INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
24–0375/2025/33:330505
http://www.fspublishers.org

Full Length Article

Prevalence of Post-harvest Fungal Pathogens of Apples in Nagar Valley, Gilgit Baltistan, Pakistan

Johar Ali[1], Aqleem Abbas[1*], Waqar Hussain[3], Saif-Ud-Din[2], Masum Haider[3] and Abdul Razaq[3]
[1]*Department of Agriculture and Food Technology, Karakoram International University, Gilgit-Baltistan, Pakistan*
[2]*Department of Animal Sciences, Karakoram International University, Gilgit-Baltistan, Pakistan*
[3]*Department of Plant Sciences, Karakoram International University, Gilgit-Baltistan, Pakistan*
*For correspondence: aqlpath@gmail.com
Received 26 August 2024; Accepted 26 December 2024; Published online 02 March 2025

Editor: Arshad Javaid

Abstract

Fungal pathogens have posed a serious problem in post-harvest losses of apples, seriously reducing their quantity, quality, and marketability. This infection, in addition to reducing aesthetic and nutritional value, leads to a related economic loss during the storage and transportation period. However, there is a lack of studies on the fungal pathogen associated with apples of Nagar Valley, Gilgit Baltistan. The present study determines the occurrence of different fungal species in three local apple varieties: Sheribil, Gorman, and Golden. *Aspergillus* spp. were the most predominant fungal group, particularly *Aspergillus niger*, accounting for 51% of the total isolates, dominating in the Golden variety and followed by Sherbil and Golden. *Penicillium* spp. were the second most frequent and accounted for 26.8% of the total fungal isolates, with a maximum frequency noted in the Golden variety. Other fungi, like *Mucor* spp., *Alternaria* spp., *Botrytis* spp. and *Rhizopus* spp. were also isolated but in low frequencies. The present study found high fungal diversity in local apple varieties in Nagar Valley and their susceptibility to post-harvest fungal pathogens. Dominant species of fungi and their prevalence across various apple varieties can help to improve shelf-life and quality in locally cultivated apples. This monitoring and control of fungal pathogens in post-harvest systems should be the focus in each of the studied apple varieties in Nagar Valley.

Keywords: Post-harvest fungi; Apple varieties; Fungal diversity; *Aspergillus* spp.; *Penicillium* spp.; Storage management

Introduction

Apples (*Malus domestica* L.) have been cultivated in the mountainous region of Gilgit Baltistan over a long period (Mehdi *et al.* 2020; Nyamende *et al.* 2021). It lies amidst the mighty ranges of the Himalayas and Karakoram, holding an unexplored treasure of apple diversity. The ecosystem—climate and soils can be said to be favorable for growing apples. These types of apples, produced from orchards located across Gilgit Baltistan, are not only a lifeline regarding the local economy but have also begun to be recognized for their taste and quality. Gilgit Baltistan, with its diversity in agro-climatic conditions, is labeled one of the most conducive places for apple cultivation (Zambounis *et al.* 2020). Notably, these orchards located specifically in the Nagar area produce a significant volume of apples as part of Pakistan's total apple production (Abbas *et al.* 2023). The peculiar geography and climate of Gilgit Baltistan, including cold winters and warm summers, provide suitable growth conditions for the cultivation of crisp apples with a sweet and refreshing taste. The beautiful Nagar Valley, an apple orchard area, lies to the north of Gilgit on the Karakoram Highway. The valley is renowned for the production of diverse apple varieties, such as Sheribil, Gorman and Golden all of them containing unique tastes. Demand for Gilgit Baltistan apples has been growing locally and in national markets. Several of these apples are either con-sumed fresh and/or used in the manufacture of pies, jams, and juices. Apples from Gilgit Baltistan are full of flavor, and the nutrient-enriched values of the apples make them a preferred product for health-conscious consumers. Like any other agri-cultural produce, the apples in Gilgit Baltistan have their challenges, with the greatest being in the post-harvest handling stage. The apples are taken from villages in Gilgit Baltistan to far-markets for sales and consumption. Being perishable, various stages of apple transportation, marketing, storage, consumption and marketing highlight the need for careful handling. Because apples are succulent fruits, more often they become easily injured, undergo enzymatic actions, and show sensitivity

