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Research Paper

Morphological and Symptomatic Characterization of Fungi Associated with Post Harvest Decay of Major Vegetables in Nashik District, Maharashtra, India

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ABSTRACT

Post-harvest rotting of vegetables caused by fungi is a serious problem for vegetable production and food security, particularly in tropical and subtropical regions. This study primarily elucidates the morphological and symptomatic characteristics of the fungi responsible for causing decay and rotting in ten vegetables—cabbage, tomato, pumpkin, green peas, potato, cauliflower, eggplant, bitter gourd, okra, and French beans—that are commonly cultivated and sold in the Nashik district of Maharashtra. Samples of diseased vegetables were collected from local markets, and mycological analysis and study were conducted on them. Fungi were isolated on Potato Dextrose Agar (PDA), and the recovered fungi were identified solely based on macroscopic colony characteristics and microscopic reproductive morphology. Due to the lack of molecular confirmation, all isolates have been recorded at the species level. Ten distinct symptoms of rot were recorded, and the associated fungi were identified as follows: *Phytophthora sp.* (cabbage), *Phoma sp.* (tomato), *Pythium sp.* (pumpkin), *Peronospora sp.* (pea), *Phoma sp.* (potato), *Alternaria sp*. (cauliflower), *Botryotinia sp.* (brinjal), *Podosphaera sp.* (bitter gourd), *Rhizopus sp.* (okra), and *Ascochyta sp.* (French bean). A detailed morphological description and photomicrographs of a representative isolate are provided. As identification was based solely on morphological criteria and pathogenicity tests were not conducted, the role of these fungi in disease causation has not yet been established; they have merely been identified and recorded as fungi associated with the observed symptoms. These findings provide a preliminary list of the types of fungi associated with post-harvest spoilage and losses in the region, and highlight the need for molecular confirmation and the testing of Koch's postulates in future investigations.

Keywords: Post harvest decay, vegetables, associated fungi, morphological characterization, genus level identification, Nashik district.

INTRODUCTION

Vegetables are vital components of the human diet and agricultural economies worldwide; they provide essential nutrients, vitamins, minerals, and dietary fiber. India is a leading nation in vegetable production;

within the country, Maharashtra and specifically its Nashik district plays a pivotal role due to a favorable subtropical climate and vast expanses of cultivable land. However, due to their high water content and delicate structure, vegetables are highly susceptible to rapid deterioration after harvest (Snowdon 1992). At the international level, post-harvest losses in vegetables are estimated to range from 20% to 40%, and fungi are largely responsible for these losses (Agrios 2005; Smilanick 2004). In India, this loss imposes a massive economic burden and poses a threat to food security.

Nashik district (19.9975° N, 73.7898° E) has emerged as a major region for vegetable production due to its subtropical climate and highly fertile soil. Despite high productivity, the hot and humid climate creates favorable conditions for the growth of various types of fungi in the air, soil, and during storage. The post-monsoon period—characterized by high atmospheric humidity and temperatures ranging between 20 and 30 degrees Celsius—greatly promotes fungal growth. Accurate and proper identification of the fungi responsible for post-harvest decay and rotting is a prerequisite for understanding the primary cause of the disease and for developing effective, region-specific management strategies. Although molecular techniques like ITS region sequencing provide definitive taxonomic resolution, morphological characterization remains a crucial first step—especially in resource-limited settings—for documenting the diversity of cultivable fungi present on symptomatic produce.

The most common fungal species associated with post-harvest vegetable spoilage include *Alternaria, Rhizopus, Botrytis, Fusarium, Aspergillus, Penicillium*, and *Phytophthora* (Sharma et al. 2017; Singh et al. 2016). However, the mere presence of these fungi on diseased tissues does not prove their pathogenicity; fulfilling 'Koch's postulates' is essential to establish a causal relationship. Despite the agricultural significance of Nashik district, the work of systematically documenting the fungi responsible for post-harvest spoilage of vegetables in this region has remained extremely limited in scope. Most available studies focus more on field diseases than on post-harvest pathology; the resulting gap in knowledge hinders the development of targeted research. Therefore, the present study was undertaken to isolate and morphologically characterize fungi obtained from symptomatic post-harvest vegetable samples collected in the central Nashik district, thereby providing baseline survey data to guide future molecular and pathogenicity-based studies.

