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Current status of mango pre and post-harvest diseases with respect to environmental factors

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Key words: Pre and post-harvest diseases, Fungal isolation, Disease severity, Disease prevalence, Disease incidence.

Abstract

Mango is an important fruit hence plays an important role in the economy of Pakistan via export. Mango can suffer from serious losses by mango pre and post-harvest diseases. Environmental factors i.e. temperature, rainfall, and humidity have serious effect on the onset of these diseases. Mango pre harvest diseases included mango malformation, blossom blight, anthracnose and twig blight. Mongo post-harvest diseases include anthracnose, stem end rot, *Aspergillus* rot and *Alternaria* rot. A survey has been conducted in 37 orchards i.e. Muzafargarh (15), Multan (12) and Khaniwal (10) for the assessment of mango pre- harvest diseases by using severity scale of 1-5. Post-harvest disease assessment was done on collected mango fruits of respective study areas. Objective of the present study is to assess prevalence, incidence and severity of pre and post-harvest mango diseases with respect to prevalent environmental conditions in study area. Samples for pre and post-harvest diseases were collected from three study sites for the sake of causal fungal isolation. All pre and post-harvest diseases were present in all three study areas. Pre harvest disease incidence was lied in the range of 0-100%, 80-100%, 40-100%, 0-100% for blossom blight, anthracnose, mango malformation and twig blight respectively. Mango post-harvest disease incidence was found in range of 40-90%, 16-86.6%, 16-76.6% and 3.33-53.3% for anthracnose, stem end rot, *Alternaria* rot and *Aspergillus* rot respectively.

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Introduction

Mango *(Mangifera indica)* belongs to a family *Anacardiaceae* and it is recognized as king of fruits. It is one of the delicious fruit having minerals, vitamins and nutritious qualities. It is usually grown in tropical and subtropical regions of the world including subcontinent. Pakistan has 4th rank in mango exporting countries in world (Maqbool *et al.*, 2007). Different districts of Punjab, Sindh and Khyber Pukhtunkhwa are well known to produce mango. Sindhri, Langra, Chaunsa, Samar Bahisht, Anwar Ratoleetc are leading cultivated varieties in Pakistan. In many countries mango is an important constituent of the diet (Mukherjee, and Litz, 2009). However, mango is susceptible to a number of diseases at all stages of its development from the seedling to the fruits (Alemu, 2014). Crop loss may be the outcome of fungal diseases while postharvest diseases may lead to the export losses (Prakash, 2004). The mango tree and fruit is the usually host of different pathogens specifically fungi which produces post-harvest fruit rot worldwide (Diedhiou *et al.*, 2007). Malik *et al.* (2005) concluded that different diseases like tip die back, twig blight, gummosis, stem bleeding, wilting and bark cracking were prevalent in the orchards of Pakistan. Diseased trees presence in orchards and inappropriate management practices may cause the destruction of whole mango orchard (Saeed *et al.*, 2012).

Mango malformation *Fusarium* specie disease is characterized by abnormal flower and leaf development which results in reduced plant and fruit growth. Blossom blight is caused by *Colletotrichumgloeosporioides* (Kolase *et al.*, 2014). Early signs of blossom blight are the formation of black spots or circles on the mango inflorescences. Twig blight is caused by *Lasiodiplodiatheobromae* and their symptoms are shown on branches and twigs which start drying. Anthracnose affects pre- and post-harvest mango quality. It is caused by *Colletotrichumgloeosporioides* (Ploetz, and Freeman, 2009). Symptomsare characterized by semi-circular dark brown lesions along the margin of leaves and large, depressed, black wounds on fruits which ultimately lead to fruit drop off (Pitkethley, and Conde, 2007). *Lasiodiplodiatheobromae* responsible for stem end rot in which dark brown to black spots appear from the tip of mango and spread on whole fruit (Maqsood *et al.*, 2014). *Alternariaalternata*is the causal fungus of *Alternaria* rot causingbrown soft lesions with reddish margins on fruit surface. *Aspergillusniger* is the causal agent of *Aspergillus* rot which causes post-harvest losses by producing black spore fruiting body on fruit.

