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Research Article

Fungi Associated with Post-harvest Losses of Multani Mango

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Abstract

The Multani mango, renowned for its exceptional taste and aromatic profile, is a cultivar thriving in the fertile soils of Multan, Pakistan, representing a cherished tropical delicacy. Despite its esteemed status in the region's mango-producing legacy, a formidable challenge arises from its limited shelf life. Fungal pathogens associated with the mango curtail its shelf life and detrimentally impact fruit yield and quality. This study aimed to isolate and identify fungal pathogens linked to Mango anthracnose, stem end rot, side rot, and fruit fly attack symptoms. Ten mango fruits exhibiting symptoms were systematically sampled from three distinct markets in Multan, totalling 30 fruits for each disease symptom category. Isolation and identification outcomes revealed that in mangoes displaying anthracnose symptoms, *Colletotrichum gloeosporoides* was present in 100% of fruit samples, followed by *Aspergillus fumigatus* (72.72%). For mango fruits with stem end rot, *Botryodiplodia theobromae* was identified in 90.90% of samples, followed by *Colletotrichum gloeosporoides* (70%). Fruits exhibiting symptoms of fruit fly attack and side rot contained *Aspergillus flavus* in 99.90% and 88.81% of samples, respectively. These findings underscore the pivotal role of fungal pathogens in the Multani mango's shelf-life predicament, necessitating strategic interventions in cultivation and post-harvest handling. The identification of specific pathogens provides valuable insights for targeted management practices aimed at preserving the quality and extending the shelf life of this esteemed mango cultivar.

Keywords: Multani mango; *Colletotrichum gloeosporoides;* Anthracnose; Fruit fly; shelf life.

Introduction

The global cultivation of fruits is a widespread and fundamental practice, essential to diverse regions (Board, 2005). However, the economic value of fruits encounters a substantial challenge in the form of reduced shelf life attributed to post-harvest diseases (Coates and Johnson, 1997). Despite the recommended daily consumption of 230 g of fruits for a healthy adult, current per capita intake falls below 160 g/day, underscoring a significant gap in fruit consumption patterns (Sánchez-Moreno *et al.*, 2006). With its origins in India and Southeast Asia, Mango has emerged as a pivotal tropical fruit, with cultivation expanding to various corners of the world, encompassing Africa, the

Americas, and the Caribbean(Yadav and Singh, 2017).

In Pakistan's tropical and subtropical regions, mango (*Mangifera indica* L.) holds a prominent agricultural presence, covering an extensive cultivation area of 170.1 ha (Anon., 2012). Ranking second among fruits produced in the country, with an impressive production of 1729.9 thousand tonnes and the fourth-highest exporter, mango plays a crucial role in Pakistan's agricultural landscape (Maqbool et al., 2007; Anonymous, 2012). Globally, Pakistan is fourth in mango production, following India, China, and Thailand (FAO, 2010). Multani mangoes, hailing from Pakistan's fertile region of Multan, boast varieties like Chaunsa, Sindhri, and Anwar Ratol. Recognized for their unique flavours and aromas, these mangoes are locally and globally prized(Naz *et al.*, 2014). The distinct taste of Multani mangoes reflects the rich agricultural heritage of the region. However, the mango industry in Pakistan faces substantial challenges, particularly concerning postharvest losses attributed to diseases and insect pests (Ishaq et al., 2004; Iqbal et al., 2004).

The susceptibility of mango fruits to post-harvest diseases induces physiological alterations conducive to pathogen development, resulting in significant economic losses (Ecker et al., 1996). Effective handling and storage practices at optimal temperatures are crucial for extending the shelf life of mangoes, as storage below 12°C may induce chilling injury in specific cultivars (Medlicott, 2003). Post-harvest treatments, including fungicidal dips, have shown relative efficacy in safeguarding mangoes against pathogenic infections (Swart et al., 2002).

Pakistan boasts a diverse range of mango varieties, including Sindhri, Chaunsa, Saharni, Alphonso, Langra, Anwar Ratol, Dosehri, Fajri, Fazli, and Neelam, with some varieties finding markets in the USA, EU, Middle East (Kuwait/Saudi Arabia/UAE), and South-East Asia (Malaysia/Singapore, China-Hong Kong). Notable varieties like Sindhri, Sammar Bahist, and Chaunsa hold significant export value (Amin & Hanif, 2002; Nafees et al., 2013).

