Comparison of Incidence, Prevalence and Severity of Post-Harvest Fungal Diseases in Pakistan improved integrated management orchards and conventional practices blocks

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Abstract-A comprehensive survey was undertaken in 12 orchards with the objective to record disease distribution during 2013 for assessing the status of major post-harvest diseases of mango in the orchards (farmer and demo block) of Punjab and Sindh province of Pakistan. The data regarding prevalence, disease incidence and severity was collected and the associated fungal pathogens were isolated through tissue segment method on general and specific media. Total of n=60 fruits from each location were studied and predominant fungus was Colletotrichum spp. and Lasiodiplodia spp. in every case. Each fruit was scored on the 0–5 point scale 0% no disease, 1-5% trace, 6-25% mild, 26-50% moderate, 51-71% severe and 76-100% very severe disease. In both provinces Diseases observed were generally the same but incidences, severity and prevalence varied according to management practices employed by individual farmers. Disease incidence was relatively less in 2013 than the surveys of 2011 and 2012 in demo block as compared to farmer block. Disease incidence was highest in Multan (Punjab) i-e 90% during 2013. In farmer block anthracnose disease incidence was ranged from 1-70 % , stem end rot was from 15-80% while in demo block anthracnose disease incidence was 1-38% , stem end rot was from 13-60%. Present investigation also revealed that Colletotrichum spp. and Lasiodiplodia spp. were the fungal pathogens involved in anthracnose and stem end rot and these were the most prevalent post-harvest diseases that damage mango fruit.

Index terms- Post-harvest fungal diseases; Survey; Farmer block; Demo block, Mango.

1.INTRODUCTION:

Pakistani mangoes acquired a superior position in global race due to their good qualities, delicacy and the delicious cultivars. According to statistical analysis Pakistan is the fourth largest producer of mango fruit with exact value of 1,784,300 million tons [1]. Mango production is unfavorably hampered by the several biotic diseases in Pakistan. Among which post-harvest diseases are the main risk to the mango cultivation. Post-harvest losses occur because of infection either by bacteria or fungi or certain physiological disorders [2].

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The post-harvest diseases of mango fruit reduce fruit quality as well as cause severe economic losses, as they lead to the entirely unmarketable fruits. Technologies used for post-harvest processing, packaging, transportation, handling, storage and consumption in Pakistan are traditional which caused 20-40 percent loss of fruit and vegetables. Regardless of the fact that conditions for growing mango is favorable in Pakistan, predominantly in regions of Punjab and Sindh but diseases have rendered yield of mango crop significantly low [3]. Nearly 5% fruit is lost due to the post-harvest diseases, while this figure can be 100% if the conditions are suitable for disease development [4]. During 2007-2008, 20% decline was witnessed in export because in international market, Pakistan received lowest rates (per kg) because of poor quality.

Figure 1: Disease symptoms

The stem end rot has been reported to be caused by several fungi *including Lasiodiplodia theobromae, Dothiorella dominicana, Phomopsis sp.*, and *Alternaria alternate*. The initial symptoms are fruit becomes water soaked and eventually extend internally into the fruit. Later, the affected area enlarges to form a circular brownish black patch which under humid atmosphere extends rapidly and turns the whole fruit black within 2-3 days. Anthracnose caused by *Colletotrichum sp.* is regarded as one of the single most significant threat affecting a vast host range which includes fruits, vegetables and cereals and is considered as most dominant disease of the mango mainly in the humid production areas where the disease incidence may reach to 100%[5]. As the mango is harvested green and most of the mango rots appear when mango starts turning color; at that time mango is placed either in storage or selling points of the markets. In Pakistan for the improvement of mango handling after harvesting for local and export purpose, it is necessary to improved agronomic practices for orchard management. Orchard management has been started in Sindh and Punjab but at initial stages and in few orchards. The purpose of management is to meet the World Trade Organization (WTO) requirements for mango fruit export by the farmers who are planning to export their fruit. So it is necessary to increase interest of farmers in mango fruit export and their attention should be diverted towards the adoption of improved agricultural / agronomical practices for orchard management

and proper fruit handling at the time of harvest [6](Sohail et al. 2011).

