Pak. J. Agri. Sci., Vol. 57(X), xxx-xxx; 2020
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI: 10.21162/PAKJAS/19.8336
http://www.pakjas.com.pk

DYNAMICS OF STEM END ROT DISEASE OF MANGO FRUIT AND ITS MANAGEMENT

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Stem end rot (SER) is a fungal disease complex that poses a major threat to the delivery of quality mangoes from Pakistan to export markets. The dynamics of this disease remain unclear, but must be elucidated so that effectiveness of disease management can be improved. The present study sought to describe the infection process and delineate a disease cycle of SER under Pakistani growing conditions. Our study discovered three possible routes of infection that could lead to SER: 1) infection at bloom stage; 2) the pathogen may be endophytic within the twigs and branches; and 3) airborne spores of the pathogen invade the pedicels and xylem and then grow into the ripening fruit. Culturing of symptomatic samples from selected orchards revealed colonization by five fungal pathogens, of which *Lasiodiplodia theobromae* was most prevalent. A pathogenicity assay revealed that all five pathogens were pathogenic to mango fruit when inoculated artificially. The efficacy of fungicides - Cabrio Top®, Nativo®, Scholar®, Tecto®, Amistar®, and Sportak® at several concentrations was evaluated against SER pathogens *in vitro* and *in vivo*. Results indicated that Nativo®, and Cabrio Top®, at 250 µg/mL provided statistically significant suppression of mycelial growth and SER development as compared to other tested fungicides.
Keywords: Pathogenicity, postharvest, cold storage, poisoned food technique, nativo, cabrio top.

INTRODUCTION

Pakistan, the fourth largest producer and exporter of mango, faces several challenges from severe losses due to postharvest diseases which limit its share in international markets (Ambreen *et al*., 2014). Postharvest losses of fresh mango fruits were reported to average 69% in Pakistan (Rehana *et al*., 2014) but sometimes reach 100% under disease-favorable environments (Johnson, 2008; Sakalidis *et al*., 2011). Postharvest diseases of mango are economically damaging because they render the fruit unmarketable (Bally *et al*., 2009).

Stem end rot (SER) is among the most damaging postharvest diseases of mango in Pakistan. SER etiology is complex, with multiple fungal pathogens reported to be involved before and after harvest (Barkai-Golan 2001; Ambreen *et al*., 2014). Symptoms appear after fruit ripen; a soft brown decay begins from the stem end of the fruit and rapidly involves the whole fruit. The decay may also exude a straw-colored fluid from the stem end of the fruit (Johnson, 2008). In previous reports, *Lasiodiplodia theobromae*, *Colletotrichum gloeosporioides*, *Dothiorella dominicana*, *Dothiorella mangiferae*, *Phomopsis mangiferae*, *Alternaria alternata*, *Pestalotiopsis mangiferae*, *Botryosphaeria* spp., *Cytosphaera mangifera* and *Botrytis cinerea* were linked to SER in mangoes (Abdalla *et al*., 2003; Slippers *et al*., 2005; Costa *et al*., 2010; Johnson *et al*., 2012; Alam *et al*., 2017). *Lasiodiplodia theobromae* is considered to be the most important SER pathogen in Pakistan, causing severe losses in fruit quality and marketability (Ambreen *et al*., 2014). Fruit infection by *L. theobromae* has been assumed to arise via colonization of the pedicel scar of mango after harvest or by colonization of floral tissues that do not abscise after flowering and fruit set (Johnson *et al*., 1993).

Assessment of SER can be made by a rapid assay of floral and fruit parts, which helps to define the assemblage of pathogens and provides a foundation for designing effective management strategies (Slippers *et al*., 2005). Thousands of tons of fruit are wasted because of poor postharvest management and a lack of suitable storage facilities (Bally, 2006). Of several postharvest management practices that have been evaluated against SER, application of synthetic fungicides is considered to be the most important control method (Singh and Sharma, 2007). Fungicides are used alone or in combination (Ismail and Zhang, 2004), and some have

anti-sporulation activity (Kanetis *et al*., 2006). The aim of the present study was to clarify the SER disease cycle under Pakistani growing conditions and quantify SER control under several fungicide regimes.

MATERIALS AND METHODS

***Survey and orchard selection*:** The survey was conducted during 2013 and 2014 in five mango orchards (cv. Samar Bahisht Chaunsa) that were producing fruit for export in each of three Districts of Punjab Province - Rahim Yar Khan (28° 34' 27.0" N; 70° 15' 52.4" E), Multan (30° 14' 49.3" N 71° 35' 08. 11"E), and Vehari (30° 1' 60" N 72° 21' 0" E). In each orchard, five mango trees were selected arbitrarily. The age of orchards was in the range of 25 to 30 years.

