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OUTBREAK OF ANTHRACNOSE AND STEM END ROT DISEASES OF MANGO IN CHANGING CLIMTE AND THEIR MANAGEMENT THROUGH HOT WATER TREATMENT

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A B S T R A C T

Pakistan is the 6th largest producer of mangoes which are known world over for their superior traits but export of mangoes from Pakistan is quite limited due to post-harvest diseases like anthracnose and stem-end rot (SER) mainly caused by *Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae*, respectively. These diseases are aggravating day by day due to environmental changes in mango producing region of Pakistan. Quarantine of mangoes through physical methods like Hot Water Treatment (HWT) is always preferred by the foreign markets because of its high effectiveness in reducing the post-harvest diseases. During 2012-13, keeping in view the importance of HWT, a comprehensive study was conducted to standardize the temperature and dip duration levels for HWT along with a fungicide named Nativo (*Trifloxystrobin + Tebuconazole*) @ 0.3g/L of water for mango Cvs. Sindhri, S.B. Chaunsa, Fajri and Sufaid Chaunsa using newly developed HWT facility at Agricultural Mechanization Research Institute, Multan for the control of post-harvest diseases with a little focus on other fruit quality parameters as well. HWT resulted in better marketability of fruits due to uniform peel color development and visual quality rating (VQR). Control of (SER) with either of both high levels of temperature (54°C) and dip duration (06minutes) simultaneously or any of one high level along was noted in all the tested varieties with 77.27 to 85.26% disease reduction in contrast to control of anthracnose which was effectively minimized with the range of 77.24 to 90.36% through low temperature (52°C) and low dip level (03minutes) in case of Sindhri and S.B. Chaunsa while 52°C for 06 minutes and 54°C for 06 minutes showed good performance in case of Cvs. Fajri and Sufaid Chaunsa, respectively. Surely, this study developed the understanding of mango industry stake holders with efficient use of safe and cheaper physical method i.e. HWT to control post-harvest diseases to enhance the mango export from Pakistan.

Keywords: HWT, Nativo, anthracnose, stem end rot, mango cultivars.

INTRODUCTION

Mango (*Mangifera indica* L.) is a major cultivated fruit in tropical & sub-tropical regions of Pakistan. During 2012-13, its production touched the hike of 1717 thousand tons and arrested an area of approximately 171 thousand hectares in Pakistan. It ranked Pakistan as a 6th largest producer of mangoes which are known world over for their superior quality, peculiar aroma and its fabulous taste (GOP, 2014-15). Reportedly, below 10% of total production is exported from Pakistan annually due to various post-harvest issues including diseases like anthracnose and stem-end rot (SER) of mangoes becoming more prevalent due to environmental degradations in Pakistan especially in mango region (Johnson, 2011). Anthracnose is an important pre-harvest as well as post-harvest disease of mango caused by a fungus *Colletotrichum gloeosporioides*. In start symptoms appear in the form of small, irregular, light to dark brown color spots generally at fruit shoulder which gradually coalesce into bigger lesions. On young fruits, severe infection may lead to early abortion and abscission, whereas major post-harvest losses can occur

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on ripening fruits. On the other hand, stem end rot is a post-harvest disease caused by several fungi but *Lasiodiplodia theobromae* is the most prevalent. The fruit becomes brown to black while ripening at stem end. Disease progresses downwards and whole fruit become black within two- three days (Prakash, 2003). Losses can be increased when fruits are stored for prolonged period at low temperature or when fruits ripen at high temperature (>28°C).

The idea of improved quality and increased quantity of exportable mango through on farm integrated management of orchards is quite young in Pakistan (Iqbal *et al*., 2004). Resultantly, export of mangoes from Pakistan is limited to low end markets as a result of which our mangoes are fetching lowest average return in international markets. In order to reach high end markets of the world, the mangoes of Pakistan must meet WTO/HACCP/ISO requirements. For the said purpose, quarantine of mangoes through physical methods like Hot Water Treatment (HWT), Vapor Heat Treatment (VHT) or Irradiation Treatment is essentially required. As a disinfestation treatment, hot water has earned fame and adoption at a large scale because of its high efficacy in reducing the post-harvest diseases as well as its low cost (Jacobi *et al*., 1995; Esguerra *et al*., 2004; Sopee and Sangchote, 2005).