Temperature, humidity and rain are the primary environmental factors that affect the onset of different mango disease. Anthracnose, stem end rot and *Aspergillus* rot are more prevalent in moist conditions and high humidity (Iram *et al.*, 2013). In areas with heavy rainfall during flowering and fruit set anthracnose is more prevalent lead to serious losses up to 35% of fruits (Martinez *et al.*, 2009). Suitable environmental conditions for the attack of blossom blight include cool weather, heavy dew and fog. Twig blight has optimum temperature range of 20°C-30°C for the growth of *Lasidodiplo-diatheobromae* (Adeniyi *et al.*, 2011). Spores of *C. gloeosporioides* are dispersed by rain drops while spores of *Alternaria* specie are spread by wind. Disease incidence of *Alternaria* rot is dependent on relative humidity (Iram *et al.*, 2013). Storage conditions can also affect the onset of mango post-harvest diseases.

Materials and methods

Three study areas including Multan, Khaniwal and Muzafargarh were selected for assessment of pre and post-harvest diseases of mango. Total 37 orchards including 15 orchards of Muzafargarh, 12 orchards of Multan and 10 orchards of Khaniwal were surveyed for the assessment of mango pre- harvest diseases. In each orchard five trees were carefully observed. For the observation of each tree all four sides i.e. East, West, North and South were taken and from each side10 inflorescence were observed (Masood *et al.*, 2010). Mango malformation and twig blight were observed on the basis of their presence and absence (+) for presence and (–) for absence of disease. For

the pre harvest diseases assessment the effected plant parts were cut down in order to done the fungal isolation for the respective diseases. The samples were kept in brown paper bags and after proper labeling transported in cardboard bags to Environmental Mycology and Ecotoxicology Laboratory, Department of Environmental Sciences, Fatima Jinnah Women University, Rawalpindi.

In order to done post-harvest diseases assessment mango fruits were collected from these study areas. The sample size for each study area was 30. Mangoes were completely sterilized and kept at room temperature 25- 27˚C for 14 days. The black spots started to appear from 5 to 6 days. Fruit colour and ripeness data was collected on daily basis by using the scales (Table 1, Table 2).

Table 1. Grading scale for fruit colour.

Grading	Colour
1	Green
2	Yellowish green
3	Yellow 25%
4	Yellow 50%
5	Yellow 75%
6	Yellow 100%

Table 2. Grading scale for fruit firmness.

Grading	Firmness
1	Hard
2	Sprung
3	Between Sprung and eating ripe
4	Eating ripe
5	Over ripe

Table 3. Severity scale

Scale	Percent severity
1	0–1%
2	2-5%
3	6–10%
4	11–49%
5	50–100%

Assessment of severity, incidence and prevalence of mango pre and post-harvest diseases

Separate Performa were designed to note down the disease severity by using disease severity scale of 1-5 (Corikidi *et al*., 2006) for each pre and post-harvest diseases on the basis of visual observation. These performa were evaluated to calculate the incidence, severity and percent disease index of each disease.

Prevalence

Prevalence of each disease, based on symptoms, was estimated by counting the number of location showing mango disease with respect to total locations (Tucho *et al*., 2014).

$$\% \text{ Prevalence} = \frac{\text{Locations showing mango disease}}{\text{Total Locations}} \times 100$$

Disease incidence

Disease incidence was assessed by counting the number of apparently diseased trees/mangoes per site with respect to overall plants/mangoes in an orchard (Tucho *et al*., 2014).

$$\% \text{ Disease Incidence} = \frac{\text{No. of diseased plants/mangoes}}{\text{Total no. of plants/mangoes}} \times 100$$

Percent disease index (PDI)

Percent disease index was measured by using following formula (Prabakar *et al*., 2005).

$$\text{Percent disease index} = \frac{\text{Sum of all individual ratings}}{\text{Total number of trees/mangoes observed} \times \text{maximum grade}} \times 100$$

Isolation and identification of fungi associated with mango pre and post-harvest diseases