***Collection of potential sources of inoculum*:** A total of 50 samples of each of five tissue types - dried branches, dead twigs, dried panicles, leaves entangled in the tree canopy and mummified fruits (suspecting to have pycnidia and perithecia) - were collected in the month of Oct-Nov from each of the selected orchards and assessed for the presence of SER fungi at the Plant Disease Diagnostic Laboratory, University of Agriculture, Faisalabad. The objective was to propose a disease cycle. Tissue sections were excised from samples using a sterile scalpel, followed by three replications of sterilization (dipping in 0.1% sodium hypochlorite solution for two minutes followed by rinsing with sterilized water) and plating on potato dextrose agar (PDA), incubated at 25°C for 7 days under alternating 12-h light and dark periods. The isolated fungal pathogens were purified and identified on the basis of morphological characteristics like spore color, shape and size. Frequency percentage of isolated fungi was calculated by following formula:

$$\text{Frequency \%} = \frac{\text{Number of isolated fungi}}{\text{Total number of isolates}} \times 100$$

***Collection of asymptomatic samples*:** Isolations on PDA were made from 50 samples (asymptomatic) each of floral buds, young panicles, senescent flowers, young fruit with spent floral parts, developing fruit and mature fruit that were collected from each orchard between flowering and harvest. Tissue samples (1 to 2 cm) were excised with a sterile scalpel and surface sterilized as described previously, before plating on PDA and incubated at 25°C for 7 days under alternating 12-h light and dark periods. The isolated fungal pathogens were purified and identified on the basis of morphological characteristics like spore color, shape and size.

***Assessment of postharvest diseases*:** One hundred fully mature mango fruit (cv. Samar Bahisht Chaunsa) were collected from each selected orchard. After harvesting and de-sapping (allowing latex-like sap in fruit to exude by placing the peduncle end of the fruit downward for one hour immediately after harvest) in the orchards, fruit were brought to the Post Harvest Research and Training Center, University of Agriculture, Faisalabad in a cool chain van (10°C) and stored at 33°C (designated as ambient storage) for 2 weeks and at 12°C (designated as cold storage) for 4 to 5 weeks. After removal from cold storage, these fruits were placed at 33°C (4 days) for ripening and fruit from both experiments were evaluated separately for disease incidence. Disease incidence of isolated fungi was calculated using the following formula ((Feng *et al*., 2014).

$$\text{Disease incidence (\%)} = \frac{\text{Number of symptomatic fruit}}{\text{Total number of fruit}} \times 100$$

***Isolation, purification and identification of SER pathogens*:** Fruits exhibiting SER symptoms after ripening were brought to the Plant Disease Diagnostic Laboratory, University of Agriculture, Faisalabad. Samples of the infected fruit tissues from stem end portion were cut into small pieces (1-2 cm), surface sterilized, dried on sterilized blotter paper, placed in petri dishes containing PDA medium, and incubated at 25°C for 4 to 5 days under alternating 12-h light and dark periods. The hyphal tip transfer method was used for purification of isolated fungal pathogens. The purified fungal pathogens were identified on the basis of morphological characteristics like spore color, shape and size (Barnett and Hunter, 1998).

Pathogenicity assay: Based on the fungal species that were identified after isolation from symptomatic mango tissues, a single isolate was selected for each of six species - *Colletotrichum gloeosporioides, Lasiodiplodia theobromae, Phomopsis mangiferae, Alternaria alternata,* C*ytosphaera mangiferae* and *Botrytis cinerea.*

***Preparation of spore suspension*:** Spore suspension ($1x10^4$ spores/ml) was prepared from purified cultures grown on PDA and incubated at 25°C for 7 days under alternating 12-h light and dark periods. After mycelia and conidia were scraped from the agar surface with a sterile glass rod, 5 ml of 0.05% (w/v) Triton X-100 was added in 500 ml sterilized water and shaken vigorously for 10 minutes on a mechanical shaker. The resulting suspension was passed through two layers of cheese cloth. The concentration of suspensions was adjusted to $1x10^4$ spores/ml using a haemocytometer (Sivakumar *et al*., 1997).

***Inoculations of mango fruit*:** Unripe but mature mango fruit (cv. Samar Bahisht Chaunsa) were selected for *in vitro* pathogenicity tests. Fruit were de-sapped and surface sterilized with 1% NaOCl solution for 5 minutes, then rinsed with sterilized water and allowed to air dry. Fruit were pricked with a sterilized scalpel to a depth of 4 mm at the stem end portion, then inoculated with 50 µl of the spore suspension using a microliter syringe (Awa *et al*., 2012). Fruit in the control treatment were inoculated with sterilized water only. Inoculated fruit in each treatment were placed separately into plastic crates and incubated at ambient temperature (33°C) for 9 days and in cold storage (12°C) for 21 days at 70 to 80% relative humidity at the Post-Harvest Research and Training Centre, Institute of Horticultural Sciences, University of Agriculture, Faisalabad. The experiment was run using three replications and each replicate consisted of 15 mangoes.