In Pakistan this technology is being adopted on limited scale by the exporters as no operational parameters for hot water treatment are optimized/standardized especially for water bath temperature and dipping time for different varieties of mangoes. So, it was very imperative to conduct the comprehensive study for the purpose of optimization/standardization of dip temperature and time for a particular variety with fruits for the control of a particular disease with little focus on other fruit quality parameters through a hot water treatment facility newly developed at Agricultural Mechanization Research Institute (AMRI), Multan. This study was also imperious to tone down the ongoing as well as the upcoming environmental deprivations so that quantity and quality of mangoes could be enhanced up to the extent of potential of varieties in Pakistan.

MATERIAL AND METHODS

Experimental Material: Fruits of different mango varieties viz. Sindhri, S.B. Chaunsa, Fajri and Sufaid Chaunsa were procured from mango orchards of Multan having normal agricultural practices during the season for a particular variety throughout the study. Fruits of the desired varieties were harvested at their half maturity stages having the following brix (B°) levels tested through hand refract meter randomly.

Sr. No.	Mango Variety	Brix (b°) Level	Fruit Weight (g)
1.	Sindhri	6.5-7.0	420.6
2.	S.B. Chaunsa	7.0-8.0	406.9
3.	Fajri	7.0-8.0	601.5
4.	Sufaid Chaunsa	6.5-7.0	588.7

Fruits of uniform size of each variety with pedicle showing some other physical parameters of fruit maturity like color and shoulder development were harvested. After harvesting, ten fruits of each variety were selected at random to calculate their average fruit weight. Then de-sapping of fruits were started with the complete de-stemming inside the container having 0.5% lime water. After de-sapping washing with simple tap water was done followed by drying of fruits for some time on the iron tables at ambient temperature (Malik *et al*., 2013).

Hot Water Treatment: Fruits were treated with hot water treatment facility present at Agricultural Mechanization Research Institute, Multan with the following some major features within 24 hours of harvest but not sooner than 04 hours, 375 fruits of the same size of each variety were completely immersed in hot water containing a fungicide named Nativo (*Trifloxystrobin + Tebuconazole* @ 0.3 g/L of water as recommended by (Malik *et al*., 2013) for the evaluation of each of two temperature (52°C & 54°C) and dip levels (03 minutes & 06 minutes) keeping 05 treatments with 03 replications and adopting standard statistical methods i.e. CRD for the control of post-harvest diseases during 2012 and 2013.

Packing and Ripening: Packing was done in corrugated boxes without wrapping of fruits in any type of paper and then the fruits were transferred for ripening at 1±25°C with the maintenance of humidity level more than 80% with humidifier.

DATA COLLECTION AND ANALYSIS

The inspections were made daily and data of anthracnose and stem end rot (SER) of mango were recorded on natural disease incidence basis and adopting the following specific disease severity scale as devised by (Homes *et al*., 2009) as the fruits became fully ripe.

RATING SCALE	RATING
0	Nil
1	Less than 01 cm^2
2	2-3 cm^2 (approximately 3%)
3	3-12 cm^2 (approximately 10%)
4	12 cm^2 (approximately 10-25%)
5	More than 25%

The change of peel color was assessed by using color index scale of 01-05 (Where 1:100% green, 2: 75% green, 3:50% green, 4:25% green and 5:100 yellow) as described by (Miller and McDonald, 1991). Similarly, the marketability of all fruits was classified with the use of Visual Quality Rating (VQR) as followed 1: inedible, 2: edible but not marketable, 3: poor, 4-5: fair, moderate defects, 6-7: good, minor defects and 8-9: excellent (Lizada *et al*., 1986).