Before the isolation process media bottles, distilled water, Petri plates and surgical blades were sterilized in the autoclave at 121˚C for 20 minutes and then dried at 90˚C in hot air oven. From each area sample causal fungus was isolated. The fungus was isolated from the infected mango tree leaves, twigs and inflorescence as well as from infected mango fruit

part following tissue segment method. Infected diseased samples along with healthy tissues were washed with tap water and then cut into small pieces. These small pieces were surface sterilized and washed with sterilized distilled water. Then these pieces were put on the whatman filter paper for drying andevenly spaced in petri dishes (9 mm diameter) containing solidified media. These plates were placed in an incubator adjusted at 28°C for three days for mycelium growth. After fungal isolations fungal identification were done by compound microscope.

Results

Data of average temperature, relative humidity and annual rainfall of last four years were provided by National Agromet Center Islamabad to assess the current status of mango diseases in prevalent environmental conditions (Table 4).

Table 4. Current status of average temperature, relative humidity and annual rainfall of Multan.

Year	Average Temperature (°C)	Relative Humidity	Average Rainfall (mm)
2011	32.1	53.6	15.5
2012	32.1	49.5	19.9
2013	32.0	52.9	18.8
2014	32.5	54.75	21.5

Pre and Post-harvest fungal pathogens were isolated from diseased mango trees and fruit samples for the sake of pre and post-harvest disease assessment. Isolated causal fungal pathogens were fungal were identified up to genus level by morphological studies based on colony and spore characters of fungi (Table 5).

Table 5. Morphological analysis of fungal pathogens of mango pre and post-harvest diseases.

Diseases	Causal Fungal Agent	Colony characteristics	Spore Characters
Anthracnose/ Blossom blight	*Colletotrichumgloeosporioides*	Dense Whitish mycelium formed with black and orange color fruiting bodies	Smooth, hyaline and sub-cylindrical with round end spores.
Twig blight/ Stem end rot	*Lasiodiplodiatheobromae*	Colony color was observed as white at initial stage of growth, darken from the centre which turned to dark grey after 3-4 days and finally black after one week.	Thick walled, bicelled and dark brown colored spores
Mango malformation	*Fusarium*mangiferae	White, grey, light green in some culture	Kidney shape, elongated, pointed ends, curvy
Alternaria rot	*Alternariaalternata*	Black color colony	Conidia obclavate to ellipsoidal, with a short, cylindrical beak,
Aspergillus rot	*Aspergillusniger*	Black color colony	Sporangiophores of *Aspergillusniger*were prominent under microscopes with short circular to semicircular sporangi spores

Severity of mango pre-harvest diseases

Disease severity of blossom blight and Anthracnose in Multan, Khaniwal and Muzaffargarh was found to be in range of 1-5. Mango malformation was observed in all orchards of Multan, Khaniwal and Muzaffargarh while twig blight was absent in 2 orchards of Multan and 1 orchard of Muzaffargarh and present in all orchards of Khaniwal (Table 6).

Table 6. Disease severity of mango pre harvest diseases of selected study areas.

Locations	Orchards	Disease Severity of Blossom blight	Disease Severity of Anthracnose	Presence +/ Absence – of Mango Malformation	Presence +/ Absence – of Twig Blight
Multan	1	0	1-3	+	-
	2	0	1-3	+	-
	3	1-4	1-3	+	+
	4	1-5	1-5	+	+
	5	1-5	2-4	+	+
	6	1-5	3-4	+	+
	7	1-5	2-4	+	+
	8	1-5	1-3	+	+
	9	1-4	1-4	+	+
	10	1-2	1-3	+	+
	11	1-5	1-4	+	+
	12	1-4	1-2	+	+
Khaniwal	1	1-5	2-4	+	+
	2	1-5	2-5	+	+
	3	1-5	2-5	+	+
	4	1-5	2-5	+	+
	5	1-5	2-5	+	+
	6	1-5	2-5	+	+
	7	1-5	1-4	+	+
	8	1-5	1-4	+	+
	9	1-4	2-5	+	+
	10	1-5	1-5	+	+
Muzaffargarah	1	1-5	1-4	+	+
	2	1-5	1-5	+	+
	3	1-5	1-3	+	-
	4	1-5	1-4	+	+
	5	1-5	1-3	+	+
	6	2-4	1-2	+	+
	7	2-5	1-3	+	+
	8	1-4	1-4	+	+
	9	1-5	2-5	+	+
	10	2-4	2-4	+	+
	11	2-4	3-5	+	+
	12	2-4	3-5	+	+
	13	2-4	1-4	+	+
	14	2-4	3-5	+	+
	15	1-4	1-2	+	+

