collected from Rythubazar, C-Camp, Kurnool. The collected samples comprised banana (*Musa acuminata*), papaya (*Carica papaya*), orange (*Citrus sinensis*), carrot (*Daucus carota*), ivy gourd (*Coccinia grandis*), okra (*Abelmoschus esculentus*), beetroot (*Beta vulgaris*), spinach (*Spinacia oleracea*), brinjal (*Solanum melongena*), and tomato (*Lycopersicon esculentum*). All samples were aseptically transferred to the laboratory in sterile polythene bags for further examination.

Sample Preparation

Fruits and vegetables were cut from the lesion edges using a disinfected knife. The samples were surface-sterilized using 0.1 g/L sodium hypochlorite solution (2% chlorine) for 5 minutes, rinsed thrice with distilled water, and homogenized in a blender. The homogenate was serially diluted with 8.5% NaCl normal saline solution.[7]

Culture Media Preparation

Potato Dextrose Agar (PDA) is a widely used medium for culturing fungi, including molds and yeasts. To prepare PDA, 200 g of peeled, chopped potatoes are boiled in 500 mL of distilled water for 30 minutes, then filtered and diluted to 1000 mL. Next, 20 g of dextrose and 15 g of agar are added and dissolved by heating. The pH is adjusted to 5.6 ± 0.2, and 0.3 g/L of chloramphenicol may be added to prevent bacterial growth. The medium is sterilized at 121°C for 15 minutes, cooled to 45–50°C, poured into sterile Petri dishes, and stored at 4°C until use. PDA provides essential nutrients for fungal growth and is widely applied in microbiology and plant pathology.[8,9]

Isolation of Fungi

The isolation of fungi involves collecting samples from spoiled fruits and vegetables preparing them for culture. Solid samples are mixed with sterile water or saline and diluted to reduce microbial load. A small amount of the sample is then spread on Potato Dextrose Agar (PDA) or Sabouraud Dextrose Agar (SDA) plates containing antibiotics to prevent bacterial growth. Plates are incubated at 25–30°C for 5–7 days, allowing fungal colonies to develop. Distinct colonies are picked and transferred to fresh media for purification. Fungal identification is done using Lactophenol Cotton Blue (LPCB) staining and microscopic examination based on spore and hyphal structures. Pure cultures can be stored at 4°C on PDA slants for short-term use or preserved long-term in glycerol at –80°C. This method is widely used in microbiology and plant pathology for fungal identification and study.[8,10]

Identification of the Isolated Fungi

Fungal isolates were identified after incubation at 30°C for 6–7 days. Small 1 cm^2 blocks of Potato Dextrose Agar (PDA) were transferred to sterile slides, stained with 0.5% lactophenol blue, and examined under a light microscope at 10 × magnifications. Key features such as colony growth, hyphal structure, reproductive structures (sporangia/ conidia), pigmentation, and spore type were recorded for identification following standard methods.[8,11]

Pathogencity Test

The pathogenicity test was conducted to determine whether the isolated fungi caused fruit spoilage.[12,13] Healthy fruits were surface sterilized with 90% ethanol, and 4 mm incisions were made using a sterile cork borer. Fungal cultures from Potato Dextrose Agar (PDA) were cut with a similar cork borer and placed into the incisions under sterile conditions. The inoculated wounds were sealed with petroleum jelly to prevent contamination. Control fruits with incisions but no fungal inoculation were kept in sterile polythene bags with moistened cotton wool to maintain humidity. All samples were incubated at 28°C for 5 days, and symptoms were observed after 72 hours. Fungi were then re-isolated from infected fruits and compared with the original isolates to confirm their role in spoilage.

Statistical Significance

All the data are expressed as mean ± SEM. Comparison of the obtained values for the above parameters and the extracts with control group, was done through making use of ANOVA followed by Dunnett’s test. The values of $p < 0.05$ and $p < 0.01$