Mean disease intensity in different treatments was analyzed by analysis of variance as two factorial combinations of treatments and diseases. Mean were compared at P=0.05% by LSD test (Steel and Torrie, 1980).

Determination of Associated Fungi with diseased fruits: Three diseased fruits of each variety showing each of SER and anthracnose symptoms were selected at random for lab analysis to make isolations and identification of associated pathogens in both diseases. Forty-eight fruits and ten pieces of 03-05mm long exercised for each diseased fruit. These pieces were surface disinfected in 01% NaOCl solution for the duration of 02 minutes rinsed twice with sterilized de-ionized water dried on sterilized blotting papers and placed in sterilized 9cm (Pyrex) glass Petri dishes containing potato dextrose agar medium (PDA). The plates containing tissue pieces were incubated at 25±1°C temperature (Prusky *et al.,* 1983). The plates were examined after 3-5 days of incubation. The tissue bits yielding different fungi were undergone identification based on the characteristics specific for the fungus (Saleem and Nasir, 1991).

RESULTS AND DISCUSSIONS

Post-harvest heat treatment was used to regulate the ripening of fruits and control of decay losses due to post-harvest diseases particularly anthracnose and stem-end rot during ripening. It resulted in better marketability of fruits due to uniform peel color development and visual quality rating (VQR). It also managed the infections as mangoes held in hot water along with fungicide resulted in reduction of post-harvest diseases and disorders compared with untreated fruits during ripening at 25±1°C.

Peel Color: Maximum peel color index was observed in T_4 by 96.28, 91.20, 49.32 and 88.28 percent on mangoes of Cv. Sindhri, S.B. Chaunsa, Fajri and Sufaid Chaunsa respectively where the fruits were treated at 54°C for the duration of 6 minutes. This treatment also differed significantly from the rest of treatments in all the tested varieties except T_2 in case of Fajri. On the other hand, the untreated fruits showed the minimum peel color index (40.76 to 86.26%) with significant difference between the treated and untreated fruits of each variety (Table 1).

Visual quality rating (VQR): A significantly difference was noted among the treated and untreated (control) fruits of all the varieties as for as visual quality rating is concerned. However, VQR dropped gradually in all the treatments during the observation days and on the last observation date, the mangoes under control treatment turned almost unmarketable by 24.41, 29.38, 24.83 and 28.50 percent VQR indexes in Cvs. Sindhri, S.B. Chaunsa, Fajri and Sufaid Chaunsa respectively (Table 1). While other treatments for VQR of mangoes of all the varieties were found very much correlated with the incidence of diseases. The treatments performed well in the control of diseases in a particular variety also responded to enhance VQR in the same variety. For instance, T_1 and T_3 remained very effective for the control of anthracnose and stem-end rot by 90.36 and 85.26percent respectively for Cv. Sindhri. The same treatment also showed the best performance regarding VQR by 40.26 and 43.26 percent in T_1 and T_3respectively (Table 1 & Figure 1). Similarly, the same trend was noted in all the varieties. The best treatments viz. T2 for S.B. Chaunsa, T_4 for Fajri and SufaidChaunsa reflected significant differences from the other treatments except T_1 and T_3 in case of Cv. Sindhri where these stood non-significant with each other (Table 1).

Anthracnose: Maximum decrease in anthracnose over control for the mangoes of Cv. Sindhri, S.B. Chaunsa, Fajri and Sufaid Chaunsa was observed by 90.36, 83.27, 77.24

and 79.18 percent in T_1, T_2 and T_4, respectively (Figure 1, 2, 3 & 4). All the treatments behaved significantly as compared to control treatments in all the varieties. Similarly, a significant difference was examined among all the treatments in case of Cvs. S.B. Chaunsa and Fajri but for Cv. Sindhri T_2& T_3 while for Cv. Sufaid Chaunsa T_1& T_2 showed non-significant performance for the control of anthracnose (Table 2).

