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Identification of Fungi Associated with Post-Harvest Losses of Apples in Hunza Valley, Gilgit Baltistan

Samreen Alam[1†], Kulsoom[1†], Nilma Hassan[1†], Aqleem Abbas[1*], Saif-ud-Din[2], Waqar Hussain[3], Sartaj Ali[1], Mir Muhammad Nasir Qayyum[1] and Muhammad Shah Zaman[4]

[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*
[4]*Department of Agriculture, Research Division, Gilgit-Baltistan, Pakistan*
[*]For correspondence: aqlpath@gmail.com
[†]Contributed equally to this work and are co-first authors
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Abstract

Postharvest losses due to fungal pathogens pose a significant challenge, impacting the quality, quantity, and market value of agricultural commodities throughout the food supply chain. Despite the substantial consequences of these losses, a critical knowledge gap exists, particularly in identifying the fungal pathogens responsible for postharvest diseases in apples in Nasir Abad, Hunza Valley, Gilgit Baltistan (GB). In this study, approximately 15 fungal isolates were recovered from stored apples. Each isolate, labeled from "Asp-A-1" to "Asp-A-15," was characterized and assessed for its impact on apple fruits. The growth rates of *Aspergillus* sp. isolates were investigated. Asp-A-2 emerged as the fastest-growing isolate with a mean radial growth of 59.3 mm, while Asp-A-6 exhibited the slowest growth rate at 39.27 mm. The remaining isolates demonstrated intermediate growth rates, providing valuable insights into their varied growth characteristics. Pathogenicity testing was conducted to evaluate the impact of fungal isolates on both wounded apple fruits (WAF) and non-wounded apple fruits (NWAF). Among the isolates, Asp-A-2 exhibited the highest pathogenicity, causing significant damage with WAF and NWAF values of 91.7% and 25.4%, respectively. Asp-A-8 also demonstrated notable pathogenicity, with WAF and NWAF values of 86.4 and 22.9%. Isolates Asp-A-7 and Asp-A-14 displayed moderate pathogenicity, while Asp-A-1 and Asp-A-10 showed lower levels of pathogenic impact. Asp-A-12 and Asp-A-13 exhibited similar levels of pathogenicity. In conclusion, these findings contribute to understanding the complex interactions between *Aspergillus* sp. isolates and apple fruits, offering insights into potential strategies for managing postharvest losses in apple production in Gilgit Baltistan.

Keywords: Postharvest losses; Fungal pathogens; Apples; Nasir Abad; Hunza valley; Gilgit Baltistan; Sustainability

Introduction

Apples, known scientifically as *Malus domestica* (L.), have a rich history in the mountainous region of Gilgit Baltistan (GB) (Ilyas *et al.* 2007). This picturesque region, nestled between the towering peaks of the Himalayas and the Karakoram Range, is home to a unique variety of apples that thrive in its cool climate (Abbas *et al.* 2023). These apples, cultivated in orchards across GB, play a vital role in the local economy and are gaining recognition for their exceptional taste and quality. GB, with its diverse agro-climatic conditions, is an ideal environment for apple cultivation (Khan *et al.* 2020). The orchards in this region, particularly in areas like Nagar and Hunza, contribute significantly to Pakistan's overall apple production (Mehdi *et al.* 2020). The unique geography and climate of GB, with cold winters and moderate summers, create the perfect conditions for the cultivation of apples with a crisp texture and a sweet, refreshing flavor (Wiehle *et al.* 2021). The Hunza Valley in Gilgit-Baltistan is famous for its diverse apple varieties, each boasting a unique flavor profile and culinary purpose. The demand for these apples is skyrocketing, not just within Pakistan but also in international markets. Beyond being enjoyed fresh, Hunza Valley (or GB) apples are incredibly versatile, finding their way into delicious pies, jams and juices. Their distinctive taste, combined with their rich nutritional value, makes them a highly sought-after choice for health-conscious consumers. However, like all agricultural products, GB apples face challenges, particularly after harvest. These

