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Response of Various Apple Cultivars against Post Harvest Rottening

Sidra Muqeet*[1], Amer Habib[2], Muhammad Zeeshan Mansha[2] and Amina[3]

[1] Directorate of Pest Warning and Qualty Contol of Pesticides, Punjab
[2] Department of Plant Pathology, University of Agriculture, Faisalabad
[3]Horticultural Research Institute, AARI, Faisalabad
*Corresponding author's Email: sidrarana61@yahoo.com

Abstract: In Pakistan, Apples are grown in Baluchistan, Kashmir and Northern areas. More than a dozen cultivars are grown in Pakistan, a significant amount of apples is lost due to poor postharvest conditions. Apple cultivars, which are popular in apple growing areas, vary in their response to post harvest rottening. Current study was conducted in Seed Health Testing Laboratory, Department of Plant Pathology, University of Agriculture, Faisalabad to screen out apple cultivars against rottening fungi (*Penicillum expansum, Botrytis cinerea, Aspergillus niger, Aspergillus flavus*), five most important cultivars (Royal gala, Kandhari, Red delicious, Golden delicious and Kulu) were collected from market, and fungi causing rottening were isolated. Healthy fruits collected from the market were inoculated with *"Pencillum expansum"*, the most common among isolated fungus species. The apple cultivar response against this species ranged from highly resistant to highly susceptible. Royal gala found to be highly resistant and Red gala found to be highly susceptible.
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1. Introduction

Apple belongs to rose family. Apple (*Pyrus domestica* L.) is one of the most important tree fruit of the world. It is an important part of human diet (Khanizadeh *et al.,* 2008).

In Pakistan, Apples are grown in Murree Hills (Rawalpindi), Northern areas, Kashmir and Quetta. Its important varieties which are grown in Pakistan include Top Red, Red Spur, Red Delicious, Oregon Spur, Golden Delicious, Super Gold, Double Red, Apple Elite, Stark Crimson, Red Rom Beauty, Royal Gala, Red Chief and Mondial Gala (Ali *et al.,* 2004).

It is estimated that about 17% of apples produced in Balochistan are lost during postharvest operations (Shah *et al.,* 2002). In Pakistan, apples kept under the conditions of cold storage for 22 weeks, losses were found to be 28 percent (Ilyas *et al.,* 2007).

Cultivars may also differ in fruit physiology and anatomy (Saleh *et al.,* 2009), including ethylene production, texture, fruit flesh firmness (Larrigaudiere *et al.,* 1997; Stow *et al.,* 2000; Johnston *et al.,* 2001; Nilsson and Gustavsson, 2007) and water loss during storage (Khan and Ahmad, 2005).

The postharvest losses may also depend on storage conditions. Among the external conditions, temperature and relative humidity during postharvest handling operations are the most important factors influencing the storage performance of apple (LeBlanc *et al.*, 1996), which affect the fruit flesh firmness, juice content, weight loss, pH, soluble solids content (SSC), and other quality (Tu *et al.*, 2000).

A number of fungi cause large losses during and after harvest of fruits in apple during storage and shipping for export purposes. During storage, more than 90 species of fungi are reported which cause apple rot (Jones and Aldwinckle, 1991). Climate and storage environment are important factors effecting the relative significance of each pathogen. These pathogens enter the apple fruits through injuries or openings in the growing season or after harvesting (Biggs, 1995; Roberts, 1994) and enhance the losses during the storage (Sholberg and Haag, 1996). Moreover, harvesting with no care, characterized by undeveloped and over mature fruit, is another severe source of post- harvest losses (Ingle *et al.,* 2000). The most significant kinds of fungi which cause post-harvest diseases are: Penicillium spp, Aspergillus, Alternaria spp, and *Botrytis cinerea.*

The most important reasons which affect the harvested apple fruits, while handling, exporting, storing or transporting them, *Penicillium granulatum*, and *Penicillium viridicatum* and *Penicillium expansum* fruit rot problems.

