*INT. J. BIOL. BIOTECH.*, 13 (4): 587-592, 2016.

# PREVALENCE AND DETECTION OF FUNGI ASSOCIATED WITH POST- HARVEST ROTS OF BANANA IN KARACHI

**M. Talha Azeem[1], Saleem Shahzad[1] and Nasreen Sultana[2]**

[1]*Department of Agriculture and Agribusiness Management, University of Karachi, Karachi-75270, Pakistan*
[2]*Crop Disease Research Institute, PARC, Karachi-75270, Pakistan*

## ABSTRACT

Three post-harvest rots *viz*. Crown rot, Anthracnose and Cigar end rot were found to be more prevalent in the banana hands collected from Storage godowns, whole sale and Local markets. Crown rot disease was the most common disease affecting banana hands in more than 90% of the localities followed by anthracnose and cigar end rot. A total of 26 fungi were isolated from the rotted banana hands of which *Colletotrichum musae* and *Verticillium theobromae* caused anthracnose and cigar end rot when inoculated singly and in combination with *Fusarium pallidoroseum, Lasiodiplodia theobromae* produced crown rot only.

**Key-words**: Banana, post-harvest diseases, crown rot, anthracnose, cigar end rot, pathogenesis

## INTRODUCTION

Banana is the staple food for more than 400 million people across the globe and stands as the fourth most important food product and is amongst the five most consumed fruits on the planet. More than 1000 varieties of banana are known to be cultivated worldwide (UNCTAD, 2013).The Basrai variety of Cavendish sub group produced outstanding results in Pakistan, especially in Sindh province. Nearly 98% of total plantation present in Sind belongs to the similar subgroup (Khashkeli, 2011). Up till 2012, Banana production in Pakistan was around 115,552 tons with an area harvested of around 27238 Hectares (FAO, 2013). It is the second most important horticultural crop of Sind, cultivated on almost 66 produced in Sind annually and represents 85% of country's total banana production (SDF, 2009). Major areas of Banana production in Sindh include Thatta, Khairpur, Badin, Matiari, Mirpur Khas, Naushero Feroze, Sanghar, Nawab Shah and Jamshoro. However, Hyderabad, Sukkur, Ghotki, Umerkot and Karachi are also included in Banana producing areas but with small area of cultivation (CABI, 2008). It is reported that Pakistan exported 58.8 Thousand Tons of Banana in 2012 (FAO, 2014).

Post-harvest conditions and problems of fruits are the result of combined effect of uncared handling, uncontrolled indigenous factors and changing physiological states (Meah and Khan, 1987; Wilson *et al.*, 1997). Post-harvest diseases are reported to destroy 10-30% of the total yield of crops especially in developing countries (Agrios, 2000; Ilyas *et al.*, 2007; Kader, 2002). In Pakistan post-harvest losses are up to 35-40% of the total production (SDF, 2009).

Rot producing fungi *viz.Lasiodiplodia theobromae*, *Colletotrichum musae*, *Fusarium moniliformae* and *Verticillium theobromae* were reported from banana during transport and storage in Pakistan (Ilyas*et al.*, 2007). Crown rot is a disease complex and is the significant most post-harvest disease of Banana. The most common pathogens associated with crown rot include *Colletotrichum musae*, *Fusarium roseum*, *Fusarium pallidoroseum* and *Lasiodiplodia theobromae*. Other species that cause crown rot includes *Cephalosporium* sp., *Verticillium theobromae*, *Ceratocystis paradoxa* and *Phomopsis* sp. (Lukezic *et al.*, 1967; Meredith, 1965; Ogawa, 1970; Ploetz *et al.*, 1994; Snowdon, 1990).Anthracnose is caused by *Colletotrichum musae*. It is commonly found on wounds but it is capable of attacking healthy fruits as well. Usually the pathogen colonizes the necks of the fingers when they are damaged.(Meredith, 1971, Ploetz *et al*., 1994; Snowdon, 1990).Another important post-harvest disease of banana called as Cigar end rot is caused by *Tachysphaera fructigena* and *Verticillium theobromae.* The fruits become subject to attack by the pathogen in their more immature stages (Wardlaw, 1961)

The assessment of banana post-harvest diseases and survey of markets and storage godowns in Karachi was carried out to study the extent of different post-harvest rots affecting the banana fruit.