Table 7. Severity, incidence, prevalence and percent diseases index of mango post-harvest diseases in selected areas.

Location	Diseases	Disease Severity	Incidence %	Percent Disease Index	Prevalence
Multan	Anthracnose	1-5	90	76	100
	Stem end rot	1-4	86.6	72	100
	Alternaria Rot	1-4	76.6	60	100
	Aspergillus rot	1-5	53.3	48	100
Muzaffargarh	Anthracnose	1-5	40	49	100
	Stem end rot	1-5	33.3	37	100
	Alternaria Rot	1-4	26.6	35	100
	Aspergillus rot	4-5	6.6	24	100
Khaniwal	Anthracnose	1-5	40	46	100
	Stem end rot	1-5	16	30	100
	Alternaria Rot	1-5	16	29	100
	Aspergillus rot	1-4	3.33	22	100

Prevalence and incident of pre-harvest diseases in study areas

Blossom blight and twig blight were more prevalent in Khaniwal and Muzaffargarh showing 100% prevalence and it was least in Multan with 83 % prevalence. Anthracnose and Mango Malformation were 100% prevalent in all three study areas (Fig. 1.).

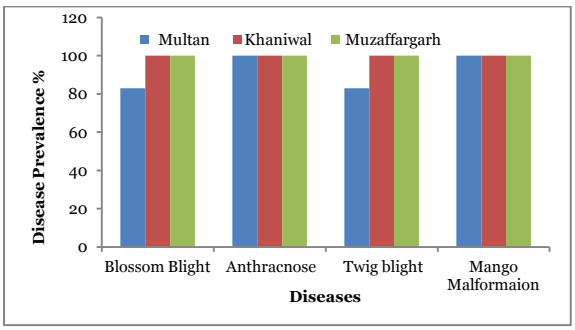

Fig. 1. Disease prevalence in selected study areas.

Incidence of pre harvest mango diseases in study areas

Disease incidence of blossom blight and anthracnose in Multan was found to be 80-100 %. Mango malformation was observed in all 12 orchards of Multan with disease incidence of 40-100% while twig blight was absent in 2 orchards and showed disease incidence of 40-100% (Fig. 2.). Khaniwal has highest disease incidence (100%) of blossom blight and Anthracnose. Mango malformation and twig blight was observed in all 10 orchards of Khaniwal with disease incidence of 60-100% for both diseases (Fig. 3.). In Muzaffargarh disease incidence of blossom blight was 60-100 % and for anthracnose it was 80-100%. Mango malformation was observed in Muzaffargarh with disease incidence of 40-100% whereas for twig blight disease incidence range of 0-100% was observed (Fig. 4.).

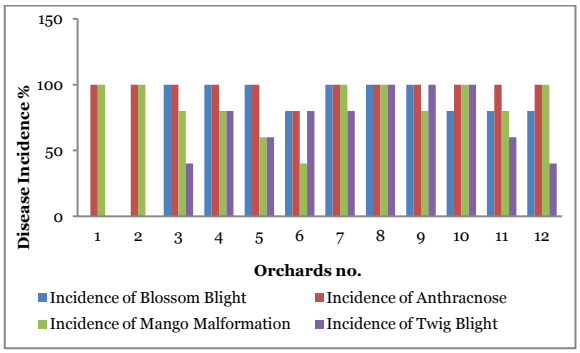

Fig. 2. Disease incidence in Multan.

