Available Online at EScience Press
Plant Protection
ISSN: 2617-1287 (Online), 2617-1279 (Print)
http://esciencepress.net/journals/PP

ASSESSMENT AND IN VITRO CONTROL OF POMEGRANATE FRUIT ROT DISEASE IN GILGIT DISTRICT, PAKISTAN

[a]Nazakat Hussain, [b]Hasnain Abbas, [b]Shahab-Ud-Din, [b]Heera Ali, [c]Aqleem Abbas, [d]Sagir Hussain, [b]Salim Khadim, [b]Shakeel Hussain, [b]Waqar Hussain, [e]Saif Ud Din, [b]Abdul Razaq

[a] Department of Plant Pathology, College of Agriculture, University of Sargodha, Pakistan.
[b] Department of Plant sciences, Karakoram International University, Gilgit Baltistan, Pakistan.
[c] Department of Agriculture and Food Technology, Karakoram International University, Gilgit Baltistan, Pakistan.
[d] State Environmental Protection key Liboratory of wetLand Ecology and vegetation Restoration, School of Environment, NorthEast Normal University, Changchun 130117, China.
[e] Department of Animal sciences, Karakoram International University, Gilgit Baltistan, Pakistan.

ARTICLE INFO

Article history
Received: 2nd June, 2023
Revised: 9th July, 2023
Accepted: 15th july, 2023

Keywords
Fruit rot
Pomegranate
Aspergillus niger
Biological
Chemical
Management

ABSTRACT

The pomegranate fruit rot disease, referred to as "heart rot" or "black heart," stands as a significant issue among pomegranate diseases in Gilgit Baltistan (GB). This condition is defined by the presence of black rot within the fruit core, which extends from the calyx area. Importantly, the outer peel and tough rind maintain their healthy appearance. Several varieties of pomegranate fruit such as Sweet, Doom, Sour, and Kandhari are found in different localities of GB. Among these varieties, the sweet type locally known as isakolii is widely affected by fruit rot. The objective of this study was to determine the primary causal agent responsible for the occurrence of fruit rot disease in pomegranates in the Gilgit district. Additionally, the study aimed to develop effective management strategies for the disease under laboratory conditions. Although many fungal species belonging to genera, *Penicillium*, *Aspergillus*, *Botrytis*, and *Rhizopus*, have been isolated from pomegranate fruits, however, *Aspergillus niger* was the primary causal agent of fruit rot. In this study, two methods were carried out to manage fruit rot: biological control using *Trichoderma harzanium* in dual culture technique, resulting in a 60% inhibition of the fungus after seven days, and chemical control using food poisoning technique in culture method. The growth inhibition % on day seven was calculated as 27 ± 4.05, indicating the effectiveness of Tebuconazole 25.9 WP, which showed a decrease over time to inhibit the Aspergillus niger. The growth inhibition % for the Thiophanate Methyl treatment was calculated as 15.923 ± 3.27 after seven days. The study provides primary information regarding the prevalence of fungal disease infections in Gilgit and can help guide future studies toward mitigating fungal disease epidemics.

Corresponding Author: Hasnain Abbas
Email: hasnainabbas.shafaei@gmail.com

INTRODUCTION

The pomegranate (*Punica granatum*) is indigenous to Iran and is grown globally, including in the Himalayan regions of Pakistan and India. The pomegranate fruit holds medicinal properties and is recognized for its antioxidant, anti-tumoral, anti-hepatotoxic effects, as well as its

potential in improving cardiovascular health. Cultivation of pomegranate fruit takes place in various areas of Gilgit-Baltistan, Pakistan, such as Gollapor Vellay, Diamer, Gilgit, and Ghizer (Idrees et al., 2021). Pomegranate, a significant fruit crop, originated from Iran and its surrounding regions and is presently cultivated in both tropical and temperate zones. Pomegranate is renowned for its numerous inherent health benefits (Xiang et al., 2022). Pomegranate belongs to the *Punica* genus in the Lythraceae family. It has two species: *P. granatum* L. and *P. protopunica* Balf (Anand and Reddy, 2009). There are approximately 50 commercially cultivated varieties out of 500 globally distributed varieties (Kahramanoglu and Usanmaz, 2016).