Stem-end rot: For the control of stem-end rot high level of temperature or dip duration were required generally as the results of study revealed. In case of Cv. Sindhri maximum disease reduction over control was noted in T_3 by 85.26percent where the mangoes were treated at 54°C for 3 minutes (Figure 1). The fruits of Chaunsa (SB) showed the best performance against SER by 78.73percent disease decrease when the fruits were dipped for 6 minutes at 52°C in T_2 (Figure 1). Similarly, T_4 with the temperature level of 54°C and 6 minutes dip duration level controlled SER by 77.27 and 77.35 percent over control for Cvs. Fajri andSufaid Chaunsa respectively (Figure 3 & 4). T_3 and T_2 in case of Sindhri and S.B. Chaunsa while T_4 in case of Fajri and Sufaid Chaunsa behaved significantly amongst the rest of treatments (Table 2).

DISCUSSIONS

Hot water treatment (HWT) is comparatively inexpensive and safe than other post-harvest treatments like irradiation and vapor heat treatment as it is evident from the work of Lonsdale, (1993) who tried to control the post-harvest decay of mango by combining mild (0.75 KGy) irradiation with HWT. It presented excellent control of post-harvest diseases but was found to be phototoxic causing lenticels damage. Further, HWT is quicker and easier to implement, kills surface parasitic organisms, cleans the fruits surface and costs only 10% of the vapor heat treatment (Collin *et al*., 2007).

On the other hand, a wide range of pre-harvest factors influence the development of the post-harvest diseases. These factors may have a direct influence on the development of disease by reducing/increasing inoculums source or by discouraging/encouraging infection. Alternately, they may affect the physiology of the produce in a way that impacts on disease development after harvest (Coates *et al*., 2001).

Generally, in Pakistan pre-harvest management for the post-harvest diseases is always very poor. In this whole scenario, hot water treatment stands only the best option along with fungicide application in the hot dip to control the post-harvest diseases as its utility without fungicide provides inadequate disease control (Johnson *et al*., 1997).

In the post-harvest situation, fungicides are often applied to control infections already established in the surface tissues of produce or to protect against infections which may occur during storage and handling. In case of quiescent field infections, fungicides must be able to penetrate to the site of infection to be effective. Systemic fungicides are generally used for this purpose. In mango the penetration of systemic fungicides is enhanced by applying them as heated dips. Neither hot water treatment alone nor even systemic fungicide dip work to control the post-harvest diseases (GOP, 2014-15). If infection is well advanced at the time of post-harvest treatment then control even with fungicide will be difficult (Coats *et al*., 2011). In this study hot water treatment along with a systemic fungicide Nativo responded in a similar way towards peel color, VQR in four commercial varieties of mango viz. Sindhri, S.B. Chaunsa, Fajri and Sufaid Chaunsa. There was a significant difference between treatment and control in these varieties. This is contradictory to the finding of Anwar and Malik (2007) who tested the quality and storage life of Sindhri after HWT and did not find any significant difference between treatments and control but on the other hand, very similar to the phenomenon by Jacobi *et al*., (1995) who described that fruit softening, peeling and disease incidence varied with variety. Further, hot water treatment temperature and time may vary the results. A series of temperature are usually tested against a series of time interval to find the best hot water treatment for a particular variety. For example, Johnson *et al*., (2011) established the best hot water treatment of Australian mango variety against anthracnose and stem end rot at 52°C for 05 minutes along with a fungicide Fludioxonil. In cv. Sindhri and S.B. Chaunsa hot water treatment reduced the incidence of anthracnose at 52°C for 03minutes. In cv. Fajri the best control was achieved at high dip level (06 minutes) only while mangoes of Sufaid Chaunsa checked the anthracnose at 54°C for 06 minutes. These high levels in two cultivars for the control of anthracnose might be correlated with the firmness of the produce or stage of pathogenesis (Coates *et al*., 2011), irrespective of superficial infection nature of anthracnose. In contrast, stem-end rot pattern was different in Cvs. Sindhri, S.B. Chaunsa and Fajri. Its excellent control in these cultivars were observed at high levels of either temperature or dip duration or both in case of Cv. Sufaid Chaunsa not only for mangoes texture but common feature of SER pathogens as an abscission one barrier at the stem end prevents on delay colonization of fruit tissue. During ripening abscission of pedicel is initiated and stem-end rot pathogens which have remained quiescently under epidermis in pedicel and peduncle tissues invade fruit.