The aim of present study is to determine the effectiveness of improved agricultural/agronomic practices in the expression of postharvest diseases anthracnose and stem end rot) in demonstrated blocks and farmers blocks of different mango growing areas of Punjab (Multan, Muzafargargh and Rahim Yar khan) and Sindh (Matiyari, Mirpur khas and Tando Allah Yar), Pakistan based on the disease symptoms; their estimation in terms of incidence, severity, percent disease index and identification of the isolated pathogens and their correlation with the percent diseases index. The present investigation will be focused on famous varieties of mangoes (Sindhri and Chaunsa) in Pakistan during mango growing season 2013.

2.MATERIAL AND METHODS:

2.1.Survey and Sampling:

A survey was conducted during 2013 in the orchards and domestic markets of Punjab (Multan, Muzafargargh and Rahim Yar khan) and Sindh (Matiyari, Mirpur khas and Tando Allah Yar).Sampling was made of equal size from both the demo and the farmer block for analyzing disease trend. Sindhri and white Chaunsa variety of mango was taken for analysis as it is popular exportable variety. The sample size at each location was consisted of 60 mangoes picked randomly. After the packed fruit was transported to Environmental Mycology and Ecotoxicology Laboratory, Department of Environmental Sciences, Fatima Jinnah Women University, Rawalpindi. Sample fruit was kept at room temperature 25oC for ripening 5-7 days. Fruit colour and ripeness data was also collected on daily basis and using the following scale.

TABLE 1: RATING SCALE FOR FRUIT COLOUR AFTER HARVESTING FROM ORCHARD

Number	Colour
1	Green
2	Breaker
3	25%
4	50%
5	75%
6	100%

TABLE 2: RATING SCALE FOR FRUIT FIRMNESS

Number	Firmness
1	Hard
2	Sprung
3	Between sprung & eating ripe
4	Eating ripe
5	Over ripe

2.2.Comparison of farmer and demo block practices:

During sampling and disease assessment information regarding improved harvesting and

management practices in demo block were also gathered and trend in disease incidence were inferred from that data.

2.3. Assessment of Severity, Incidence and Prevalence of Post-Harvest Diseases of Mango

After sampling, sixty mango samples were brought to the Mycology and Ecotoxicology Laboratory, Department of Environmental Sciences, Fatima Jinnah Women University, Rawalpindi and these were categorized on the bases of symptoms of post-harvest diseases namely stem and side Anthracnose, side and Stem end rot and tear stain, subsequently assessment of disease severity, incidence and prevalence was made. For first week mangoes were left to ripen on room temperature and each fruit was assessed for its firmness, colour, and the appearance of disease on daily basis.

Prevalence was calculated with the help of following formula:

Prevalence % = Locations showing mango disease X 100÷ Total Locations

Severity of each post-harvest fungal disease was rated on 1-5 disease severity scale given below [7] .

Disease severity scale	Severity %
1	0 – 1%
2	2- 5%
3	6 – 10%
4	11 – 49%
5	50 – 100%

Disease incidence was calculated by counting visibly diseased mangoes each market in relation to total mangoes

Incidence = no. of diseased mango ÷ total no. of mangoes

Percent Disease Index:

It represents exact status of disease in each orchard combining the disease incidence and the severity and was calculated through following formula [8].

PDI = sum of all individual ratings ÷ total no. of mango graded ×100 / max. Grade

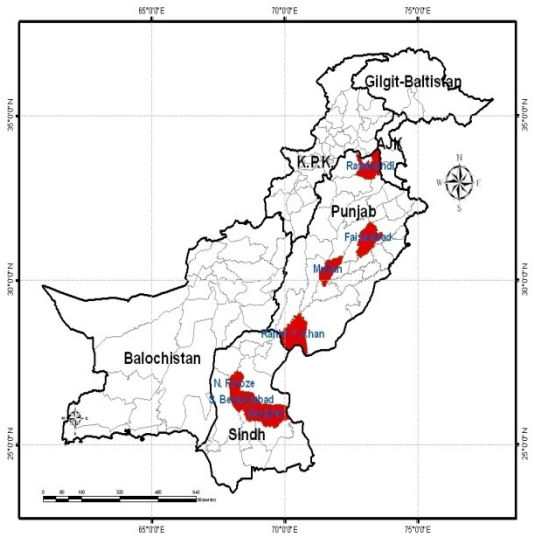
2.4. Isolation, Characterization and Identification of fungi associated with Mango Post Harvest Diseases:

Before the isolation process media bottles, distilled water; Petri plates, syringes and surgical blades were sterilized in the autoclave for 20 minutes at 121˚C and dried in hot air oven at 90˚C. Isolation of fungi from diseased mango was made on both general media i-e potato dextrose agar and specific media. After isolation morphological studies were conducted for fungal identification which includes microscopic studies at 10X, 40X and 100X magnifications of compound microscope and macroscopic characterization.

3. Results And Disscussion:

3.1. **Survey and Sampling:** White Chaunsa and sindhri (mango variety) is regarded as the exporter's commodity so these were targeted from each location and 60 mangoes of each variety were collected randomly (Figure 1). During survey it was observed that techniques for harvesting were mostly manual but as compared to last year's De sapping was quiet improved and no specific storage transport facilities were available for distribution to markets and also there in markets. The mode of transport was cart, van and truck in traditional and nontraditional mango growing areas. For transportation 1-3 days were required from orchard to far markets while in case of mango growing areas 1-3 hour required. Among the samples major diseases of mango fruit were observed like side and stem anthracnose, side and stem end rot, tear stain, sooty blotch and dendritic spots.

FIGURE 2: SAMPLING AREAS



3.2. Practices in demo blocks:

Plant Canopy management and pruning was done on regular basis. One and half feet height rings were made inside 2 feet away from the main trunk of plant. Dried and unwanted branches removed from the mango plants up to 5 to 9 inches away from healthy portion. Fungicides and fertilizers were applied at regular intervals. Due to this Yield in all areas were increased from 50 to 100 boxes each containing 12 mangoes.

3.3. Prevalence, Severity and Incidence of Post-Harvest Diseases:

During the survey , it was found that side anthracnose, side and stem end rot diseases were 100% prevalent in mango fruits of all markets while sooty blotch and dendritic were absent in all locations. The range of disease severity of was varied in all sampling areas. At Multan the disease severity was highest and ranged from 0-5 severity scale. The incidence of disease was more in farmer block as compared to demo block (Table 2). Percent disease index of anthracnose and stem end rot were high in farmer block of Tando Allah Yar (Sindh) and Multan (Punjab).was high at Tando AllahYar followed by Multan and Rahim Yar khan.

Overall disease prevalence, incidence and severity were highest in farmer block Multan (Punjab).

TABLE 3: INCIDENCE, SEVERITY AND PREVALENCE OF MANGO POST-HARVEST DISEASE IN FARMER BLOCK AND DEMO BLOCK

Diseases	Locations	Farmer block			Demo block		
		Incidence	Severity	Prevalence	Incidence	Severity	Prevalence
	Rahim Yar khan	28	1	100	3.33	1	100
	Multan	46.47	1-4	100	4.2	1	100
Side	Mirpur khas	20	1-2	100	8.33	1	100
Anthracnose	Matiyari	1.66	1	100	38.33	1-3	100
	Muzafargargh	21.6	1-2	100	1.66	1	100
	Tando Allah Yar	70	1-5	100	38.33	1-3	100
	Rahim Yar khan	6.66	1	100	1.66	1	100
	Multan	15.49	1-2	100	36.61	1-3	100
Side Stem	Mirpur khas	3.33	1	100	3.33	1	100
end rot	Matiyari	25	1-2	100	11.66	1	100
	Muzafargargh	21.6	1-2	100	11.66	1	100
	Tando Allah Yar	6.66	1	100	1.66	1	100
	Rahim Yar khan	3.33	1	83	3.33	1	50
	Multan	47.88	1-4	83	0	0	50
Stem	Mirpur khas	3.33	11	83	0	0	50
anthracnose	Matiyari	8.33	1	83	15	1-2	50
	Muzafargargh	21.6	1-2	83	3.33	1	50
	Tando Allah Yar	5	1	83	0	0	50
	Rahim Yar khan	33.33	1	100	6.66	1	100
Stem end rot	Multan	73.23	1-5	100	88.73	1-5	100
	Mirpur khas	15	1-2	100	13.33	1	100
	Matiyari	43.33	1-4	100	36.66	1-3	100
	Muzafargargh	45	1-2	100	25	1-2	100
	Tando Allah Yar	80	1-5	100	41.66	1-4	100
	Rahim Yar khan	3.33	1	33	0	0	33
	Multan	88.73	1-5	33	88.73	1-5	33
Tear stain	Mirpur khas	1.66	0	33	0	0	33
	Matiyari	0	0	33	0	0	33
	Muzafargargh	0	0	33	0	0	33
	Tando Allah Yar	0	0	33	0	0	33
	Rahim Yar khan	0	0	0	0	0	0
	Multan	0	0	0	0	0	0
Sooty blotch	Mirpur khas	0	0	0	0	0	0
	Matiyari	0	0	0	0	0	0
	Muzafargargh	0	0	0	0	0	0
	Tando Allah Yar	0	0	0	0	0	0