## MATERIALS AND METHODS

A survey on the post-harvest handling and diseases of banana was conducted in the local markets, whole sale market and storage godowns in Karachi during January to September 2013. The main objective of survey was to identify the major post-harvest diseases and their causal organisms. The sample size consisted of 8-10 hands

randomly collected from the storage godowns, whole sale market and local markets. The environmental conditions including temperature and RH% were also recorded. Diseased fruits were then brought to the Plant Pathology Laboratory, Department of Agriculture and Agribusiness Management for the isolation of rot producing fungi.

Diseased tissues were cut into 2-3 cm long pieces, surface sterilized with 2% NaOCl and placed in Petri plates containing Potato Dextrose Agar (PDA), Czapek Agar (CzA) and Carrot Agar (CA). The Petri plates were incubated at $28^0$C for 5-7 days and fungi growing on diseased tissue were isolated in pure culture. The fungi were identified after reference to Barnett and Hunter (1998), Domsch *et al*. (1980), Ellis (1971), Kulwant (1991), Nelson *et al*., (1983), Raper and Fennel (1965) and Watanabe (2002). Frequency of Colonization (%) and Abundance (%) were calculated using the following formulae:

$$\textbf{Frequency of Colonization (\%)} = \frac{\text{No. of segments from which a given species was isolated}}{\text{Total number of segments used for isolation}} \times 100$$

$$\textbf{Abundance (\%)} = \frac{\text{No. of banana hands colonized by a particular fungus}}{\text{Total number of banana hands sampled}} \times 100$$

Pathogenic potential was confirmed by Koch's postulates (1884) for which healthy banana hands carrying around 8 fingers each were first surface sterilized with 2% NaOCl solution for 2 minutes, washed with sterile distilled water and dried on sterilized blotter paper. With the help of a 5mm sterile cork borer wounds were created in each hand. To plug the wounds a 5mm diameter culture discs cut from an actually growing culture of isolated fungi were used both individually as well as in combination. Intact banana hands with the application of plug of simple agar were set as controls. The banana hands were tied in plastic bags and incubated at room temperature of 25 ± 2°C. The tests were replicated thrice.

## RESULTS

In a survey of storage godowns, whole sale market and local market of bananas in Karachi carried out during January to September 2013, a total of 90 samples of banana hands including 45 samples from storage godowns, 27 from whole sale markets, and 18 from local markets were collected. Most of the banana samples were carrying symptoms of major post-harvest rots. The sample size consisted of 9 hands of banana each carrying 8-10 fingers randomly collected from the above mentioned localities. During the survey, it was observed that no proper packing facilities were existing. Banana bunches were brought by means of trucks in an open environment. Later on, the bunches were cut down into hands and packed into open wooden boxes in extremely poor sanitary conditions in open areas. Most of the storage godowns were not maintained properly except a few. On the basis of symptoms, three post-harvest rots *viz.* Crown rot, Anthracnose and Cigar end rot were found to be more prevalent in the banana hands collected from Storage godowns, Whole sale market and Local market(s). Crown rot disease was the most common disease affecting banana hands in more than 90% of the localities visited followed by anthracnose and cigar end rot. Crown rot symptoms included blackening and softening of the tissues at the crown surface. The crowns were invaded by white and greyish molds with the blackening and rotting of the finger stalks as well. Anthracnose symptoms included dark spots having white or yellowish margins. Cigar end rot was the least prevalent post-harvest disease with symptoms of darkening of the perianth tissues.

A total of 26 fungi were isolated from the affected banana hands. Of these, *Fusarium pallidoroseum, F. solani, Aspergillu tamarii, Colletrrichum musae, A. niger, Verticillium theobromae* and *Lasiodiplodia theobromae* were more frequently isolated (Table 1). However, *F. pallidoroseum, C. musae, L. theobromae* and *V. theobromae* were the most abundant.

Pathogenesis of fungi *viz. F. pallidoroseum, L. theobromae, C. musae* and *V. theobromae* was conducted. Single inoculation of banana hands with *Colletotrichum musae* and *Verticillium theobromae* produced the symptoms of Crown rot, Anthracnose and Cigar end rot. However, banana hands inoculated singly with *Fusarium pallidoroseum* and *Lasiodiplodia theobromae* showed the symptoms of crown rot only. Above mentioned fungal isolates when used in combinations were found to produce symptoms of crown rot in banana hands. All the fungi were successfully re-isolated which confirmed their pathogenicity (Table 2). Statistically, significant differences in % area rotted were present between the control and the treatments (*Fusarium pallidoroseum+ Colletotrichum musae + Lasiodiplodia theobromae+ Verticillium theobromae* (Crown rot), *Colletotrichum musae* (Anthracnose) and *Verticillium theobromae* (Cigar end rot) on the $7^{th}$ day (Table 3).