MATERIALS & METHODS

Study Area and Sample Collection

Samples of ten commonly consumed vegetables were collected: cabbage (*Brassica oleracea var. capitata*), tomato (*Solanum lycopersicum*), pumpkin (*Cucurbita pepo*), pea (*Pisum sativum*), potato (*Solanum tuberosum*), cauliflower (*Brassica oleracea*), eggplant (*Solanum melongena*, bitter gourd (*Momordica charantia*), okra (*Abelmoschus esculentus*), and hyacinth bean (*Lablab purpureus*). Samples exhibiting visible symptoms of post-harvest rot—such as discoloration, water-soaked lesions, soft and dry rot, visible fungal growth, and foul odor—were collected from the premises of the Nashik Rural Agricultural Produce Market Committee (APMC) and three local markets in the central part of Nashik district. Samples were collected during the post-monsoon period (October–December); during this time, atmospheric humidity levels are high, and fungal infestation is generally more prevalent. Approximately 10–15 infected samples of each vegetable were collected, placed in sterilized polythene bags, and appropriately labeled; subsequently, to prevent cross-contamination, these samples were transported to the laboratory within 2–4 hours.

Disease Incidence Calculation

The incidence of disease on each vegetable was calculated as the percentage of samples exhibiting symptoms of a specific type of decay out of the total samples examined.

Surface Sterilization and Isolation of Fungi

Standard Operating Procedures (SOPs) for isolation were followed. Symptomatic tissue fragments (approximately 5 mm²) were excised from the margins of the lesions; subsequently, they were surface-sterilized with a 1% sodium hypochlorite solution for 1–2 minutes, rinsed three times with sterile distilled water, and placed on streptomycin-supplemented Potato Dextrose Agar (PDA) medium. The plates were incubated at a temperature of 25 ± 2°C for 3 to 7 days. To obtain pure cultures from the fungal colonies that developed on the plates, they were sub-cultured onto fresh PDA medium.

Morphological Identification of Fungi

The identity of the pure cultures was verified based on their macroscopic and microscopic morphological characteristics. Macroscopic examination involved noting the colony color (upper and lower surfaces), texture (cottony, woolly, or powdery), colony margin, growth rate, and the presence or absence of exudates or pigments. For microscopic examination, a small portion of mycelium from 3–5-day-old cultures was immersed in 'Lactophenol Cotton Blue' and examined under a compound microscope (at 40× to 100× magnification). The observed characteristics included hyphal septation and branching, reproductive structures (sporangia, sporangiophores, conidiophores, conidia, pycnidia), spore morphology (shape, dimensions, color, septation), and specialized structures (chlamydospores, sclerotia, rhizoids). The isolated samples were classified into genera through a comparative study of standardized mycological keys and descriptions (Barnett & Hunter 1998; Snowdon 1992). As no molecular (DNA barcoding) analysis was conducted, no attempt was made to identify the specimens at the species level; all isolates have been recorded only at the genus level (e.g., Phytophthora sp.). This fungus is said to be associated with the observed symptoms; however, its pathogenicity has not been experimentally verified.

Data Recording and Analysis

Information regarding the extent of the disease and morphological characteristics was presented in the form of tables. Photomicrographs were captured using a microscope and a mobile phone camera.