Table 1. Effect of hot water treatment on peel color and VQR of Sindhri, s.b. Chaunsa, Fajri and Sufaid Chaunsa varieties of mango (mean percent index, 2012-2013).

Treatments	Temperature and dip level	Peel color index (%)				Visual quality rating (vqr) %			
		Sindhri	S.b. Chaunsa	Fajri	White chaunsa	Sindhri	S.b.chaunsa	Fajri	Sufaid chaunsa
T_1	52°C for 03 minutes	91.35c	86.75d	44.50b	80.73c	40.26ab	37.33b	31.29b	37.16b
T_2	52°C for 06 minutes	94.33b	89.65b	78.66a	84.33b	37.29b	38.96a	31.88b	33.14c
T_3	54°C for 03 minutes	93.85b	88.18c	45.25b	83.55b	43.26a	36.28bc	29.08c	34.25c
T_4	54°C for 06 minutes	96.28a	91.20a	49.32a	88.28a	37.83b	35.50c	36.30a	39.91a
T_5	Control (Untreated)	86.26d	80.80e	40.76c	71.91d	24.41c	29.38d	24.83d	28.50d
	LSD	1.873	1.300	1.883	1.239	30.046	1.625	2.121	1.979

Table 2. Effectiveness of HWT along with fungicide in control of Anthracnose and Stem-end rot of Mango CVS. Sindhri, s.b. Chaunsa, Fajri and Sufaid chaunsa (mean percent disease index, 2012-2013)

Treatments	Temperature and dip level	Anthracnose intensity (%)				Stem-end rot intensity (%)			
		Sindhri	S.b. Chaunsa	Fajri	White chaunsa	Sindhri	S.b. Chaunsa	Fajri	Sufaid chaunsa
T_1	52°C for 03 minutes	3.23a	7.56e	13.63b	15.18b	11.1b	10.81c	14.88b	15.32b
T_2	52°C for 06 minutes	7.56c	11.46d	8.6e	13.61b	8.76c	7.91d	12.33c	13.01c
T_3	54°C for 03 minutes	6.76c	13.86c	12.11c	11.15c	5.83d	15.18b	13.91bc	13.06c
T_4	54°C for 06 minutes	9.0b	16.53b	10.0d	7.11d	10.27bc	16.00b	8.81d	8.03d
T_5	Control (Untreated)	33.53a	45.20a	35.86a	34.15a	39.56a	37.20a	38.76a	35.46a
	LSD	1.422	1.743	0.885	1.857	1.937	1.819	1.660	0.966

Table 3. Fungi associated with post-harvest diseases of mango fruits.

Sr. No.	Nature of disease	Fungi associated	Infection frequency %
1	Anthracnose	*Colletotrichum gloeosporioides* *Asperigillus niger*	55.83 (30-75) 44.16 (26-70)
2	Stem-End Rot	*Lasiodiplodiatheobromae* *Alternaria alternata.*	84.64 (45-73) 15.36 (0-27)

Colletotrichum gloeosporioides, *Asperigillus spp.* were found as the most associated fungi with the symptomatic fruit of all the cultivars affected with anthracnose with 55.83 and 44.16% infection frequency, respectively. Similarly, *Lasiodiplodia theobromae*, *Alternaria alternata* were very much related in cause of stem-end rot of mango with 84.64 and 15.36% infection frequency, respectively. This diagnosis is enormously correlated with the findings of Johnson, (2011).

