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# Potential of Fungicides and Plant Activator for Postharvest Disease Management in Mangoes

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## ABSTRACT

Mango fruit quality, marketability and shelflife are adversely affected by postharvest disease development. The fungicides and plant activators suppress the disease development in fruits and vegetables. These studies were targeted to evaluate the potential of pre and postharvest fungicidal applications and on-tree foliar spray of a plant activator viz. a viz. salicylic acid for postharvest disease management in two commercial mango cultivars (Sindhri & Samar Bahisht Chaunsa) of Pakistan. *Alternaria alternata* (49.3% in Sindhri; 58.5% in Chaunsa), *Phomopsis mangiferae* (20.0% in Sindhri; 18.2% in Chaunsa) and *Botryodiplodia* sp. (3.9% in Sindhri; 2.7% in Chaunsa) were found to be associated with mango fruit stem end rot (SER) under agro-ecological conditions of Punjab, Pakistan. *Penecillium sp.* and *Aspergillus* sp. (*A. niger* & *A. flavus*) were identified to be associated with secondary infections in the diseased mango fruit tissues. Among the postharvest diseases, fruit side rots were predominant (5-10% affected area) followed by stem end and distal end rots (<5% affected area). Postharvest fungicidal treatments gave significantly better disease control as compared to preharvest applications. Postharvest hot water (52°C; 5min) application of Tecto (1.8 mL/L) a.i. Thiabendazole (TBZ) individually and in combination with Sportak (0.5 mL/L) a.i. Prochloraz (tank mix) resulted in significantly lower postharvest disease development than other pre and postharvest treatments. Pre and postharvest fungicidal applications significantly affected fruit peel color development. Relatively better color development was observed in the fruit subjected to postharvest fungicidal treatments as compared to preharvest applications. Non significant effect of both pre and postharvest fungicidal treatments was observed on biochemical quality attributes (TSS & titratable acidity). On tree foliar sprays of salicylic acid (250, 500 & 1000 µM) in cvs. Sindhri and Samar Bahisht Chaunsa at the time of panicle pushing/flower opening, fruit set and stone hardening could not perform well against postharvest disease development. 

**Key Words:** *Mangifera indica*; Postharvest diseases; Pathogens; Control measures

## INTRODUCTION

Quality of fresh produce is one of the key factors having significant relationship with the consumer acceptability and marketability; and has always been a major concern of stakeholders from production level to marketing (Shewfelt, 1999). Postharvest disease development is a major constraint to the quality and shelf life of mango fruit thereby limiting its domestic and export marketing (Bally *et al.,* 2009) as well as resulting in heavy economic losses (Barkai-Golan, 2001; Narayanasam, 2006). Like other fresh commodities, mango has also been found prone to postharvest fruit decay due to rapid disease development during storage and ripening (Prusky *et al.,* 2009). Anthracnose (caused by *Colletotrichum gloeosporioides*) is regarded one of the major postharvest diseases of mango (Bally *et al.,* 2009). Stem end rot (SER) and black spots (i.e., *Alternaria* rot) have also been reported to cause significant postharvest decay in mango (Prusky *et al.,* 2009). Recently, mango sea-freight trial shipments from Pakistan to Germany also indicated SER as the major concern for high postharvest losses (Malik *et al.*, 2010).

Stem end rot of mango is a complex disease caused by variety of pathogens. Studies on SER of mango were made in Burma, Ceylon, the Philippines, Mauritius and USA (Srivastava, 1972). Later, due to rapid increase in the severity and incidence of this infection in the mango growing countries, its management became a worldwide concern to ensure the postharvest fruit quality during the supply chains (Johnson *et al.,* 1993). According to various reports regarding the organisms associated with SER; *Phomopsis mangiferae*, *Botryodiplodia theobromae* and various *Dothiorella* species (i.e., *D. manqiferae, D. dominicana* & *Dothiorella*'long') and *Diplodia* spp. are involved in mango fruit SER in different growing regions of the world. The role of *C. gloeosporioides, Cytosphaeria manqiferae, Lasiodiplodia theobromae and Pestalotiopsis* sp. has also been described by various researchers

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(Sangchote, 1987; Bagshaw, 1989; Johnson *et al.,* 1991b). Most of these pathogens get invaded into the plant tissues; colonize the fruit peduncle during panicle emergence and flowering; penetrate into the fruit tissues during fruit development and maturation period by endophytic hyphal growth and cause SER during ripening (Johnson *et al.,* 1991a, 1992, 1993; Everett, 2001).