To cite this paper: Ali J, A Abbas, W Hussain, Saif-Ud-Din, M Haider, A Razaq (2025). Prevalence of post-harvest fungal pathogens of apples in Nagar Valley, Gilgit Baltistan, Pakistan. *Intl J Agric Biol* 33:330505. https://doi.org/10.17957/IJAB/15.2310

even to fungal attacks, singly or in combination (Pagès *et al.* 2020). Physical injuries can lead to increased evaporation from the fruit skin surface (Khadiri *et al.* 2024), resulting in moisture loss, making the injured fruits more susceptible to microbial attacks, especially through the activity of fungus that causes disease. Diseased fruits have been observed to undergo more rapid respiration than their healthy counterparts (Argenta *et al.* 2021). Hence, it is important to develop effective strategies to uphold apple fruits' quality and shelf life throughout the supply chain. Apples face fungal contaminations at the storage and transport stages. Researchers have lately proved that apples are susceptible to postharvest decay by the action of different fungal species, with just a limited number causing commercial losses (Hasan *et al.* 2022). The genotype of the apple determines the chances of fungal decay, pre-and post-harvest managing practices, and environmental factors. Fungi are the most commonly occurring organisms associated with post-harvest infections of apples and other crops (Khan and Javaid 2021, 2022, 2023). Latent infections are introduced before harvest or infection through wounds before or after harvesting. The former happens through perfect fruit tissue or natural skin openings like crack marks and lenticels, and the latter through wounds caused by insects, hail, physiological disorders, and mechanical factors. Several fungi include *Botrytis* spp., *Penicillium* spp., *Mucor* spp., *Monilia* spp. and *Aspergillus* spp. play a major role in apples' post-harvest decay since these fungi infect the fruit only through wounded tissues (Singh and Aravind 2021). On the other hand, other fungi, especially those of the *Colletotrichum* spp. and *Alternaria* spp., may cause latent infection. These complexities need to be understood to formulate proper strategies that would help to minimize the amount of post-harvest losses and to enhance the qualitative and quantitative improvement of apple production (Nyamende *et al.* 2021). These fungi have been largely recognized due to their involvement in the decay and rot of apples. Moreover, *Aspergillus* spp. and *Penicillium* spp. have been reported to cause potential infectious or allergic threats to the consumers of apples (Xu 2022). The enormous diversity of fungi linked to post-harvest losses of apples underlines the complexity of these post-harvest diseases in this popular fruit. Therefore, control of these fungal pathogens is important to maintain the quality and safety of apple produce. Most of these fungal pathogens infect fruits through wounds or at the time of flowering, remain latent, and express themselves during storage. It is not a challenge for apple yield reduction only, but fruit quality is likely to get compromised due to the production of mycotoxins by these pathogens. For this reason, to manage or maintain postharvest losses to a minimum, apple producers in Gilgit Baltistan use multiple controls. Commonly, fungicide is applied and supplemented with cultural practices like cleaning and sanitation by removing debris and infected fruits and reducing handling and wounding during the harvest and most importantly, during the storage of fruits. However, proper records of fungal pathogens responsible for post-harvest losses in apples in Gilgit Baltistan are not available. Hence, the present study is conducted with the objectives; (1) To isolate and identify the fungal pathogen responsible for post-harvest losses in apples, (2) To determine the frequency of occurrence of various fungi associated with apples.

Materials and Methods

Samples and study area

The apple samples used in this study were collected from three distinct varieties: Gorman, Golden, and Sheribal as shown in Fig. 1. Sheribal is yellow, sweet and juicy and known for its balanced flavor and crisp texture. Golden is characterized by its excellent taste, golden greenish skin colour, sweetness, and rich aroma. Gorman is reddish and has sweetness, juiciness and an overall appealing taste. Thirty apple samples were collected from the Nagar Valley, Gilgit Baltistan, during November and December 2023. A total of 10 apples per sample were selected, with each apple showing visible lesions or signs of spoilage.