Lesion diameter of all inoculated mangoes was recorded periodically, i.e., after 3, 6 and 9 days from ambient storage and after 7, 14 and 21 days from cold storage.

***Relation between the pedicel length and disease severity*:** Mature green fruit of cv. Samar Bahisht Chaunsa were used to study the relationship of pedicel length to SER severity. Subsamples of 15 fruits were established in which the length of pedicel was either 0, 1, 2, 3 or 4 cm, and three replicate subsamples of fruit with each pedicel length were assessed. Fruit were pricked at the stem end portion with a sterilized needle to a depth of 4 mm and inoculated by microliter syringe with 50 µl of the prepared spore suspension ($1x10^4$ spores/ ml) comprised of an equal mixture of four major SER pathogens: *L. theobromae, C. gloeosporioides, A. alternata* and *P. mangiferae*. The fruit were then incubated in a moist chamber at room temperature (33°C) for 96 hours, after which disease severity was checked at ripened stage and treated fruit were rated on 1 to 5 scale, where 1=0%, 2=1-25%, 3=26-50%, 4=51-75%, and 5=76-100% fruit area affected (Sivakumar *et al*., 2002).

***Effect of fungicides on suppression of mycelial growth*:** Fungicides Nativo® (trifloxystrobin & tebuconazole), Cabrio Top® (metiram) Scholar® (fludioxonil); Tecto® (thiabendazole), Amistar® (azoxystrobin), and Sportak® (prochloraz) were tested *in vitro* to assess suppression of mycelial growth of SER pathogens using the poisoned food technique (Grover and Moore, 1962). Each of the fungicides was tested at 50, 100, 150, 200 and 250 µg/ml. Dilutions were prepared from stock solutions of 1 g of each fungicide in 100 ml sterile distilled water. To prepare 9-cm-diameter PDA plates, 1 ml of each fungicide solution was transferred to each plate before agar was poured. After solidification the plates were inoculated with a 5-mm-diameter mycelial plug from a 7-day-old culture of each fungus. The experiment was conducted with three replications per treatment; a treatment without fungicide was used as a control. The plates were incubated at 25°C for 7 days under alternating 12-h light and dark periods. Percent mycelial growth inhibition was calculated as given below:

$$\text{Mycelial growth inhibition} = \frac{X-Y}{X} \times 100$$

X = Radial growth of control plate; Y = Radial growth of fungicide treated plate

***Impact of fungicides on SER caused by L. theobromae*:** Based on the results of the *in vitro* tests, 200 and 250 µg/ml of Nativo and Cabrio Top were selected to be assessed for impact on SER development on harvested fruit. For this purpose, undamaged, mature fruits (cv. Samar Bahisht Chaunsa) of comparable size and free from any pesticides were used. Fruits were surface sterilized by dipping in 1% sodium hypochlorite solution for 5 min followed by rinsing twice in sterilized distilled water. Based on results of preliminary studies, conidial suspensions of *L. theobromae* was prepared and adjusted to a concentration of $1x10^4$ spores/ml using a haemocytometer. Fruit were pricked with a sterilized scalpel to a depth of 4 mm at the stem end portion. Immediately after wounding, these fruit were injected with 50 µl of conidial suspension and 50 µl of each fungicide concentration by using separate microliter syringes. Control fruits were injected with sterile distilled water only. The inoculated and treated fruits of each treatment were placed into plastic crates and covered by plastic sheets at ambient temperature (33°C) for 9 days. The experiment was run using three replications for each treatment and a single replicate was comprised of 15 fruits. Nine days after storage, disease severity of inoculated and treated fruits was rated on 1 to 5 scale as reported above (Sivakumar *et al*., 2002).

***Statistical analysis*:** Experiments were conducted under a completely randomized design. The collected data were statistically analyzed using Statistix® 8.1 software. Analysis of variance was employed to test the overall significance of the data, and Tukey's test ($P \leq 0.05$) was used to compare differences among treatment means (Steel *et al*., 1997).

RESULTS

***Sources of inoculum*:** Almost all the orchard samples were colonized by at least one pathogen, and frequently two or more pathogens were isolated. *P. mangiferae* was not recovered from dead twigs, dried branches, or leaves entangled in the tree canopy. Overall, *L. theobromae* was the SER pathogen most frequently isolated from the collected samples (Table 1). Based on our data for sources of inoculum, we proposed a disease cycle of SER under Pakistani growing conditions (Supplementary Fig. 1). The pathogen survived as black pycnidia and perithecia in wart-like stomata on dead twigs, branches, dried panicles and mummified fruits.