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apples travel long distances from remote villages to reach markets and consumers. Their susceptibility to damage and spoilage throughout the entire journey – from harvesting and transportation to marketing, storage, and finally, consumption – highlights the critical need for proper handling practices. As succulent fruits, GB apples are especially vulnerable to physical injuries, enzymatic breakdown, and attacks by pathogens, both individually and in combination (Johnston *et al.* 2002; Prusky 2011; Radenkovs and Juhnevica-Radenkova 2018). Physical injuries and damage to the fruits can lead to the loss of moisture through accelerated surface evaporation (Hussein *et al.* 2020). Subsequently, these injured fruits become susceptible to pathogenic attacks, particularly by fungal pathogens, resulting in diseases. The diseased fruits undergo rapid respiration rates compared to their intact, healthy counterparts (Yahia *et al.* 2019). This heightened fruit respiration and increased metabolic activity contribute to senescence, ultimately leading to storage decay or rot. Hence, there is a need to develop effective strategies to preserve the quality and longevity of apple fruits throughout the entire supply chain. Fungal pathogens that affect apples after harvest constitute a significant portion of the economic losses experienced in apple production. The annual losses attributed to post-harvest diseases caused by fungal pathogens range widely, typically falling between 5 and 35%. In the context of developing countries, the extent of losses is even more pronounced, ranging from 20 to 50% before the apples reach the consumers (Yahia *et al.* 2019; Argenta *et al.* 2021a). Hence, post-harvest losses encompass reductions in quality, quantity, and market value, which are a significant concern for apple growers in the region (Porat *et al.* 2018; Radenkovs and Juhnevica-Radenkova 2018; Yahia *et al.* 2019; Argenta *et al.* 2021a). Fungal pathogens emerge as significant concerns for apples during storage and transportation. Research has highlighted the susceptibility of apple fruit decay caused by various fungal pathogens (Morales *et al.* 2010; Shewa *et al.* 2022). The probability of fungal decay hinges on factors such as the apple genotype, pre- and post-harvest management practices, and environmental conditions. Postharvest fungal losses predominantly result from latent infections established before harvest or infections through wounds, occurring either before or after harvesting. Latent infections infiltrate the intact fruit tissue or natural skin openings like lenticels, while wound infections take place through injuries induced by insects, hail, physiological disorders, and mechanical factors. Several notable fungi, including *Botrytis* sp., *Penicillium* sp., *Mucor* sp., *Monilia* sp. and *Aspergillus* sp., play crucial roles in causing postharvest decay in apples by infecting the fruit through wounded tissues. Conversely, other fungi like *Colletotrichum* sp. and *Alternaria* sp. can initiate infections through latent means. These fungi have been widely acknowledged for their role in causing decay and rot in apples. Among these fungi, *Aspergillus* sp. has been isolated, posing potential threats of infections or allergies to apple consumers, as reported by many researchers. Hence, the diverse range of fungi associated with post-harvest losses of apples underscores the complex nature of post-harvest diseases affecting this popular fruit. Most of these fungal pathogens can infect the fruit through wounds or during the flowering period, leading to latent infections that may only become apparent during storage. The challenge is not only in the reduction of apple yields but also in the potential compromise of fruit quality due to the production of mycotoxins. Understanding and addressing these fungal pathogens is crucial for preserving the quality and safety of apple produce (Dwiastuti *et al.* 2021). To mitigate post-harvest losses, apple growers in GB employ various strategies Fungicide spraying is a common practice, supplemented by cultural practices such as proper sanitation (removing debris and infected fruits) and careful handling during harvesting and storage (Hussein *et al.* 2020). The challenge lies in managing these diseases effectively, given the ability of fungi to produce secondary inoculum and the importance of preventing wounds during the harvest. Despite the growing importance of apple cultivation in GB, there is a need for more research on the fungal pathogens responsible for post-harvest losses in apples from Nasirabad Hunza. Keeping in view the economic importance of apples and the postharvest losses caused by fungal pathogens, the objectives of this study are to identify and characterize the fungal pathogens responsible for postharvest diseases in apples in Nasir Abad, Hunza Valley and Gilgit Baltistan. Additionally, the study aims to investigate the growth rates of various *Aspergillus* sp. isolates, providing valuable insights into their growth characteristics and potential impact on stored apples. Another objective is to assess the pathogenicity of these fungal isolates on both wounded and non-wounded apple fruits, determining their respective levels of damage to better understand their effects.