Isolation and identification of post-harvest fungi

The collected samples were transported to the Plant Sciences Laboratory for fungal isolation. The apples were incubated in cardboard boxes lined with moist paper towels to maintain high humidity, with the boxes covered to create conducive environment for pathogen growth. Incubation was carried out at room temperature (25°C) for two weeks. After two weeks, the fungal-infected apples were cut from the advancing edges of the lesion with a sterilized blade. The cut pieces of the lesion were disinfected with 75% ethyl alcohol and washed with sterilized distilled water. After washing, a 0.5 g cut piece was homogenized in 4.5 mL sterilized distilled water using a sterile glass rod in a test tube and a drop of homogenate was placed in the center of potato dextrose agar (PDA) containing ampicillin (0.5 g L^{-1}) to inhibit bacterial growth. The Petri dishes were then incubated at 25°C for 5 days, and the frequency of occurrence of various fungi was calculated. The colonies were transferred to PDA media to obtain a pure culture. The fungal colonies were identified by colony morphology and morphological keys as described previously (Klich 2002). The fungal isolates were maintained at 4°C.

Statistical analysis

The frequency of occurrence of various fungi associated with apples was calculated using the following formula (Głos *et al.* 2022):

$$Frequency\ (\%) = \frac{Number\ of\ occurrences\ of\ a\ specific\ isolate}{Total\ number\ of\ isolates\ in\ all\ samples} \times 100.$$

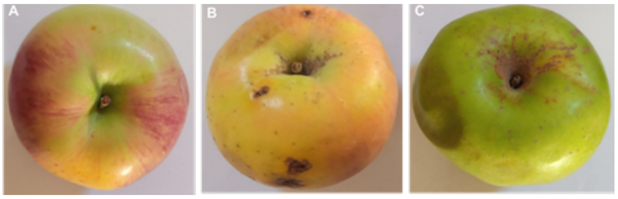

Fig. 1: Three local apple varieties of apples from Nagar Valley

Table 1: Frequency of occurrence of fungal isolates in three local varieties of apples

	Frequency of occurrence (%)			
Fungal spp.	Sheribil	Gorman	Golden	Total
Penicillium spp.	9.9	3.5	13.4	26.8
Aspergillus niger	9.2	4.2	16.2	29.6
Mucor	3.5	2.8	6.3	12.7
Alternaria	1.4	0	3.5	4.9
Aspergillus flavus	4.9	2.1	7.7	14.8
Aspergillus spp.	2.8	0.7	2.8	6.3
Rhizopus spp.	1.4	0.7	2.1	4.2
Botrytis spp.	0	0	0.7	0.7
Total	33.1	14.1	50	100

Table 2: Frequency of occurrence of fungal general associated with apples

Fungal Genus	Total Isolates	Frequency of occurrence (%)
Aspergillus spp.	73	51.4
Penicillium spp.	38	26.8
Mucor spp.	18	12.7
Alternaria spp.	7	4.9
Rhizopus spp.	6	4.2
Botrytis spp.	1	0.70
Total	142	100

Results

Frequency of occurrence

The frequency of occurrence of various fungal species in three local apple varieties, Sheribil, Gorman and Golden is shown in Table 1. *Aspergillus* spp. emerged as the dominant fungal group, appearing in all three varieties. It was most frequent in Sheribil, where it accounted for 2.8% of the isolates and, in total contributed 6.3% of the overall fungal population. *Aspergillus niger* was also prevalent, particularly in Golden, where it represented 16.2% of the isolates and 29.6% across all varieties. *Penicillium* spp. was distributed across all varieties, with the highest frequency in Golden (13.4%), making up 26.8% of the total isolates. *Mucor* was found in all varieties, with the highest number in Sheribil (3.5%), and comprised 12.7% of the total fungal isolates. *A. flavus* was most common in Golden, contributing 7.7% of the isolates and 14.8% of the total. *Alternaria* was the least frequent, with 4.9% of the isolates predominantly found in Golden (3.5%). *Rhizopus* spp. was present in all varieties, contributing 4.2% of the total isolates. *Botrytis* spp. was very rare, with only one isolate (0.7%) observed in Golden. Golden exhibited the highest fungal diversity among the three varieties, accounting for 50.0% of the total isolates. This was followed by Sheribil, which had 33.1% of the total fungal population, and Gorman, which contributed 14.1%.