Postharvest diseases cause losses that are greater than generally realized because the value of fresh fruits and vegetables increases several-fold with the passing from fields to consumers (Eckert and Sommer, 1967). Postharvest losses have been estimated to range from 10 to 30% per year despite the use of modern storage facilities and techniques (Harvey, 1978). Fruit crops are attacked by a wide range of microorganisms in their postharvest phase (Snowdon, 1990; Ogawa & English, 1991). Apples are kept in storage from 4-6 months up to 1 year to ensure that fruit are available year round for consumption. While in storage, apples are highly susceptible to decay. One of the economically most important problems worldwide is blue mould decay. It is caused by Penicillium spp., among which *P. expansum* is prevalent. Blue mould decay leads to significant economic losses during storage that also impacts fruit destined for processing by its production of the carcinogenic mycotoxin patulin (Barkai-Golan, 2008).

P. expansum infects fruit primarily through wounds caused by stem punctures or bruises occurring at harvest or during postharvest handling. The fungus can also enter the fruit through natural openings, i.e. lenticels, stem ends and the calyx end (Rosenberger *et al.,* 2006).

Symptoms occur as light colored, soft lesions. Lesions are soft due to maceration of the tissue by polygalacturonase enzyme which plays a significant role in *P. expansum* virulence (Jurick *et al*., 2010). As a lesion expands the decayed portion can easily be separated from the surrounding sound tissue. Fungal growth on the lesion surface, at first white, becomes pale blue as sporulation occurs. Although blue mould lesions mainly start from wound infections, the fungus can cause nesting in a fruit bin by growing into neighboring healthy fruit (Sommer *et al.*, 2002). *P. expansum* produces abundant conidia that are readily airborne.

Penicillium species have been studied over 200 years and the genus was first described in 1809. Initially, morphological identification methods were used, but diversity within the genus has resulted in researchers seeking alternative techniques and approaches to improve accuracy. These methods have involved biochemical analyses of secondary metabolites in conjuction with morphological examination. With the emergance of more accurate and rapid molecular identification tools scientists embraced modern technology to address diversity challanges. In order to provide a more holistic approach towards the taxonomy of complex genera, morphological analysis remains the essential component in Penicillium identification (Frisvad and Samson, 2004; Pitt and Hocking, 2009). Since several Penicillium species can cause blue mould decay and having in mind that their identification is difficult due to their morphological similarities (Remove this irrelevant), the aim of this study was to identify the causal agent of apple decay in several storage facilities using morphological. The thallus (mycelium) of the Penicillium is multinucleate, septate, and mostly colourless and conidia are oval to globose in structure and crystal or glass beads shaped (Kirk *et al,* 2008).

2. Materials And Methods

Market Survey and Sample Collection

A survey of Faisalabad and Kamalia markets was made. Five varieties were collected i.e. Royal Gala, Kulu, Kandhari, Red delicious and Golden Delicious. The specimens of Apple fruit were collected in polythene bags from two different markets and brought to the seed health testing laboratory of Department of Plant Pathology, University of Agriculture, Faisalabad. At the time of collection, the characteristic disease symptoms of fungal infection were recorded, The rotten apples were collected from the maket.

Isolation and Purification of Fungi

The fungi were isolated and identified from apple by employing the following strategy.

- About 1-2 cm diseased portion of rotten apple was cut, surface sterilized with 70% ethyl alcohol and rinsed twice in sterilized distilled water followed by drying with sterilized filter paper, then placed on 2 solidified sterilized potato dextrose agar (PDA) contained in petri plates.
- Small bits of diseased portion were directly placed without surface sterilization on sterilized PDA (Potato Dextrose Agar).

Identification of Isolated Fungi

To identify the fungi, characters of isolated fungi were carefully noted with morphological characters. Following literature was consulted for the identification of test fungi, Raper and Thom (1949), Mirza *et al.*, (1979) and Schipper and Stalper (1984). Pure culture of Blue mould *(Penicillium expansum*) was made for further processing.

Artificial Inoculation

Purified culture of isolated fungi for inoculation were made on Potato Dextrose agar (PDA) medium. Fresh fruits were injured with help of sterilized needle. Seven holes were made in 1cm circle. Circles were made with the help of marker, then fungus was applied on these holes with help of contaminated needle and these samples were placed in polythene bags and were remained on room temperature.