RESULT

Disease Incidence

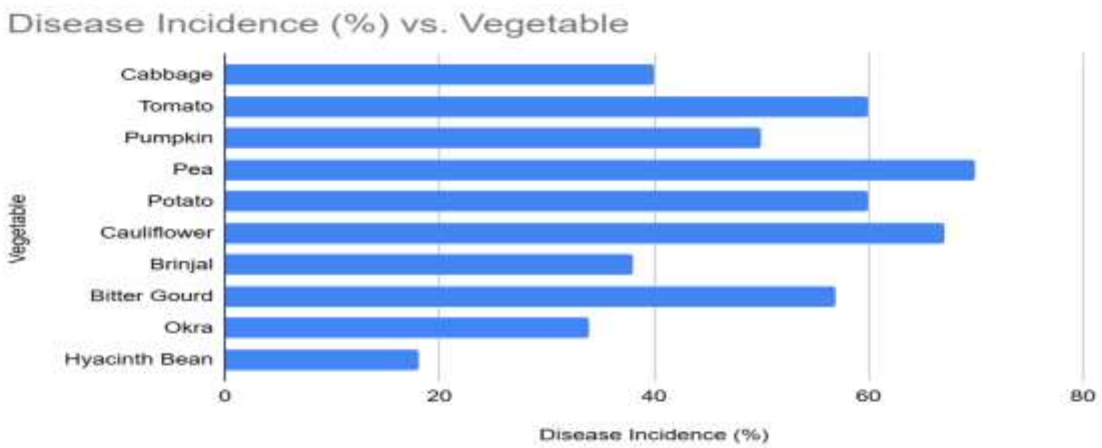

Figure 1: The incidence of the dominant decay symptoms observed on each vegetable is summarized in bar graph. Percent incidence ranged from 18% (hyacinth bean pod spot) to 70% (pea downy mildew like symptoms).

Fungal Isolates and Associated Symptoms

A single, distinct morphotype was consistently isolated from each type of lesion. The identified species and their associated characteristics are presented in Table 1. In the absence of molecular data and pathogenicity tests, these fungi are recorded as organisms associated with the observed lesions, rather than as confirmed causative agents.

Table 1. Post harvest decay symptoms and fungi isolated from major vegetables in Nashik district. Identifications are to genus level only, based on morphological characters.

Vegetable	Disease	Causal Organism	Key Morphological Features	Disease Incidence (%)
Cabbage	Phytophthora like rot	*Phytophthora sp*	Aseptate hyphae; papillate sporangia; oospores	40
Tomato	Phoma like rot	*Phoma sp.*	Pycnidia with conidia; dark, sunken lesions	60
Pumpkin	Cottony Leak	*Pythium sp.*	Coenocytic hyphae; oospores; cottony mycelium	50
Pea	Downy mildew like	*Peronospora sp.*	Branched conidiophores; violet conidia; oospores	70
Potato	Gangrene like	*Phoma sp.*	Pycnidia; 'target spot' and 'devil's thumb-print' lesions	60
Cauliflower	Alternaria like rot	*Alternaria sp.*	Septate hyphae; muriform, beaked conidia	67
Brinjal	Grey Mould like Rot	*Botryotinia sp*.	Gray conidiophores; unicellular conidia; sclerotia	38
Bitter Gourd	Powdery Mildew	*Podosphaera sp.*	Superficial mycelium; barrel-shaped conidia	57
Okra	Rhizopus Rot	*Rhizopus sp.*	Aseptate hyphae; rhizoids; black sporangia	34
Hyacinth Bean	Ascochyta Pod like Spot	*Ascochyta sp.*	Pycnidia; septate hyphae; cylindrical conidia	18

Morphological Descriptions of Isolated Fungi

The observed disease symptoms in the vegetable samples are illustrated in Figure 2 (A–J).