Frequency of occurrence of fungal genera

The distribution of fungal genera on the three local apple varieties is shown in Table 2. *Aspergillus* spp. was the most predominant genus, with a total of 73 isolates, hence representing 51.4% of the overall fungal population. This included both *Aspergillus niger* and *Aspergillus flavus*. It was followed by *Penicillium* spp., accounting for 38 isolates, that is, 26.8% of the total fungal isolates. *Mucor* was represented by 18 isolates, making up 12.7% of the total. *Alternaria* had 7 isolates, which is 4.9%, while *Rhizopus* spp. had 6 for 4.2%. *Botrytis* spp. occurred the least, having only 1 isolate or 0.7%. In short, 142 fungal isolates have been recorded, with *Aspergillus* spp. as the most predominant group and *Penicillium* spp. as the second major group.

Discussion

Post-harvest fungal pathogens are major contributors to apple decay during storage and transport. These pathogens invade through surface wounds or latent infections, leading to significant quality deterioration and economic losses in apple production. The findings of this study offer valuable insights into the fungal post-harvest diseases affecting three local apple varieties (Sheribil, Gorman and Golden), of Nagar Valley, Gilgit Baltistan. The distribution and prevalence of fungal species in the local apple varieties namely Sheribil, Gorman and Golden provide important insights into the fungal ecology associated with these crops. Among the fungal species recovered from apples, *Aspergillus* spp. are dominant fungal genus, followed by *Penicillium* spp. of the total isolates across all varieties. This study is in agreement with previous studies, that *Aspergillus* is a common inhabitant of fruit crops where it can thrive under various conditions. Among the *Aspergillus* spp. *Aspergillus niger* emerged as the most frequent species within this genus, particularly in the Golden variety. This high frequency in the Golden variety suggests that this variety is susceptible to *A. niger* growth. *Aspergillus flavus* was also prevalent, especially in the Golden variety. The notable presence of *A. flavus* highlights its potential role in the fungal community of these apple varieties. It raises questions about its interaction with other fungal species and its impact on fruit health. Previous studies revealed that *A. niger*, *A. fumigatus*, *Alternaria tenuis* and *A. tenuissima* play a substantial role in causing post-harvest losses in apples during storage and transportation globally (Ilyas *et al.* 2007). Hence, *Aspergillus*, a major genus of fungi, has been consistently identified as a key player in causing such post-harvest losses, as supported by prior research (Gupta and Saxena 2023).

As aforementioned, *Penicillium* spp. was the second most prevalent genus. Its highest frequency in Golden followed by Sheribil variety indicates that this variety supports a diverse fungal community. The widespread occurrence of *Penicillium* spp. across all apple varieties indicates its adaptability to a range of environmental conditions, consistent with its established ability to thrive in diverse habitats. This genus is well-known for its role in the spoilage of various fruits, including apples. Blue mould decay is a significant post-harvest fungal disease globally, with *Penicillium expansum* being the primary culprit associated with this condition in apples and pears. Other species, such as *Penicillium solitum*, *P. commune*, *P. verrucosum*, *P. chrysogenum*, *P. regulosum* and *P. crustosum* can also be found on decayed apples and pears. *Penicillium* is among the largest and most significant toxigenic fungal genera, encompassing over 400 species worldwide (Visagie *et al.* 2014). Notable species responsible for apple decay include *P. expansum, P. solitum, P. crustosum, P. italicum, P. digitatum, P. commune, P. verrucosum, P. rugulosum, P. polonicum, P. chrysogenum, P. allii* and *P. sumatraese* (Habib *et al.* 2021). Mechanical damage such as punctures, bruises, and cracks on the apple fruit, whether from harvesting or during post-harvest handling, often serves as the entry points for *Penicillium* and *Aspergillus* spp. Besides these two fungal genera, enzymatic activities and metabolic processes contribute to the degradation of apple fruit quality (Tarabay *et al.* 2018). The impact of this pathogen is contingent upon various factors, including climate and storage conditions. Typically, these pathogens gain entry into apple fruits through wounds or lenticular openings, both during the growing season and post-harvest, thereby intensifying the losses experienced during storage (Yahia *et al.* 2019). Similarly, other fungi like *Mucor*, *Alternaria*, and *Rhizopus* spp. were also prevalent in the Golden variety, with their occurrence being notably higher compared to Sheribil and Gorman. In our study, the lowest frequency of *Botrytis* spp. associated with apples. *B. cinerea* is an important postharvest pathogen because of the conducive conditions prevailing throughout the postharvest handling chain, including injuries, high humidity, senescing plant tissue, and high sugar content. Further low frequency of *Botrytis* spp. observed in the apples may be attributed to the absence of moist conditions during flowering. Without favorable moisture, the chances for spores to establish and persist as latent infections are greatly minimized. Under normal circumstances, *Botrytis* can remain dormant in the flower remnants and later manifest as small rots or blemishes near the calyx as the fruit matures. However, due to the dry conditions during blossom time, the pathogen likely had fewer opportunities to infect and establish itself, leading to its reduced presence in the ripened fruit (Abdelhalim *et al.* 2023). In our study, *Mucor* is not the dominant pathogen associated with apples; however, in another study, *Mucor* causes rot in pome and stone fruit in the USA and other countries. For example, *M. piriformis* is the primary cause of *Mucor* rot in apples and pears; however, *M. Macedo* and *M. racemosus* have also been reported to cause decay (Sutton *et al.* 2014). Post-harvest fungal infections in apples typically occur through wounds or natural openings that provide entry points for pathogens (Simonato *et al.* 2021). During harvest, handling, and storage, apples may develop wounds such as bruises, punctures, or cracks, which create favorable conditions for fungi like *Penicillium* spp., *Rhizopus* spp. and *Mucor* spp. to establish and proliferate (Leng *et al.* 2023). Additionally, natural openings like the calyx or stem end can serve as entry sites for fungal spores (Argenta *et al.* 2021). Once inside the fruit, these fungi can rapidly colonize the tissues, leading to decay. Environmental factors such as humidity, temperature and oxygen levels in storage facilities further influence fungal growth and the severity of post-harvest diseases. Moreover, latent infections that initially establish during the flowering or early fruit development stages can remain dormant until favorable conditions arise post-harvest, causing decay during storage and distribution. Thus, the combination of physical damage, natural entry points, and storage conditions plays a key role in the onset and progression of post-harvest fungal diseases in apples.