Pathogenicity and Screening Test

Initially screening of the indigenous commercially cultivated varieties was done to measure their ability to resist against the disease during post-harvest. In this trial different apple cultivars Royal

gala, Red delicius, Golden delicious, Kulu and Kandhari were used. In this screening method fungus were inoculated with contaminated needle to check their results and efficacy. The seeing results were assessed on the basis of disease rating scale. In order to determine whether the fungal isolated were responsible for causing rots, pathogenicity tests were carried out in the Laboratory. Pure culture was reproduced in laboratory on PDA medium. Tap water was used for washing healthy and fresh apples. A similar set of apple was inoculated with each isolate without making injuries. In each case a control was also used for inoculation and treated similarly except that no inoculum was used. The experiment was conducted in Complete Randomized Design (CRD). There were three replications of each treatment, in each replication four apple fruits were used. After this inoculated apple with or without injuries were kept at room temperature in polythene bags for observations. The reisilations were made from the inoculated apple fruit were examined morphologically for the companion with the fungi isolated if they yielded isolated identical in all respects to the isolates used in inoculate healthy apples to confirm the screening of isolates obtained from disease samples.

Data recording

Fruit decay was recorded after 6 days kept in polythene bags in room temperature. Disease rating scale developed by Cuero *et al.*, 1987 was used.

Table 1: Disease Rating Scale

Score	Symptoms	Remarks
0	No visible symptoms	Immune
1	Mould growth covered less than ¼ of apple surface	Resistant
2	Mould growth covered greater than ¼ but less than ½ of apple surface	Moderately Resistant
3	Mould growth covered ½ or more but less than ¾ of apple surface	Susceptible
4	Mould growth covered ¾ or more of apple surface	Highly Susceptible

Statistical analysis

The experiments i.e screening of apple cultivars, were carried out in the laboratory condition ns designed according to completely randomized design with factorial arrangement (Steel *et al.*, 1997). The data was analyzed by statistical techniques and means were tested for significance.

3. Results and Discussion

Screening of Various Apple Cultivars

In the screening trial of different cultivars Royal gala, Red delicious, Golden delicious, Kulu and Kandhari were tested against post-harvest rottening of apple fruit by different fungi. Rottening fungi were isolated from these apple cultivars. The species isolated from each cultivar are shown below in table-2. The species *Penicillium expansum* was isolated from all apple cultivars, while *Botrytis cinerea* was isolated from Red delicious, Golden delocious, Kulu and Kandhari varieties except Royal gala. *Aspergillus niger* was isolated from three cultivars Royal Gala, Golden delocious and Kulu *Aspergillus flavus* was isolated from only two cultivars namely Red delicious and Golden delicious.

As *Penicillium expansum* was isolated from all the apple cultivars therefore this was used to study the fruit rottening by artificial inoculation. Five varieties of apple were screened by artificial inocculation with *Penicillium expansum* and their response was observed according to scoring scale given in table 3. Royal gala showed resistant response because *Penicillium expansum* covered about 2 cm fruit area and covered less than one forth of fruit surface. Red delicious showed highly susceptible response because mould growth (*Penicillium expansum*) covered about 13 cm fruit area and covered more than ½ of apple surface and showed more mould growth. Golden delicious showed susceptible response because mould growth covered about 7cm area and more than ½ cm and less than ¾ of fruit surface. Kulu and Kandhari also showed susceptible response because mould growth covered about 7cm area and more than ½ cm and less than ¾ of fruit surface.

Table 2. Association of post-harvest fungi with different apple cultivars

Fungi	Apple Cultivars				
	Royal Gala	Red Delicious	Golden Delicious	Kulu	Kandhari
Penicillium expansum	+	+	+	+	+
Botrytis cinerea	-	+	+	+	+
Aspergillus flavus	-	+	+	-	-
Aspergillus niger (Black rot)	+	-	+	+	-

+= Isolated - = Not isolated

Table 3. Response of different Apple cultivars after Artificial Inoculation of P*enicillium expansum* after 6 days

Score	Symptoms	Rating Score	Remarks
Royal Gala	Mould growth covered about 2cm area (less than ¼ of apple surface)	1	Resistant
Red Delicious	Mould growth covered about 13cm area (more than ½ of apple surface)	4	Highly Susceptible
Golden Delicious	Mould growth covered about 7cm area (more than ½ and less than ¾ of fruit surface)	3	Susceptible
Kulu	Mould growth covered about 7cm area (more than ½ and less than ¾ of fruit surface)	3	Susceptible
Kandhari	Mould growth covered about 7cm area (more than ½ and less than ¾ of fruit surface)	3	Susceptible