Materials and Methods

Study area and sampling

The research was conducted in the Laboratories of the Department of Plant Sciences and the Department of Agriculture and Food Technology at Karakoram International University (KIU), Gilgit. Apples were gathered from five randomly selected orchards from Nasir Abad, Hunza Valley, Gilgit, during the ripening stage. The study area has been shown in Fig. 1. About 10–15 apples were randomly selected from each orchard, which were then transported to KIU Laboratories for fungal isolation. Nasirabad is a town located in the Hunza District of Gilgit-Baltistan, Pakistan.

Fungal pathogens isolation and identification

For fungal isolation, apples were moist incubated in cardboard boxes with lids, lined with moist paper towels to

maintain high humidity, and incubated at room temperature (25°C) for two weeks to facilitate pathogen growth. Subsequently, the fruits with obvious rotting symptoms were surface sterilized with 75% ethyl alcohol for 3 min, followed by rinsing in sterile water. Thin sections (2 cm) were cut lengthwise along the lesion and individually placed onto PDA for 7 days at 25°C. This medium was prepared by combining 200 mL of juice extracted from 200 g of potatoes, along with 20 g of dextrose and 15 g of agar per liter. The colonies were transferred to PDA media after 1 and 3 days of cultivation, and the process was repeated until a pure culture of strains was achieved. The fungal colonies were identified by colony morphology and morphological keys as described previously (Klich 2002). The fungal isolates identified were maintained at 4°C.

Morphological observation and growth rate

Fungal isolates were cultivated on PDA. The inoculated agar plates were then kept in darkness at a temperature of 25°C. Subsequently, a small agar piece (5 mm in diameter) containing fungal mycelia was carefully excised from the periphery of a well-established 7-day-old colony. This agar piece was inverted and positioned at the center of a new PDA plate, which was then subjected to further incubation at a temperature of 25°C in the absence of light. After an additional 7 days, the radial growth of the fungal colonies was measured, and the mean growth rate was subsequently calculated based on these measurements (Sam *et al.* 1992; Spotts *et al*. 1999; Natale *et al.* 2001; Nunes *et al.* 2001; Jijaki *et al.* 2004).

Pathogenicity test

All the fungal isolates underwent pathogenicity testing on mature apple fruits. The experiment involved two distinct treatment groups: (i) wounded apple fruits (WAF), which were subjected to a 3 mm wound in the equatorial zone, and (ii) non-wounded apple fruits (NWAF). Inoculation was carried out by placing a 5-mm-diameter plug from a 5-day-old mycelial culture of isolates onto both intact and wounded pomegranate fruits. A 5-mm-diameter PDA plug served as the untreated control. The treated fruits were then kept in containers with lids, maintaining a moist incubation environment at room temperature (25°C). The Disease Severity Index (DSI) was evaluated for 5 days post-inoculation, with three replications, each consisting of three fruits, and the experiments were conducted twice. The assessment of DSI for all inoculated fungi utilized a modified rot index scale, ranging from 0 to 5. This scale categorized the severity of symptoms, with 0 indicating no symptoms and 5 indicating more than 75% of the fruit's internal or external surface being infected. The scale also included intermediate levels (1 to 4) corresponding to different percentages of infection on the fruit surface.

Statistical analysis

For the analysis of fungal isolates, a descriptive analysis was performed to provide an overview of the distribution of isolates within sweet and tart apple varieties. The radial growth rates of fungal isolates were scrutinized using ANOVA to detect potential significant differences among *Penicillium* and *Aspergillus* isolates. The LSD test was employed for further identification of significant differences. In the context of pathogenicity data, a one-way ANOVA was utilized to evaluate the impact of fungal isolates on both wounded (WAF) and non-wounded (NWAF) apples. The significance level (alpha) for all tests was set at 0.05. Statistical analyses were performed using Statistix 8.1 software.