In the past, various attempts have been made worldwide to ascertain the pathogen, mode and source of infection, stage of infection and development of SER (Johnson *et al.,* 1993). Furthermore, various pre and postharvest disease management studies have been reported in literature including fungicidal treatments (Sanders *et al.,* 2000; Mortuza *et al.,* 2003), hot water dips and vapor heat treatments (Esguerra *et al.,* 2004; Sopee & Sangchote, 2005), emulsion coatings (Diaz-Sobac *et al.,* 2000). Biological control (Carrillo-Fasio *et al.,* 2005) has also been investigated. Recently, disease control by inducing host resistance and activating the defense mechanisms in plants (especially herbaceous plants) and harvested fresh produce has also been explored (Johnson & Hofman, 2009). Salicylic acid is a well known natural inducer of disease resistance in plants (Sticher *et al.,* 1997) and its performance against mango anthracnose has been reported (Zainuri *et al.,* 2001; Zeng *et al.,* 2006).

In Pakistan, comprehensive studies are lacking in evaluating the causes of SER and solutions of postharvest disease development in mango grown under the agro-ecological conditions of Punjab. These studies were conducted on two commercial mango cultivars (Sindhri & Samar Bahisht Chaunsa) of Pakistan to identify the fungi associated with SER and to evaluate the fruit disease control potential of fungicides (Tecto & Sportak) and a plant activator (salicylic acid).

## MATERIALS AND METHODS

**Study-1: Isolation of pathogens and pathogenicity confirmation:** The diseased fruits of cv. Sindhri and Samar Bahisht Chaunsa were sampled from different mango orchards and were used for disease isolations to identify different pathogens involved in postharvest diseases. Isolations were made from small pieces of decayed portion of each infected fruit along with healthy areas, disinfected in 1 % NaOCl solution (Iqbal *et al.,* 2010), placed into the petri plates containing agar-agar medium (CAS 9002-018-0; Research Organics Inc., USA) (Ploetz & Gregory, 1993) and colonies of microbes were examined and identified under microscope after 7 days of incubation at 25°C (Iqbal *et al.,* 2008). The frequency of microorganism from each locality was estimated as colonization percentage. Pathogen confirmations were done using the inoculation method as adopted by Ko *et al.* (2009) with some modifications i.e., using glass bell jars for incubation instead of plastic bags.

**Study-2: Fruit Disease Management**

**Pre and postharvest application of fungicides:** This study included pre and postharvest application of different fungicidal treatments i.e., control, Carbendazim (450 mg/L), Tecto (1.8 mL/L) a.i. Thiabendazole (TBZ), Sportak (0.5 mL/L) a.i. Prochloraz and Tecto (1.8 ml/L) + Sportak (0.5 mL/L) on mango cultivar Samar Bahisht Chaunsa. Preharvest application was done as foliar spray with control having no fungicide application. The postharvest treatments were applied in hot water (52°C; 5 min dip) including hot water wash as control treatment.

For pre-harvest application, 15 mango trees were selected at a commercial orchard in Multan District (30°12′N, 71°26′E), Punjab, Pakistan and subjected to the fungicidal treatments 15 days prior to fruit harvest. Each tree was considered as a treatment. After 15 days of treatment application, the fruits were harvested, graded, packed in corrugated cardboard boxes and shifted to Postharvest Research and Training Centre (PRTC), Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan. For postharvest application, mango fruits were harvested from the untreated trees from the same orchard (on the harvest date of preharvest fungicidal application trial). The harvested fruits from different trees were pooled into a single lot and fruits free from apparent sign of diseases and injuries were selected, packed and shifted to Faisalabad, where postharvest hot water fungicidal treatments (same treatment combinations as included in the preharvest treatment plan; 5 min dip) were applied.

The fruits of both pre and postharvest trials were subjected to cold storage (12±1°C; 80-85%RH) for 21 days. The data were collected regarding disease development and other physical parameters on weekly basis during storage. At the end of storage, the fruits were shifted to ambient conditions for ripening. The data was collected at removal and ripe stage and subjected to statistical analysis under Randomized Complete Block Design (RCBD) for preharvest trial and Completely Randomized Design (CRD) for postharvest trial.