Table 1. Frequency (%) of isolation of fungal pathogens from symptomatic organs of mango in orchards in Pakistan.

Sources of inoculum	Frequency of isolated fungi (%)				
	L. theobromae	***C.gloeosporioides***	***P. mangiferae***	***A. alternata***	***C. mangiferae***
Dead twigs	55.0 a	14.0 c	0.0 e	17.3 b	8.6 d
Dried branches	36.6 a	19.3 c	0.0 e	21.3 b	14.0 d
Leaves entangled in tree canopy	27.6 b	12.6 d	0.0 e	32.3 a	19.6 c
Dried panicles	40.6 a	13.0 c	18.0 b	15.0 c	6.0 d
Mummified fruits	34.3 a	16.0 c	19.6 b	13.0 d	11.3 e

*Means within a row that are followed by the same letter do not differ significantly according to the Tukey's test ($P \leq 0.05$). n=250

Colonization began at the flowering stage and reached the stem end of fruit several weeks after flowering. Blossom blight symptoms were also observed after colonization by the pathogen. Wounds and breaks in the cuticle were hypothesized to be routes of entry. The spores of *L. theobromae* can be liberated by the onset of rain and infect the fruit. Ascospores landing on the fruit surface, particularly on the broken pedicels that exude sticky latex, can invade the vascular tissue of the mango fruit within a few hours, but no symptoms are visible until 4 to 5 days later. Infections on immature fruit remain latent until the fruit begin to ripen after harvest, when they resume activity and trigger SER symptoms (Supplementary Fig. 1).

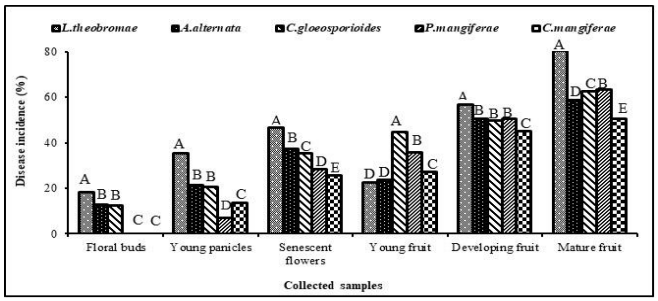


Figure 1. Incidence of fungal pathogens associated with mango fruit-related tissues sampled between flowering and harvest. Means within a cluster of bars that are followed by the same letter do not differ significantly according to the Tukey's test ($P \leq 0.05$). n=250

***Asymptomatic samples*:** During flowering and fruit set, incidence of colonization by *A. alternata, C. gloeosporioides, P. mangiferae* and *C. mangiferae* increased as the flowers senesced and young fruit formed. Early sampling showed that incidence of all fungi during flowering and fruit set was present, suggesting that colonization started after panicle emergence and then spread to the fruit pedicel. The incidence of *L. theobromae* associated with pedicel tissues declined after peak flowering and remained significantly low for about 1.5 weeks after which it increased again (Fig. 1).

***Pathogens recovered from stored fruit*:** Six different fungi - *Lasiodiplodia theobromae, Colletotrichum gloeosporioides, Phomopsis mangiferae, Alternaria alternata, Cytosphaera mangiferae* and *Botrytis cinerea* - were associated with stored mango fruit showing SER symptoms. Incidence data showed that *L. theobromae* and *C. gloeosporioides* were the most prevalent SER pathogens in the evaluated orchards. In cold storage fruit, maximum disease incidence was recorded for *L. theobromae* (37.6%), followed by *C. gloeosporioides* (24.8%) and *B. cinerea* (15.2%). After ambient storage, incidence (33.6%) for *L. theobromae* was recorded, followed by *A. alternata* (29.7%) and *C. gloeosporioides* (18.8%) (Fig. 2).

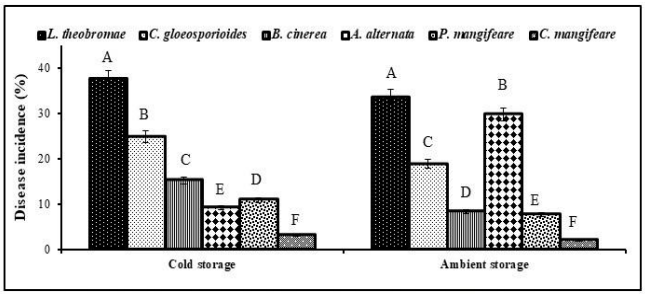


Figure 2. Incidence of fungal pathogens of stem end rot (SER) isolated from symptomatic mango fruit (cv. Samar Bahisht Chaunsa) that were collected from different geographical districts in Pakistan, including Multan, Vehari and Rahim Yar Khan, and exposed to cold storage (12° C) or ambient storage (33° C) until fruit ripening. Means within a cluster of bars that are followed by the same letter do not differ significantly according to the Tukey's test ($P \leq 0.05$). n=100

***Pathogenicity assay*:** All of the inoculated fungal pathogens produced SER symptoms as fruits ripened, no matter which

Table 2. Mean length (mm) of lesions in mango (cv. Samar Bahisht Chaunsa) fruit after artificial inoculation at stem end portion with one of six fungal pathogens associated with stem end rot (SER).