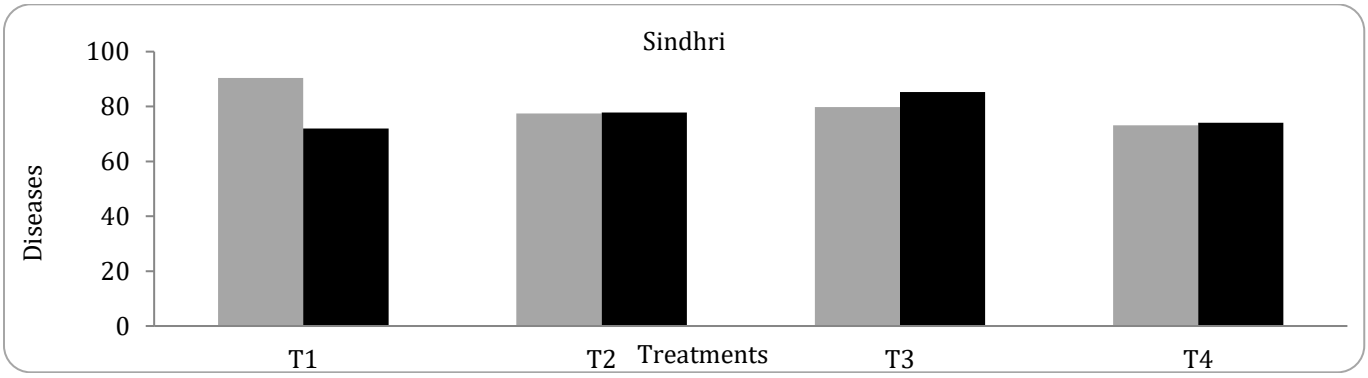


Figure 1. Disease decrease percentage of Anthracnose and Stem-End Rot in Different Cultivars of Mango after HWT.

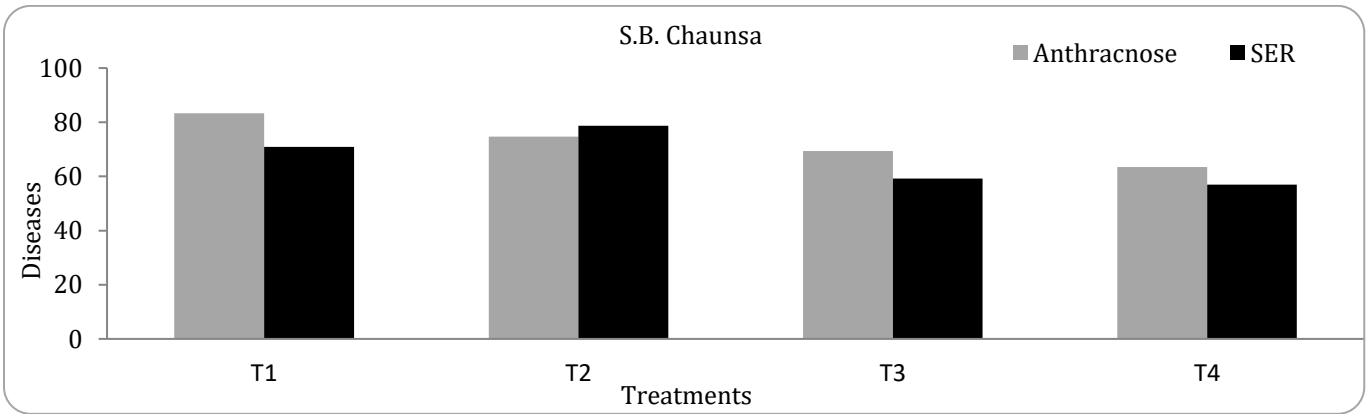
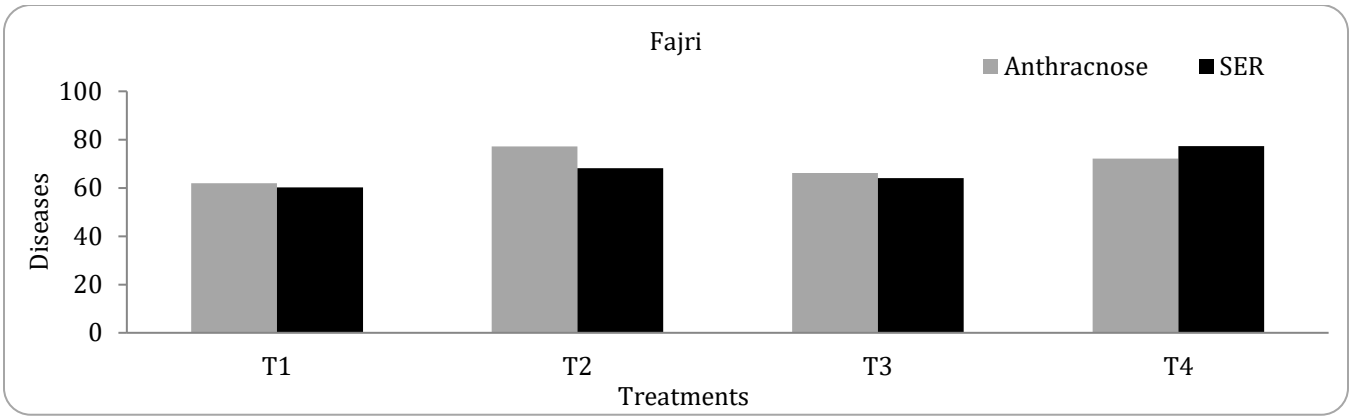