Stem end rot, a significant post-harvest affliction, is caused by various fungi, including *Botryodiplodia theobromae*, manageable through controlled dipping solutions. Studies in Taiwan identified Phomopsis mangiferae as a causative agent for stem end rot in cultivars such as Kiet and Irwin (Ko et al., 2009). Other reported pathogens associated with stem end rot include *Dothiorella dominicana, Colletotrichum gloeosporioides*, and *Aspergillus niger* (Gangolly et al., 1957; Kurup et al., 1967; Pathak & Sarivastava, 1968; Vock, 1978). Anthracnose poses formidable challenges for participants in the global trade of mangoes, as the pathogen infiltrates the fruit through natural openings and wounds, particularly during inflorescence and within the xylem. The pathogen persists asymptomatically in the stem tissue until the fruit reaches ripening stages (Syed, Lodhi, Rajput, Kumbhar, & Khanzada, 2017).

In addition to anthracnose and stem end rot, mango fruits are susceptible to other dark grey to black rots, typically rounded, and slightly sunken manifestations (Hamd Meer *et al.*, 2013). While not deeply penetrating the flesh, these rots contribute to the overall post-harvest challenges faced in mango preservation. Complicating matters is that the fruit fly occasionally punctures mango fruits, potentially introducing spores of the anthracnose-causing fungus, further complicating the management of post-harvest diseases in mangoes (Arauz, 2000).

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Identifying the fungi, associated with mango, is imperative for devising effective control strategies. So, the objective of this study was to identify fungal pathogens which were associated to Mango anthracnose, stem end rot, side rot, and fruit fly attack symptoms in Multani mangoes.

Methodology

Samples collection and categorization

The specimens were meticulously transported to the Mango Pathology Laboratory at CDRI, employing appropriate packaging with paper and further encapsulation within cardboard. Subsequent to visual inspection and symptomatology assessment, the samples were classified into four distinct categories: 1) Mangoes displaying anthracnose symptoms, 2) Mangoes exhibiting symptoms of stem end rot, 3) Mangoes manifesting side rot symptoms, and 4) Mangoes subjected to fruit fly infestation. Within each category, fruits exhibiting severe, moderate, and minor symptoms were selected for in-depth investigations, while any fruit displaying more than 50% signs of decay were excluded from the study. In addition, a subset of healthy fruits was included for latent infection analysis, and these were stored under refrigerated conditions, following the protocol outlined by Zainab and Shinkafi (2016).

Mango sample analysis

Direct slide technique

In this methodological approach, samples enclosed in carefully packaged paper were employed to investigate fungal growth on live specimens. The aim was to identify distinctive signs and structures that provide preliminary insights into the factors contributing to infection. A representative set of 10 fruits for each symptom category earmarked for study was systematically selected. The procedure involved applying a strip of adhesive tape, approximately 1.5 inches in length, onto the infected area of each fruit. Subsequently, the tape was delicately removed and carefully placed on a glass slide containing a water droplet. Systematic observations were conducted under a compound microscope at magnifications of 10X, 20X, 40X, and 100X, enabling the discernment of potential causes of infection. Each sample underwent meticulous examination across ten slides, ensuring a robust dataset for subsequent detailed analyses.

Standard isolation with slight modification

Prior to analysis, all fruit samples underwent a cleansing process achieved through a thoroughly spray of methylated spirit. Subsequently, the samples were precisely sectioned into 5mm pieces and stirred on paper for drying. These sample fragments were then transferred to 90 mm petri dishes containing Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA). Unlike conventional methods involving bleach or 1% Clorox washing, our approach utilized initial inoculation on PDA, with subsequent fungal purification on MEA. This rigorous process was applied to each category, with a total of 4 Petri plates were used for each sample (Adjou, René, Edwige, & Soumanou, 2021). The fungal colonies from MEA were then picked with a sterile needle and examined under the compound microscope.