**Foliar application of salicylic acid on mango trees:** This study aimed to evaluate the efficacy of different dozes of salicylic acid (a plant activator) to suppress postharvest disease development in full bearing trees (25-30 years old) of two commercial mango cultivars (Sindhri and Samar Bahisht Chaunsa). The study was laid out under RCBD with four treatments i.e., control (untreated), salicylic acid (250 µM salicylic acid (500 µM) and salicylic acid (1000 µM) and three replications for each cultivar (taking one tree per replicate per treatment unit). The treatments were applied to the selected trees as foliar sprays at three critical stages i.e., at panicle pushing/flower opening, fruit setting and stone hardening.

The fruits (15 fruits per replicate in a treatment unit) were harvested at proper harvest maturity, graded, packed in cardboard boxes and shifted to Faisalabad and subjected to low temperature storage (12±1°C; 80-85%RH) for 28 days followed by ripening under ambient conditions. At ripe stage, the observations were made regarding disease development and fruit quality.

**Data collection:** The data regarding the extent (severity) of different postharvest diseases as well as various fruit quality parameters was collected using a rating scale showing %age of disease affected area i.e., 0= Nill, 1 = <5%, 2 = 5-10%, 3 = 11-25%, 4 = 26-50%, 5 = 51-75% and 6 = 76-100%. Peel color was rated as 1= 100% green, 2= 1-25% yellow, 3= 26-50% yellow, 4= 51-75% yellow and 5= 75-100% yellow; and textural softness as 1= hard, 2= sprung, 3= slightly soft, 4= eating soft and 5= over ripe (Malik & Singh, 2005). Total soluble solids (TSS) were determined using digital refractometer (ATAGO, RX 5000, Japan) (Amin *et al.,* 2008), whereas for the determination of titratable acidity (TA), N/10 NaOH titration method (Hortwitz, 1960) was used. TSS/acid ratio was also calculated as a biochemical fruit quality parameter.

**Statistical analysis:** Data were subjected to statistical analysis under respective experimental designs using statistical soft ware MSTAT- C (Michigan State University, USA) (Russel & Eisensmith, 1983). Analysis of variance techniques was employed to test the evaluate the differences among the studied factors, Least Significant Difference (LSD) test ($P \leq 0.05$) was used to compare the differences among treatment means (Steel *et al.*, 1997).

## RESULTS AND DISCUSSION

**Pathogens isolated from the stem end rotten tissues:** These studies indicated that fruit SER in mango cvs. Sindhri and Samar Bahisht Chaunsa, under agro-ecological conditions of Punjab, Pakistan, was found to be caused by three different fungi. *A. alternata* was identified as the major organism associated with SER (49.3% & 58.5% frequency in cvs. Sindhri & Chaunsa, respectively) followed by *P. mangiferae* (20.0% & 18.2%) and *Botryodiplodia* sp. (3.9% & 2.7%) (Table I). These three fungi along with variety of other fungi have been reported to cause mango stem end decay in various growing countries of the world (Mansour *et al.,* 2006; Ko *et al.,* 2009). It has been well-established that SER in mango is complex fungal disease caused by combined activity of the variety of fungi (Prusky *et al.,* 2009). These pathogens not only result in the stem end decay; but some other fruit rots (i.e., side rot) are also caused. Furthermore, many of these fungi have also been reported to be associated with world wide decline of mango trees (Darvas, 1993; Ploetz *et al.,* 1996; Pernezny & Ploetz, 2000; Jiskani, 2002; Mahmood & Gill, 2002; Al-Adawi *et al.,* 2003). Hence, adopting proper control strategies for these pathogens at preharvest level (i.e., from panicle emergence to maturation) will also minimize the decline in mango. Considerable population of some other pathogenic microbes including *Aspergillus sp., Penecillium sp.* and *Xanthomonas sp.* etc., was also isolated from SERten fruit tissues (26.8% frequency in Sindhri & 20.6% in Chaunsa) (Table I). Upon pathogenicity confirmation, these microbes were found as the source of secondary infection in both cultivars, hence, adding up to the destruction caused by primary pathogens.