Treatments	Lesion diameter (mm)					
	Ambient storage			Cold storage		
	3 days	6 days	9days	7 days	14 days	21 days
L. theobromae	24.7 a	45.8 a	74.6 a	14.6 a	28.7 a	54.6 a
C. gloeosporioides	18.9 b	32.6 b	54.6 b	13.5 a	22.6 b	37.6 b
A. alternata	14.7 c	29.6 c	44.5 c	9.0 b	17.7 c	29.8 c
P. magiferae	6.7 e	14.0 d	24.4 d	5.4 d	11.6 e	18.6 e
C. mangiferae	8.4 d	13.2 d	19.2 e	7.5 c	13.1 d	27.4 d
B. cinerea	4.5 f	8.7 e	14.6 f	2.6 e	8.5 f	15.0 f
Control	0.0 g	1.2 f	2.0 g	0.0 f	0.6 g	1.0 g

Means in columns followed by the same letter differ significantly according to the Tukey's test ($P \leq 0.05$). Fruits were stored under ambient (33 ° C; during 9 days) or cold (12 ° C; during 21 days) conditions. n=45

pathogen was used as inoculum. The pathogens were re-isolated from infected fruit to fulfill Koch's postulates. For each pathogen, lesion diameter was increased with duration of the storage period in both storage environments. *L. theobromae* produced larger lesion diameters (74.6 mm in ambient storage; 54.65 mm in cold storage) than the other pathogens, whereas *B. cinerea* exhibited the smallest lesion diameters. Negligible symptoms were observed on control fruit (Table 2).

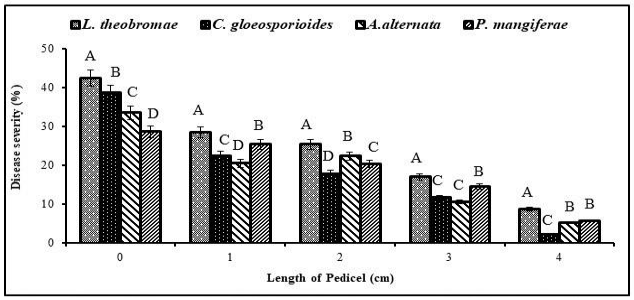


Figure 3. Effect of length of pedicel on SER development on artificially inoculated fruit incubated in a moist chamber after 96 hours. Means within a cluster of bars that are followed by the same letter do not differ significantly according to the Tukey's test ($P \leq 0.05$).n=45

***Relation between the pedicel length and disease severity*:** Fruit harvested without the pedicel attached developed SER symptoms much earlier than fruit with the pedicel attached. With increased pedicel length, there was a progressive delay in SER symptom appearance. SER symptoms appeared much later in fruit which had 4-cm-long pedicels compared to other lengths. A delay in ripening of fruit was also observed with increased pedicel length (Fig. 3).

***In vitro efficacy of fungicides*:** Nativo and Cabrio Top at 250 µg/ml reduced mycelial growth most effectively for four of the six fungi tested (*C. gloeosporioides, L. theobromae, P. mangiferae,* and *C. mangiferae*). All other fungicides were statistically equivalent in reducing mycelial growth. Mycelial growth of *A. alternata* was suppressed when Nativo and Cabrio Top were applied even at the relatively low dose of 150 µg/ml. Complete reduction of mycelial growth of *B. cinerea* was observed by application of Scholar at 250 µg/ml (Fig. 4).

***Impact of fungicides on SER disease development*:** Nativo and Cabrio Top applied at 250 µg/ml concentration gave good control and significantly reduced SER severity on mango fruit. Increased disease severity resulted in softening and rotting of fruit tissue. Maximum disease reduction (77.7 %) was observed in fruit treated with Nativo at 250 µg/ml, followed by Cabrio Top at the same concentration with 71.1% (Table 3).