Results and Discussion

Identification through direct slide methods

With the direct method, all fruit samples containing anthracnose symptoms or fruit fly

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damage symptoms had mixed colonies of *Aspergillus's* Sp., *Alternaria* Sp and *Colletotrichum* sp. While fruit having SER symptoms with the direct method only *Alternaria* sp. and *Botryodiplodia* sp. were seen under the microscope. Side rots shown three species of *Aspergillus.*

Isolations through standard procedure with slight modification

Mixed fungal colonies were observed because of isolation procedures employing a standard protocol with slight modifications. Subsequent isolation and identification analyses elucidated that among mangoes manifesting anthracnose symptoms, *Colletotrichum gloeosporoides* was detected in 100% of fruit samples, with *Aspergillus fumigatus* identified in 72.72% of cases. Mango fruits afflicted with stem end rot predominantly harbored *Botryodiplodia theobromae*, identified in 90.90% of samples, followed by *Colletotrichum gloeosporoides* at 70%. Additionally, fruits exhibiting symptoms indicative of fruit fly infestation and side rot contained *Aspergillus flavus* in 99.90% and 88.81% of samples, respectively (Table 1 and 2).

Table 1. Fungi isolated from mango showing anthracnose and stem end rot symptoms.

Category	Code No.	C. g.	Alt	Bot	A. fl.	A. f.	A. n.
Anthracnose	An-1	+	+	-	-	+	+
	An-2	+	+	-	-	-	+
	An-3	+	_	-	+	+	+
	An-4	+	+	-	+	+	+
	An-5	+	_	-	+	+	+
	An-6	+	_	-	+	-	+
	An-7	+	+	-	-	+	+
	An-8	+	-	-	+	+	+
	An-9	+	+	-	-	-	+
	An-10	+	+	+	_	+	+
	An-3	+	-	-	+	+	+
Stem End Rot	SER-1	+	-	+	+	-	+
	SER-2	+	-	+	+	-	+
	SER-3	+	+	+	+	-	+
	SER-4	-	-	+	-	+	-
	SER-5	-	+	+	-	+	-
	SER-6	+	+	+	-	+	-
	SER-7	+	+	+	-	+	-
	SER-8	+	-	+	+	-	+
	SER-9	+	-	+	-	+	+
	SER-10	+	+	+	-	-	-

Where C.g. *Colletotrichum gloeosporoides*; Alt *Alternaria; Botryodiplodia theobromae;* A. fl. *Aspergillus flavus;* A. f. *Aspergillus fumigates;* A. n. *Aspergillus niger.*

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Table 2. Fungi isolated from mango showing side rot and stem fruit fly attack symptoms.

Category	Code No.	C. g.	Alt	Bot	A. fl.	A. f.	A. n.
Side Rot	SR-1	-	+	-	+	+	+
	SR-2	-	+	+	-	+	+
	SR-3	+	+	+	-	+	+
	SR-4	-	-	+	-	+	+
	SR-5	-	+	-	+	+	+
	SR-6	+	+	+	+	+	+
	SR-7	+	-	+	+	+	+
	SR-8	+	+	+	-	+	+
	SR-9	-	+	+	+	-	+
	SR-10	+	+	-	-	+	-
Fruit Fly	FF-1	+	-	-	+	+	-
	FF-2	-	-	-	+	+	-
	FF-3	-	-	-	+	+	-
	FF-4	+	-	-	+	+	-
	FF-5	+	-	-	-	+	+
	FF-6	+	-	-	-	+	+
	FF-7	+	+	-	-	+	-
	FF-8	-	-	-	+	+	-
	FF-9	+	-	-	+	+	+
	FF-10	-	-	-	-	+	-

C.g. *Colletotrichum gloeosporoides*; Alt *Alternaria; Botryodiplodia theobromae;* A. fl. *Aspergillus flavus;* A. f. *Aspergillus fumigates;* A. n. *Aspergillus Niger*

Colletotrichum gloeosporoides exhibited ubiquitous presence in all examined isolates, albeit with varying frequencies. This widespread occurrence could be attributed to its rapid growth rate. Conversely, *Botryodiplodia theobromae*, a less frequently identified fungus, suggested a distinct pattern of presence. Its infrequent detection implied its prevalence within the orchards, particularly on trees and in pedicels, potentially contributing to stem end rot. However, the limited transfer of this fungus to the fruits suggested a preference for non-fleshy substrates. In contrast, various *Aspergillus* species, including *flavus, fumigatus,* and *Niger*, were consistently identified across all categories associated with physical damage. The observed prevalence of *Aspergillus* sp. in these scenarios suggests its proclivity to invade fruits under conditions of external injury.