**Disease Management Strategies**

**Performance of fungicidal applications against postharvest disease development:** Carbendazim has been used widely during the last three decades to control various pre and postharvest diseases in horticultural crops (Mortuza *et al.,* 2003). Use of carbendazim in mango has also been reported (Stovold & Dirou, 2004; Khanzada *et al.,* 2005). However, its use has been restricted by some markets of the world due to its hazardous residual effects [acute (WHO, 1996) and chronic (Mantovani, 1998)] on the health of consumers. The modern food safety concerns have led to withdraw the approval for the use of carbendazim on fruit crops (Anonymous, 2009). Under this perspective, the efficacy of two chemicals Tecto a.i. (Thiabendazole/TBZ) and Sportak (a.i. Prochloraz) was compared with carbendazim both at pre- and postharvest levels. Significant effect of the treatments was recorded on anthracnose and body rots (SER, side rot, distal end rot & soft rot) (Table II).

Comparatively, postharvest fungicidal applications gave significantly better disease control as compared to the preharvest treatments (Fig. 2) (as earlier described by Barkai-Golan, 2001). Postharvest application of Tecto (1.8 mL/L) alone and in combination with Sportak (0.5 mL/L) gave significantly better disease control (0.24 & 0.27 disease score, respectively) as compared to other pre and postharvest treatments (Fig. 1a & b). In case of preharvest applications, least extent (severity) of disease was observed in the fruit of combined Tecto and Sportak spray followed by carbendazim. However, none of the treatments gave complete disease control. The reason may be that preharvest treatments were applied once (15 days before harvest) 90-100 days after fruit set, whereas the inoculum has been reported to invade the mango trees at panicle emergence, flowering and fruit set (Johnson *et al.,* 1991a, 1992, 1993; Everett, 2001). The extent of SER, side rot and distal end rot was significantly less in the fruit subjected to postharvest fungicidal treatments as compared to those of preharvest applications. However, non-significant difference was found among the two times of fungicidal applications regarding anthracnose and soft rot (Fig. 2). Among the postharvest diseases, fruit side rot was found as a major disease (with highest extent) followed by SER and distal end rot respectively. The extent of anthracnose and soft rot was found minimum (Fig. 2). Relatively lesser disease incidence was recorded in all postharvest treatments as compared to preharvest applications (Fig. 1a & b). The lower incidence of disease in postharvest control seems to be the impact of hot water treatment, which has also been reported to reduce disease incidence (Buganic, 1997).

As none of the tested fungicidal combinations could give complete disease control (Fig. 1a & b; Table II), therefore, some modifications in the studied strategies are suggested for better postharvest disease management. Combining the preharvest fungicidal applications at initial fruit development stages with subsequent postharvest treatments need to be tested in future.

**Table I: Frequency of the fungi isolated from mango cvs. Sindhri and Samar Bahisht Chaunsa**

| Type of pathogen | Isolated pathogens/microbes | Frequency (%) | | Appearance of mature culture |
|---|---|---|---|---|
| | | Sindhri | S.B. Chaunsa | |
| Primary | *Alternaria alternata* | 49.27b | 58.50a | Dark brown to black |
| | *Phomopsis mangiferae* | 19.97d | 18.23d | Reddish brown to black |
| | *Botryodiplodia* Spp. | 3.93e | 2.73e | Dotted black |
| Secondary | *Others (Aspergillus sp., Penecillium sp., Xanthomonas sp.* etc.*)* | 26.80c | 20.63d | Hyaline, hyaline to brown, light brown, dark brown |

Means not sharing a letter are significantly different from each other ($P \leq 0.05$; LSD= 4.136; n =30)

**Table II: Performance of pre and postharvest fungicidal treatments against postharvest disease development in cv. Samar Bahisht Chaunsa after 21 days of storage**