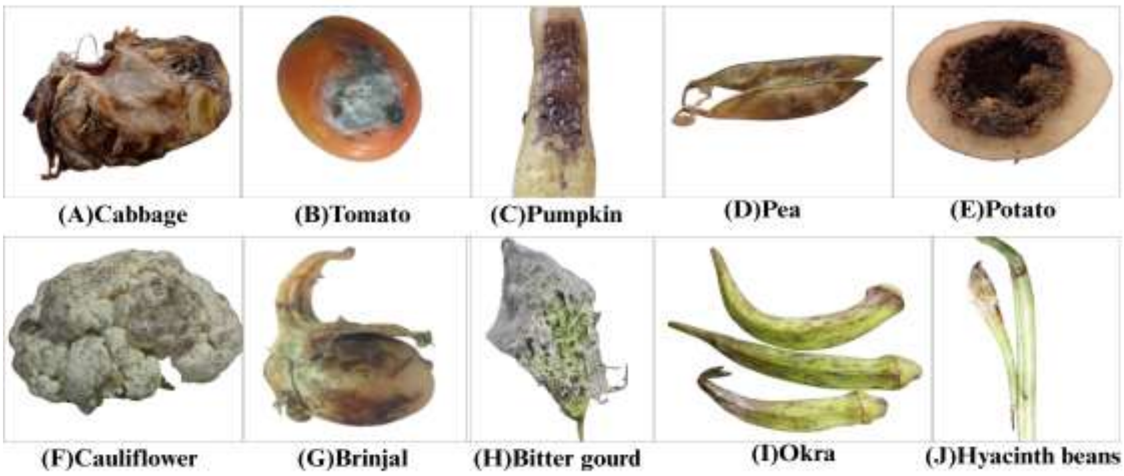

Figure 2: Symptoms of Post Harvest fungal infection in Vegetable Samples collected from Nashik District Maharashtra (A) Cabbage showing Grey blotches on wrapper leaves. (B) Tomato showing firm sunken lesions (C) Pumpkin showing Soft dark lesions (D) Pea showing mealy blotches (E) Potato showing dark sunken lesions (F) Cauliflower showing Black leaf spots (G) Brinjal showing Grey mould lesions (H) Bitter gourd showing white powdery layer (I) Okra showing soft water soaked lesions (J) Hyacinth beans showing reddish brown lesions on pod

Image

The microscopic structures of the three predominant fungal genera are shown in Figure 3.A-C

(A) *Alternaria sps.* **(B) *Rhizopus sps.*** **(C) *Phytophthora sps.***

Figure 3: Photomicrographs of fungal structures observed under light microscope (40×–100×) after lactophenol cotton blue staining. (A) Conidia of *Alternaria sp.* showing multicellular, muriform spores isolated from cauliflower, (B) Sporangiophore bearing sporangium of *Rhizopus sp.* isolated from okra, (C) Sporangial structures of *Phytophthora sp.* showing ovoid to lemon-shaped sporangia isolated from cabbage.

1. Cabbage – Phytophthora like rot (Phytophthora sp.)

Symptoms: Gray, water-soaked spots appear on the outer leaves; the rotting process begins at the stalk and extends to the head, characterized by firm, brown-to-dark-gray tissue and a distinct sour odor.

Morphology: Colonies grown on PDA appeared white to cream-colored and web-like (resembling a spider's web). The hyphae were non-septate and hyaline (4–8 microns in diameter). The sporangia were papillate, ranging from lemon-shaped to ovoid (30–60 × 20–35 microns). Thick-walled oospores (30–40 microns) were present. These characteristics are consistent with the *Phytophthora* species.

2. Tomato – Phoma like rot (Phoma sp.)

Symptoms: Firm, sunken lesions (spots) ranging from dark brown to black in color, primarily found near the stalk end; minute black 'pycnidia' are visible on their surface.

Morphology: Colonies grown on PDA medium appeared gray to olive-colored. The pycnidia were dark-colored, spherical, and 'ostiolate' (possessing an opening). The conidia (spores) were hyaline (colorless), unicellular, and small (3–6 microns). Pycnidial conidiomata and small conidia are characteristic features of the *Phoma* fungus.

3. Pumpkin – Cottony leak (Pythium sp.)

Symptoms: Soft, dark, rapidly spreading lesions exuding copious fluid and emitting a slight marshy odor. The affected area was covered with abundant white, cottony mycelium.

Morphology: The hyphae were non-septate and coenocytic (5–8 microns in diameter). Sporangia were filamentous, and thick-walled, spherical oospores (20–25 microns) were observed. The colonies were fast-growing and cottony in appearance; these characteristics are essential for the identification of *Pythium*.

4. Pea – Downy mildew like (Peronospora sp.)

Symptoms: Yellowish spots appear on the pods, which later turn glossy brown and exhibit greenish-yellow stripes; a white, woolly growth is also visible inside the pods.

