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ISOLATION, IDENTIFICATION, AND PATHOGENICITY OF FUNGAL PATHOGENS ASSOCIATED WITH POSTHARVEST SPOILAGE OF APPLE (*PYRUS MALUS* L.) IN NAVI MUMBAI MARKET

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ABSTRACT

Apple (*Pyrus malus L.*) is a widely cultivated fruit globally and in India, but significant postharvest spoilage has been reported under market storage conditions, particularly in the APMC Fruit Market of Vashi, Navi Mumbai. The current study conducted over two consecutive seasons from January to February 2025 and January to February 2026. Regular inspections revealed frequent rotting, and eleven fungal pathogens were isolated from decayed fruits using Czapek's Dox Agar. They were *Colletotrichum acutatum, Venturia inaequalis, Monilinia fructicola, Botrytis cinerea, Alternaria alternata, Aspergillus fumigates, A. flavus, Sclerotina fructigena, Rhizopus stolonifer, Mucor piriformis,* and *Penicillium expansum.* Among them, *Penicillium expansum* showed the highest incidence (90%), while *Monilinia fructicola* had the lowest (30%). Spoilage was more severe in humid conditions and in fruits with surface injuries, and pathogenicity tests confirmed that all isolated fungi could infect healthy apples.

KEYWORDS: Apple fruits, postharvest, isolation, fungal pathogens, Pathogenicity, spoilage.

INTRODUCTION

Fruits are essential components of the human diet, providing vital nutrients such as vitamins and minerals that support overall health. However, postharvest diseases, including fruit scab and decay, lead to significant economic losses. Apples (*Pyrus malus* L.), rich in sugars and nutrients with a naturally low pH, are particularly vulnerable to fungal infections that cause rapid spoilage (Singh and Sharma, 2007). The global consumption of fruits and vegetables has increased substantially in recent decades (Barth *et. al.,* 2009), further emphasizing the importance of minimizing postharvest losses. Fungal contamination often occurs during handling, transportation, and storage, reducing both the quality and market value of fruits (Arya, 2004).

Apple, a pomaceous fruit belonging to the family Rosaceae, is among the most widely cultivated and consumed fruits worldwide. Postharvest apples, especially varieties like Red Delicious, are highly susceptible to infection by various fungal pathogens such as *Colletotrichum acutatum*, *Venturia inaequalis*, *Monilinia fructicola*, *Botrytis cinerea*, *Alternaria alternata*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Sclerotinia fructigena*, *Rhizopus stolonifer*, *Mucor piriformis*, and *Penicillium expansum*. Among these, *Penicillium expansum* is frequently reported as the most dominant species in storage facilities and fruit markets, including the APMC Fruit Market in Vashi, Navi Mumbai. It is estimated that 20–25% of harvested fruits are lost due to postharvest pathogens, even in developed countries (Al-Hindi *et.al.* 2011). Studies on other fruits, such as *Carica papaya*, *Citrus sinensis*, *Ananas comosus* and *Lycopersicon esculentum*, also highlight the widespread impact of fungal spoilage (Akinmusire, 2011). Since infection can spread rapidly from a single contaminated fruit to others during storage and transport, understanding the causative agents of decay is crucial (Jay, 2003). Therefore, the present study aims to identify fungal pathogens responsible for postharvest spoilage of apples in the APMC Fruit Market of Vashi, Navi Mumbai.

MATERIALS AND METHODS

Apple fruit samples were collected from various storage facilities in the APMC Fruit Market, Vashi, Navi Mumbai. Infected fruits showing visible signs of decay were carefully examined, while healthy and diseased samples were stored separately in clean polyethylene bags and transported to the Research Laboratory, Department of Botany, K. V. Pendharkar College, Dombivli, for further analysis.

Isolation of fungal pathogens associated with diseased fruits was performed according to standard procedures (Aneza, 2003). Small portions of infected tissue were surface-sterilized with a 0.01% mercuric chloride ($HgCl_2$) solution and thoroughly rinsed with sterile distilled water. The sterilized segments were then aseptically transferred onto Czapek's Dox Agar medium in Petri plates and incubated at 27 ± 2 °C for seven days, with regular monitoring for fungal growth. Emerging colonies were sub-cultured onto agar slants to obtain pure cultures for further study. Identification of fungal isolates was performed using microscopic examination based on morphological characteristics such as hyphal structure, septation, and spore formation, following standard taxonomic keys (Rangaswami and Mahadevan, 1998; Agrios, 2008).

Pathogenicity of the isolated fungi was confirmed using Koch's postulates. A small incision was made on healthy apple fruits, and a loopful of fungal culture was inoculated aseptically. The inoculated fruits were wrapped in cellophane bags to maintain humidity and observed periodically for symptom development. Fungi were re-isolated from infected tissues to confirm their identity.

The frequency of occurrence of each fungal species associated with postharvest decay was calculated using the formula as per Van der Plank (1963).

$$F = \frac{\text{Total number of infected fruits}}{\text{Total number of fruits}} \times 100$$

Where **F** represents the percentage frequency of occurrence.