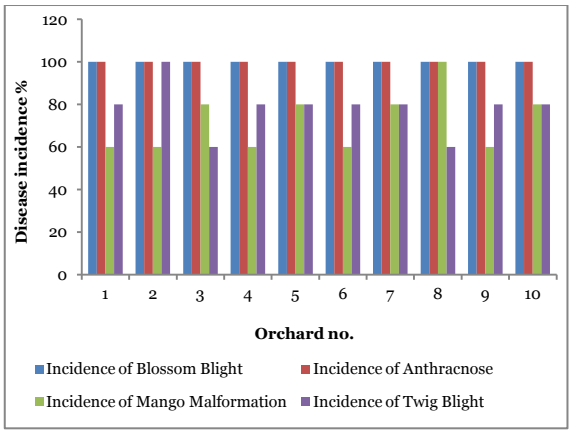

Fig. 3. Disease incidence in Khaniwal.

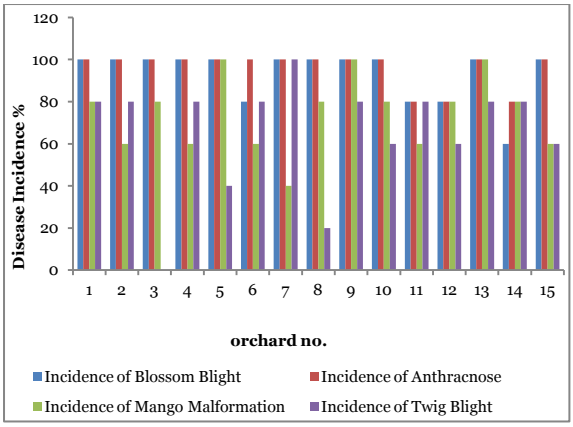

Fig. 4. Disease incidence in Muzaffargarh.

Percent disease index of blossom blight and anthracnose in study area

Blossom blight and anthracnose hadwide range of percent disease index in Multan, Khaniwal and Muzaffargarh orchards calculated with the help of observed diseases severity (Fig. 5, Fig. 6, Fig. 7.).

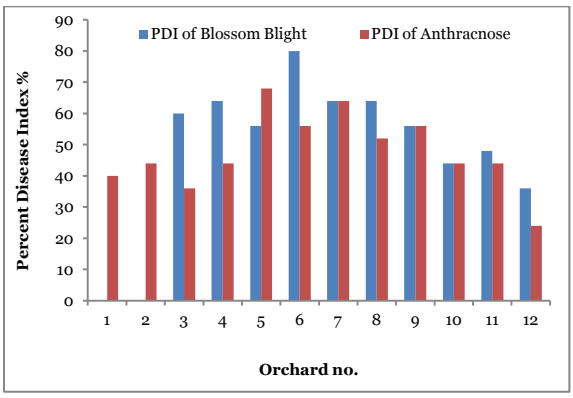

Fig. 5. Percent disease index of blossom blight and anthracnose in Multan orchards.

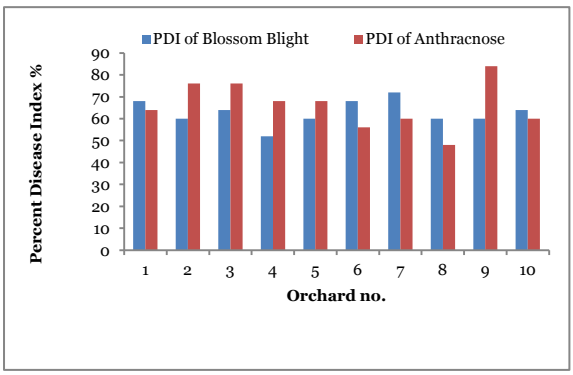

Fig. 6. Percent disease index of blossom blight and anthracnose in Khaniwal orchards.