Conclusion

This study revealed the very significant effect of post-harvest fungi, dominated by *Aspergillus* spp. and *Penicillium* spp., on local apple varieties. The Golden variety was observed to be the most vulnerable, where nearly half of all the isolated fungal populations were recovered from this variety. The dominance of *Aspergillus* spp. and *Penicillium* spp. requires the adoption of an effective management strategy against post-harvest losses; future research in this area needs to be directed toward understanding molecular resistance or susceptibility characteristics of different apple varieties against this specific fungal pathogen. In addition, such studies must be carried out using eco-friendly and sustainable post-harvest treatment regimens implemented with biocontrol agents or natural antifungal compounds instead of chemical treatment. Such a study on more varieties and spreading them to other regions would help fine-tune these management practices, contributing to the general reduction of postharvest apple losses. Therefore, this would require collaboration by the researchers, farmers, and industry players in implementing these strategies to improve postharvest quality on a larger scale.

Acknowledgements

We thank the reviewers for their constructive comments.

Author Contributions

AA planned the experiments, JA, WH, SUD, MH and AR interpreted the results, AA and JA made the write up and JA statistically analyzed the data and made illustrations.

Competing Interests

The authors declare no conflict of interest.

Consent for publication

Not applicable.

Data Availability

Not applicable.

Ethics approval

Not applicable.

Funding Source

No external funding.

References

Abbas A, M Mubeen, W Younus, Q Shakeel, Y Iftikhar, S Bashir, MA Zeshan, A Hussain (2023). Plant diseases and pests, growing threats to food security of Gilgit-Baltistan, Pakistan. *Sarhad J Agric* 39:510–520

Abdelhalim A, GA El-Kot, AA Elzaawely (2023). Isolation, morphological characterization, and phylogenetic identification of Botrytis cinerea associated with postharvest gray mold of apple fruits. *J Sustain Agric Environ Sci* 2:91–103

Argenta LC, STD Freitas, JP Mattheis, MJ Vieira, C Ogoshi (2021). Characterization and quantification of postharvest losses of apple fruit stored under commercial conditions. *HortScience* 56:608–616

Głos H, H Bryk, M Michalecka, J Puławska (2022). The recent occurrence of biotic postharvest diseases of apples in Poland. *Agronomy* 12:399-406