| Time of application | Treatments | Anthracnose | Rots | | | | Total Disease Severity |
|---|---|---|---|---|---|---|---|
| | | | Stem End Rot | Side Rot | Distal End Rot | Soft Rot | |
| Preharvest | Control | 0.13ab | 0.40c | 2.57b | 1.53a | 0.33 | 4.96A |
| | Carbendazim (450mg/L) | 0.27a | 0.77b | 1.77c | 0.83b | 0.03 | 3.67BC |
| | Tecto (1.8mL/L) | 0.07b | 0.80b | 2.87a | 0.43c | 0.13 | 4.30AB |
| | Sportak (0.5 mL/L) | 0.00b | 1.10a | 2.60b | 0.10cd | 0.10 | 3.90B |
| | Tecto (1.8 mL/L) + Sportak (0.5 mL/L) | 0.00b | 1.20a | 1.90c | 0.00d | 0.00 | 3.10C |
| | *LSD Value* | *0.1575* | *0.1684* | *0.2382* | *0.3420* | *NS* | *0.7921* |
| Postharvest | Control | 0.00 | 0.70a | 0.73a | 0.50a | 0.57a | 2.49A |
| | Carbendazim (450mg/L) | 0.00 | 0.17c | 0.17b | 0.17b | 0.00b | 0.51B |
| | Tecto (1.8mL/L) | 0.00 | 0.10c | 0.07b | 0.07bc | 0.00b | 0.24C |
| | Sportak (0.5 mL/L) | 0.00 | 0.43b | 0.00b | 0.07bc | 0.10b | 0.60B |
| | Tecto (1.8 mL/L) + Sportak (0.5 mL/L) | 0.00 | 0.17c | 0.10b | 0.00c | 0.00b | 0.27C |
| | *LSD Value* | --- | *0.1191* | *0.1975* | *0.1191* | *0.1458* | *0.2306* |

Means not sharing a letter are significantly different from each other ($P \leq 0.05$; n =30)

**Table III: Physico-chemical quality of mango cv. Samar Bahisht Chaunsa in response to various pre and postharvest fungicidal applications (21 days storage at 12±1°C; 80-85%RH followed by 5 days ripening at ambient conditions)**

| Time of Application | Treatments | Peel color (score) | Textural softness (score) | TSS (°Brix) | Titratable acidity (%) | TSS/Acid Ratio |
|---|---|---|---|---|---|---|
| Preharvest | Control | 1.30a | 1.66b | 20.80 | 0.72 | 28.94 |
| | Carbendazim (450mg/L) | 0.66b | 1.56b | 18.65 | 0.67 | 27.62 |
| | Tecto (1.8mL/L) | 1.50a | 2.36a | 18.33 | 0.66 | 27.40 |
| | Sportak (0.5 mL/L) | 1.23a | 1.93ab | 21.45 | 0.74 | 28.77 |
| | Tecto (1.8 mL/L) + Sportak (0.5 mL/L) | 1.16a | 2.40a | 22.35 | 0.72 | 30.64 |
| | *LSD Value* | *0.4294* | *0.4837* | *NS* | *NS* | *NS* |
| Postharvest | Control | 1.40c | 2.03 | 20.80 | 0.72 | 28.94a |
| | Carbendazim (450mg/L) | 2.10a | 2.00 | 20.70 | 0.73 | 28.37a |
| | Tecto (1.8mL/L) | 1.86ab | 2.36 | 20.45 | 0.79 | 25.96b |
| | Sportak (0.5 mL/L) | 1.70bc | 2.00 | 20.05 | 0.78 | 25.68b |
| | Tecto (1.8 mL/L) + Sportak (0.5 mL/L) | 1.93ab | 2.16 | 21.65 | 0.83 | 25.82b |
| | *LSD Value* | *0.3420* | *NS* | *NS* | *NS* | *2.107* |

Means not sharing a letter are significantly different from each other ($P \leq 0.05$; n =30)

**Effect of fungicidal applications on fruit quality:** Significant effect of both, the pre and postharvest fungicidal applications was observed on fruit peel color (Table III). Relatively better color development in the fruit subjected to postharvest fungicidal treatments seems to be the result of hot water treatment (Anwar & Malik, 2008). Non significant effect of both pre and postharvest fungicidal treatments was observed on biochemical quality attributes (TSS & TA) (Table III). This indicates that tested fungicidal treatment combinations did not have any negative impact on the physico-chemical fruit quality of mango.

**Effect of salicylic acid on postharvest disease development:** Use of plant activators for disease control has been reported in herbaceous plants (Dann & Deverall, 2000); however, inadequate information is available regarding the response of perennial crops towards plant activators. Mango tree and fruit anthracnose caused by *C. gloeosporioides* has been previously reported to be minimized by the pre and postharvest application of plant activators (Zainuri *et al.,* 2001; Zeng *et al.,* 2006). Salicylic acid has been reported to be effective in this regard (Zainuri *et al.,* 2001; Zeng *et al.,* 2006; Johnson & Hofman, 2009). During the current studies, recurrent foliar sprays of different salicylic acid concentrations (i.e., 250, 500 & 1000 µM) on Sindhri and Samar Bahisht Chaunsa trees at three fruit developmental stages (including panicle pushing/flower opening, fruit set & stone hardening) could not prove effective against postharvest disease development