Along with figs, dates, olives, and grapes, the pomegranate is among the world's first five cultivated crops. It is now grown in sub-tropical, tropical, temperate, and hilly climates up to 1800 meters above sea level all over the world. However, plant physiology and growth habits are influenced by the environment (Dinesh and Sankaran, 2016). Mediterranean nations, India, Iran, and the United States lead pomegranate production. Pomegranate output globally is estimated to be approximately 1.5 million tons. The top five producers are India, Iran, China, the United States, and Turkey. Following India, the United States has the largest production area (0.125 million hectares) and the maximum output (18.5 tons per hectare) (18.3). Iran is the country that exports the most, at 60,000 tons each year (Kahramanoglu and Usanmaz, 2016). However, Pakistan's warm temperate Himalayan areas are regarded as the fruit's second point of origin (Nasir et al., 1972), and it is classified as an insignificant fruit with only a few recognized varieties and ranks 10th in fruit output. Nevertheless, it is a popular fruit that thrives in semi-arid, mild-temperate to subtropical climates and is naturally acclimated to chilly winters and hot summers. Despite their minor economic value, pomegranates are extensively spread across the world's tropical and subtropical climates (Meshram et al., 2010).

The pomegranate tree, by nature, has many trunks. In orchards, plants are often grown with a single trunk, creating a big shrub or small tree with a mature height of 12-20 feet. In colder climates, trees can be trained to have numerous trunks to decrease the chance of total tree loss. Pomegranate plants have tiny, thin, oblong leaves with short stems that are more or less spiky and deciduous (Morton, 1987). Pomegranate cuttings and suckers are easy to grow, allowing valuable genetic resources to be passed down for generations (Dinesh and Sankaran, 2016). Pomegranate cultivars exhibit a wide range of pomological and chemical characteristics (Dinesh and Sankaran, 2016). The pomegranate has long been utilized for its medicinal benefits, with plant components such as flowers, bark, leaves, and roots being used to cure a variety of ailments, such as eye irritation, tapeworm removal, relief from mouth and throat pain, and assistance with digestion (Sarma et al., 1990).

The juice and peel of pomegranates are high in antioxidant-rich tannins and flavonoids. Pomegranate juice is abundant in Vitamin C, potassium, pantothenic acid, and polyphenols. Pomegranate seeds generate unique oil, with around 80% consisting of a highly uncommon 18-carbon fatty acid called punicic acid. The oil also comprises the isoflavone genistein, the phytoestrogen coumestrol, and the sex steroid estrone (Longtin et al., 2003). Pomegranate extracts possess antibacterial, anthelminthic, and antioxidant properties, making them ideal for cancer therapy (Cheng et al., 2017).

Pomegranate fruits of various varieties, such as Sweet, Doom, Sour, and Kandhari, can be found in different parts of Gilgit-Baltistan (GB). Pomegranate trees are prone to various fungal, bacterial, and viral diseases (Ertuğrul et al., 2020). Among the fungal diseases, pomegranate fruit rot (also known as heart rot or black heart) is a serious disease that impacts pomegranate production in GB. Arils in diseased fruits start to rot when they are opened, turning from brown (soft) to black (dry). The outer peel and hard rind of infected fruits, however, continue to appear healthy. When hit, fruits with heart rot emit a dull sound compared to the lively sound made by fruits with healthy hearts. They may also be identified by the color of their skin and their weight (Abudabos and Yehia, 2013).

In the last five years, a major problem of fruit rot has been noticed in the sweet variety of pomegranate in Gilgit district (Avenot et al., 2008). Many fungi, such as *Alternaria* spp., *Aspergillus* spp., *Penicillium* spp., *Botrytis* spp., and *Rhizopus* spp., have been shown to cause fruit rot. It is not always apparent which fungi are the disease causative agent(s) or what function other fungi play in the disease spectrum. Commonly, it is believed that fruit infection develops in the orchard during blossoming (Zhang and McCarthy, 2012). *Aspergillus* spp. can enter

the center of the pomegranate and cause disease in two ways: first, during the bloom by dispersing spores through storm or rain droplets, and second, by invading through breaking of the leathery skin. The spore enters the pistil of an open flower, develops into the tunnel (a connection between the pistil and the loculus), and then enters into the loculus, where it lies dormant until the maturing fruit can sustain it. Later, it restarts development in the subordinate loculus, causing aril rot as it advances into the higher loculus, eventually infecting the whole fruit (Ezra et al., 2013). The present study was therefore, conducted to monitor the major fungal pathogens responsible for pomegranate fruit rot in Gilgit district and to explore *in vitro* management using biological and chemical strategies.