were considered to indicate a significant difference between the groups

Results

Isolation and Identification of Fungal Isolates

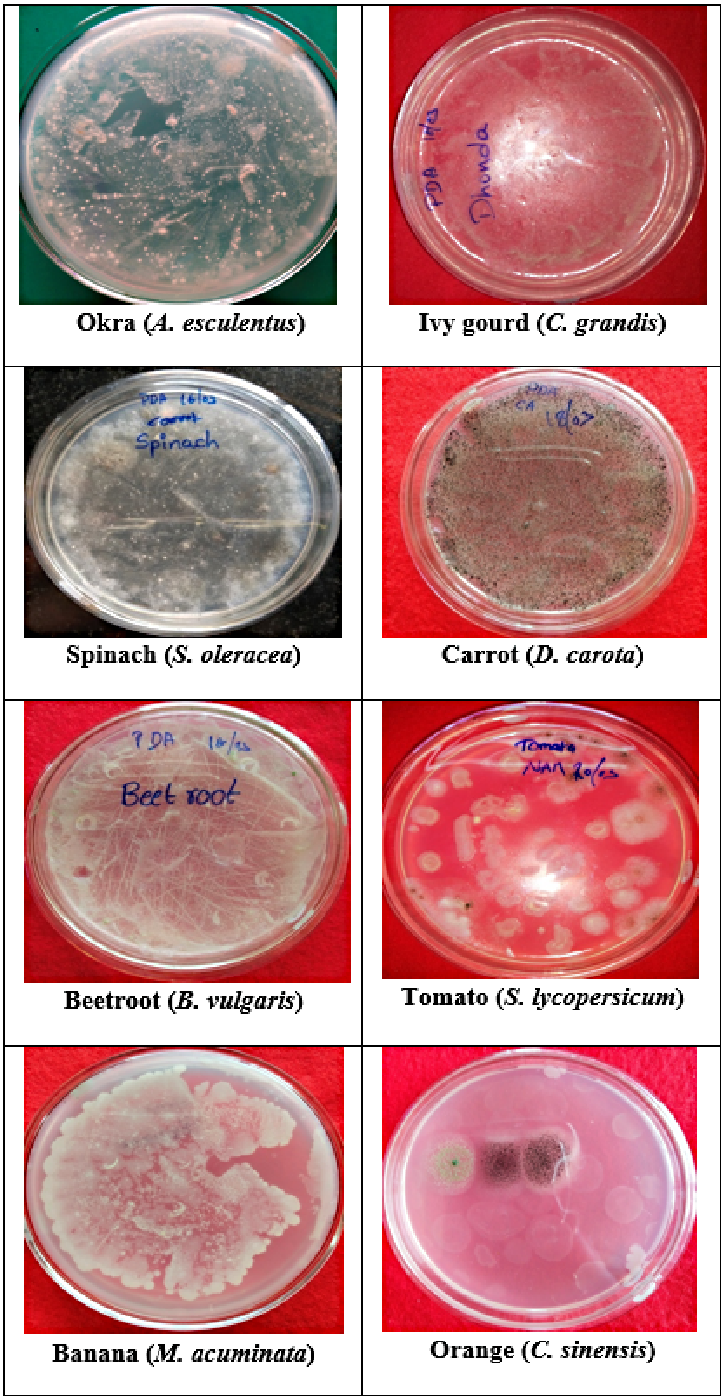

Plate-1: Isolated fungal colonies of different vegetables and fruits

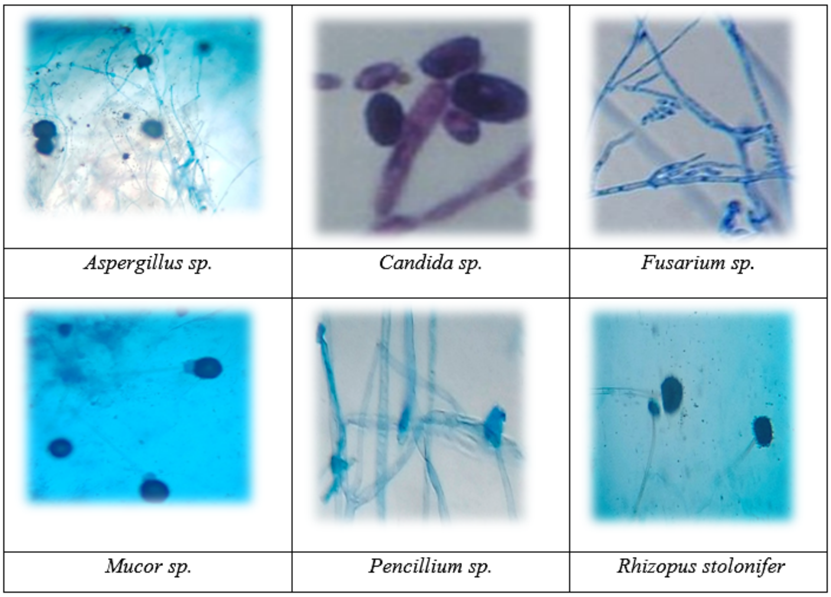

Plate-2: Microscopic observation of Fungal isolates

Table 1: Identification of fungal isolates from spoiled fruits and vegetables

Vegetables & Fruits	Spoilage Characteristics	Colony Appearance	Microscopic Features	Identified Organism
Okra *(A. esculentus)*	White cottony fibres, moist	White-grey cottony colonies, irregular spread	Non-septate hyphae, rhizoids, sporangiophores	*R. stolonifer*
	Whitish moist flat fibres	Shiny, woolly to cottony colonies	Hyaline septate hyphae, conidiophores, phialides	*Fusarium* sp.
Spinach *(S. oleracea)*	White cottony fibres, water-soaked	White-black cottony spread	Septate hyphae, conidia, vesicles	*Aspergillus* sp.
	Water-soaked depression	Shiny, smooth cream-white colonies	Septate hyphae, short inflated conidiophores, black-walled conidia	*Candida* sp.
Beetroot *(B.vulgaris)*	Greenish sunken depression	White-black smooth scattered colonies	Unbranched conidiophores, swollen vesicles, septate hyphae	*Aspergillus* sp.
Ivy gourd *(C. grandis)*	Wrinkled, spongy white deposits	Black colonies, dense cottony mycelia	Broad non-septate hyphae, rhizoids, sporangia	*Rhizopus* sp.
Carrot *(D. carota)*	Black moistwater-soaked, spores visible	White-black dense mycelial growth	Septate hyphae, conidiophores, vesicles	*Aspergillus* spp.
Tomato *(S. lycopersicum)*	Wrinkled, depressed	Whitish turning brown-black	Non-septate mycelia, branching sporangiosphores	*Rhizopus stolonifer*

	Wrinkled, depressed	White-grey cottony fibers	Prostrate hyphae, sporangiophore, sporangium	*Mucor* spp.