	Rahim Yar khan	0	0	0	0	0	0
	Multan	0	0	0	0	0	0
Dendritic	Mirpur khas	0	0	0	0	0	0
	Matiyari	0	0	0	0	0	0
	Muzafargargh	0	0	0	0	0	0
	Tando Allah Yar	0	0	0	0	0	0

A comprehensive comparison was made between the farmer and the demo blocks where improved practices were implemented and clear decrease in disease incidence was observed (Table 4).

TABLE 4: COMPARISON OF DISEASE INCIDENCE BEFORE AND AFTER MANAGEMENT PRACTICES.

Cluster	Diseases incidence before practices					Diseases incidence after practices				
	MSD	MMD	ANTH	TDB	SER	MSD	MMD	ANTH	TDB	SER
Matiyari	7%	30%	10%	10%	5%	5%	20%	8%	8%	5%
Mirpurkhas	50%	5 %	20%	40%	5 %	2%	3 %	7%	10%	4 %
T. Allahyar	10%	20%	18%	15%	4%	8%	15%	12%	12%	4%
Multan	10%	25%	15%	20%	20%	7%	20%	10%	17%	10%
Muzafargarh	20%	25%	20%	20%	10%	15%	18%	15%	10%	5%
Rahim Yar khan	10%	20%	18%	15%	5%	7%	10%	10%	10%	2%

*MSD= Mango Sudden Death, MMD= Mango Malformation, ANTH= Anthracnose, T DB= Tip Die back SER=Stem end rot

3.4. Identification and characterization of post-harvest fungal pathogens of mango:

Most dominant fungi were found to be *Colletotrichum gleosporiodes* that was isolated from the diseased mangoes sections of anthracnose to the greater extent while *C. acutatum* to the lesser extent. Lasiodiplodia theobromae was isolated from the diseased mangoes and responsible for stem end rot diseases.

Isolates that were made for sporulation on Potato Dextrose Agar showed typical morphology of *Lasiodiplodia* cultures. It was observed during study that initially the colony color was white which turned to grey within 2-3 days and finally black on maturation and fluffy in texture.The presence of shiny black pycnidia was also observed on the culture plates with maturation which varies in their location some have centered while some have

scattered or peripheral arrangement and these was not grouped. Mycelium was hyaline initially but with time it took shape of twigs with dark brown appearance and septation was also observed. The conidia were also hyaline initially with no septation but after week or 10 days conidia became 1-septate, darker brown in color, walls were bit thicker and ellipsoidal in shape mostly thicker from center as well as upper portion. The size of conidia was ranged from 18-30µm length and 13-15.6µm in width.

Isolates of Colletotrichum gloeosporioides were sporulated on Potato Dextrose Agar showed Two types of colonies One with white mycelium and shiny orange conidial masses which covered most of the area other with white mycelium with no visible conidial masses.