RESULTS AND DISCUSSION

Eleven fungal pathogens were isolated from infected postharvest apple fruits collected periodically from various storage facilities in the APMC fruit market in Vashi, Navi Mumbai. The fungal species identified were *Colletotrichum acutatum, Venturia inaequalis, Monilinia fructicola, Botrytis cinerea, Alternaria alternata, Aspergillus fumigatus, Aspergillus flavus, Sclerotinia fructigena, Rhizopus stolonifer, Mucor piriformis,* and *Penicillium expansum*. The percentage frequency of occurrence (Table 1) revealed that *P. expansum* had the highest incidence (90%), followed by *A. fumigatus* and *A. flavus* (70% each), *M. piriformis* (60%), and *C. acutatum* and *B. cinerea* (50% each). *V. inaequalis, A. alternata, S. fructigena,* and *R. stolonifer* each showed an occurrence rate of 40%, while *M. fructicola* recorded the lowest frequency (30%). These findings indicate that *P. expansum, A. fumigatus,* and *A. flavus* are

the predominant fungi responsible for post-harvest decay of apple fruits in storage and market conditions.

Pathogenicity tests (Table 2) confirmed that all isolated fungal species were capable of inducing rot in apple fruits. The symptoms observed in artificially inoculated fruits were consistent with those recorded during initial isolation and identification, based on morphological, colonial, and microscopic characteristics. Infected fruits exhibited slight discoloration, tissue softening, and progressive decay. Re-isolation of the pathogens from infected fruits yielded the same organisms, thereby fulfilling Koch's postulates and confirming their pathogenic role in fruit spoilage. Each fungal species produced distinct rot symptoms, indicating variability in infection patterns and disease expression.

Similar findings have been reported by Akintobi *et a*l. (2011), who isolated nine fungal species from pawpaw fruits, of which five were significantly associated with fruit decay in both ripe and unripe samples. The fungal species identified in the present study *C. acutatum, V. inaequalis, M. fructicola, B. cinerea, A. alternata, A. fumigatus, A. flavus, S. fructigena, R. stolonifer, M. piriformis,* and *P. expansum*, are widely recognized as common agents of post-harvest spoilage in fruits and vegetables (Booth, 1976; Amadi and Oso, 1996; Amadi, 2009; Oyetunji *et al*., 2012; Amadi *et al*., 2014).

Post-harvest contamination of agricultural produce is influenced by multiple factors, including pre-harvest infections in the field, handling practices during harvesting, and methods of packaging, transportation, and storage. The variation in fungal load observed in this study may be attributed to differences in sanitation levels and environmental conditions within storage facilities and market areas. Mechanical injuries or wounds on fruits serve as major entry points for pathogens, thereby increasing susceptibility to microbial invasion during transit and storage.

Pathogenicity testing remains a critical criterion for establishing the causal relationship between fungal isolates and disease development. In this study, all isolates were confirmed to be pathogenic on apple fruits, supporting earlier reports by Peter *et al*. (2002), Amadi *et al*. (2009), and Renu and Lal (2009), which identified *Fusarium* and *Aspergillus* species as major causative agents of rot in fruits such as watermelon, carrot, and guava. Furthermore, the dominance of *P. expansum* (90% prevalence) highlights its high virulence and significant role in post-harvest apple spoilage. Comparable observations have been made by Mathew *et*

al. (2010), who reported *Aspergillus niger* and *Rhizopus stolonifer* as major pathogens in post-harvest diseases of guava.

CONCLUSION

Apple fruits are highly susceptible to mechanical injury, which significantly predisposes them to microbial infection and subsequent spoilage. Therefore, careful attention must be given during harvesting, handling, packaging, and transportation to minimize physical damage such as bruising, cuts, and abrasions. Reducing such injuries can limit entry points for pathogenic microorganisms, thereby extending the shelf life of fruits and maintaining their quality for longer, particularly during the marketing season.

Furthermore, proper sanitation and maintenance of storage facilities are essential to reduce the accumulation and spread of fungal inoculum. Contaminants originating from the field can persist and proliferate under favourable storage conditions, increasing the risk of postharvest decay. Regular cleaning, adequate ventilation, and appropriate temperature and humidity control in storage environments can significantly lower microbial load. Implementing these preventive measures will contribute to minimizing postharvest losses and improving the overall availability and marketability of apple fruits.

Table 1: Percentage frequency of fungal pathogens isolated from the infested apple Fruits.

Fruit sample	Total number of samples	Infected fruits	Fungal Pathogens	Frequency (%)
Pyrus malus	10	05	*Colletotrichum acutatum*	50
	10	04	*Venturia inaequalis*	40
	10	03	*Monilinia fructicola*	30
	10	05	*Botrytis cinerea*	50
	10	04	*Alternaria alternata*	40
	10	07	*Aspergillus fumigates*	70
	10	07	*Aspergillus flavus*	70
	10	04	*Sclerotina fructigena*	40
	10	04	*Rhizopus stolonifer*	40
	10	06	*Mucor piriformis*	60
	10	09	*Penicillium expansum*	90

Table 2: Pathogenicity Test.

Sr. No.	Fungal Pathogens	Fruit inoculated	Pathogenicity Test
1.	*Colletotrichum acutatum*	*Pyrus malus*	+
2.	*Ventur iainaequalis*		+
3.	*Monilinia fructicola*		+
4.	*Botrytis cinerea*		+
5.	*Alternaria alternata*		+
6.	*Aspergillus fumigates*		+
7.	*Aspergillus flavus*		+
8.	*Sclerotina fructigena*		+
9.	*Rhizopus stolonifer*		+
10.	*Mucor piriformis*		+
11.	*Penicillium expansum*		+

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