Table 1. Frequency of colonization (%) of fungi isolated from diseased banana fruits collected from different localities.

| | Fungi | Frequency of Occurrence (%) |
|---|---|---|
| 1 | *Absidia* sp. | 2.88 |
| 2 | *Aspergillus candidus* | 3.85 |
| 3 | *Aspergillus flavus* | 1.92 |
| 4 | *Aspergillus fumigatus* | 4.33 |
| 5 | *Aspergillus niger* | 6.25 |
| 6 | *Aspergillus* sp. | 1.92 |
| 7 | *Aspergillus tamarii* | 7.69 |
| 8 | *Chaetomium* sp. | 1.92 |
| 9 | *Cladosporium* sp. | 1.92 |
| 10 | *Colletotrichum musae* | 6.73 |
| 11 | *Colletotrichum* sp. | 3.85 |
| 12 | *Curvularia lunata* | 1.92 |
| 13 | *Drechslera indica* | 3.85 |
| 14 | *Fusarium moniliformae* | 4.81 |
| 15 | *Fusarium oxysporum* | 0.96 |
| 16 | *Fusarium pallidoroseum* | 10.57 |
| 17 | *Fusarium proliferatum* | 0.96 |
| 18 | *Fusarium solani* | 7.69 |
| 19 | *Lasiodiplodia theobromae* | 4.81 |
| 20 | *Nigrospora oryzae* | 0.96 |
| 21 | *Penicillium citrinum* | 2.88 |
| 22 | *Penicillium* sp. | 0.96 |
| 23 | *Rhizopus stolonifer* | 3.85 |
| 24 | *Ulocladium* sp. | 2.88 |
| 25 | *Verticillium theobromae* | 5.77 |
| 26 | *Verticillium alboatrum* | 3.85 |

Table 2. Pathogenicity of four fungal isolates to banana

| | *Fusarium pallidoroseum* | *Colletotrichum musae* | *Lasiodiplodia theobromae* | *Verticillium theobromae* |
|---|---|---|---|---|
| Crown rot symptoms | ✓ | ✓ | ✓ | ✓ |
| Anthracnose symptoms | × | ✓ | × | × |
| Cigar end rot symptoms | × | × | × | ✓ |

✓ Produced rot symptoms with successful re-isolation; × Rot symptoms were not produced with unsuccessful re-isolation

Table 3.Disease severity of inoculated rot producing fungi.

| Treatments | % Area rotted (% ± SE) |
|---|---|
| **Crown rot:** *Fusarium pallidoroseum* + *Colletotrichum musae* + *Lasiodiplodia theobromae* + *Verticillium theobromae.* | 32.7± 0.61 b |
| **Anthracnose:** *Colletotrichum musae* | 43.2± 0.26 c |
| **Cigar end rot:** *Verticillium theobromae* | 47.1 ± 0.37 d |
| **Control:** No inoculum | 27.9± 0.17 a |

## DISCUSSION

During the present study, crown rot disease was the most prevailing post-harvest rot affecting nearly 90% of banana hands existing in all the localities *viz.* storage godowns, whole sale market and local markets. It was observed that no proper packaging facilities were existing and banana bunches were brought by trucks. Later the banana bunches were cut into hands and packed in open wooden boxes in extremely poor sanitary conditions in open areas where bulk of contaminated discarded banana parts were spread all over the surface. The temperature at storage godowns was not properly maintained as most of the godowns were maintaining the temperature of 67-68$^0$F against the required temperature of 54-60 $^0$F (Kader, 1994). Other important prevailing post-harvest rots of banana were anthracnose and cigar end rot.