Gupta S, S Saxena (2023). Endophytes: Saviour of apples from post-harvest fungal pathogens. *Biol Contr* 182:105234

Habib W, M Masiello, H Chahine-Tsouvalakis, ZA Moussawi, C Saab, ST Tawk, L Piemontese, M Solfrizzo, AF Logrieco, A Moretti, A Susca (2021). Occurrence and characterization of *Penicillium* species isolated from post-harvest apples in Lebanon. *Toxins* 13:730-741

Hasan AM, QA Marzani, DN Abdulrahman (2022). Management of fungal postharvest diseases of apple and pear using plant extracts. *J Duhok Univ* 25:66–78

Ilyas MB, MU Ghazanfar, MA Khan, CA Khan, MAR Bhatti (2007). Postharvest losses in apple and banana during transport and storage. *Pak J Agric Sci* 44:534–539

Khadiri M, H Boubaker, SE Lasli, A Farhaoui, S Ezrari, N Radouane, M Radi, L Askarne, EA Barka, R Lahlali (2024). Unlocking nature's secrets: Molecular insights into postharvest pathogens impacting Moroccan apples and innovations in the assessment of storage conditions. *Plants* 13:553-572

Khan IH, A Javaid (2023). *Penicillium citrinum* causing postharvest decay on stored garlic cloves in Pakistan. *J Plant Pathol* 105:337-337

Khan IH, A Javaid (2022). *Penicillium echinulatum* causing blue mold on tomato in Pakistan. *J Plant Pathol* 104:1143-1143

Khan IH, A Javaid (2021). Molecular characterization of *Penicillium expansum* associated with blue mold disease of apple in Pakistan. *Pak J Bot* 53:2299–2303

Klich MA (2002). *Identification of common Aspergillus species*. Centraalbureau voor schimmelcultures (Available at: https://cir.nii.ac.jp/crid/1571980075214285952

Leng J, Y Ma, J Liu (2023). Recent advances in research on biocontrol of postharvest fungal decay in apples. Crit Rev Food Sci Nutr 63:10607–10620

Mehdi A, A Sohail, M Mazahir, KS Abbasi, RM Amir, W Ali, M Asim (2020). Assessment of physico-chemical and phyto-chemical properties of six apple varieties cultivated in district Nagar and Hunza Gilgit-Baltistan, Pakistan. *Pure Appl Biol* 9:1627–1636

Nyamende NE, FR Domtchouang, ZA Belay, Z Keyser, A Oyenihi, OJ Caleb (2021). Alternative postharvest pre-treatment strategies for quality and microbial safety of 'Granny Smith' apple. *Heliyon* 7:1-9

Pagès M, D Kleiber, F Violleau (2020) Ozonation of three different fungal conidia associated with apple disease: Importance of spore surface and membrane phospholipid oxidation. *Food Sci Nutr* 8:5292–5297

Simonato B, M Lorenzini, G Zapparoli (2021). Effects of post-harvest fungal infection of apples on chemical characteristics of cider. *LWT* 138:110620-110626

Singh KP, T Aravind (2021). Postharvest diseases and disorders of apple: Perspectives for integrated management. *In*: *Postharvest Handling and Diseases of Horticultural Produce*, pp:267–280. CRC Press

Sutton TB, HS Aldwinckle, AM Agnello, JF Walgenbach (2014). *Compendium of Apple and Pear Diseases and Pests.* APS Publishers

Tarabay PA, H Chahine-Tsouvalakis, ST Tawk, N Nemer, W Habib (2018). Reduction of food losses in Lebanese apple through good harvesting and postharvest practices. *Ann Agric Sci* 63:207–213

Visagie CM, J Houbraken, JC Frisvad (2014). Identification and nomenclature of the genus *Penicillium*. *Stud Mycol* 78:343–371

Xu J (2022). Assessing global fungal threats to humans. *MLife* 1:223–240

Yahia EM, JM Fonseca, L Kitinoja (2019). Postharvest losses and waste. *In*: *Postharvest Technology of Perishable Horticultural Commodities*, pp:43–69. Elsevier

Zambounis A, I Ganopoulos, A Tsaftaris, D Valasiadis, P Madesis (2020). Metagenomics analysis of fungal communities associated with postharvest diseases in pear fruits under the effect of management practices. *Arch Microbiol* 202:2391–2400