Brinjal *(S. melongena)*	Large sunken depression	White-black cottony colonies	Septate hyphae, conidia, vesicles	*Aspergillus* spp.
Pawpaw *(C. papaya)*	Large sunken depression	White colony with black conidia	Long conidiophore, vesicle, conidia	*Aspergillus* spp.
	Wrinkled, water-soaked depression	Whitish colony turning brown-black	Non-septate mycelia, ovoid sporangiosphore	*Rhizopus* spp.
Orange *(C. sinensis)*	Dark brown discoloration, gas formation	White with black edges	Septate hyphae, long conidiophore, conidia	*Aspergillus* spp.
	Spongy with gas production	White-grey filamentous colonies	Branched conidiophores, brush -like conidia clusters	*Penicillium* spp.
Banana *(M. acuminata)*	Dry, wrinkled, rough, blackish, moist	White-grey fluffy mycelium	Mycelium with sporangia and spores	*Mucor* spp.

A total of six fungal genera, namely *Aspergillus*, *Fusarium*, *Mucor*, *Rhizopus*, *Penicillium*, and *Candida*, were isolated from four fruit and six vegetable samples. *Mucor* sp. was identified in banana (M. *acuminata*), while *Aspergillus* sp. and *Rhizopus* sp. were recovered from pawpaw (*C. papaya*). Similarly, *Aspergillus* sp. and *Mucor* sp. were found in orange (*C. sinensis*), and *Aspergillus* sp. was detected in carrot (*D. carota*). *Rhizopus* sp. was isolated from ivy gourd (*C. grandis*), whereas *Rhizopus* sp. and *Fusarium* sp. were found in okra (*A. esculentus*). Beetroot (*B. vulgaris*) harbored *Aspergillus* sp., and both *Aspergillus* sp. and *Candida* sp. were detected in spinach (*S. oleracea*). *Aspergillus* sp. was also isolated from brinjal (*S. melongena)*, while tomato (*S. lycopersicum*) yielded *Rhizopus* sp. and *Mucor* sp. (Plate1, 2 & Table 1).