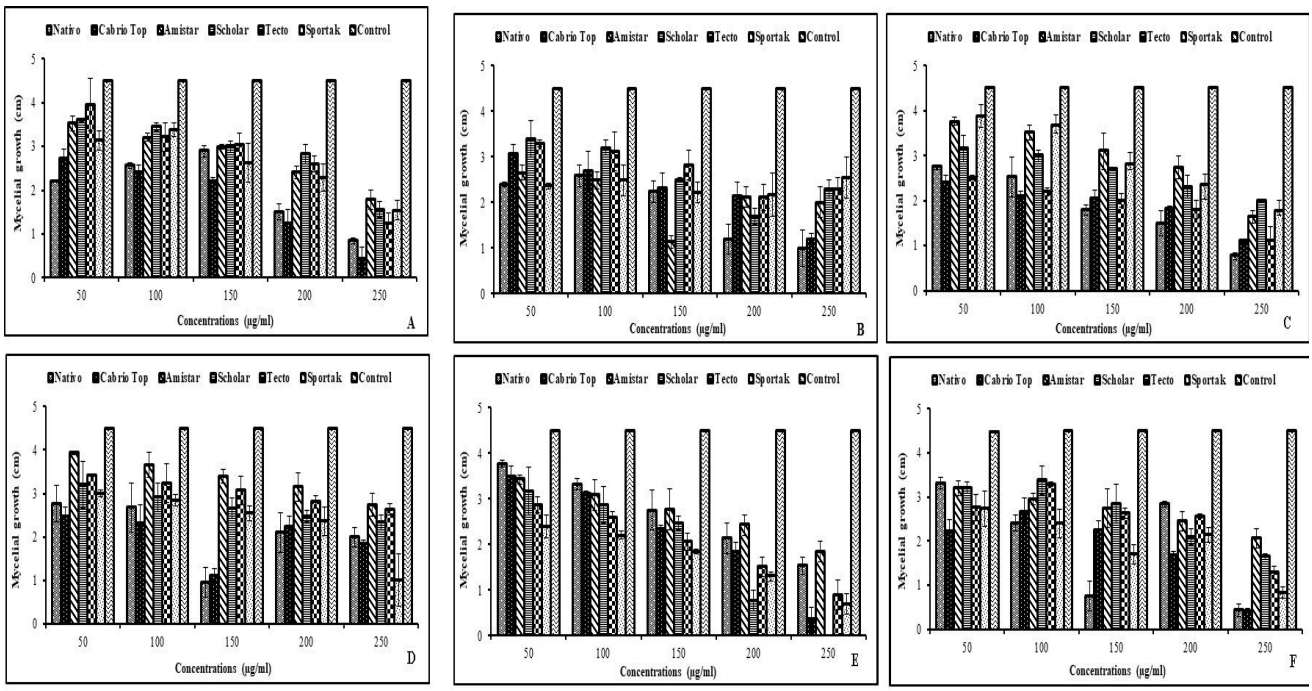


Figure 4. Effect of various concentrations of fungicides on mycelial growth of A) *L. theobromae*; B) *C. gloeosporioides*; C) *P. mangiferae*; D) *A. alternata*; E) *B. cinerea*; and F) *C. mangiferae* grown on PDA after 7 days of incubation at 25±2 ºC. Vertical bars represent ± S.E. of means and are invisible when the values are smaller than the symbol. n=3.