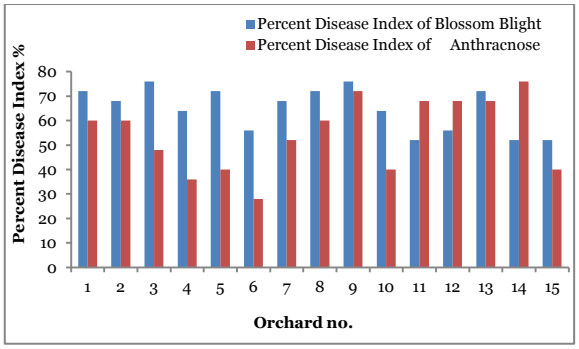

Fig. 7. Percent disease index of blossom blight and anthracnose in Muzaffargarh orchards.

Assessment of post-harvest diseases

Severity of anthracnose ranges from 1-5 in all three study areas while severity of stem end rot ranges from 1-4 in Multan, 1-5 in Muzaffargarh and Khaniwal. *Alternaria* rot has severity range of 1-4 in Multan and Muzaffargarh orchards while it was 1-5 in Khaniwal. Multan and Muzaffargarh has severity range of 1-5 while Khaniwal has 1-4 for *Aspergillus* rot. Observed four post-harvest diseases were present in all three study areas so they showed 100% prevalence.

Analysis of variance (ANOVA) was used to analyze disease incidence and percent diseases index of post-harvest diseases in three study areas. Results were highly significant at P< 0.05 and they showed that values of disease incidence and percent disease index (PDI) differ with different locations.

Incidence and percent disease index of post-harvest diseases in selected study areas

Four observed post-harvest diseases has different incidence in different locations. Multan has highest incidence of anthracnose, stem end rot, *Alternaria* rot, *Aspergillus* rot while Khaniwal has the least incidence of all diseases (Figure 8). Multan has highest percent disease index (PDI), Muzaffargarh has second highest PDI value while Khaniwal has least value of PDI for all observed post-harvest diseases.

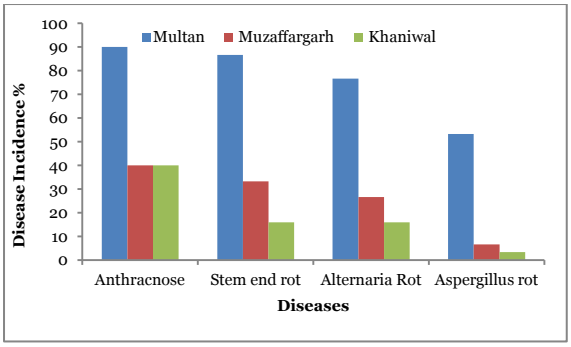

Fig. 8. Comparison of post-harvest disease incidence in selected study areas.

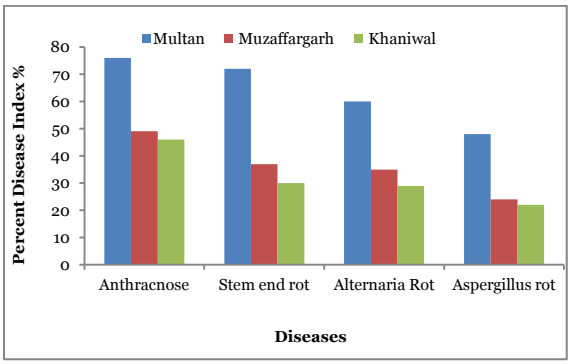

Fig. 9. Comparison of percent disease index of post-harvest diseases in selected study areas.

Discussion

Anthracnose is one of the most prevalent and common disease, which could lead to premature fruit drop and decline in quality of ripped fruits by shortening their storage lifetime. Pre and post anthracnose of mango was found to be 100% prevalent in South West and causal agent was identified as *Colletotrichumgloeosporioides* (Tucho *et al.*, 2014). These results coincide with the results of present study in which it was 100% prevalent in study areas. Disease incidence was found to be 41 - 72.1% on leaf and 36.2-76% on mango fruit while it was recorded 80-100% incidence on leaves, 40-90% on fruits in present study. A study of Naqvi *et al.* (2014) concluded that blossom blight is caused by *Colletotrichum gloeosporioides* and maximum disease incidence was recorded in 43.66 % in Band Bosan while in present study maximum disease incidence was recorded as 100% in selected areas.