MATERIALS AND METHODS

In the current study, a survey was conducted to collect samples of fungal pathogens from various locations in GB. The survey was designed to identify potential sources of fungal pathogens, such as diseased fruit and plant debris. The samples were collected from different areas, including agricultural fields, nurseries, and gardens, to ensure a diverse range of sources. Visual inspection was also conducted during the field survey. Subsequently, the samples were shifted to the fungal culture bank and plant disease diagnostic laboratory at the College of Agriculture, University of Sargodha, Punjab, Pakistan.

Survey and sample collection

A survey was conducted in various areas of Gilgit District, namely Heramosh, Jalalabad, and Nomal, to collect diseased pomegranate fruits. Approximately 180 pomegranate trees were randomly selected, and samples were collected specifically from the sweet variety (Isakolii) showing symptoms of soft rot disease. To ensure preservation during transportation to the laboratory, these fruits were placed in polythene bags. The polythene bags were carefully labeled with detailed information about the collection time, date, and place of each fruit. The sampling process took place in August and September of the year 2022.

Figure 1: Infected tree of sweet variety of pomegranate in Heramosh Gilgit.

Media preparation and isolation of the fungi

Potato Dextrose Agar (PDA) media were used to isolate pathogenic fungi from diseased samples. PDA is a selective and differential culture medium commonly employed for fungal growth. The medium consists of potato infusion, dextrose, and agar, providing a nutritious environment for the fungi to thrive.

To prepare the PDA medium, dextrose, potato extract, and agar were mixed in 1000 ml of distilled water, stirred well, and autoclaved for 20 minutes at 121ºC. The sterilized medium was then poured into clean Petri plates and left to solidify.

Infected fruit tissue was cut into small pieces (2 cm) using a knife or scissors, treated with 0.1% Chlorox for 2 minutes, and washed thrice with distilled water to remove any residues. The infected pieces were then placed on the PDA medium using sterilized forceps and incubated in a temperature-controlled environment at 25-28°C for 3-7 days.

The growth of pathogens was monitored, and spores were collected for further analysis. Temporary slides were prepared from the spores and studied under a

microscope to observe their morphological characteristics (Moller and DeVay, 1968).

Purification and identification of the pathogen

The hyphal tip was used for the purification of the pathogen. In this process, a hyphal tip from the fungal culture was picked with the help of a sterilized wire loop and then placed in the center of a Petri plate, keeping the medium at 25-30°C for 4-5 days. Pure fungal culture was preserved on PDA plates and stored at 4°C for future work. The fungal isolates were identified based on cultural and morphological characteristics. Cultural characteristics such as colony color, colony diameter, sporulation, and growth diversity on different culture media were noted. The identification and characterization of pathogens were compared with the literature reported by Geiser et al. (2007).

Figure 2: Fungal colony and isolation of the fungus.

Figure 3: Pure culture of *Aspergillus niger.*

Morphological characterization

The identification of the pathogens in the collected samples was based on their morphological characteristics, which included the length and size of hyphae, the shape and color of conidia, and the appearance of asexual spores. These features were observed and recorded from prepared slides using a light microscope (Pitt et al., 1997).

***In vitro* Biological and chemical control**

Biological control

The study aimed to assess the effectiveness of different biocontrol agents against *Aspergillus niger* under laboratory conditions. In this study, *Trichoderma harzianum* was used as the biocontrol agent against *A. niger*, a pathogen responsible for the soft rot of pomegranates. The double-culture technique was employed to test the antagonistic effect of *T. harzianum* on *A. niger* (Rajendiran et al., 2010).

Evaluation of fungicides

This study aimed to assess the effectiveness of various fungicides in controlling fungal pathogens that cause plant diseases, specifically in pomegranate plants. The evaluation process involved testing the fungicides against a particular fungal pathogen in a laboratory setting and

measuring their impact on the growth and spread of the pathogen. The study's outcomes can assist in identifying the most efficient fungicides for managing and controlling fungal diseases in pomegranate plants. Two fungicides (Table 1) were evaluated against the fungus using the poisoned food technique, and three replicates of each fungicide were used (Amin et al., 2014).