Aspergillus niger and *Colletotrichum gloeosporoides* were ubiquitously present in all examined mango samples, signifying their widespread prevalence and potential involvement in the initiation of infection. The well-established association of *Botryodiplodia theobromae* with stem end rot, as documented by Meah et al. (1991), underscores its specific contribution to this disease manifestation. *Aspergillus fumigatus* exhibited consistent presence across various symptomatic conditions, suggesting its opportunistic nature in colonizing weakened fruit. The concurrent occurrence of multiple fungi within each symptom category implies potential interactions among them. For instance, the observed softening effect of *Colletotrichum gloeosporoides* on the fruit

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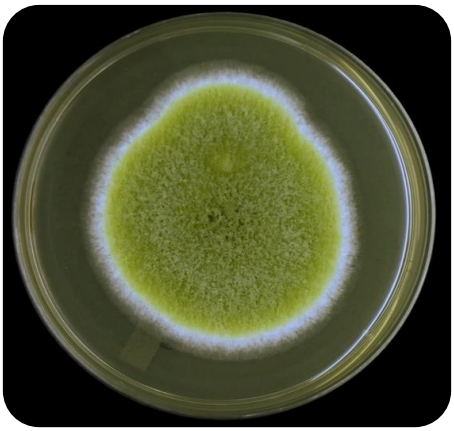
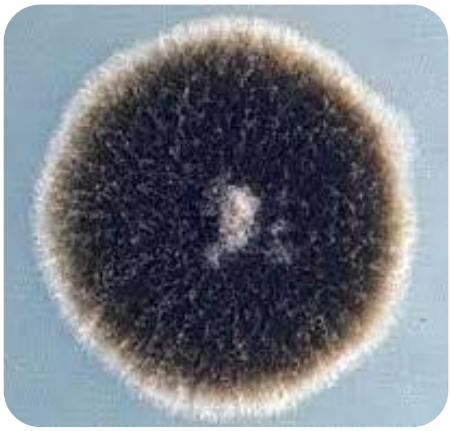
(Muirhead & Gratitude, 1986) may render it more susceptible to secondary colonization by Aspergillus spp. or *Botryodiplodia theobromae*.



Aspergillus flavus

Aspergillus fumigatus

Aspergillus Niger



Alternaria alternate

Subsequent investigations could explore the synergistic or antagonistic effects of these fungi on mango spoilage. The specific association of certain fungi with distinct symptoms, such as *Botryodiplodia theobromae* with stem end rot, suggests their prospective utility as diagnostic markers for early disease detection. This finding aligns with prior investigations by several researchers, implicating Colletotrichum gloeosporioides as the primary causative agent of mango diseases (Than et al., 2008; Kim et al., 2008; Sangeetha and Rawal, 2009; Jayasinghe and Fernando, 2009). Earlier reports, including those by Johnson (2008), have implicated various fungal species worldwide in postharvest diseases leading to fruit rot during ripening. Okereke et al. (2010) similarly isolated these fungal species from infected mangoes in their study. Maqsood et al. (2014) identified *C. gloeosporioides, Lasiodiplodia theobromae, Alternaria alternata, Aspergillus niger,* and *Dothiorella domonicana* in Sindhri mango fruits, with *C. gloeosporioides* being the predominant species in Pakistan. In India, Rajmane and Korekar (2016) reported *Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, Botryodiplodia theobromae, C. gloeosporioides, Penicillium chrysogenum*, and *R. stolonifer* associated with mango fruit spoilage. Abdullah et al. (2016) found *Alternaria alternata, A. aculeatus, Aspergillus flavus, A. japonicas, A. niger, A. parasiticus, Eurotium amstelodami, Mucor circinelloides, Penicillium viridicatum, Rhizopus arrhizus, Trichoderma koningii, T. harzianum*, and *Verticillium tenerum*

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associated with postharvest rot disease of mango in Yemen. All identified fungi in these studies were implicated as pathogens causing deterioration in mango fruits.

Conflict of Interest

The authors have not declared any conflict of interest.

Authors Contributions

All the authors contributed equally to the manuscript.

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