Mango Malformation is a serious problem of mango and has become a regulating factor in the setting up of economically viable orchards. In present study this disease was found to be widely distributed in Punjab province with 100% prevalence. This result matches with the results of the study of Iqbal *et al.* (2004) which also showed 100% prevalence of mango malformation in their study area.

A study was planned by Iqbal *et al.* (2007) for the assessment of incidence, prevalence and intensity of mango twig blight which is one of the major diseases predominant in mango growing areas of the Punjab. This study concluded that twig blight is 55.0 % prevalent in Punjab while in present study it is found to be 81% prevalent. One of the major reasons behind post-harvest losses of mango during storage conditions is stem end rot. In vitro studies (Maqsood *et al.*, 2014) were carried out to identify responsible fungal pathogens of stem end rot and *Lasidodiplodiatheobromae* was isolated as causal fungal pathogen similar to present study. Maximum disease incidence of stem end rot was found as 70% while it was 86.6% in the present study.

Assessment of mango post-harvest diseases as done (Meer *et al.*, 2013).In that study *Alternariaalternata* was isolated as causal fungal pathogen of *Alternaria* rot similar to the present study. *Alternaria* rot was found maximum in Shujabad with incidence 16.66%, severity range 1-3, percent disease index of 3.33% and it was 80% prevalent in their study areas. However in percent study Multan has the maximum disease incidence 76.6%, severity range 1-4, percent disease index 60% of *Alternaria* rot and it was 100% prevalent in selected sites. *Aspergillus* rot incidence was found to be 3.33-53.3%, severity range of 1-5, percent disease index of 22-48% in present study while according to Meer et al. (2013) their study areas had diseases incidence of *Aspergillus* rot range from 0- 16.66%, severity range of 1-5, and percent disease index of 0-3.33.

Awa *et al.* (2012) carried out a study to determine the fungal pathogen responsible for major post-harvest diseases caused post-harvest mango fruit rotting during ripening. Different fungi species were

recovered including *Colletotrichumgloeosporioides*, *Botryodiplodiatheobromae* and *Aspergillusniger* associated with fruit rot of ripen mango. This study coincides with the results of present study as its result proposed Colletotrichum gloeosporioides as the causal agent of anthracnose of mango whereas *Botryodiplodiatheobeomae* and *Aspergillusniger* showed rotting symptoms of fruits by causing stem end rot and *Aspergillus* rot respectively.

Conclusion

Present study was aim to assess the mango pre and post-harvest diseases with respect to environmental factors. It was found that all assessed mango pre and post-harvest disease are prevalent in the selected study areas. Mango pre harvest diseases which were assessed include anthracnose, blossom blight, twig blight and mango malformation and their isolated fungal pathogens were *Colletotrichum gloeosporioides* (for bothanthracnose and blossom blight) *Lasiodiplodiatheobromae, Fusarium*mangiferae respectively. Mango post-harvest diseases which were observed in the study areas in present study include stem end rot, *Alternaria* rot, *Aspergillus* rot, anthracnose and their isolated causal fungal agents were *Colletotrichumgloeosporioides, Lasiodiplodia-theobromae, Alternaria alternate, Aspergillusniger* respectively. All pre harvest diseases were present in all three study areas and showed incidence range of 0-100%, 80-100%, 40-100%, 0-100% for blossom blight, anthracnose, mango malformation and twig blight respectively. Multan has the highest disease incidence for each post-harvest disease i.e. 90%, 86.6%, 76.6% and 53.3% for anthracnose, stem end rot, *Alternaria* rot and *Aspergillus* rot respectively. Environmental factors i.e. temperature, humidity and rainfall have significant effect on the inception of fungal diseases because fungal pathogens grow better in moist and warm conditions. Poor orchard management conditions can also count for the pathogen attacks like poor sanitation conditions, lack of care for disease management in the form of pruning and presence of dead fallen leaves etc. In these types of conditions fungal pathogens grow better and attack healthy trees.

Acknowledgments

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