To prepare the different concentrations of fungicides, a stock solution was first created with a concentration of 1000 parts per million (ppm) for each fungicide. This was achieved by dissolving 0.5 g of the fungicide in 500 ml of distilled water. The resulting stock solution was then utilized to prepare solutions with varying concentrations for the study.

To create the different concentrations of each fungicide, the stock solution was utilized to prepare three varying concentrations. For 100 parts per million (ppm) concentrations, 10 ml of the stock solution was mixed with 90 ml of distilled water. Likewise, for 200 ppm concentration, 20 ml of the stock solution was combined with 80 ml of distilled water. For the highest concentration of 300 ppm, 30 ml of the stock solution was mixed with 70 ml of distilled water.

Before pouring, the PDA was supplemented with the appropriate concentration of each fungicide. Three plates were prepared for each concentration. After the plates had solidified, a mycelial plug of the target fungus was placed in the center of each plate, and subsequently, the plates were placed in an incubator at 25ºC. The diameter of the colonies was measured and recorded after 3, 5, and 7 days. The percent inhibition was then calculated using a formula.

$$\text{Inhibition (\%)} = \frac{C - T}{C} \times 100$$

where, C = Colony diameter in control, and T = Colony diameter in treatment

Table 1. Fungicides used in the study.

Sr. No	Fungicide	Active ingredient	Company name
1	Thiophanate	Thiophanate	Roko
2	Tebuconazole	Tebuconazole	Syngenta

Biocontrol of *Aspergillus niger*

After isolation and *in vitro* identification, the fungus that caused rotting problems in pomegranate was determined to be a filamentous fungus commonly found in soil. Bio-control of the fungus was conducted using a dual culture method with *Trichoderma harzianum*. A 5 mm mycelial plug was taken from the margin of a 5-7-days-old culture of *A. niger* on PDA and placed on a fresh PDA plate (3 cm from the center). Subsequently, a 5 mm mycelial disc obtained from the margin of a 5-7-day-old culture of *T. harzianum* was placed 3 cm away from the inoculum of the pathogen. Seven days after incubation at 28 ℃, the growth of the pathogen in dual culture and control plates was measured, and the percentage of pathogen inhibition was calculated (Ram et al., 2018). The percentage inhibition of the fungus after a 7-day reading in the dual culture method was found to be 30% (Figure 5), which was calculated using the formula;

$$\text{Inhibition (\%)} = \frac{C - T}{C} \times 100$$

Chemical control

In vitro chemical control of the fungus was carried out using thiophanate methyl 50%WP and Tebuconazole 25.9 WP through the food poisoning method. Concentrations of 100 ppm were prepared from the stock solution and three replicates of chemically amended PDA plates were created. A mycelial plug of the fungus was placed at the center of each plate, and then they were incubated at 25ºC. The colony diameter was recorded after three, five, and seven days. The percentage inhibition of the fungus was calculated using the formula:

$$\text{Inhibition (\%)} = \frac{C - T}{C} \times 100$$

Statistical analysis

The lesion size data from the infected pomegranates for each pathogen were subjected to a one-way analysis of variance (ANOVA) for statistical analyses. A descriptive test was employed to determine the differences, and significance was considered if the P-value was ≤ 0.05. Similarly, the inhibition rate between pathogens was analyzed using the same methods to identify any significant differences.

RESULTS

Identification of causal agent of fruit rot

The samples afflicted with the disease were examined using a basic compound microscope, and approximately

180 samples/isolates were analyzed. Identification was performed by evaluating morphological traits. The findings revealed that about 80% (144 isolates) of the diseased samples harbored *Aspergillus niger* (Figure 4), establishing it as the primary cause responsible for pomegranate fruit rot.