Results

Number of fungal isolates collected from apples

There are 15 fungal isolates recorded in this study, as shown in Table 1. Each isolate is represented by a unique identifier, ranging from "Asp-A-1" to "Asp-A-15," and they all belong to the genus *Aspergillus* sp.

The growth rate of fungal isolates associated with apples

The growth rate of *Aspergillus* sp. isolates' growth rates on a PDA nutrient medium at 25°C after 7 days of incubation are shown in Fig. 2. Among the isolates, Asp-A-2 demonstrated a quick-growing pattern with a mean radial growth of 59.3, standing out as the isolate with the highest growth rate. Alongside Asp-A-2, isolates Asp-A-7, Asp-A-8, and Asp-A-14 also exhibited rapid growth. On the other hand, Asp-A-6 displayed the lowest growth rate with a mean radial growth of 39.27, categorizing it as the isolate with the slowest growth. The remaining isolates, including Asp-A-1, Asp-A-3, Asp-A-4, Asp-A-5, Asp-A-9, Asp-A-10, Asp-A-11, Asp-A-12, Asp-A-13, and Asp-A-15, demonstrated average growth rates, clustering around the overall mean of 48.68. These results provide insights into the varied growth characteristics of *Aspergillus* sp. isolates under the specified incubation conditions. The colony characteristics of some of these fast-growing fungal isolates are shown in Fig. 3.

Pathogenicity testing

The pathogenicity of fungal isolates on apples and the impact on both wounded fruits (WAF) and non-wounded apple fruits (NWAF) was systematically investigated as shown in Table 2. The results presented in the table reveal distinct variations in the pathogenic effects of different fungal isolates on both categories of fruits. Among the isolates, Asp-A-2 exhibited the highest pathogenicity, causing significant damage with a WAF value of 91.7 and an NWAF value of 25.4. Following closely, Asp-A-8

Table 1: Fungal isolates from the stored apples

Sr. No	Isolates	Genus
1	Asp-A-1	*Aspergillus* sp.
2	Asp-A-2	*Aspergillus* sp.
3	Asp-A-3	*Aspergillus* sp.
4	Asp-A-4	*Aspergillus* sp.
5	Asp-A-5	*Aspergillus* sp.
6	Asp-A-6	*Aspergillus* sp.
7	Asp-A-7	*Aspergillus* sp.
8	Asp-A-8	*Aspergillus* sp.
9	Asp-A-9	*Aspergillus* sp.
10	Asp-A-10	*Aspergillus* sp.
11	Asp-A-11	*Aspergillus* sp.
12	Asp-A-12	*Aspergillus* sp.
13	Asp-A-13	*Aspergillus* sp.
14	Asp-A-14	*Aspergillus* sp.
15	Asp-A-15	*Aspergillus* sp.

demonstrated notable pathogenicity as well, with WAF and NWAF values of 86.4 and 22.9, respectively. Isolates, Asp-A-7 and Asp-A-14 displayed moderate pathogenicity, resulting in WAF values of 83.4 and 79.7 and NWAF values of 16.4 and 14.8, respectively. Asp-A-1 and Asp-A-10 showed further reductions in pathogenic impact, with WAF and NWAF values of 78.1 and 11.5 and 74.4 and 8.6, respectively. Interestingly, Asp-A-12 and Asp-A-13 exhibited comparable levels of pathogenicity, both recording WAF values of 74.3. However, Asp-A-12 had a slightly higher NWAF value of 6.5, while Asp-A-13 showed an NWAF value of 5.3.