Morphology: The conidiophores are branched (150–300 microns) and emerge through the stomata. The conidia are oval (20–30 × 15–20 microns), purple to brown in color, and occur singly. The oospore walls are thick (30–40 microns). These structures are characteristic features of *Peronospora*.

5. Potato – Gangrene like (Phoma sp.)
Symptoms: Small "target spots" or large, dark, sunken lesions ("Devil's thumbprint") found at the site of the wound, with black pycnidia present on their surface.
Morphology: Colonies on PDA ranged from gray to blackish in color. The pycnidia were dark and spherical (100–200 µm). Conidia were hyaline and ellipsoidal (4–6 × 1.5–2 µm). Morphologically, this pycnidial fungus resembles *Phoma.*

6. Cauliflower – Alternaria like rot (Alternaria sp.)
Symptoms: Very dark; spots on leaves and stems appear nearly black—sometimes zonate—and have a velvety texture under humid conditions.
Morphology: Colonies ranged from dark olive-green to black. Conidiophores were dark-colored; conidia were large, muriform (possessing both transverse and longitudinal septa), club-shaped, and often formed chains, measuring 20–50 × 10–20 µm. These characteristics are typical of *Alternaria.*

7. Brinjal – Grey mould like rot (Botryotinia sp.)
Symptoms: Water-soaked, brownish-grey lesions with reddish-purple margins; abundant grey-brown conidial clusters and small black sclerotia were observed under high humidity.
Morphology: Colonies were grey and feathery. Conidiophores were long and branched, bearing clusters of hyaline, unicellular conidia (8–14 × 5–9 µm). Irregular black sclerotia (1–5 mm) formed in older cultures. This isolate belongs to the species Botryotinia (the teleomorph of *Botrytis*).

8. Bitter Gourd – Powdery mildew (Podosphaera sp.)
Symptoms: A white, powdery growth appears on the leaf surface, causing the leaves to turn yellow, then brown, and eventually wither.
Morphology: The mycelium develops superficially, forming a mat-like layer. The conidia are hyaline, barrel-shaped (25–35 × 12–18 microns), and produced in chains. The chasmothecia (cleistothecia) are spherical (70–100 microns) and possess characteristic appendages. These features resemble those of *Podosphaera*.

9. Okra – Rhizopus rot (Rhizopus sp.)
Symptoms: Soft, water-soaked lesions spread rapidly, causing the fruit to burst and leak juice while emitting a sour, fermented odor. A thick, white fungal growth appears, bearing spherical sporangia that transition in color from white to black.
Morphology: The colonies were fast-growing, cottony, and grey-brown in color. The hyphae were non-septate (5–15 microns) and possessed well-developed rhizoids. Large, black sporangia (100–300 microns) filled with sporangiospores (5–10 microns) were borne on sporangiophores (500–2000 microns). This isolate belongs to the *Rhizopus* genus.

10. Hyacinth Bean – Ascochyta like pod spot (Ascochyta sp.)
Symptoms: Irregular reddish-brown lesions on the pods, dark rot at the stalk end, and minute brown pycnidia frequently arranged in concentric circles.
Morphology: Colonies ranged from gray to brown in color. Pycnidia were spherical, dark, and ostiolate (100–150 microns). Conidia were hyaline, cylindrical, and septate (10–18 × 3–5 microns). The presence of pycnidial conidiomata and septate conidia classifies this fungus within the *Ascochyta* genus.

DISCUSSION

This study presents a morphological inventory of fungi consistently isolated based on the characteristic symptoms of post-harvest decay in ten major vegetables from Nashik district. The isolated microorganisms belong to the genera *Phytophthora, Phoma, Pythium, Peronospora, Alternaria, Botryotinia, Podosphaera, Rhizopus*, and *Ascochyta*; this indicates the involvement of various types of fungi (mycoflora) in the spoilage of vegetables under different conditions in the local market. This study revealed significant variations in the incidence of the disease; the prevalence was highest in peas (70%) and cauliflower (67%). This is likely attributable to their delicate tissue structure, high water content, and susceptibility to mechanical injury during handling.