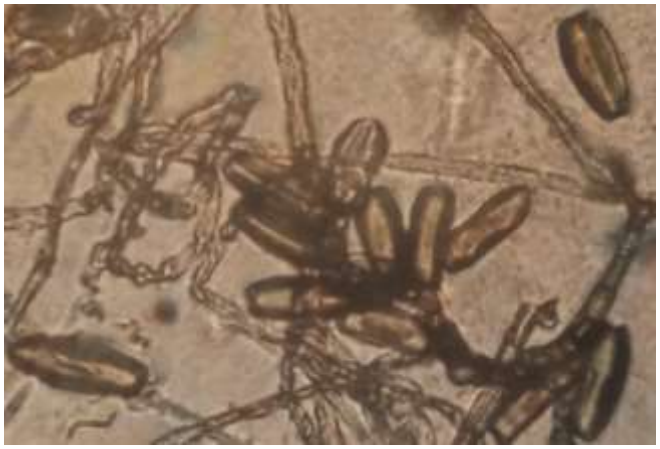

Figure 4. Spores of *Aspergillus niger.*

Biocontrol of *A. niger*

The results showed that the pathogen, *A. niger*, grew to 50 mm in diameter after seven days when cultured alone without any bio-control agent. However, when co-cultured with *T. harzianum*, the pathogen's growth was reduced to 20 mm in diameter, indicating a significant inhibition of 60% (Table 2). This suggests that *T. harzianum* has strong antifungal properties and can effectively prevent *A. niger* from infecting pomegranate fruits.

Treatment with Tebuconazole 25.9% WP

The antifungal activity of Tebuconazole 25.9 WP was evaluated by measuring the growth of the fungus over seven days. The fungal colonies were grown in two groups: a control group (without fungicide treatment) and a treatment group (with fungicide treatment). The fungal growth in the control group increased steadily over time, while the fungal growth in the treatment group was suppressed. On day 3, the fungal colony growth in the control group was 80 ± 0.00, while the colony growth in the treatment group was significantly lower at 15 ± 1.00. This indicated that Tebuconazole inhibited fungal growth by 61.25 ± 1.25% on day 3. On day 5, the fungal colony growth in the control group was 90 ± 0.00, while the colony growth in the treatment group was again significantly lower at 17 ± 1.00. This indicated that Tebuconazole inhibited fungal growth by 71.11 ± 1.11% on day 5. On day 7, the fungal colony growth in the control group was 90 ± 0.00, while the colony growth in the treatment group increased to 57 ± 3.60.

Table 2: Effect of *T. harzianum* on the growth of *Aspergillus niger*.

Treatments	Pathogen growth (mm)	Percentage inhibition (%)
Control	50	0
T. harzianum	20	60

The fungal growth inhibition percentage on day 7 was calculated to be 27 ± 4.05, indicating that the efficacy of Tebuconazole declined over time. In summary, the results suggest that Tebuconazole has some antifungal activity against the tested fungus, especially on days 3 and 5. However, the fungicide efficacy decreased over time, and further studies are needed to determine the optimal dosage and application frequency for controlling fungal growth in pomegranate fruit (Table 3).

Treatment with Thiophanate methyl 50%WP

The antifungal activity of thiophanate methyl against the fungus was evaluated over seven days. The growth of fungal colonies was monitored in two groups: one with fungicide treatment and one without (control). The control group showed a consistent increase in fungal growth over time, while the treatment group showed a

significant reduction. On day 3, the fungal colony growth in the control group was 90 ± 0.00, whereas in the treatment group, it was substantially lower at 61.6 ± 3.51. This indicated a growth inhibition of 21.53 ± 3.76%, demonstrating the effectiveness of thiophanate methyl on day 3. On day 5, the fungal colony growth in the control group remained at 90 ± 0.00, while in the treatment group; it further decreased to 57 ± 3.60. This represented a growth inhibition of 27 ± 3.17%, indicating the sustained effectiveness of thiophanate methyl on day 5. On day 7, the fungal colony growth in the control group was 80 ± 0.00, while in the treatment group, it dropped to 51.3 ± 3.21. The growth inhibition percentage on day 7 was calculated to be 15.92 ± 3.27, suggesting a decline in the effectiveness of thiophanate methyl over time. These results indicated that thiophanate methyl effectively inhibited the growth of the tested fungus, especially on days 3 and 5. However, its effectiveness diminished throughout the experiment. Further studies are needed to determine the optimal dosage and frequency of application for controlling fungal growth in pomegranates (Table 4).