**Table IV: Post-storage disease incidence in mango cvs. Sindhri and Samar Bahisht Chaunsa in response to different salicylic acid concentrations (28 days storage at 12±1°C; 80-85%RH followed by 5 days ripening at ambient conditions)**

| Cultivar | Treatments | After storage | At ripening |
|---|---|---|---|
| Sindhri | Control | 31.47b | 78.17b |
| | 250 µM | 43.07a | 88.63a |
| | 500 µM | 39.50a | 90.23a |
| | 1000 µM | 33.27b | 81.20b |
| | *LSD Value* | *4.542* | *5.531* |
| SB Chaunsa | Control | 56.67 | 100.00a |
| | 250 µM | 46.47 | 98.04a |
| | 500 µM | 43.33 | 100.00a |
| | 100 0µM | 43.33 | 86.67b |
| | *LSD Value* | *NS* | *8.156* |

Means not sharing a letter are significantly different from each other ($P \leq 0.05$; n =30)

**Fig. 1: Effect of pre (a) and postharvest (b) fungicidal applications on postharvest disease development (severity) in ripe cv. Samar Bahisht Chaunsa after 21 days of storage followed by 5 days of shelf (n =30)**

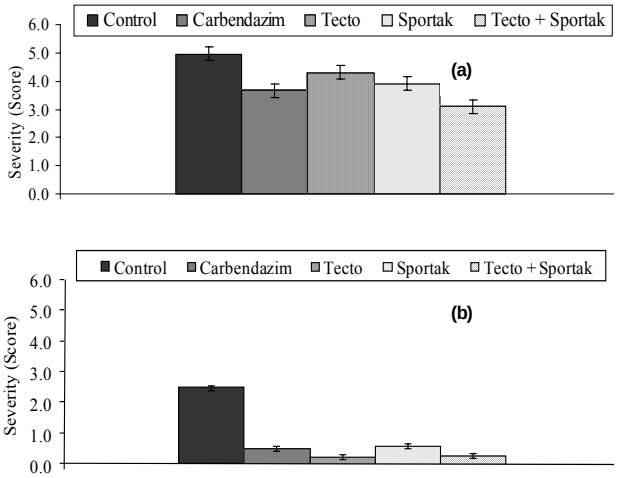
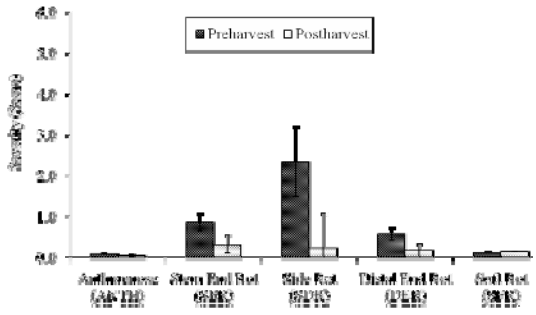



**Fig. 2: Extent of different diseases in pre and postharvest fungicidal treated fruits after 21 days of storage (n =30)**




in both cultivars (Table IV). This could be due to the more prevalence of other inoculums (i.e., *A. alternata, P. mangiferae* etc.) as compared to *C. gloeosporioides* in the mango orchards of Pakistan (Table I) causing significantly higher fruit body rots with least anthracnose incidence. Another consideration is that only one year application may not be sufficient to induce the needed level of host resistance against pathogens involved in postharvest disease development.

## CONCLUSION

*A. alternata, P. mangiferae* and *Botryodiplodia* spp. were the main pathogens associated with SER of mango under the agro-ecological conditions of Punjab province of Pakistan. Among the postharvest diseases, fruit side rot was identified as the major disease followed by SER, while fruit anthracnose incidence was minimal as compared to other diseases. Postharvest hot water fungicidal dips (Tecto @1.8 mL/L alone or in combination with Sportak @ 0.5 mL/L; 52°C; 5 min) gave better disease control as compared to preharvest treatments. However, application of salicylic acid (250, 500 & 1000 µM) from panicle emergence to stone hardening (3 sprays) could not induce resistance in the trees of mango cultivars (Sindhri & Samar Bahisht Chaunsa) against postharvest disease development. In future, combining the appropriate fungicidal sprays during flowering/fruit set with postharvest hot water fungicidal treatments management may be tested. Furthermore, new products (fungicides & plant activators) should also be investigated.

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