Figure 2. Disease decrease percentage of Anthracnose and Stem-End Rot in Different Cultivars of Mango after HWT.



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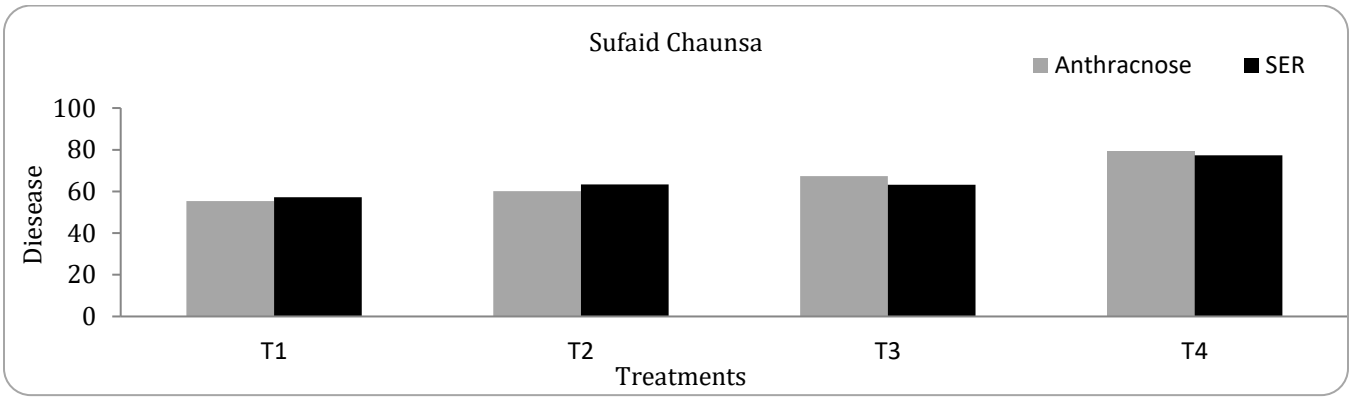

Figure 4. Disease decrease percentage of Anthracnose and Stem-End Rot in Different Cultivars of Mango after HWT.

CONCLUSION

Colletotrichum gloeosporioides and *Lasiodiplodia theobromae* are the main fungi associated with anthracnose and stem-end rot of mangoes in Pakistan. Hot Water Treatment (HWT) has been found the best option to regulate the fruit ripening and control of decay losses in mangoes. The temperature (54°C) and dip duration (06 minutes) are the best level for the control of anthracnose and stem-end rot in case of cvs. Fajri and Sufaid Chaunsa while for cvs. Sindhri and S.B. Chaunsa have been evaluated as (54°C) for 03 minutes & (52°C) for 06 minutes, respectively.

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