Table 3. Effect of treatment of Tebuconazole on the growth of *Aspergillus niger.*

	Colony Growth		
Time interval	In control	In fungicide treatment	%Inhibition
Day 3	80 ± 0.00	15 ± 1.00	61.25 ± 1.25
Day 5	90 ± 0.00	17 ± 1.00	71.11 ± 1.11
Day 7	90 ± 0.00	57 ± 3.60	27 ± 4.05

Table 4. Effect of treatment of Thiophanate methyl 50%WP on the growth of *Aspergillus niger.*

	Colony growth		
Time interval	In control	In fungicide treatment	%Inhibition
Day 3	90 ± 0.00	61.6 ± 3.51	21.53 ± 3.76
Day 5	90 ± 0.00	57 ± 3.60	27 ± 3.17
Day 7	80 ± 0.00	51.3 ± 3.21	15.92 ± 3.27

DISCUSSION

We conducted the first study on monitoring pre-harvest rot of pomegranate caused by *Aspergillus niger* in the Gilgit district, Pakistan. Our research may have significant implications for enhancing fruit productivity and health in the study area. Moreover, it can provide a basis for further investigation on pre-harvest monitoring of pomegranate rot. We tested the antifungal activity of Tebuconazole 25.9 WP and Thiophanate methyl 50%WP on pomegranate fruit infected by the fungus. Both fungicides effectively inhibited the fungal growth in the treatment groups compared to the control groups, indicating that they controlled the disease. However, the effectiveness of the fungicides decreased over time, as shown by the increased fungal growth in the treatment groups on day 7 compared to days 3 and 5. The decrease in effectiveness may be due to various factors, such as the degradation of the fungicides, the emergence of resistant fungal strains, or the regrowth of the pathogen from the infected fruit. Further studies are needed to identify the factors affecting the effectiveness and to develop strategies to overcome them.

In addition, the bio-control experiment results showed that *T. harzianum* effectively inhibited *A. niger*'s growth. The inhibition percentage was 60%, indicating that *T. harzanium* has potential as a bio-control agent for controlling *A. niger* on pomegranate fruit. However, further studies are needed to determine the optimal dosage and application frequency for achieving the best results. Overall, the results suggest that chemical and biological methods can control fungal growth on pomegranate fruit. However, further studies are needed to determine the best strategies for managing fungal growth under different environmental conditions and to ensure the safety of methods for consumers and the environment. The findings of the current study showed that the sweet variety had the highest disease incidence. The sweet variety (isakoli) is susceptible to various diseases, but notable ones include fruit rot, leaf spots, leaf blight, flower bud rot, and damping-off. *A. niger* caused fruit rot, which was prevalent in GB, affecting 60-75% of pomegranates (Li et al., 2009). Pomegranate

losses before harvest, such as rotting, have been a severe issue in Gilgit-Baltistan for the past five years. The survey's objectives in the Gilgit district included a physico-chemical examination of several common pomegranate varieties that are commonly produced in Gilgit-Baltistan and an evaluation of the pre-harvest losses of the sweet variety, which are typically and readily affected by pathogens. The survey found that Nomal, Gilgit, and Danyore had the lowest mean pre-harvest losses, whereas Haramosh and Jalalabad had the highest losses on average, as shown in Figure 1 (G.N.M. Kumar, 1990). We discussed fruit rot with farmers while conducting a field survey. They exchanged information that was essentially identical to what was previously reported. Fruit rotting is more severe during the rainy season or when rain has been abundant (Onur et al., 1988).

Our findings suggest that pathogens can be controlled under *in vitro* conditions by using *Trichoderma* spp. These fungi were able to inhibit the growth of pathogens on PDA in a dual culture, and exhibited mycoparasitic activity by deforming, bubbling, and decomposing the hyphae of the pathogens. They may also produce antifungal phenolic compounds (Saba Banday et al., 2008). Plant-pathogenic fungi are a common problem, and chemical treatments are often ineffective (Anand and Jayarama, 2009). Moreover, the high cost of using fungicides to manage diseases caused by soil-borne fungi is a limiting factor in crop production efficiency. Biological control may be the best alternative in those situations. *Trichoderma* is mainly used as a fungal biological control agent, and is known for its strong competition against plant-pathogenic fungi (Chet et al., 1981; Papavizas, 1985).