Meredith (1971) discussed some of the transport and storage diseases of bananas including crown rot and anthracnose. Irtwange (2006) reported microbiological, mechanical and physiological factors as the major cause of losses in perishable crops. Thompson and Burden (1995) reported the increase of bananas to disease attack due to the long transportation and storage periods at the markets. The major losses in banana often occurs during their transportation to the final market, mainly because of ripening delays, physical defects and post-harvest rots such as crown rot and anthracnose that occur during the transportation (Lassois *et al*., 2010). Ilyas *et al*. (2007) reported approximately 40% post-harvest losses in banana during transportation in Pakistan. Kader (2002) stated that quality and handling of fresh produce is the major concern during transportation. He pointed out that loss during the transportation can be minimized by giving attention to the vehicles, equipment, infrastructure and proper handling practices. Sanitary conditions must be maintained in all areas dealing with post-harvest handling and packaging. It is reported by Spurr (1994) that inoculum sources and development of microbial communities on the fruit surface depends on the surrounding aerial environments. The left over material after sorting and cutting of banana bunches like extraneous bunch parts, leaves and soil can act as substrates for rot producing pathogens (Sholberg and Conway, 2004). Finlay *et al*. (1992) reported that crown rot incidence can be reduced by 50% if trimming and cutting of banana clusters is performed in a clean environment rather than in the open environment.

It was found that the most abundant post-harvest rot producing fungi isolated from different localities of Karachi city *viz*. storage godowns, whole sale market and local markets included *Fusarium pallidoroseum*, *Colletotrichum musae*, *Lasiodiplodia theobromae* and *Verticillium theobromae.* Ilyas*et al*., (2007) reported the isolation of rot producing fungi *viz*. *Colletotrichum musae*, *Lasiodiplodia theobromae* and *Verticillium theobromae* from banana during the transportation and storage. The isolated fungi upon inoculation were found to be the cause of rot production in banana hands and fingers. Similar symptoms of crown rot, anthracnose and cigar end rot were produced as characterized by Snowdon (1990), Ocran (2005) and Ploetz *et al*. (1994) who also reported *Lasiodiplodia theobromae*, *Colletotrichum musae*, *Fusarium pallidoroseum* and *Verticillium theobromae* as the fungi responsible for crown rot which upon inoculation proved their pathogenicity. Inoculation of banana fruit with *Colletotrichum musae* and *Verticillium theobromae* singly confirmed the report of Snowdon (1990) and Thangamani *et al*. (2003) as the causal agent of anthracnose and cigar end rot in banana.

## REFERENCES

Agrios, N.G (2000). *Plant Pathology*. Academic Press Inc., pp.803.

Barnett, H. L. and B.B. Hunter (1998). *Illustrated Genera of Imperfect Fungi*. 4th ed. APS Press, St. Paul, Minnesota.

CABI.(2008). *Survey Report on Technological & Social Constraints Of Banana Production In Sind, Pakistan*. CABI South Asia, Rawalpindi, Pakistan.

Domsch, K. H., W. Gams, W. and T.H Anderson (1980). *Compendium of Soil Fungi.* Vol.1. Academic Press, New York.

Ellis, B (1971). *Dematiaceous Hyphomycetes*. Commonwealth Mycological Institute, Kew, Surrey, England.

FAO.(2013). Production statistics of banana in Pakistan. FAO Statistics Division, FAO. Rome. Available from www.faostat3.fao.org.[ Accessed on 10th Dec. 2014].

FAO.(2014). *Banana market review and Banana Statistics 2012-13*. FAO, Rome.

Finlay, A.R., C. Lubin and A.E. Brown (1992).The banana stalk as a source of inoculum of fungal pathogens which cause crown rot. *Tropical Science*, 32: 343-352.

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 Journal of Agricultural Sciences*, 4(3): 534-539.

Irtwange, S.V. (2006). Application of modified atmosphere packaging and related technology in post harvest handling of fresh fruits and vegetables. *Agricultural Engineering International: the CIGRE journal*, Makurdi, Nigeria, 4(8): 1-13.

Kader, A.A (2002). *Post-harvest Technology of Horticultural Crops.*University of California, Agriculture and Natural Resources. Pub.3311.

Kader, A.A. (1994). Post harvest handling of tropical fruits. *ACIAR proceedings*, 50(1): 239-249.

Khashkeli, M.A. (2011). Controlling diseases in banana cultivation. Available from : www.Dawn. com. [ Accessed on 18th March 2012].

Koch, R. (1884). Die Aetiologie der Tuberkulose. *Mittheilungenausdem Kaiserlichen Gesundheitsamte*, 2: 1–88.