Table 2: Frequency of occurrence of each isolates in Vegetable & fruit samples

Fruit/Vegetable	Fungal Species	Frequency (%) Mean ± SEM
Okra *(A. esculentus)*	*Rhizopus* sp., *Fusarium* sp.	74.2 ± 0.70*, 26.8 ± 0.66
Spinach *(S. oleracea)*	*Aspergillus* sp., *Candida* sp.	64.3 ± 0.46**, 26.7 ± 0.70*
ivy gourd *(C. grandis)*	*Rhizopus* sp.	59.3± 0.66*
Beetroot *(B. vulgaris)*	*Aspergillus* sp.	72.5 ±1.77
Carrot *(D. carota)*	*Aspergillus* sp.	66.3± 0.24*
Tomato *(L. esculentum)*	*Rhizopus* sp., *Mucor* sp.	48.7 ± 0.40, 52.3 ± 0.70*
Brinjal *(S. melongena)*	*Aspergillus* sp.	57.4 ±0.46**
Pawpaw *(C. papaya)*	*Aspergillus*, *Rhizopus* sp.	33.7± 0.28* 66.3%± 0.44
Orange *(C. sinensis)*	*Aspergillus* sp., *Mucor* sp.	68.4± 0.68**, 42.6± 0.66
Banana *(M. acuminata)*	*Mucor* sp.	74.6± 0.99

All the data are expressed as mean ± SEM, n=6, * $p < 0.05$ and ** $p < 0.01$ when compared with control group One way ANOVA followed by Dunnett's tes

Among the isolated fungi, *Aspergillus* was the most common, causing major spoilage in fruits and vegetables. It was most frequently found in spinach (*S. oleracea*), beetroot (*Beta vulgaris*), carrot (*D. carota*), brinjal (*S. melongena)*, papaya (*C. papaya*), and orange (*C. sinensis*). In okra (*A. esculentus*), *Rhizopus* sp. was more common than *Fusarium* sp., while *Aspergillus* sp. was found more often than *Candida* sp. in spinach. *Mucor* sp. dominated in tomato (*S. lycopersicum*), *Rhizopus* sp. was more frequent than *Aspergillus* sp. in papaya, and *Aspergillus* sp. was more common than *Mucor* sp. in orange. *Candida* sp. was the least frequent, found only in spinach (Table 2).

Table 3: Pathogenicity test

Fungal Species	Inoculated Fruit/Vegetable	Observed Spoilage
Aspergillus sp.	Spinach, beetroot and carrot	Cottony growth, discoloration
Rhizopus sp.	Okra, pawpaw	Sunken lesions, spongy texture
Mucor sp.	Tomato, banana	Moist, rough surface
Fusarium sp.	Okra	White, moist growth
Candida sp.	Spinach	Watery lesions
Penicillium sp.	Orange	Gas production, spongy texture

Pathogenicity tests confirmed that these fungi caused spoilage, as they reproduced decay symptoms when reintroduced into healthy fruits.

Discussion

In this study, *Aspergillus* sp. and *Rhizopus* sp. isolated from pawpaw (*C. papaya*) were responsible for soft rot in pawpaw and tomato are common pathogens which is consistent with findings by Baiyewu *et al.* (2007).12 The isolation of R. stolonifer and *Mucor* sp. from tomato (*S. lycopersicum*) supports previous reports by Efiuvwevwere (2000),[18] Chuku *et al.* (2008),[19] and Purseglove (1977),[20] who identified *Fusarium* sp. and *R. stolonifer* as major causes of tomato soft rot. The colonization of fruits and vegetables by spoilage microorganisms is a critical stage in microbial deterioration. Fungal spoilage is influenced by multiple factors occurring at different handling stages, from pre-harvest to consumption. These factors include the physiological and physical condition of the produce and environmental conditions such as temperature and humidity. Efiuvwev were (2000)[18] also reported that high moisture and relative humidity promote fungal growth in farm produce, reducing their storage life. The predominance of *Aspergillus* spp. across multiple samples suggests its widespread occurrence in fruits and vegetables, potentially posing risks related to spoilage and mycotoxin contamination.[14 & 15] The identification of *Fusarium* sp. further highlights the importance of monitoring fungal contamination due to its potential impact on food safety and agricultural productivity.[16]

Conclusion

Fungal contamination is a major cause of fruit and vegetable spoilage. Proper post-harvest handling and timely fungicide application can mitigate fungal infections and reduce mycotoxin risks. Further studies should explore insect-mediated fungal transmission. To minimize spoilage, fruits and vegetables should be consumed fresh or cooked. Creating storage conditions unfavorable for fungal growth can enhance food safety.

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Conflict of Interest

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This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Permission to Reproduce Material from other Sources

Not Applicable

Author Contributions

- **Bommana Kavitha:** Colletion of Data, Methodology, Writing – Original Draft.
- **Guneti Sri Ranganayakulu:** Writing – Review & Editing and met thoroughly the manuscript, offered valuable insights and suggestions to improve it.

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