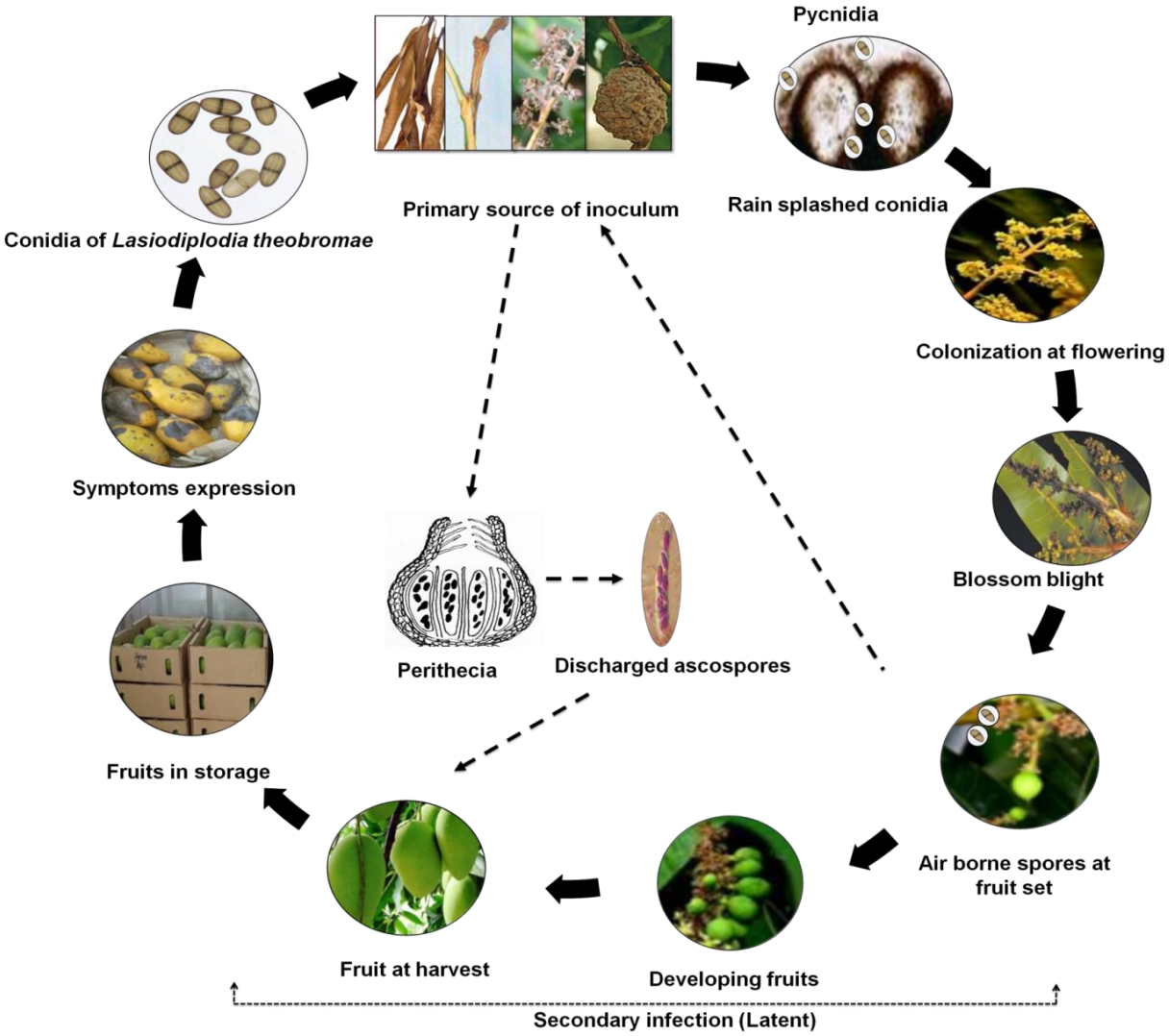
[1]**Table 3. Effect of fungicide concentration on stem end rot (SER) development of mango (cv. Samar Bahisht Chaunsa) after 9 days of incubation at 33° C.**

Treatments	Concentration (µg/ml)	Disease Severity*	*Disease Reduction (%)
Nativo	200	1.4 C	68.8
Nativo	250	1.0 D	77.7
Cabrio Top	200	1.8 B	60.0
Cabrio Top	250	1.3 C	71.1
Control	---	4.5 A	---

***=** Square root transformed disease severity (mean of three replications) was measured on a 1-5 qualitative scale. Means in a column followed by the same letter do not differ significantly according to the least significant difference test (P =0.05). n=45.
[1] * Data are in comparison to the non-treated control

DISCUSSION

In the present study, we determined that *L. theobromae* was the most aggressive SER pathogen in Pakistani mango production. This pathogen has been reported to affect a wide range of other tropical fruits, including avocado, cocoa, citrus, banana, yam, melon, and papaya (CABI, 2005; Wang *et al.*, 2007). However, little information has been published regarding etiology or epidemiology of stem end rot. Gaining a clearer understanding of the infection process can set the stage for development of more effective control strategies. We found that, under Pakistani production conditions, *L. theobromae* colonizes the inflorescence and peduncle several weeks after flowering and remains quiescent until fruit reach maturity and begin to ripen.



Supplementary figure 1. Disease cycle of stem end rot (SER) of mango, represented by *Lasiodiplodia theobromae*, the most prevalent SER pathogen in Pakistan.

SER pathogens were found throughout the dried twigs, branches, leaves and panicle samples observed, as has been reported for avocado and mango in Australia (Johnson and Hyde, 1992). The SER pathogens dominated the fungal populations recovered from the samples. In other studies, primary inoculum appeared to originate from dead twigs and bark (Sangchote, 1991; Govender, 2005; Cook *et al*., 2009). As the fruit starts to mature, hyphae of *L. theobromae* start to grow from the peduncle to the fruit after initial symptoms of blossom blight (Johnson, 2008; Ko *et al*., 2012). The exact mode of entry has not been conclusively determined; however, natural openings and sunburn-damaged epidermis could be routes of infection (Slippers *et al*., 2005). Our study revealed at least three routes of infection that could lead to SER: 1) infections at bloom stage that remain quiescent until fruit starts to ripen; 2) the pathogen may be endophytic within the twigs and branches of tree and then grow into the fruit; and 3) airborne spores of *L. theobromae* invade the pedicels and xylem, then grow into the maturing fruit.