Discussion

The findings of this study offer valuable insights into the fungal postharvest diseases affecting apple varieties in Nasir Abad, Hunza Valley, GB. By investigating the isolation, growth rates, and pathogenicity of fungal isolates associated with postharvest losses, this research sheds light on the complex dynamics within the apple ecosystem. Specifically, the pathogenicity and growth characteristics of 15 *Aspergillus* sp. isolates (Asp-A-1 to Asp-A-15) on stored apples were thoroughly examined. Among these isolates, Asp-A-2 emerged as the fastest-growing and most pathogenic, highlighting its potential as a significant threat to apple storage. Isolates Asp-A-7, Asp-A-8, and Asp-A-14 also demonstrated rapid growth, while Asp-A-6 showed the slowest growth rate. These varied growth rates and pathogenic impacts underscore the diverse strategies employed by different *Aspergillus* isolates in colonizing and damaging apple fruits. The study emphasizes the importance of understanding the specific interactions between fungal pathogens and apple varieties to develop effective postharvest management strategies. Additionally, the observed differences in pathogenicity between wounded and non-wounded apple fruits suggest that the external skin of apples provides some resistance to fungal invasion. This finding indicates that disease management strategies should consider the protective role of the fruit skin.

Table 2: The pathogenicity of fungal isolates after inoculation onto the apple fruits

Isolates	[1]WAF	[2]NWAF
Asp-A-2	91.7a	25.4a
Asp-A-8	86.4b	22.9b
Asp-A-7	83.4c	16.4c
Asp-A-14	79.7d	14.8d
Asp-A-1	78.1e	11.5e
Asp-A-10	74.4f	8.6f
Asp-A-12	74.3f	6.5g
Asp-A-13	71.3g	5.3h
Control	0h	0i

[1]WAF = Wounded Apple Fruits, [2]NWAF = Non Wounded Apple Fruits

The fungal isolates belonging to the genus *Aspergillus* sp. were characterized and identified, forming the foundation for understanding their behavior in subsequent experiments. The growth rate assessment revealed substantial variations among these fungal isolates. Asp-A-2 displayed the highest growth rate, indicating its quick-growing pattern. Notably, Asp-A-7, Asp-A-8, and Asp-A-14 also demonstrated rapid growth, while Asp-A-6 exhibited the slowest growth rate. The remaining isolates displayed average growth rates, emphasizing the diversity in growth characteristics among *Aspergillus* sp. Isolates. In the pathogenicity testing, Asp-A-2 emerged as the most pathogenic isolate, causing significant damage to both wounded fruits and non-wounded apple fruits. Following closely, Asp-A-8 exhibited notable pathogenicity. Asp-A-7 and Asp-A-14 displayed moderate pathogenicity, while Asp-A-1 and Asp-A-10 showed further reductions in pathogenic impact. Asp-A-12 and Asp-A-13 exhibited comparable levels of pathogenicity, with slight differences in their impact on NWAF. These isolates belonged to *Aspergillus* genus. In the preceding findings, it is evident that a multitude of fungal species, including *Aspergillus niger*, *A. fumigatus*, *Alternaria tenuis*, *A. tenuissima*, *Cladosporiums herbarum*, *Helminthosporium tetramera*, *Mucor racemosus*, *Penicillium expansum*, *Pencillium italicum*, and *Rhizopus nigricans*, play a substantial role in causing post-harvest losses in apples during storage and transportation globally. The literature highlights the considerable diversity, with over 90 fungal species identified as contributors to apple decay during storage (Ilyas *et al.* 2007).

Among these fungi, *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 (Porat *et al.* 2018; Springael *et al.* 2018; Tarabay *et al.* 2018; Argenta *et al.* 2021a; Shewa *et al.* 2022). This fungal genus, widely encountered and well-documented, holds significance in the deterioration of various fruits, including apples. The distinctive characteristic of *Aspergillus* sp. is their propensity to infect and exploit wounds or injuries present on the surface of fruits, earning them the designation of "wound pathogens." *Aspergillus's* impact on post-harvest losses in apples is intricately tied to its ability to capitalize on fruit vulnerabilities, particularly wounds or injuries sustained