CONCLUSIONS

In Gilgit-Baltistan, there have been no published reports on pomegranate rot. However, the recent study has shed light on this issue, identifying *Aspergillus niger* as the primary pathogen responsible for fruit rot in the region. The significance of this study lies in its contribution to our understanding of pomegranates in this specific geographic location, which can prove valuable for agricultural researchers and students studying the area. By pinpointing potential factors that might contribute to pomegranate rot, such as different cultivars, agro-climatic conditions, and environmental factors, researchers can develop targeted strategies to prevent or mitigate fruit rot in the future.

The primary objective of the study was to add to the existing knowledge about pomegranates and their susceptibility to rot in Gilgit-Baltistan, while also providing essential information on the fruit's nutritional composition. Moreover, the study underscores the significance of preserving local genetic resources, as local cultivars are often better adapted to the specific environmental conditions of Gilgit-Baltistan, and they may possess unique properties that make them valuable sources of nutrients or other beneficial compounds. Furthermore, the study offers valuable insights into the nutritional composition of pomegranates in the region, which can be beneficial for researchers and the general public alike. Pomegranates are known to be a rich source of nutrients, including vitamins, minerals, and antioxidants, and their specific composition may vary depending on factors such as cultivar, growing conditions, and maturity. By disseminating this information, the study aims to promote the consumption of pomegranates as part of a healthy diet.

ACKNOWLEDGMENTS

We express our sincere appreciation for the cooperation extended by Dr. Salman Ahmad from the Department of Plant Pathology, College of Agriculture, University of Sargodha. We would also like to extend our gratitude to Karakoram International University for providing internet facilities. Additionally, we acknowledge the cooperation of the orchid owners from Heramosh, Jalalabad, and Nomal villages.

AUTHORS' CONTRIBUTION

NH and HA conceived the idea and designed the study; NH, SUD and HA isolated and identified the fungi and conducted the research trials; SH, SK and SH helped in conducting research trials; SUD and WH arranged and analyzed the data; NH, HS and AA prepared the draft and proofread the manuscript.

CONFLICT Of INTEREST

The authors declare no conflict of interest.

REFERENCES

Abudabos, A.M., Yehia, H.M., 2013. Effect of dietary mannan oligosaccharide from *Saccharomyces cerevisiae* on live performance of broilers under *Clostridium perfringens* challenge. Italian Journal

of Animal Science 12(2), e38.

Amin, M., Fitsum, S., Selvaraj, T., Mulugeta, N., 2014. Field management of anthracnose (*Colletotrichum lindemuthianum*) in common bean through fungicides and bioagents. Advances in Crop Science and Technology 3, 19-25.

Anand, S., Reddy, J., 2009. Biocontrol potential of *Trichoderma* sp. against plant pathogens. International Journal of Agriculture Sciences 1(2), 30.

Avenot, H.F., Sellam, A., Karaoglanidis, G., Michailides, T.J., 2008. Characterization of mutations in the iron-sulphur subunit of succinate dehydrogenase correlating with boscalid resistance in *Alternaria alternata* from California pistachio. Phytopathology 98(6), 736-742.

Banday, S., Dar, G.H., Fatima, N., 2008. Influence of biocontrol agents on plant growth and white root rot of apple. Plant Disease Research 23(2), 46-50.

Cheng, J., Park, J.H., Karimi, H.R., Shen, H., 2017. A flexible terminal approach to sampled-data exponentially synchronization of Markovian neural networks with time-varying delayed signals. IEEE Transactions on Cybernetics 48(8), 2232-2244.

Chet, I., Harman, G.E., Baker, R., 1981. *Trichoderma hamatum*; its hyphal interactions with *Rhizoctonia solani* and *Phytium* spp. Microbial Ecology 7, 29-38.

Dhiman, S., Reji, G., Diganta, G., Das, N.G., Indra, B., Bipul, R., Talukdar, P.K., Lokendra, S., 2009. Diversity, spatio-temporal distribution and biting activity of mosquitoes in Tripura state, India. Entomon 34(4), 223-32.

Dinesh, M.R., Sankaran, M., 2016. Perspective of future challenges and options in fruit production and utilization. International Journal of Innovative Horticulture 5(1), 1-13.