As the apples are stored for long time and 10-30% losses have been reported during storage by Harvey 1978 and Ilyas *et al.* 2007. Large number of species have been reported that causes post harvest rottening (Snowden, 1990, Jones and Aldwinckle, 1991, Ogawa and English, 1991). Blue mould (*Penicillium expansum)* has been reported as the major cause of decay. (Barbai-Golan, 2008). In current study isolated mould species showed prevalence of 4 species viz- *Penicillium expansum, Botrytis cinerea, Aspergillus flavus and Aspergillus niger*. It was shown by the above given results that *Penicillium expansum* was found on all the varieties studied and papered as major cause of rottening thus reducing their shelf life. Rosenberger *et al.,* 2006 and Jurick *et al.,* 2010 reported that *P. expansum* enters the fruit through holes and wounds and causes symptoms of the decay (Biggs, 1995, Roberts, 1994, Sholberg and Haag, 1996 and Ingle *et al.,* 2000). As the varieties when inocculated artificially showed varying response to this mould species therefore it is suggested that different varieties should be evaluated for their shelf life and research work may be conducted to work out efficient post harvest handling technique. Royal gala which is an important variety in Pakistan (Shah *et al.,* 2002, Ali *et al*., 2004) was found more resistant as compared to other varieties against *Penicillium expansum.* There is also need to find out resistance level of all the varieties against different fungal species for efficient handling and reducing losses during storage

References

1. Khanizadeh, S., R. Tsao, D. Rekika, R. Yang, M.T. Charles and H.V. Rupasinghe. 2008. Polyphenol composition and total antioxidant capacity of selected apple genotypes for processing. Journal of Food Composition and Analysis, 21(5):396-401.
2. Ali, M.A., H. Raza, M.A. Khan and M. Hussain. 2004. Effect of different periods of ambient storage on chemical composition of apple. Fruit Int. J. Agric. Biol. 6(2):568–571.
3. Shah, N.A., S. Khan, M.A. Kasi and S.M. Khair. 2002. Postharvest and cold storage losses in apple of Balochistan. Asian J. Plant Sci. 1(1):65-66.
4. Ilyas, M.B., M.U. Ghazanfar, M.A. Khan, C.A. Khan and M.A.R. Bhatti. 2007. Post harvest losses in apple and banana during transport and storage. Pakistan J. Agri. Sci. 44(3): 534.
5. Saleh, A.M., O. Ghafir, N. Benissa, and M. F. El-Nady. 2009. Physiological and anatomical comparison between four different apple cultivars under cold storage conditions. Acta Biol. 53(1):21-26.
6. Larrigaudiere, C., J. Graell, J. Salas and M. Vendrell. 1997. Cultivar differences in the influence of a short period of cold storage on ethylene biosynthesis in apples. Post. Biol. Technol. 10:21–27.
7. Stow, J., C.J. Dover and P.M. Genge. 2000. Control of ethylene biosynthesis and softening in Coxs Orange Pippin apple during low-ethylene, low-oxygen storage. Postharvest Biol. Technol. 18:215–225.
8. Johnston, D.S., E.W. Hewett, N.H. Banks, F.R. Harker and M.L.A.T.M. Hertog. 2001. Physical change in apple texture with fruit temperature: Effect of cultivar and time of storage. Post. Biol. Technol. 16:107-118.
9. Nilsson, T. and K.H. Gustavsson. 2007. Postharvest physiology of aroma apples in relation to position on the tree. Postharvest Biol. Technol. 43:36–46.
10. Khan, M.A., and I. Ahmad. 2005. Morphological studies on physical changes in apple fruit after storage at room temperature. J. Agric. Social Sci. 1(2):102–104.