The periodic isolation of morphologically similar fungi from specific decay symptoms is consistent with findings from other post-harvest surveys. For instance, the high prevalence of *Rhizopus* species on various types of vegetables is consistent with their well-established role as aggressive saprophytes and wound invaders in hot and humid climates (Agrios 2005; Smilanick 2004). The recovery of oomycetes such as *Phytophthora* and *Peronospora* from market samples indicates that field infections can persist and even proliferate after harvest, underscoring the need for continuity in pre- and post-harvest disease management.

Critical limitations: identification and causality

The most important limitation of this work is that all identifications made were based solely on morphological criteria. Although each isolate clearly belongs to a well-known species based on observed characteristics, species-level identification was intentionally avoided, as morphological characteristics alone are often insufficient to resolve species groups within genera such as *Phoma*, *Alternaria*, or *Fusarium*. Many of these species often include various cryptic species that can only be identified by DNA sequence analysis, particularly based on the internal transcribed spacer (ITS) region and, if necessary, on protein coding genes such as EF 1α or β tubulin. Therefore, the species-level names mentioned here should be considered a starting point, and any attempt to assign species names without molecular confirmation would be scientifically inaccurate.

A second, and equally serious, limitation is the lack of pathogenicity tests. Koch's hypotheses have not been met; therefore, the relationship between a given fungus and observed symptoms does not confirm causality. These isolated fungi may be primary pathogens, secondary invaders colonizing tissues already damaged by other factors, or necrotic parasites. This is a fundamental distinction, as it determines whether further research should focus on the isolated fungus as a target for control. Consequently, it would be more appropriate to describe the fungi reported here as agents of caries associated with the mycoflora, rather than as confirmed agents.

Comparison with prior work and future directions

The morphological characteristics of the isolates match published descriptions of the corresponding species (Snowden 1992; Ellis 1971; Barnett and Hunter 1998). These findings demonstrate that post-harvest samples can be reliably classified to the species level using standard mycological techniques, providing an important support in regions where molecular diagnosis is not yet possible. However, to progress from a survey of related fungi to the study of disease etiology, future research must include two important elements:

1. Molecular confirmation: Genomic DNA should be extracted from each isolate, and the ITS region (and additional loci if necessary) should be amplified and sequenced. Phylogenetic analysis can then assign distinctions to species or operational taxonomic units with precision that is important for epidemiological and management studies.
2. Pathogenicity testing: It is essential to inoculate each isolate onto healthy,

surface-sterilized vegetable tissues under controlled conditions that simulate the storage environment. Before declaring any fungus as a pathogen, it is mandatory to fulfill Koch's hypotheses, i.e., the appearance of symptoms similar to those found in the field, re-isolation of the inoculated fungus, and its re-identification and verification.

Only after the causal agents have been unambiguously identified and their pathogenicity confirmed can research meaningfully evaluate control measures, whether chemical, biological, physical, and integrated. Management strategies mentioned in the literature (induced resistance, biological control agents, physical treatments, etc.) are promising avenues, but their importance and utility in the local context cannot be determined from current information. Therefore, it would be premature to discuss these tools in detail and so that discussion has been omitted.Molecular and pathogenic characterization of the fungal isolates reported in this survey is a priority for further research.

CONCLUSION

In this survey, the main culturable fungi associated with post-harvest decay symptoms on ten different important vegetables in Nashik district were successfully isolated and characterized at the species level. The species *Phytophthora, Phoma, Pythium, Peronospora, Alternaria, Botryotinia, Podosphere, Rhizopus* and *Ascochyta* were identified based on morphological characteristics, providing a preliminary list of post-harvest fungal flora in the region. However, since identification is limited to the species level and pathogenicity tests have not been performed, none of these fungi can be designated as the causative pathogen. Future research should combine DNA-based species identification and Koch's theories to verify the etiology. The data presented here are a necessary preliminary step towards that goal and will help prioritize isolates for molecular and pathogenic characterization.

Declaration by Authors

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