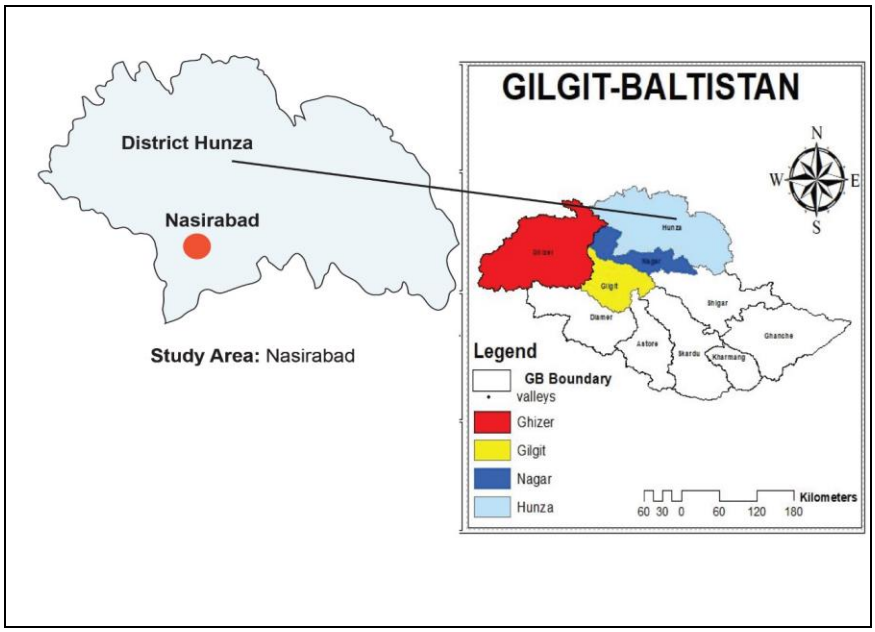

Fig. 1: Map of the study area in Gilgit-Baltistan, Pakistan. The red dot indicates the sampling location, Nasirabad, within the District of Hunza. Apple samples were collected from orchards in Nasirabad to isolate and characterize fungal pathogens responsible for postharvest diseases. The scale bar represents distances in kilometers

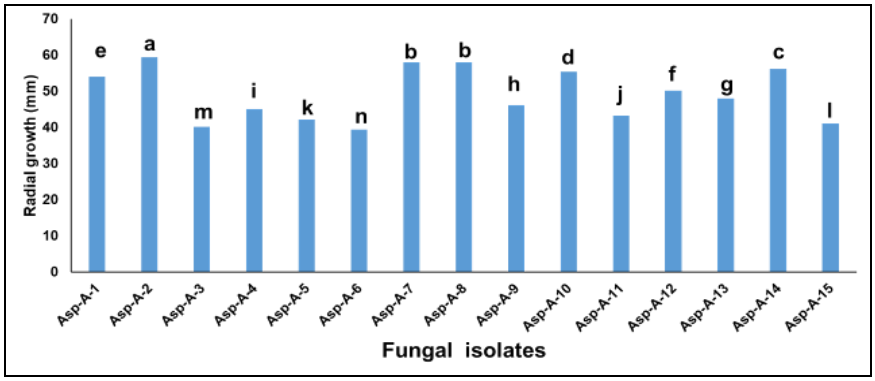

Fig. 2: Radial growth of fungal isolates growing on a PDA medium at 25°C

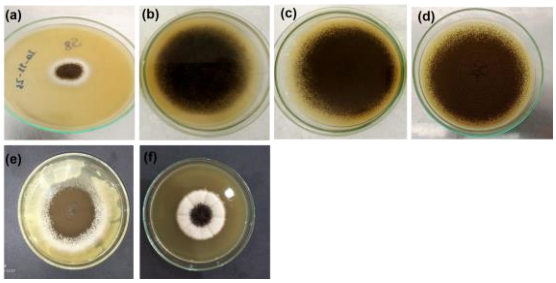

Fig. 3: Colony characteristics of some of the fungal isolates grew on potato dextrose agar for 7 days. (**a**) Asp-A-6, (**b**) Asp-A-7, (**c**) Asp-A-8, (**d**) Asp-A-2, (**e**) Asp-A-14, (**f**) Asp-A-10