11. LeBlanc D. I., R. Stark, B. MacNeil, B. Goguen and C. Beraulieu. 1996. Perishable food temperature in retail stores. New Development in refrigeration for Food Safety and Quality. Int. Inst. Commission. 6:42-57.
12. Tu, K., B. Nicolai and J.D. Baerdemaeker. 2000. Effects of relative humidity on apple quality under simulated shelf temperature storage. Scientia Horticulturae. 85(3):217-229.
13. Jones, A.L. and H.S. Aldwinckle, 1991. Compendium of Apple and Pear Diseases. The American Phytopathological Society. St. Paul, Minnesota, USA.
14. Ogawa, J. M. and H. English. 1991. Diseases of temperate zone tree fruit and nut crops. Davis, CA: University of California, Division of Agriculture and Natural Resources.
15. Biggs, A.R. 1995. Detection of latent infection in apple fruit with paraquat. Plant Dis. 79:1062-1067.
16. Roberts, R.G. 1994. Integrated biological control of post-harvest disease management strategies. Hort. Sci. 29:758-762.
17. Sholberg, P.L. and P.D. Haag. 1996. Incidence of post-harvest pathogens of stored apples in British Columbia. Can. J. Plant Pathol. 18:81-85.
18. Ingle, M., M.C. D'Souza, and E.C. Townsend. 2000. Fruit Characteristics of York apples during development and after storage. Hort. Sci. 35(1):95-98.
19. Eckert, J.W. and N.F. Sommer. 1967. Control of diseases of fruits and vegetables by postharvest treatment. Ann. Rev. Plant Pathol. 5:391-432. Experimental Station, University of California, Berkeley Publications, NE.
20. Harvey, J.M. 1978. Reduction of losses in fresh fruits and vegetables. Ann, Rev. Phytopathol.
21. Snowdon, A.L. 1990. Color atlas of post-harvest diseases and disorders of fruits and vegetables, Vol. 1: General introduction and fruits. Boca Raton, FL: CRC Press.
22. Barkai-Golan, R. 2008. Penicillium mycotoxins. *In* R. Barkai-Golan and Paster, N. (Eds) Mycotoxins in components. J. Nutr. 2001. 131:955–962, San Diego, USA: Elsevier.
23. Rosenberger, D.A., C.A. Engle, F.W. Meyer and C.B. Watkins. 2006. *Penicillium expansum* invades apples through stems during controlled atmosphere storage. Online. Plant Health Progress. doi:10.1094/PHP-2006-1213-01-RS.
24. Jurick, W.M., I. Vico, V.L. Gaskins, W.L. Garrett, B.D. Whitaker, W.J. Janisiewicz, and W.S. Conway. 2010. Purification and biochemical characterization of polygalacturonase produced by *Penicillium expansum* during postharvest decay of 'Anjou' Pear. Phytopathol. 100:42-48.
25. Sommer, N., R.J. Fortlage and D.C. Edwards. 2002. Postharvest diseases of selected commodities. In A. Kader (Ed.), Postharvest technology of horticultural crops (3rd ed.). Davis, CA: University of California, Agricultural and Natural Resources.
26. Frisvad, C.J. and A.R. Samson. 2004. Polyphrasic taxonomy of *Penicillium* subgenus *Penicillium*: A guide to identification of food and air borne terverticillate Penicillia and their mycotoxins. Stud. Mycol. 49:1-174.
27. Pitt, J.I. and A.D. Hocking. 2009. Fungi and food spoilage. New York, NY: Springer.
28. Kirk, P.M., P.F. Cannon, D.W. Minter and J.A. Stalpers. 2008. Dictionary of the Fungi (10th ed.). Wallingford, UK: CABI. 505.
29. Raper, K.B. and C. Thom. 1949. A manual of the Penicillia. Raton, FL, USA. pp 123-133.
30. Mirza, S.A., J.G. MacGregor and M. Hatzinikolas. 1979. Statistical descriptions of strength of concrete. Journal of the Structural Division. 105(6):1021-1037.
31. Schipper, M.A.A. and J.A. Stalpers. 1984. The Rhizopus microsporus group. Stud Mycol, 25:20-34.
32. Cuero, R.G., J.E. Smith and J. Lacus. 1987. Interaction of water activity, temperature and substrate on mycotoxin production by *Aspergillus flavus*, *Penicillium viridicatum* and *Fusarium graminearum* in irradiated grains. Trans. Bri. Mycol. Soc. 89:221-226.
33. Steel, R.G.D., J.H. Torrie, and D.A. Dickey. 1997. Principles and procedures of statistics: a biometrical approach: McGraw-Hill College. New York, USA.

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