Kulwant S.J.C. (1991). *An illustrated manual on identification of some Seed borne Aspergilli, Fusaria, Penicillia and their Mycotoxins.* Hellerup, Denmark.: Danish Government Institute of Seed Pathology for Developing Countries and Department of Biotechnology, The technical university of Denmark.

Lassois, L., M.H. Jijakli, M. Chillet and L.D.L. De Bellaire (2010). Crown rot of Bananas: Preharvest factors involved in Post-harvest disease development and integrated control methods. *Plant Disease*, 94(6): 648-658.

Lukezic, F.L., W.J. Kaiser and M.M. Martinez (1967). The incidence of crown rot of boxed bananas in relation to microbial populations of the crown. *Canadian Journal of Botany*, 45: 413-421.

Meah, M.B. and A.A. Khan (1987).*Survey of diseases of some important fruit and vegetables crops of Bangladesh*.Annual progress report, Department of plant pathology. Bangladesh Agriculture University, Mymen Singh, Bangladesh, 12-13.

Meredith D.S. (1965). Tip rot of banana fruits in Jamaica. 2. *Verticillium theobromae* and *Fusarium* spp. *Transactions of the British Mycological Society*. 48: 327-336.

Meredith D.S. (1971). Transport and Storage diseases of bananas: Biology and Control. *Tropical Agriculture (Trinidad)*, 48(1): 457-532.

Nelson, P.E., T.A. Toussoun and W.F.O. Marasas (1983). *Fusarium Species. An Illustrated Manual for Identification*. The Pennsylvania State University Press, University Park and London.

Ogawa, J.M. (1970). Post-harvest diseases of banana in China (Taiwan).*FAO Plant protection Bulletin*, 18:31-42.

Ocran, J. K. (2005). *Effect of postharvest hot water and fungicide application on the severity of crown rot disease and quality of Cavendish bananas (Musa spp)*. MSc Thesis, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

Ploetz, R.C., G.A. Zentmyer, W.T. Nishijima, K.G. Rohrbach and H.D. Ohr (1994).*Compendium of tropical fruit diseases*. APS Press, USA, 88.pp 75-76.

Qureshi, M. (2011). Banana export potential remains untapped. Available from: www. Dawn. com. [ Accessed on 19th March 2011].

Raper, K.D. and D. I. Fennel (1965). *The genus Aspergillus*. Baltimore (edn.).The Williams and Wilkins Co.

SDF (2009). *Banana exporting company: developing supply chain to facilitate exports*. Sindh Development Fund (SDF), Tandosoomro, Pakistan.

Sholberg, P.L. and W.S. Conway (2004).*Post harvest Pathology*. UC Davis. Available from: www.nc.postharvest. com. [ Accessed on 21st March 2015].

Snowdon, A.L. (1990). *A colour atlas of post-harvest diseases and disorders of fruits and vegetables* Vol .I: General introduction and fruits. Wolfe Scientific Publications, London, UK, 302.

Spurr, H.W. Jr. (1994). The microbial ecology of fruit and vegetable surfaces: its relationship to postharvest biocontrol. In: Wilson C.L. and Wisnieski M.E. (eds) *Biological control of postharvest diseases–theory and practice.* ARS USDA CRC, Boca Raton, pp. 11–23

Thangamani, P.R., P. Kuppusamy and M.F. Peeran (2003). Morphological and physiological characterization of *Colletotrichum musae* the causal organism of banana anthracnose. *World Journal of Agricultural Science*, 7(6): 743-754.

Thompson, A. K., and O. J. Burden (1995). Harvesting and fruit care. In: *Bananas and plantains* (pp. 403-433). Springer, Netherlands.

UNCTAD. (2013). Banana: *Commodity Profile*. Available from www.unctad.info/en/infocomm/commodity. profile.Banana. [Accessed on 21st Dec. 2014].

Wardlaw, C.W. (1961). *Banana Diseases*. Longmans, Green &Co. Ltd., London, UK. 648.

Watanabe, T. (2002). *Pictorial Atlas of Soil and Seed Fungi* (Second Edition ed.). Florida: CRC Press.

Wilson, C.L., J.M. Solar, A. El-Ghaouth and M.E. Wisniewski (1997). Rapid evaluation of plant extracts and essential oils for antifungal activity against *Botrytis cinerea*. *Plant Disease*, 81: 204-210.

(*Accepted for publication August 2016*)