In present investigation 12 locations were targeted for survey of mango collection which was categorized into two groups (1) demo block in orchards of both Sindh and Punjab (2) farmer block of Sindh and Punjab during 2013. It was found that as compared to previous years techniques used for harvesting, picking and storage were improved that lead to a decrease in annual losses and annual production was increased from 700 to 750 boxes. [8] Prabakar et al., 2005 worked on mango fruit to check the loss extent at four stages of storage and marketing (1) retail level, (2) consumer level (3) wholesale level (4) field level. At retail level fungal spoilage was at its peak (40.79%) on the other hand at wholesale level (mandi) the loss was minimum (3.01%). Likewise their results and findings, present investigation also revealed that Colletotrichum gleosporides and L .theobromae fungi were involved in anthracnose and stem end rot of mangoes.

Percentage of diseased fruits was greater in transit variety due to surface injury during fruit transport, handling and packaging as suggested by [9]. Increased fungal incidence and abundance in transit variety may be due to physiological state of fruits. Local varieties suffer less mishandling and are greener than transit ones which show fast ripening during transport. Thus local semi-ripe fruits show low pathogenic activity for most fungi due to low sugar content and less enzymatic activity in fruits [10].

In other case, [11] results contradict from present studies results and at wholesale level there was maximum loss in fruit, about 30.6% loss was observed at this stage. The reason may be that they keep the fruit for a long time at this stage and secondly in present investigation did not compared with other stages during survey so it is difficult to say that either this loss is maximum or minimum as compared to field, consumer or retail level.

Anthracnose found in present results ranged between 3.33–63.33% while according to the [12] results anthracnose fall between 5 – 60%. Colletotrichum gleosporiodes was involved in anthracnose disease while in passion fruit

Colletotrichum passiflorae and glomerella cingulate were the causal agents. Might possible, the host of this specie is passion fruit in Kenya (Thika).

[13]Also reported that infection due to Lasiodiplodia theobromae was most frequent in Multan region but infection of mango plants sample from orchard infected with different decline disorders of mango fruit. As this area is too hot and main mango growing area and it may possible that farmers focused on production not on quality and management practices that could be the reason that mostly fungus attack in this region. As well as their use of nitrogen fertilizers was also excessive which one of the major causes of rots was.

In present investigation the morphological and cultural studies have showed no considerable differences in characteristics among isolates of Lasiodiplodia theobromea and Colletotrichum gloeosporioides that could help in strain differentiation within species. All parameters observed in this study were fitted with the descriptions in relevant references.

Identification of species causing post-harvest diseases like stem end rot , anthracnose and side rots is important in understanding epidemiology of disease as well as its control.

All fungal isolates that were originated from different mango cultivars affected with stem end rot disease exhibited morphological characteristics typical of *Lasiodiplodia theobromea* [14]. [15]Chen et al. (2010) reported conidia of the L. t*heobromae* were ellipsoid to oblong which were broadly rounded from apices, may be tapering or the truncate bases, which were widest from middle towards upper one-quarter sections, these were initially hyaline as well as aseptate but with age became one-septate or dark brown in color, size was ranged from (22.5-) 23.5-26(-27) × (12-)13-14.5(-16) µm.

The grouping of the C. gloeoporioides into two subgroups represented the complexity of species. Many studies on species differentiation of C. gloeosporioides have shown it very variable in morphological characteristics [16] (Arx, 1970) as well as cultural characteristics [17](Than et al., 2008). Different Studies also validated these results according to which spore measurement of the C. *gloeosporioides* were varied between 3.94-12.14 µm length and 1.43-2.14 µm width.

At the time of visual observations sometimes confusion occur due to initial symptoms or mask symptoms so after isolation process it can confirm either that fungi were involved or not. By this study it would be possible to study the post-harvest fungal pathogens in detail by various analyses.

4. CONCLUSTION:

The present work signifies and confirms the role of fungal pathogens and climatic factors in deterioration of mango fruits. Present investigation is just preliminary study and it forms the basis to study the post-harvest fungal pathogens of mango for their pathogenic behavior, Fungicide resistance, and genetic variability which would help in

developing effective control strategies and understanding of management of post-harvest diseases.

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