Ertuğrul, H., Murat, B., Güngör, O., Soytaş, U., 2020. The effect of the COVID-19 outbreak on the Turkish diesel consumption volatility dynamics. Energy Research Letters 1(3).

Ezra, D., Liarzi, O., Gat, T., Hershcovich, M., Dudai, M., 2013. First report of internal black rot caused by *Neoscytalidium dimidiatum* on *Hylocereus Undatus* (Pitahaya) fruit in Israel. Plant Disease 97(11), 1513.

Geiser, D.M., Klich, M.A., Frisvad, J.C., Peterson, S.W., Varga, J., Samson, R.A., 2007. The current status of species recognition and identification in *Aspergillus*. Studies in Mycology 59(1), 1-10.

Idrees, M., Khan, S., Asghar, M., Shahzaman, M., Khalid, M., Shah, H.A., Raza, M., Aslam U.A., 2021. Evaluation of different insecticides against *Virachola Isocrates* infestation in Pomegranate orchards of Gilgit-Baltistan, Pakistan. Plant Protection 5(2), 95-99.

Kahramanoglu, I., Usanmaz, S., 2016. Pomegranate production and marketing. CRC Press.

Kumar, G. N. M., Knowles, N.R., 1993. Changes in lipid peroxidation and lipolytic and free-radical scavenging enzyme activities during aging and sprouting of potato (*Solanum tuberosum*) seed-tubers. Plant Physiology, 102(1), 115-124.

Li, Y., Guo, C., Yang, J., Wei, J., Xu, J., Cheng, S., 2006. Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. Food Chemistry 96(2), 254-260.

Meshram, D.T., Gorantiwar, S.D., da Silva, J.A.T., Jadhav, V.T., Chandra, R., 2010. Water management in pomegranate. Fruit, Vegetable and Cereal Science and Biotechnology, 4(2), 106-112.

Moller, W.J., DeVay, J.E., 1968. Carrot as a species-selective isolation medium for *Ceratocystis Fimbriata*. Phytopathology 58(1), 123-24.

Morton, J.F., 1987. Fruits of warm climates. JF Morton.

Nasir, E., Ali, S.I., Stewart, R.R., 1972. Flora of West Pakistan.

Onur, M., Reynolds, A.C., 1988. A new approach for constructing derivative type curves for well test analysis. SPE Formation Evaluation 3(01), 197-206.

Papavizas, G.C., 1985. *Trichoderma* and *Gliocladium*: biology, ecology, and potential for biocontrol. Annual Review of Phytopathology 23, 23-54.

Pitt, J.I., Hocking, A.D., Pitt, J.I., Hocking, A.D., 1997. Methods for isolation, enumeration and identification. Fungi and Food Spoilage 21-57.

Rajendiran, R., Jegadeeshkumar, D., Sureshkumar, B.T., Nisha, T., 2010. *In Vitro* assessment of antagonistic activity of *Trichoderma viride* against post harvest pathogens. Journal of Agricultural Technology 6(1), 31-35.

Ratul, M.R., Keswani, C., Bisen, K., Tripathi, R., Singh, S.P., Harikesh B., 2018. Biocontrol technology: Eco-

Friendly approaches for sustainable agriculture 177-90 in Omics technologies and bio-engineering. Elsevier.

Sarma, S.D., He, S., Xie, X.C., 1990. Localization, mobility edges, and metal-insulator transition in a class of one-dimensional slowly varying deterministic potentials. Physical Review B 41(9), 5544.

Xiang, Q., Li, M., Wen, J., Ren, F., Yang, Z., Jiang, X., Chen, Y., 2022. The bioactivity and applications of pomegranate peel extract: A Review. Journal of Food Biochemistry 46(7), e14105.

Yassin, M.T., Mostafa, A.A.-F., Al-Askar, A.A., Sayed, S.R.M., Rady, A.M., 2021. Antagonistic activity of *Trichoderma harzianum* and *Trichoderma viride* strains against some fusarial pathogens causing stalk rot disease of maize, in vitro. Journal of King Saud University-Science, 33(3), 101363.

Zhang, L., McCarthy, M.J., 2012. Black heart characterization and detection in pomegranate using NMR relaxometry and MR imaging. Postharvest Biology and Technology 67, 96-101.