Early colonization of floral remnants and fruit-pedicel tissues led to premature fruit drop; therefore, this is considered to be the primary infection phase for SER fungi in mature mango fruit. Sampling conducted near harvest revealed that colonization was confined to the outer tissue of the pedicel and the fruit surface adjacent to the stem end. This observation suggests that host-mediated restriction of colonization is responsible for the prevention of disease spread and delay of symptom development in fruit until harvest (Johnson *et al*., 1993). The incidence of *Botryosphaeria dothidea* in the peduncle and stem end tissues increased as the fruit matured. In avocado fruit, these levels rose more quickly in peduncle tissue than in stem end tissue (Peterson, 1978).

Pathogenicity assays verified that all pathogens isolated from mango fruit produced lesions on the stem end fruit. However, *L. theobromae* was found to be the most aggressive and virulent pathogen, with the largest lesions on inoculated fruit. Studies from Taiwan and Australia indicated that *L. theobromae* produced lesions that developed faster and reached a larger size than those of other SER pathogens when artificially inoculated into mango fruit (Rehana *et al*., 2014; Sakalidis *et al*., 2011; Ni *et al*., 2012).

Retaining a portion of the pedicel on harvested fruit reduced development of SER during the course of ripening, in comparison to fruit from which the pedicel was removed. Longer pedicels on harvested mangoes tended to suppress disease development, whereas fruit with no pedicel developed the highest SER incidence. These findings suggested that the pedicel plays a key role in SER resistance, and that retaining it on harvested fruit can protect these fruit from SER. Hassan (2006) investigated the development of anthracnose in sap-filled and de-sapped pedicels; he reported that SER was suppressed by retaining 2- to 3-cm-long pedicels on mango fruit. Karunanayake *et al*. (2015) studied anthracnose development in mango fruit with stalks with different lengths and concluded that longer pedicels slowed disease development. On the other hand, Johnson *et al*. (1993) reported considerably higher severity of SER symptoms in fruit having pedicels than in those lacking pedicels, and proposed that the retained pedicel would harbor endophytic SER pathogens longer than fruit without pedicels.

Storage diseases can destroy entire mango shipments if fruit are not treated with fungicide prior to transport (Mansour *et al*., 2006). Postharvest disease control in mango is usually attempted by adopting pre-harvest and post-harvest practices such as application of fungicides, strict orchard hygiene management, and temperature management during storage and shipping. Although several fungicides are labeled for pre- and post-harvest SER management, many pathogens have developed resistance to the most frequently used fungicides (Combrink *et al*., 2012). In the present study, therefore, we selected fungicides whose modes of action differ from those presently used in the Pakistani mango industry.

The *in vitro* fungicide assay results revealed that the quinone outside inhibitor (QoI) fungicides Nativo, Cabrio Top and Scholar were highly effective against SER-associated pathogens at higher concentrations (i.e., 150, 200 and 250 µg/mL). Excellent activity of QoI fungicides has been observed against SER of avocado and SER, soft brown rot and anthracnose of mango (Zhang, 2012; Swart *et al*., 2009; Zeming *et al*., 2003; Meijiao *et al*., 2004; Sundravadana *et al*., 2006, Amin *et al*., 2011).

The *in vivo* results supported the findings of the *in vitro* results. Nativo and Cabrio Top suppressed *in vitro* mycelial growth and SER development on inoculated fruit. Similar results have been obtained when mango fruit were artificially inoculated with *L. theobromae* inoculum and then treated with different fungicides (Swart *et al*., 2002; Rehana *et al*., 2014).

***Conclusion*:** *Lasiodiplodia theobromae* appeared to survive on diseased panicles, dead twigs and on leaf litter. Airborne inoculum was responsible for primary infections in inflorescence and developing fruit. Therefore, the hypothesis that removing tree litter from the orchard floor can reduce SER incidence merits testing in field experiments. Based on our results, it is recommended to retain a portion of the pedicel on harvested fruit in order to suppress SER during ripening. Nativo and Cabrio Top showed promise for control of SER and should be applied at early fruit development followed by postharvest treatments. Therefore, future strategies for controlling SER disease of mango should aim to interrupt the infection process in time to suppress SER pathogens and ultimately reduce disease incidence after ripening.

***Acknowledgment*:** Authors are thankful to ACIAR (Aus Aid) for providing financial support under "Mango value chain improvement project phase II". We are also grateful to Post-Harvest Research and Training Center, Institute of

Horticultural Sciences, University of Agriculture, Faisalabad for providing access to their cold storage facility.

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