during harvesting, handling, or transportation. These wounds provide entry points for Aspergillus to establish infections and initiate the deterioration process. The consequences of such infections extend beyond mere physical damage, as *Aspergillus* is known for its enzymatic activities and metabolic processes that contribute to the degradation of 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 lenticel openings, both during the growing season and post-harvest, thereby intensifying the losses experienced during storage (Yahia *et al.* 2019). Moreover, the findings of this study highlight a notable difference in pathogenicity between non-wounded apple fruits (NWAF) and wounded apple fruits (WAF). Among the fungal isolates, Asp-A-2 exhibited the highest pathogenicity, causing significant damage to both WAF and NWAF. Following closely, Asp-A-8 demonstrated notable pathogenicity as well. Isolated Asp-A-7 and Asp-A-14 displayed moderate pathogenicity and Asp-A-13 exhibited the lowest pathogenicity on apples on both WAF and NWAF. The lower pathogenicity observed in NWAF as compared to WAF suggests a potential resistance of the external fruit skin to the tested pathogens. This dual functionality of the fruit skin becomes evident, serving both as a protective barrier against pathogens and playing a crucial role in maintaining structural integrity by withstanding internal growth pressures and regulating fruit expansion. The results align with previous research indicating that skin characteristics contribute significantly to variations in vulnerability observed among different pomegranate varieties. Understanding these distinct skin properties is crucial for effective disease management and overall preservation of fruits, emphasizing the importance of tailoring strategies based on the unique attributes of the skin in various apple varieties (Ewekeye *et al.* 2016; Doryanizadeh *et al.* 2017; Radenkovs and Juhnevica-Radenkova 2018).

In a recent study conducted on post-harvest treatment of grapes with essential oils and plant extracts significantly reduced the detrimental effects of *Rhizopus stolonifer* and *Aspergillus niger* (Shamsullah *et al.* 2023). Hence, this study, underscores the significant issue of postharvest losses caused by fungi in apple production. There is need to adopt sustainable practices to minimize postharvest losses, arguing for a more prudent approach over increasing production to compensate for these losses. Besides fungal pathogens, other factors which contribute to the post-harvest losses in apples and waste of apple fruits such as, physiological disorders, mechanical injuries, and deterioration in appearance, texture, and flavor, leading to consumer dissatisfaction also need to be addressed (Argenta *et al.* 2021b). The multifaceted nature of these factors underscores the need for comprehensive strategies and interventions throughout the postharvest handling and distribution processes. Addressing these issues not only aligns with sustainability goals but also ensures the efficient utilization of resources and the delivery of high-quality apples to consumers.

Conclusion

This study enhanced our understanding of fungal postharvest diseases in apples from Nasir Abad, Hunza Valley, GB. By isolating and characterizing 15 *Aspergillus* sp. isolates, it shed light on their growth rates and pathogenicity. Asp-A-2, the fastest-growing and most pathogenic isolate, stands out as a significant threat. Other isolates like Asp-A-7, Asp-A-8, and Asp-A-14 also showed rapid growth, while Asp-A-6 grew the slowest. These insights highlight Aspergillus's role in global postharvest losses, exploiting fruit wounds to cause decay. The study underscored the need for tailored disease management strategies, considering the protective role of fruit skin. To sustainably address postharvest losses, a holistic strategy is advocated, focusing on managing fungal pathogens and other factors like physiological disorders and mechanical injuries. This research paved the way for innovative approaches to improve apple preservation and reduce economic losses.

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Author Contributions

SA, K, NH, Data collection, software; formal analysis; validation, investigation; resources; AA, Conceptualization, Supervision, original draft preparation; writing—review and editing; WH, AR, SUD, MMNQ, SA, MSZ, Technical assistance; SA, K, NH contributed equally to this publication.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper

Data Availability

Not applicable

Ethics Approval

Not applicable.

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